Environmental Health Perspectives
Vol. 23, pp. 1-8, 1978

Field Concentrations and Persistence of Polybrominated Biphenyls in Soils and Solubility of PBB in Natural Waters

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Soil samples were collected from 28 fields which had received manure from Michigan's most highly contaminated dairy herds. The number of fields in each concentration range of PBB in soil were: 2, not detectable; 15, 0.0 to 8.0 ppb; 6, 14-102 ppb, and 5, 153 to 371 ppb. Plant tissue sampled from the 10 most highly contaminated fields contained no detectable PBB. No evidence of significant degradation of PBB was noted after 1 year incubation in soil. When ^{14}C hexabromobiphenyl and heptabromobiphenyl isomers were incubated in soil <0.2% of the ^{14}C was volatilized. Also gas chromatographic analysis of soil extracts showed no difference in recovery of the six major PBB isomers between sterilized and nonsterilized soil. Analysis of these extracts by thin layer chromatography and autoradiography showed no ^{14}C-PBB intermediates. Photodegradation products of the major hexa- and heptabromobiphenyl isomers showed more but still minor (~3%) biodegradation in soil. Much of the photodegradation products appeared bound to soil, since these products could not be extracted from soil. Photodegradation does not appear to be a significant fate of PBB in manures spread on fields since no change was noted in the relative concentrations of isomers in soil samples from our field survey. Studies with distilled, tap, river, and soil waters showed that PBB solubility was markedly influenced by water composition.

Introduction

Some unexpected soil pollution occurred in Michigan as a result of the accidental addition of an industrial flame retardant, FireMaster BP-6 (a mixture of polybrominated biphenyls, or PBBs), to livestock feed in place of magnesium oxide (*1–4*). This incident led to contamination of many Michigan farm soils through disposal of PBB-tainted manure, milk, feed, and dust cleanings from buildings, etc. This contamination posed questions about the fate of PBBs in soils, including the potential for recycling PBB to farm animals, wildlife and man.

In our preliminary greenhouse studies, we found no PBB in the tops of orchard grass and carrots grown in soils amended with high concentrations of PBB (*5*). Some traces (20–40 ppb) of PBB were found associated with carrot roots. More recently, additional laboratory and greenhouse studies have confirmed that PBB was not transferred from contaminated soil to plant tops (*6*). Therefore, significant contamination of clean animals by recycling PBB through feed crops is unlikely.

We have also investigated the degradation (*5*) and retention (*7*) of PBBs in soils. Initial degradation studies showed PBBs to be extremely persistent, with only one pentabromobiphenyl isomer showing any significant disappearance after 24 weeks of incubation in soil. Leaching and adsorption experiments with the major hexabromobiphenyl isomer in PBB suggested that PBBs should not leach below the depth of incorporation in surface soils.

This study was undertaken to determine the concentration of PBBs in Michigan soils to which PBB contaminated manures had been applied, to further evaluate the degradation of PBBs in soils by using ^{14}C-PBB and to determine how the solubility of PBB was influenced by the composition of different natural waters.

Materials and Methods

Field Samples

Thirty Michigan farms were selected from a list provided by the Michigan Department of Agriculture of dairy herds which had >5 ppm PBB in their milk (on a fat basis). One poultry farm and 26 dairy farms were visited in April 1976. Fields which had

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received the greatest quantity of contaminated manure 2–3 years earlier were sampled. A 2.5-cm diameter soil probe was used to collect soil from the surface 20 cm. A soil core was taken from 20 random locations in the field; the soil from the cores was thoroughly mixed, and two duplicate subsamples of 50 g each were taken for extraction and analysis. Additional samples were also taken on some farms from milk dumping areas, feedlots, holding lots, manure piles not yet spread, and gardens.

In August 1976, the 10 manured fields which were found to have the highest PBB concentrations were revisited. Plants were sampled from 15 random locations within each field and a soil core was taken from around the roots of the sampled plants. The plant and soil samples were a composite for each field. Two corn fields which had not been contaminated with PBB were sampled similarly to serve as controls in the event that some indigenous plant compounds produced peaks with retention times similar to PBB. All soil samples were air-dried and passed through a 2-mm sieve before extraction; plant samples were refrigerated until extracted for analysis.

PBB Substrates for Soil Incubation

^{14}C-PBB (lot 872-244) was synthesized and purified according to our specifications by New England Nuclear Corp., Boston, Mass. The product contained the two major isomers of FireMaster BP-6, approximately 65% 2,2′,4,4′,5,5′-hexabromobiphenyl (*5*) and 35% 2,2′,3,4,4′,5,5′-heptabromobiphenyl (*8*). Identity was confirmed by finding identical retention times to standards run (*5*) on Dexsil and SE-30 gas chromatographic columns. No other components were detected by gas chromatography. The specific activity was 14.1 μCi/mg. This ^{14}C-PBB was mixed 1:1 with FireMaster BP-6, lot 6244A, in ethanol to achieve a final concentration of 21 ppm for addition to soil.

Since PBB is easily photodegraded by sunlight, products from ultraviolet photolysis of PBB were also added to soil. The photolyzed ^{14}C-PBB solution was prepared by irradiating a mixture of 50 ppm FireMaster BP-6 and 9.4 ppm of ^{14}C-PBB in hexane for 30 min in a Hanovia photolysis vessel (Ace Glass Incorporated, Vineland, N. J.). The exposed solution turned light yellow and showed some precipitate. The clear fraction was decanted and concentrated to 25 ml. This photolyzed material was diluted in ethanol (14×) to achieve a radioactive concentration similar to the nonphotolyzed sample before addition to soil.

Soil Incubation

A 25-g portion of Brookston sandy loam soil which had passed through a 2-mm sieve was placed in a 250-ml Erlenmeyer flask. The soil was not allowed to air dry. Half of the flasks were sterilized as follows prior to addition of the two PBB substrates. Each of these flasks received 2 ml water and, 1 hr later, 3 ml of propylene oxide was distributed dropwise on the soil surface. The flasks were covered with foam plugs and placed in a hood for 3 days to allow the sterilant to volatilize. Sterilization was confirmed by inoculating a loopfull of soil on a trypticase agar slant and finding no growth after one week of incubation.

Sterilized and nonsterilized flasks received either 1 ml of the filter sterilized ^{14}C-PBB solution or 1 ml of the ultraviolet irradiated ^{14}C-PBB solution distributed dropwise on the soil. All flasks were then sealed with a sterilized rubber stopper. A 2-ml sterilized plastic beaker which contained 1 ml of 1*N* NaOH was suspended above the soil to trap respired $^{14}CO_2$. All samples were incubated in the dark at 28 ± 1°C for 0, 3, 6, 9, or 12 months. The $^{14}CO_2$ traps were counted periodically as described elsewhere (*9*). Four replicates of each treatment were extracted for ^{14}C and GLC analysis after each incubation period.

Solubility of PBB in Natural Waters

Five waters varying in inorganic and organic content were selected for preliminary studies to determine whether composition significantly influenced PBB solubility. These waters were: (a) distilled water, glass distilled from water in which the trace organic matter had been removed by permanganate oxidation; (b) tap water, from cold water tap in laboratory; (c) Red Cedar water, collected from the Red Cedar River which flows through the MSU campus; (d) Spinks water, extracted from a Spinks loamy sand soil (1.10% organic C); and (e) Brookston water, extracted from a Brookston sandy loam soil (3.14% organic C). Water was extracted from the two soils by shaking 100 g soil with 1 liter distilled water, allowing the mixture to settle, and filtering the supernatant through Whatman GF/C glass fiber filters. These waters had the following pH and specific conductance (in μmhos), respectively: distilled (6.3, 2); tap (8.0, 619); Red Cedar (8.3, 681); Spinks (6.3, 68); and Brookston (7.1, 61).

To 1 liter of each water was added 1 ml of an acetone standard containing 1000 ppm of FireMaster BP-6 and 1 ml of acetone containing 13.9 μg of

the ^{14}C-PBB isomers described above. The PBB-treated water was shaken on a rotary shaker at 150 rpm for 24 hr. Following shaking, an aliquot of each water was centrifuged at 10,000*g* in a stainless steel tube for 3 hr. The remaining portion of each water and the centrifuged water in their tubes were placed in a constant temperature chamber (28 ± 1°C) to stand undisturbed.

The centrifuged (10,000*g*) and uncentrifuged (1*g*) waters were sampled periodically by inserting a pipet approximately 1 cm below the surface and removing a 1-ml aliquot for PBB analysis by liquid scintillation counting. Two aliquots were taken at each sampling time for duplicate analyses.

Analyses

PBB was extracted from soil with three 40-ml portions of hexane–acetone (9:1, v/v). This extraction mixture was found to be better than the benzene-isopropanol mixture used previously (*5*), since it reduced the amount of soil organic matter extracted yet recovered as much of the PBB. Before extraction the soil samples were moistened and vibrated on a minishaker to ensure moisture uniformity. The extraction procedure was the same as used previously except that the soil-solvent mixture was allowed to stand in the flask for 1 hr rather than overnight. Plant samples were macerated, extracted with hexane–acetone, and the extract cleaned-up by passage through Florisil as described by Chou et al. (*6*).

Concentrated soil and plant extracts were analyzed on a Beckman GC-5 gas chromatograph equipped with an electron capture detector (*7*) and a 2% SE-30 on 100/120 mesh Gas-Chrom Q column operated at 250°C with carrier flow of 40 ml/min. Minimum detectable PBB concentrations were 0.1 ppb (wt/dry wt soil) for soil and 0.3 ppb (wt/plant wet wt) for plants.

The ^{14}C was assayed by liquid scintillation counting. A 1-ml aliquot of water or concentrated hexane–acetone extract was counted in 15 ml Bray's solution (*10*). The $^{14}CO_2$ trapped in 1*N* NaOH was counted in Bray's solution containing 4% Cab-O-Sil. All counts were corrected for quenching by external standardization and for machine efficiency.

For thin-layer chromatographic analysis, the hexane–acetone extracts were concentrated to 0.5 ml; 10 μl was spotted on 250 μ precoated Kieselguhr G plates (Analtech, Inc.) pretreated with paraffin according to the method of de Vos and Peet (*11*). The precoated plate was soaked in petroleum ether (bp 40–60°C) containing 8% of liquid paraffin until the adsorbent layer was saturated with the solvent. The plates were developed in paraffin-saturated acetonitrile–acetone–methanol–water (20:9:20:1, v/v). After development the plates were examined for ^{14}C spots by autoradiography with Kodak No-Screen X-ray film, exposure time 10 days.

Results and Discussion

Field Samples

The PBB concentrations found in manured soils and in miscellaneous samples from highly contaminated farms in Michigan are shown in Tables 1 and 2. Concentrations in cropped fields which had received PBB-contaminated manure ranged from not detectable to almost 200 ppb for the April sampling. The presence of PBBs was confirmed in most samples by the presence of the other isomer peaks in the chromatogram. For samples with PBB concentrations too low to see the other peaks, the presence of PBB was confirmed by the ultraviolet sensitivity method of Erney (*12*). Since these cropped field samples came from the most highly contaminated

Table 1. PBB concentrations in soils and plants of fields which received PBB contaminated manure from Michigan's most highly contaminated dairy herds.

		PBB concentration, ppb[a]		
		Soil samples		Plant
Farm code	Crop	April 1976	August 1976[b]	Tissue[b]
T	Corn	178	371	ND[c]
U	Alfalfa	170	297	ND
N	Corn	167	173	ND
Z (4 A)	Corn	15	64	ND
Z (20 A)	Corn	153	285	ND
W	Corn	102	63	ND
M	Sudax	98	69	ND
P	Corn	37	224[d]	ND
F	Corn	34	9.1	ND
K	Alfalfa	24	64	ND
D		14		
C,G,J,L,O,R,S,X,Y,		8.0 to 1.0		
AA,B,E,H,I,Q		0.9 to 0.1		
A,V		ND[c]		
Control farm No. 1	Corn		ND	ND
Control farm No. 2	Corn		ND	ND

[a] On dry (soil) or wet (plant) weight basis; from all field studies the average coefficient of variation for subsampling of composite samples and analysis was 20%.

[b] Soil samples taken from root environment of the sampled plants.

[c] Not detectable, detection limit 0.1 ppb for soils and 0.3 ppb for plant tissue.

[d] Contaminated manure had been applied to field prior to second sampling.

Table 2. PBB concentrations in manure samples and soils from miscellaneous areas on highly contaminated dairy farms in Michigan.

Farm code	Source of manure or soil samples	PBB concentration, ppb[a]
T	Manure pile	1650
Z	Manure pile	1340
Y	Manure crusts, free stall barn	28.0
X	Manure crusts in field	1.6
M	Milk dump area	940
AA	Milk dump area	465
F	Milk dump area	335
D	Field receiving milk	4.2
U	Holding lot	1570
P	Holding lot	143
M	Holding lot	15.6
K	Feedlot	790
N	Area receiving feedlot runoff	60.0
W	Feedlot	6.9
R	Ditch bank containing feedlot soil	3.9
N	Garden	35.3
J	Garden	11.0
H	Garden	0.6

[a] On dry weight basis; the average coefficient of variation for subsampling of composite samples and analysis was 20%.

farms in Michigan, we expect the great majority of farm soils which were contaminated to have <10 ppb of PBB with most having "nondetectable" levels.

Several miscellaneous areas had considerably higher PBB levels (Table 2), particularly where contaminated milk had been dumped and in feedlots and holding lots. Two manure piles, which had never been spread, were sampled and found to contain approximately 1.5 ppm of PBB. These manure levels probably represent some of the highest concentrations which occurred, since these two farms had highly contaminated herds.

No PBB was detected in plant tissue sampled from the ten fields which contained the highest PBB concentrations (Table 1). Plant samples included seven corn, two alfalfa, and one sudax. The second soil sampling illustrates the variability one can encounter when sampling field soils for trace quantities of an added chemical. For example, one does not need to encounter much PBB-contaminated manure residue in a random soil sample to significantly change the resulting PBB concentration. Therefore, one could expect to find some variability when resampling PBB contaminated fields.

Soil Degradation

The recovery of the two major PBB isomer (hexa- and heptabromobiphenyls after various periods of incubation in Brookston sterile and nonsterile soil is shown in Table 3. Whether analyzed by ^{14}C or gas chromatography the data clearly show no detectable biodegradation after 1 year. It is also striking that the ^{14}C and GLC analysis of each peak showed virtually identical quantities on each date. GLC data for the recovery of non-^{14}C-labeled isomers is shown in Table 4. The only possible evidence for degradation is for the 5-Br-I isomer, which does not confirm our previous suggestion which indicated that only the 5-Br-II isomer might have been subject to slight biodegradation (*5*). Of interest is the significant loss of extractability with time of all isomers (Tables 3 and 4) in the sterile as well as nonsterile treatments.

Total ^{14}C collected in 1*N* NaOH is shown in Table 5. Though slightly more label was trapped from nonsterilized soil, the amount of additional label volatilized in the viable treatment was insignificant. Soils incubated with photodegradation products of ^{14}C-PBB showed enhanced though still only minor conversion to $^{14}CO_2$. Products created by the photodecomposition of PBB by ultraviolet irradiation are apparently more volatile than nonirradiated PBB as shown by the increase in ^{14}C collected from sterilized soils, especially at the first sampling. The microbial activity which occurred in the nonsterilized treatments appeared to increase the amount of ^{14}C volatilized, suggesting that some of the ^{14}C-degradation products of ultraviolet irradiation may be metabolized. PBB irradiated by ultraviolet light forms lower brominated biphenyls (*13*). The persistence of PBBs and the possible degradation of the ultraviolet products is consistent with the evidence reported for PCBs which shows that the more heavily chlorinated moieties (penta or greater) are resistant to degradation though many lesser chlorinated biphenyls are metabolized (*14–16*).

The extractability of the ^{14}C photodegraded PBB is shown in Table 6. Much of the added material was not extracted, in marked contrast to PBB (Table 3). Apparently the photodegraded products are more reactive with the soil organic matter thereby preventing their extraction. The early loss of extractability (0 and 3 months) is supportive of this explanation.

Since partially degraded PBB would not yield $^{14}CO_2$, we also examined soil extracts for other ^{14}C products by TLC-autoradiography (Fig. 1). The TLC system used was shown to separate the isomers of FireMaster (detection by ultraviolet). No intermediates of PBB degradation could be found. From the autoradiogram it is apparent that the two ^{14}C-PBB isomers were the only ^{14}C products in the soil. The autoradiogram of ultraviolet-treated ^{14}C-PBB extract from soil (Fig. 2) also showed little

Table 3. Recovery of the two major isomers (hexa- and heptabromobiphenyls) after incubation of 0.8 ppm of these labeled isomers in Brookston sandy loam soil.[a]

Incubation time, months	Percent of the original recovered[b]					
	^{14}C extracted[c]		6-Br-I by GLC[d]		7-Br-I by GLC	
	S	NS	S	NS	S	NS
0	93.3	92.7	91.5	90.9	89.6	89.1
3	86.8	85.3	87.0	86.2	85.3	82.9
6	88.5	87.3	89.5	85.4	87.1	86.2
9	85.5	84.8	83.8	87.8	82.2	83.9
12	84.7	84.3	85.6	84.9	82.1	83.8

[a] S = sterilized soil; NS = nonsterilized soil.
[b] Each value is the mean of four replicates.
[c] % of added ^{14}C recovered.
[d] The isomer abbreviation used in the tables and text is the same as we defined previously (*5*), e.g., 5-Br-I = the first major pentabromobiphenyl isomer on the chromatogram.

Table 4. Recovery of non-^{14}C-isomers after incubation of 0.4 ppm PBB in Brookston soil.[a]

Incubation time, months	Percent of original recovered[b]							
	5-Br-I		5-Br-II		6-Br-II		6-Br-III	
	S	NS	S	NS	S	NS	S	NS
0	87.1	86.3	89.4	88.0	83.4	84.6	79.4	80.7
3	80.3	79.2	78.2	76.3	80.5	80.0	74.7	73.7
6	77.4	79.5	75.7	73.7	81.3	80.6	71.3	70.6
9	78.9	75.4	76.1	74.5	82.5	79.3	72.1	70.2
12	77.6	71.4	72.7	70.3	79.9	78.5	70.7	71.4

[a] S = sterilized soil; NS = nonsterilized soil.
[b] Each value is the mean of four replicates.

Table 5. Percent of original ^{14}C volatilized from ^{14}C-PBB amended Brookston soil.

Treatment	Substrate	Percent of original ^{14}C trapped in NaOH[a]			
		3 months	6 months	9 months	12 months
Sterilized	^{14}C-PBB	0.03	0.07	0.10	0.12
Nonsterilized	^{14}C-PBB	0.04	0.08	0.14	0.17
Sterilized	UV-^{14}C-PBB	4.43	5.72	6.17	6.76
Nonsterilized	UV-^{14}C-PBB	6.24	7.20	7.96	9.94

[a] Each value is the mean of four replicates.

Table 6. Percent of added ^{14}C extracted from ultraviolet-irradiated ^{14}C-PBB added to Brookston soil.

Treatment	Percent of ^{14}C recovered in hexane-acetone extracts[a]				
	0 month	3 months	6 months	9 months	12 months
Sterilized	76.1	36.5	34.1	32.9	30.6
Nonsterilized	72.1	35.4	32.0	30.6	28.9

[a] Each value is the mean of four replicates.

difference between the original amendment and the extract from the soil incubation. The marked change in the PBB isomers due to the ultraviolet irradiation is clearly shown by Figure 2. Virtually none of the original ^{14}C-labeled isomers remains. Most of the label is at the solvent front, the position where lesser brominated forms would be expected to run. The label at the origin and the streaking indicates that some of the ^{14}C-PBB products may have complexed with the soil organic matter which is consistent with the low extraction efficiency of these photolyzed products.

We examined the fate of the photodegraded PBB since the higher brominated forms are readily degraded by ultraviolet light to lesser brominated forms which could be more toxic. It can be reasoned that the PBB in contaminated manures spread on soil surfaces might show some photo-

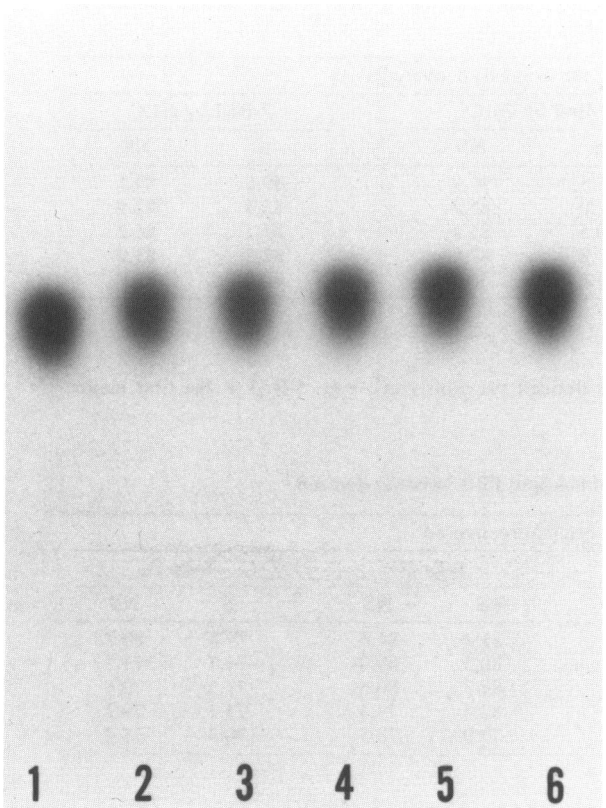

FIGURE 1. Autoradiogram of TLC plate showing ^{14}C-PBB standard and ^{14}C in extracts after incubation in soil: (1, 6) ^{14}C-PBB standard; (2, 3) extracts from sterilized soil after 6 and 12 months incubation, respectively; (4, 5) extracts from nonsterilized soil after 6 and 12 months incubation, respectively.

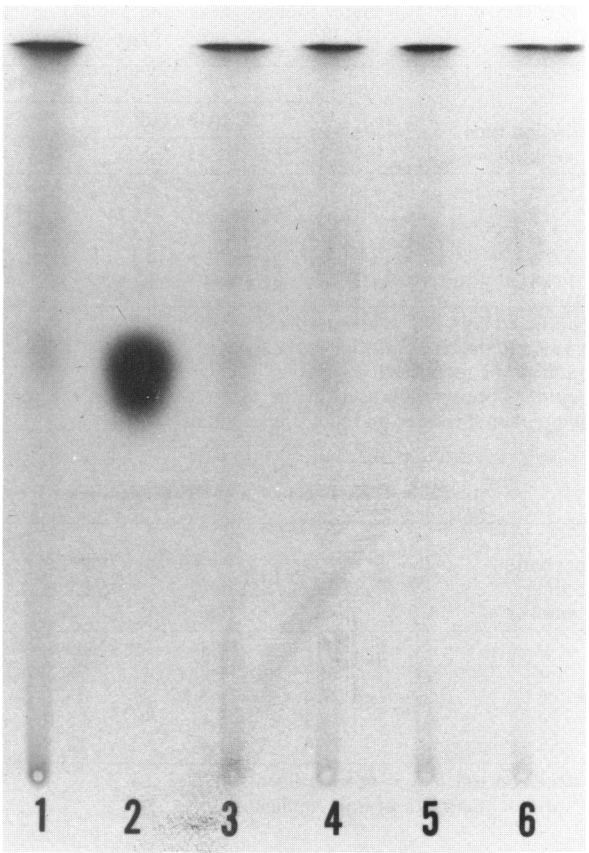

FIGURE 2. Autoradiogram of TLC plate showing ^{14}C-PBB standard, ultraviolet-irradiated ^{14}C-PBB, and ^{14}C in extracts after incubation in soil: (1) ultraviolet-irradiated ^{14}C-PBB standard; (2) ^{14}C-PBB standard; (3, 4) extracts from sterilized soil after 6 and 12 months incubation, respectively, (5, 6) extracts from nonsterilized soil after 6 and 12 months incubation, respectively.

degradation. However, this does not appear to have been a significant reaction in the field since the ratios of peaks 5-Br-I:6-Br-I:7-Br found in most of the soils surveyed (2–5:100:40–50) did not vary significantly from the PBB standard (3:100:43). Other types of samples surveyed (Table 2) showed no striking differences in peak ratios except for the samples from the milk dump areas where the major heptabromo isomer (7-Br) was markedly reduced or absent. The above findings are consistent with metabolism of PBB in cows; most of the PBB is directly excreted in the manure, thus resulting in little change in isomer composition, while the PBB reaching the milk is reduced in the heptabromo isomer content (*17*).

PBB Concentrations in Water

The mobility of a chemical like PBB in soils will largely be governed by its solubility in water and its adsorption, or interaction, with soil particles. Figure 3 shows data from some preliminary studies comparing the concentrations of PBB which stayed in solution after the addition of 14 ppb PBB to laboratory and naturally-occurring waters. Following gravitational settling, significant differences were obtained between the resulting PBB concentrations in tap and distilled waters (10–20 ppb) compared to 100–200 ppb, about 200 ppb, and about 400 ppb for the Red Cedar, Spinks, and Brookston waters, respectively. Fine particulates and water-soluble organics present in the latter three waters no doubt contributed to the higher PBB concentrations which remained in solution or in suspension, with time. These materials have been shown to result in higher, gravitationally stable concentrations of DDT in water (*18*), another highly water-insoluble compound.

However, fine particulates in suspension apparently do not dominate PBB concentrations in natural waters. Data for the centrifuged (10,000*g*) portions of these waters indicate that PBB removed

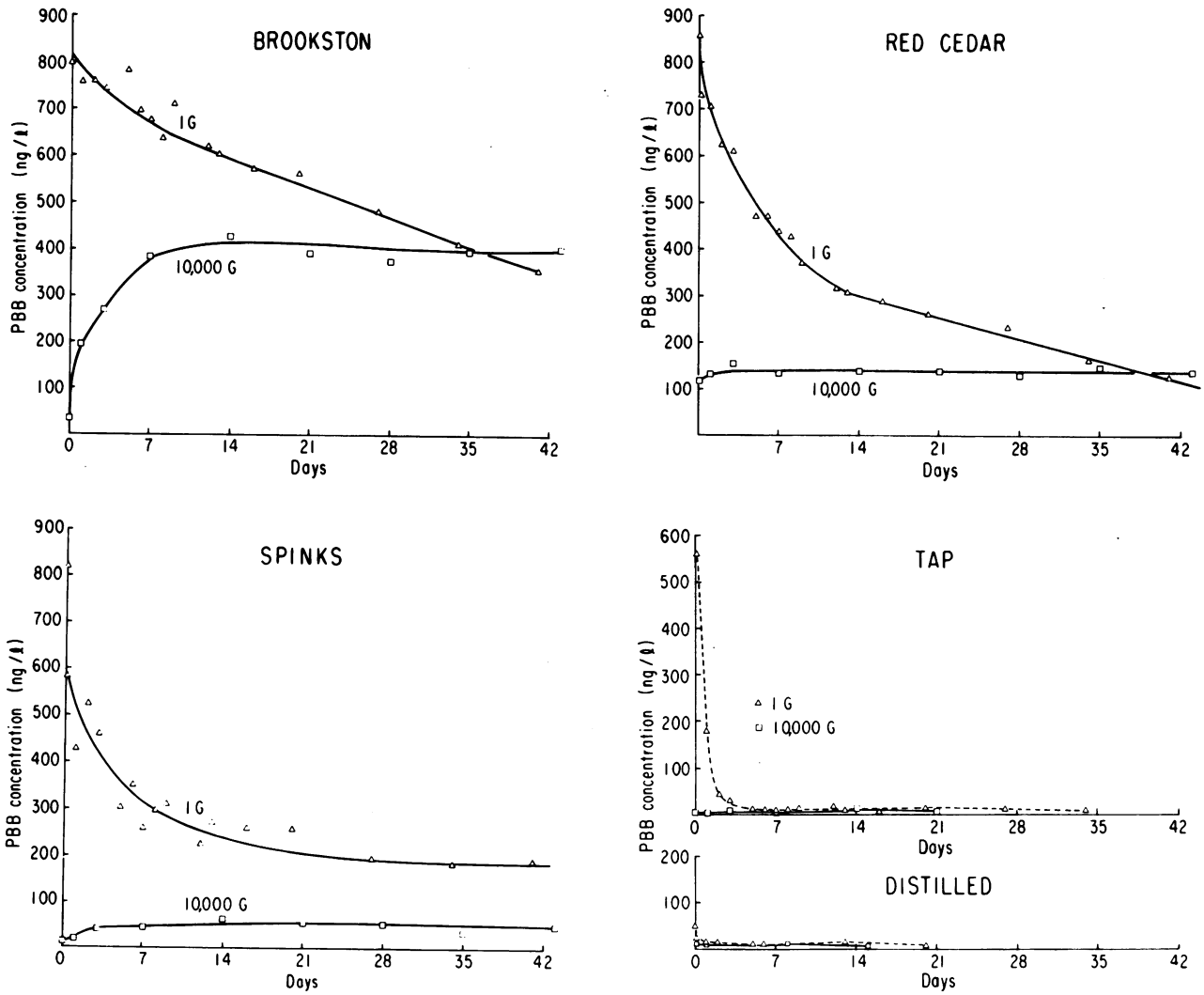

FIGURE 3. PBB solubility in different laboratory and natural waters: (Δ) PBB remaining in solution against the force of gravity; (□) PBB in solution after centrifuging at 10,000*g*.

by centrifugation will come back into solution, or suspension, to varying degrees depending on the nature of the water.

Soluble organics in solution likely have a major effect on the amount of PBB remaining in each water with time. The Brookston water extract could be expected to contain the largest quantities of these organics, since this soil has a high organic matter content. Also, the precipitation of humic acid and other organic compounds were visually observed with time for all three of the natural waters which coincides with the corresponding loss of PBB from solution for these waters.

The environmental implications of the different PBB solubilities observed (Fig. 3) should depend on the particular circumstances. For example, with soil concentrations of PBB such as those reported in Table 1, the attraction of PBB by soil organic matter particles would likely be so dominant that little, if any, PBB would reside in and move with the soil water. However, where high quantities of solid PBB are present like in a landfill, the organic content of the water passing through would likely effect PBB mobility.

Summary

Since PBBs are not degraded, are not leached, are not taken up by plants (*5*), and are not volatilized (because of their low vapor pressure), we expect PBB to be a rather permanent component of contaminated soils. Because PBB is bound to soil, wherever contaminated soil moves, whether by

wind or water erosion or animal ingestion and migration, traces of PBB (if present) can be expected to follow. However, because of the low PBB concentrations in soil and the low quantities of soils moved by these means, any serious contamination of animals, wildlife or aquatic environments seems unlikely. As a precautionary measure, areas with much higher levels of contamination, such as manure piles, milk disposal areas and feedlots should be managed to minimize runoff, erosion, and animal contacts.

We thank Marilyn Maternoski for technical assistance during the course of this study. The research was supported in part by Biomedical Sciences Support Grants, NIH. Published as Journal Article No. 8364 of the Michigan Agricultural Experiment Station.

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