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Absence of Plant Uptake and Translocation of Polybrominated Biphenyls (PBBs)

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Studies of polybrominated biphenyl (PBB) uptake by plants have been conducted in hydroponic solutions and in greenhouse experiments with soil. Autoradiograms of corn and soybean seedlings grown in hydroponic solutions showed no translocation of ^{14}C-PBB from ^{14}C-PBB-treated solutions to plant tops or within the leaf from ^{14}C-PBB-treated spots on the upper leaf surface. A significant portion of the ^{14}C-PBB associated with the roots was removed when the roots were dipped in acetone. Three root crops (radishes, carrots, and onions) were grown in two soils, each treated with a mixture of FireMaster BP-6 (PBB) and ^{14}C-PBB to achieve final concentrations of 100 ppm and 100 ppb. All roots showed more PBB when grown in the soil with the lower clay and organic matter content than they did when grown in the soil with more clay and organic matter. In the latter soil (clay loam) no PBB was detected in any roots from the 100 ppb treatment. More PBB was associated with roots of carrot than of radish or onion. Corn leaf whorls containing dust from a PBB contaminated soil and washed radishes from a heavily contaminated garden showed no PBB.

Introduction

PBBs have entered Michigan soils from manures of farm animals fed PBB, from disposal of milk, carcasses, and other produce, and from effluent and dust discharges of the PBB manufacturing plant. Since most insoluble halogenated hydrocarbons are not taken up or translocated by plants, one would predict PBB to behave similarly. However, because of the importance of this conclusion to the quality of future Michigan food, we felt it prudent to thoroughly investigate this question.

In our preliminary greenhouse studies we had shown no PBB in tops of orchard grass and carrots grown in soils amended with high levels of PBB (*1*). We did, however, find traces (20–40 ppb) of PBB associated with carrot roots. We also found no detectable PBB in plants collected from the most highly contaminated Michigan fields (*2*).

This study was undertaken to evaluate PBB translocation from roots and leaves by using ^{14}C-PBB, to more thoroughly evaluate the association of PBB with root crops (carrots, radishes, and onions), and to determine if PBB could be found in root crops grown in a PBB contaminated garden or in dust on plant tops.

Materials and Methods

Corn and soybean seedlings were grown in a coarse sand–vermiculite mixture with Hoagland's solution. At 3 weeks of age the seedlings were removed, the adhering particles washed off the roots, and the plants placed in Hoagland's No. 1 hydroponic solution. For root uptake studies, ^{14}C-PBB (hexa- and heptabromobiphenyl isomers only) (*2*) was added to the hydroponic solution to achieve a final PBB concentration of 100 ppb (1.41 μCi/l). To determine translocation from leaves, a 0.5 μl water drop containing 17 μg ^{14}C-PBB (0.25 μCi) was spotted on the upper surface of a mature leaf. In the first trial, plants were exposed to PBB for 3 and 7 days, while in the second trial, plants were exposed for 4 and 8 days.

After the indicated exposure periods the plants were removed from the hydroponic solutions and

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the roots were washed by dipping them into distilled water. In the second trial, part of the plant roots that were exposed to ^{14}C-PBB were quickly dipped five times into an acetone bath and then washed with distilled water. All plants were then immediately frozen with crushed Dry Ice, freeze-dried, and later examined by autoradiography. Portion of the soybean and corn leaves and roots were macerated and the ^{14}C quantitated by liquid scintillation counting.

Radishes, carrots, and onions were grown in the greenhouse on Brookston clay loam (3.27% organic C) and Spinks loamy sand (1.31% organic C) soils amended with a mixture of 100 ppm of FireMaster BP-6 and 50 ppb ^{14}C-PBB (0.68 μCi/kg soil). The soil was prepared by mixing 4.9 g of FireMaster BP-6 and 2.35 mg ^{14}C-PBB (33.1 μCi) in 100 ml acetone with 300 g of air-dried soil in a 500 ml brown glass bottle. The soil was gently blown with N_2 to remove excess acetone then mixed in a twin shell dry blender overnight. The PBB-treated soil was diluted with 48.7 kg of untreated soil in a mixer overnight to achieve the desired concentration of 100 ppm. A 48-g portion of 100 ppm soil was further diluted with 48 kg of untreated soil to achieve the 100 ppb concentration. From our previous experience this procedure gave a satisfactory distribution of PBB in soil.

PBB-amended soil (5 kg) was placed in a plastic container lined with a polyethylene bag. The radish, carrot, and onion seeds were planted approximately 1.25 cm below the soil surface and nutrient solutions were added as needed. The moisture levels of the soils were maintained at 29 and 13% (approximately field capacity) for the Brookston and Spinks soils, respectively, by weighing the container and adding the required amount of distilled water daily. After 6, 9, and 10 weeks the radishes, carrots, and onions, respectively, were carefully removed from the container. The edible roots or bulbs were washed with tap water, then dried with paper towels. A few of the plants grown on the 100 ppm PBB-treated soils were immediately frozen with liquid nitrogen, freeze-dried, and later examined by autoradiography.

Plant roots were cut into small pieces and extracted with hexane–acetone (1:1 v/v) in a Waring Blendor equipped with a glass mixing container. Three successive 100 ml portions of hexane–acetone were used to extract each plant sample and then combined. The hexane–acetone was then dried with anhydrous Na_2SO_4 and concentrated to 10 ml on rotary flash-evaporator. This concentrate was passed through a Florisil column to remove interfering components. The PBB was eluted with 200 ml benzene-hexane (1:1, v/v).

Concentrated extracts from the above samples were analyzed on a Beckman GC-5 gas chromatograph equipped with an electron-capture detector (*3*) and a 2% SE-30 on 100/120 mesh Gas-Chrom Q column operated at 250°C with a carrier flow of 40 ml/min. Minimum detectable PBB concentration in plant material was 0.3 ppb on a wet weight basis.

Since dust on leaf surfaces might result in PBB contamination of plant tops, corn leaf whorls, which commonly show a concentration of dust, were collected from a corn field which had a high concentration of PBB in the soil (102 ppb). Approximately 5-cm sections of leaves, including the whorl, were cut from the lower leaves of stalks, and 50 g of the plant tissue plus dust was extracted and analyzed as above.

In our survey of PBB concentrations in Michigan field soils (*2*), we found a garden planted in the contaminated field which contained the highest PBB concentration encountered (371 ppb). This garden was also heavily amended with and located near a highly contaminated manure pile (1650 ppb). From this garden, 50 g of radishes were collected, washed, extracted and analyzed as above.

Results and Discussion

The hydroponic study and greenhouse experiment were designed to favor the greatest uptake of PBB. Corn and soybeans were selected for the hydroponic studies since both are major crops in Michigan. The results of the ^{14}C uptake studies are shown in Figures 1-4. Autoradiograms of corn and soybean seedlings grown in the presence of ^{14}C-PBB showed no translocation of PBBs. PBB was found concentrated at the roots, but no PBB was translocated to the plant tops. This was confirmed by liquid scintillation counting of macerated plant tissue. Due to the insolubility of PBB in water, we expected that the PBB would be primarily associated with the root surface. A significant portion of the ^{14}C-PBB was removed when the roots were dipped in acetone (see Figs. 2 and 4), as shown by the lighter autoradiograms. The autoradiograms also showed no movement of PBB within the leaf from the site of ^{14}C-PBB application.

Three root crops, radishes, carrots, and onions, were grown in two soils which differed greatly in organic matter and clay content. The extent of PBB uptake by edible roots or bulbs was determined by autoradiography and by gas chromatographic analysis. No PBB uptake was shown on autoradiograms; however we did find trace amounts of PBB associated with the edible portions of each crop by gas chromatography (Table 1), because of the greater sensitivity of the latter method. The trace

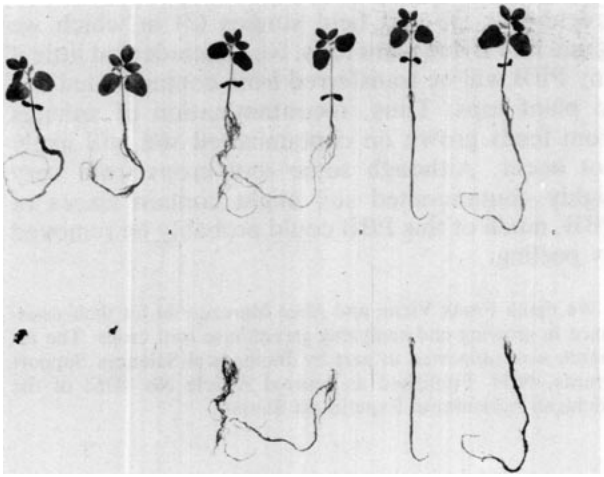

FIGURE 1. Soybean plants (top) and autoradiographs (bottom). (*A*) 7 days after leaves were treated with ^{14}C-PBB; (*B*, *C*) after root exposure to ^{14}C-PBB for 3 and 7 days, respectively.

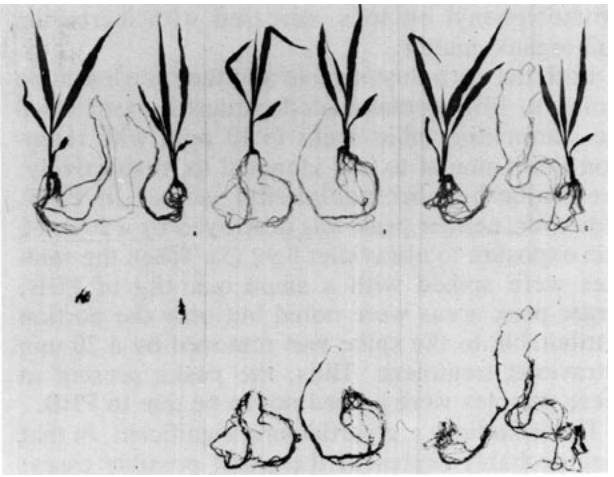

FIGURE 3. Corn plants (top) and autoradiographs (bottom): (*A*) 7 days after leaves were treated with ^{14}C-PBB; (*B*, *C*) after root exposure to ^{14}C-PBB for 3 and 7 days, respectively.

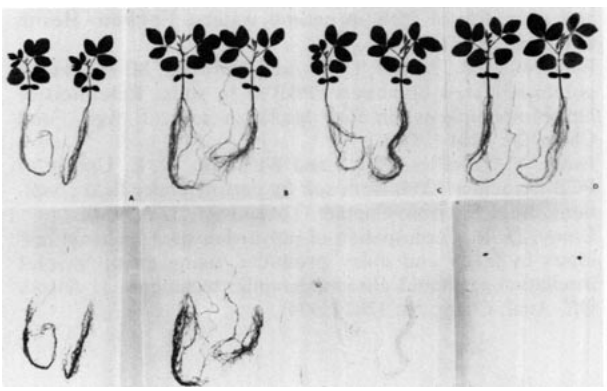

FIGURE 2. Soybean plants (top) and autoradiographs (bottom): (*A*, *B*) are after root exposure to ^{14}C-PBB for 4 and 8 days, respectively; (*C*) identical to (A) except that roots were dipped in acetone prior to autoradiography; (*D*) 8 days after leaves were treated with ^{14}C-PBB.

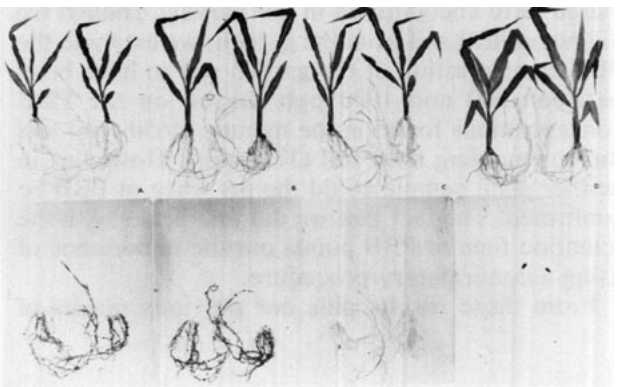

FIGURE 4. Corn plants (top) and autoradiographs (bottom): (*A*, *B*) are after root exposure to ^{14}C-PBB for 4 and 8 days, respectively; (*C*) identical to (*A*), except that roots were dipped in acetone prior to autoradiography, (*D*) 8 days after leaves were treated with ^{14}C-PBB.

amount of PBB found on roots was probably associated with root surfaces. Iwata et al. (*4*) found 97% of PCB residues in carrot roots in the peel. This observation has also been found for DDT and other organochlorine pesticides in soil in which carrots were grown.

Carrot roots showed more PBB than radish or onion (Table 1). The plants grown on the sandier, low organic matter Spinks soil showed higher PBB uptake than ones grown on the Brookston soil which had more clay and organic matter. This finding is consistent with the report of Filonow et al. (*3*) who found that the adsorption of hexa-

Table 1. PBB found associated with radish, carrot, and onion roots after 6, 9, and 10 weeks, respectively, of growth in PBB contaminated soil.

Soil type	PBB added, ppm	PBB in plant roots, ppb[a] Radishes	Carrots	Onions
Spinks loamy sand	0.1	7.2	20.4	ND[b]
	100	48.7	535	62.8
Brookston clay loam	0.1	ND	ND	ND
	100	43.7	117	33.8

[a] Based on wet weight of plant tissue; each value is the mean of three replications.
[b] ND = not detectable; detection limit 0.3 ppb.

bromobiphenyl by soils increased with increasing soil organic matter.

Both the corn-dust sample and the radish sample from the PBB contaminated garden showed small gas chromatographic peaks (<10 ppb) with retention times similar to and identical to, respectively, the major hexabromobiphenyl isomer in PBB. However, neither peak was destroyed by a 20 or 60 min exposure to ultraviolet light (*5*). When the samples were spiked with a small quantity of PBB, larger peak areas were noted but only the portion attributable to the spike was removed by a 20-min ultraviolet treatment. Thus, the peaks present in these samples were judged not to be due to PBB.

These findings are particularly significant, in that they probably represent the worst possible cases. The dust was from one of the most heavily contaminated fields (102 ppb), and the concentration of dust relative to plant biomass was far higher than animals would normally encounter. The garden with the radishes was from the most heavily contaminated farm encountered in our survey. Though we did not collect soil from the garden, we estimate the PBB concentration of the garden soil to have been between 500 and 1000 ppb [based on the PBB concentrations found in the manure (1650 ppb) and the surrounding field soil (371 ppb)]. However, in neither plant sample could the presence of PBB be confirmed. The fact that we did find peaks with the retention time of PBB points out the importance of using a confirmatory procedure.

From these results plus our previous results of greenhouse (*1*) and field studies (*2*) in which we found no PBB in plant tops, we conclude that little if any PBB will be transferred from contaminated soil to plant tops. Thus, recontamination of animals from feeds grown on contaminated soil will likely not occur. Although some root crops from very highly contaminated soil might contain traces of PBB, much of this PBB could probably be removed by peeling.

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