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Reduction of Polychlorinated Biphenyl Toxicity and Uptake of Carbon-14 Activity by Plants through the Use of Activated Carbon

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The use of soil-applied activated carbon in reducing the phytotoxicity and uptake from soil of polychlorinated biphenyls (PCB's) by a variety of crop plants was investigated. Reductions in growth parameters resulted at the highest rate of PCB for soybean [*Glycine max* (L.) Merr.] and beet (*Beta vulgaris* (L.)]. Growth parameters taken at harvest showed no apparent inhibition of corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moensch] by PCB. The activated carbon treatment substantially reduced growth inhibition caused by PCB. Treatment with soil-applied activated carbon reduced ^{14}C uptake into foliage of beet, corn, sorghum, and peanut (*Arachis hypogaea* L.) in studies using a mixture of U-^{14}C-labeled polychlorinated biphenyls mixed with unlabeled PCB and applied to soil at 20 ppm (total PCB). Activated carbon continued to be effective in reducing ^{14}C uptake over three croppings of fescue (*Festuca arundinacea* Schrib).

Land application of municipal and industrial sludge is becoming an increasingly acceptable method of disposal. Application to agricultural land is viewed as an inexpensive method to exploit the nitrogen and other nutrients of these sludges for use as fertilizer (Overcash and Pal, 1979). However, concern has been voiced over the effect of the organic chemical content of these sludges on crops, and the potential for their uptake by crops needs to be determined (Weber, 1978; Pahren et al., 1979). The PCB content of sludges has been reported to range from <0.01 to 1700 ppm dry weight (Furr et al., 1976; Lawrence and Tosine, 1976; Bergh and Peoples, 1977; Pahren et al., 1979). With application to agricultural land of 2250–22 500 kg of sludge hectare^{-1} as dictatd by nitrogen content (Black and Kronis, 1974), an application range of ≪1 to 1500 kg of PCB ha^{-1} year^{-1} could potentially occur. Since PCB's are resistant to degradation, in particular those which are highly chlorinated (Gustafson, 1970; Kalmaz and Kalmaz, 1979; Pal et al., 1980), and do not leach readily in soils (Tucker et al., 1975; Scharpenseel et al., 1977a; Moza et al., 1979a; Weber, 1980), sludge application over a 3-year period could result in PCB levels ranging from ≪1 to 1000 ppm in the upper 7.6 cm of soil. At more reasonable application rates of 11 200–44 800 kg/ha/year of sludges containing 1–200 ppm of PCB's, one could anticipate a range of <1 to 24 ppm accumulating in the upper 7.6 cm of soil over a 3-year period. PCB levels in soils receiving dried sludge have been reported to range from 0 to >50 ppm (Bergh and Peoples, 1977), and PCB levels as high as 1200 ppm have been reported in some Japanese agricultural soils (Fujiwara, 1975).

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Uptake of PCBs by various crops and weeds from soils containing low amounts of PCB's has been reported for carrots (Iwata et al., 1974), carrots and radishes (Wallnöfer et al., 1975), carrots and sugar beets (Moza et al., 1976, 1979a,c), soybean (Suzuki et al., 1977), soybean and fescue (Weber and Mrozek, 1979), and pigweed and panicum (Strek and Weber, 1980). PCB concentrations in the soils ranged from 0.05 to 100 ppm and levels in the crops reached a maximum mean (average of five isomers) of 13.9 ppm (fresh weight) in carrots grown outdoors for 72 days in soil treated with 100 ppm of Aroclor 1254 (Iwata et al., 1974). Uptake over 4 years of growth by spruce trees from soil fortified with [^{14}C]PCB-treated sludge totaled 0.401 and 0.231 ppm (dry weight) in the needles and stems, respectively (Moza et al., 1979b), showing that plants can take up PCB's from contaminated sludge applied to soil. Disruption of growth in plants has been reported for an aquatic plant *Spirodela oligorrhiza* (Mahanty and Fineran, 1976; Mahanty and McWha, 1976) and for soybean and fescue (Weber and Mrozek, 1979). Aroclor 1242 is reported to inhibit photosynthesis in isolated spinach chloroplasts (Sinclair et al., 1977).

Volatilization appears to be an important route for the loss of PCB's from the soil, despite the moderate to low volatilities of 7×10^{-3} to 4×10^{-5} mmHg at 25 °C reported for Aroclors 1221–1260 (Pal et al., 1980). Losses from a woodland soil ranged from 79.2 to 41.5% of that applied in a single cropping season under outdoor conditions for single isomers possessing two to five chlorines (Moza et al., 1976, 1979a,c). The amount of loss through volatilization appears to depend upon the degree of chlorination, with the more highly chlorinated PCB's being lost to a lesser degree (Iwata et al., 1974; Kilzer et al., 1979) and also more readily adsorbed by the soil (Haque et al., 1974).

The effectiveness of activated carbon as an adsorbent of organic molecules has long been recognized (Mattson and Mark, 1971; Cheremisinoff and Ellerbusch, 1978). Although early investigation of this adsorptive property

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focused on agricultural chemicals, particularly on pesticides applied to agricultural lands (Ahrens, 1968; Anderson, 1968; Lichtenstein et al., 1968; Coffey and Warren, 1969; Gupta, 1976), recent work seems to be directed to investigating the activated carbon adsorption of other chemicals in both agricultural lands and other parts of the environment. Activated carbon has been shown to reduce the availability of PCB's to plants (Weber and Mrozek, 1979) and to goldfish (Shea et al., 1980) when applied to contaminated soil. Adsorption of PCB's from aqueous solution by activated carbon has been demonstrated (Lawrence and Tosine, 1976; Hiraizumi et al., 1979).

The research reported herein was conducted to determine (a) the potential effect of a polychlorinated biphenyl mixture (Aroclor 1254) on several important crop plants, (b) the potential residue levels in crops, and (c) the potential of activated carbon treatments to soil to reduce toxicity and residue levels in crop plants.

MATERIALS AND METHODS

Soil. Soil used in the experiments was taken from the 0–15-cm region of the A horizon of a virgin Lakeland sand (*Typic Quartzipsamments*; siliceous, thermic, coated, pH 4.0; cation-exchange capacity, 1.5 mequiv/100 g; 1% organic matter, 5% clay, 6% silt) obtained from a Johnston County, North Carolina, site which had not been subjected to agricultural practices for least 40 years. The soil was air-dried and sieved through a 0.5-cm screen prior to potting. Plants were grown in 350-mL styrofoam pots containing 300 g of soil, which was limed to approximately pH 6.0 with $CaCO_3$.

Aroclor Studies. Analytical-grade PCB (Aroclor 1254), obtained from the Food and Drug Protection Division Laboratory of the North Carolina Department of Agriculture (Lot AM 51, originally obtained from Monsanto Co. in 1971), was dissolved in 95% ethanol. Aliquots of the stock solution were thoroughly mixed with the soil and 3 days were allowed for solvent evaporation. This procedure yielded PCB concentrations of 0, 1, 10, 100, and 1000 ppm. All PCB and carbon concentrations in the soil reported in ppm are based on dry weights. The pots were then separated into two treatment groups; one received no carbon, while the other received 3.7 t/ha (3333 ppm) activated carbon (Nuchar SA, Westvaco, Inc.), which was thoroughly mixed with PCB-treated soil.

The pots were planted to soybean [*Glycine max* (L.) Merr Ransom], beet (*Beta vulgaris* L. Detroit Dark Red), corn (*Zea mays* L. Pioneer 3369A), and sorghum [*Sorghum bicolor* (L.) Moensch Savanna 4 19833-5134]. The crops were grown in controlled environment chambers (200 hlx, 16-h day, 30 °C day, 18 °C night), and the soil was kept at ~80% field capacity and fertilized weekly with a modified Hoagland's nutrient solutin (Weber, 1977). Height, root, and top fresh weight measurements were taken at harvest or as indicated. Cumulative water use measurements were taken periodically using unplanted pots to account for water lost to evaporation from the soil. The second and third soybean plantings followed the original planting reported in a previous study (Weber and Mrozek, 1979) and represent a continuation of this particular study.

^{14}C-Labeled PCB Studies. A ring-labeled polychlorinated biphenyl mixture resembling Aroclor 1254 [specific activity = 31.3 mCi/mmol, obtained from New England Nuclear (lot no. 872-193), average molecular weight = 326.25] was mixed with Aroclor 1254 in 95% ethanol. The labeled material was an isomeric mixture of U-^{14}C-labeled polychlorinated biphenyls ~54% chlorine by weight having a greater Cl/biphenyl range than the unlabeled material. The PCB rates used for these studies were 0 and 20 ppm, and activated carbon rates were 0 and 3.7 t/ha (3333 ppm). PCB and activated carbon applications were made according to the previously described protocol. The pots were planted to fescue (*Festuca arundinacea* Schrib. Kentucky 31), beet, sorghum, peanut (*Arachis hypogaea* L. NC-2), and corn. Plants were continuously cropped in the same pots (without additional PCB additions) under the same controlled environment chamber conditions described in the unlabeled PCB studies. The sequence of planting and days of growth were as follows: fescue-1 (50), beet (39), sorghum (39), peanut (78), fescue-2 (25), corn (13), and fescue-3 (48). The plantings followed one another in the same pots after accumulated toxins were leached out through the bottom of the pot.

At harvest, plant height and top fresh weight were determined. ^{14}C activity was extracted from freshly cut plant tops by homogenizing them in 25–35 mL of a 1:1 hexane–acetone mixture for 1 min with a homogenizer at 25 000 rpm. The extract was separated from the homogenate by suction filtration through a glass fiber filter and washed with an additional 10–15 mL of extractant. The filtrate was evaported to near dryness under a hood and then redissolved in 5 mL of hexane by sonication for 1 min. A 1-mL aliquot was removed and added to a scintillation vial containing 20 mL of cocktail (5.0 g of 2,4-diphenyloxazole, 0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene, 1000 mL of Triton X-100, and 2000 mL of toluene). For removal of quenching due to chlorophyll, 5–10 drops of sodium hypochlorite (chlorox) was added. Radioactivity was determined in a liquid scintillation spectrophotometer for 20 min and was corrected for background levels in plants and for counting efficiency. All ^{14}C content of plants is reported on a fresh weight basis. Recoveries of spiked samples ranged from 86 to 99%. Activities which were converted to ppm represent a best approximation.

Random duplicate soil samples of ~5 g were taken from the pots 1.5 years after initial treatment and continuous cropping and extracted by shaking with 20 mL of hexane for 24 h. Following removal of the supernatant by suction filtration through a glass filter, a 2-mL aliquot was taken and counted in a manner similar to that described for the plant samples. Recovery of spiked samples averaged 93 ± 1% after the first extraction; therefore, no conversion factor was used in the final calculation of recovery.

Statistical Treatment. Analyses of variance were performed on the randomized complete block experiments with the carbon and no carbon treatments analyzed as complete blocks, PCB rates as the main treatment, and PCB X carbon as an interaction effect. When the PCB X carbon interaction F test proved to be significant, LSD's were calculated for mean pairs. Analyses of variance were also performed within the carbon treatments. When the PCB treatment effect proved to be significant, differences from the respective controls within the block were indicated. Three replications were used for the unlabeled PCB studies. All tests for significant differences were made at the 0.05 level of probability of error unless otherwise noted.

RESULTS AND DISCUSSION

After the first crop of soybeans grown in the PCB-treated pots showed significant reductions in height and top fresh weight at 100 and 1000 ppm and reduction of 5-day cumulative water use at all PCB rates (Weber and Mrozek, 1979), it was decided to continue cropping the same pots. This procedure allowed us to study the longevity effect of soil-applied PCB on plants as well as the

Table I. Height and Top Fresh Weight and Cumulative Water Use for Two Crops[a] of Soybean, As Affected by Soil-Applied PCB (Aroclor 1254) and Protection by Activated Carbon Applied to Lakeland Sand

carbon, t/ha	PCB, ppm	height				top fresh weight[b]				cumulative water use (18-22 days)[b]			
		second crop		third crop		second crop		third crop		second crop		third crop	
		cm	% I[b]	cm	% I	g	% I	g	% I	mL	% I	mL	% I
0	0	35.6	0	69.0	0	11.30	0	7.04	0	239	0	149	0
	1	34.8	1	55.3[d]	20	10.83	4	7.22	−3	214	10	146	2
	10	35.6	0	49.3[d]	29	10.96	3	7.82	−11	219	8	147	−5
	100	35.0	1	53.8[d]	22	11.02	2	7.19	−2	202	15	141	5
	1000	27.9	22	56.7[d]	18	8.64	24	4.41[d]	37	126	47	101[d]	32
3.7	0	36.7	0	48.0	0	12.42	0	7.10	0	258	0	127	0
	1	37.1	−1	58.3	−21	13.11	−6	7.81	−10	253	2	130	−2
	10	36.8	0	54.5	−14	13.06	−5	7.41	−4	254	2	139	−9
	100	34.9	5	56.5	−18	12.21	2	7.15	−1	239	7	140	−10
	1000	36.9	0	59.0	−23	12.55	−1	6.53	8	235	9	95	25
	LSD 0.05	NS[c]	NS	10	21	NS	NS	NS	NS	NS	NS	NS	NS

[a] Grown for 23 and 24 days, respectively. [b] Percent inhibition compared to respective controls; negative inhibition indicates stimulation. [c] PCB X carbon effect not significant at the 5% level of probability. [d] PCB effect within carbon treatment is significant and particular mean is different from control at 5% level of probability.

Table II. Height and Top Fresh Weight at Various Times and Cumulative Water Use for Beets, As Affected by Soil-Applied PCB (Aroclor 1254) and Protection by Activated Carbon Applied to Lakeland Sand

carbon, t/ha	PCB, ppm	height at						top fresh weight[b] at				cumulative water use	
		14 days		28 days		56 days		28 days		56 days		32-53 days[a]	
		cm	% I[c]	cm	% I	cm	% I	g	% I	g	% I	mL	% I
0	0	3.2	0	5.0	0	13.5	0	5.1	0	6.3	0	392	0
	1	3.5	−9	5.0	0	14.3	−6	5.4	−6	8.3	−32	374	5
	10	3.2	0	5.0	0	12.0	11	5.3	−4	5.7	10	348	11
	100	2.8	13	5.0	0	15.6	−16	5.0	2	7.5	−19	304	22
	1000	1.2[d]	63	1.5[d]	73	0[d]	100	0[d]	100	0[d]	100	17[d]	96
3.7	0	3.0	0	5.2	0	14.9	0	5.0	0	7.4	0	414	0
	1	3.7	−23	5.3	−2	16.5	−11	6.1	−22	7.5	−1	476	−15
	10	3.8	−27	5.5	−6	16.8	−13	5.1	−2	7.5	−1	469	−13
	100	3.3	−10	5.0	4	16.5	−11	6.1	−22	7.9	−7	440	−6
	1000	3.3	−10	5.3	−2	15.1	−1	5.2	4	8.3	−12	425	−3
	LSD 0.05	0.9	28	1.4	9	3.6	27	NS[c]		3.4	54	90	23

[a] Mean of two plants, each pair harvested from the same plot at 28 and 56 days. [b] Percent inhibition compared to respective controls; negative inhibition indicates stimulation. [c] PCB X carbon effect not significant at the 5% level of probability. [d] PCB effect within carbon treatment is significant, and particular mean is different from control at 5% level of probability.

effectiveness, over time, of the activated carbon treatment to protect the crop from injury.

Inhibition of plant height caused by PCB X carbon effects were significant only for the third crop of soybean (Table I), where inhibitions of 29 and 22% were reached at the 10- and 100-ppm rates, respectively. When heights were analyzed within the carbon treatments (blocks), significant difference due to PCB treatment was reached only in the no carbon treatment of the third soybean crop where heights were significantly lower at all levels of PCB. Top fresh weight and cumulative water use inhibitions were not significant for PCB X carbon effects for either crop of soybean. However, effects of the PCB treatment on fresh weight of the third crop and cumulative water use for both the second and third crops of soybean were significant, but only at the 1000-ppm rate in the no carbon treatment. The 47% inhibition of water use at the 1000-ppm rate for the second crop of soybean was significantly lower at the 10% level of probability. Root fresh weight differences caused by PCB were not significant and are not reported.

Height inhibitions of 22 and 18% and top fresh weight reductions of 24 and 37% at the 1000-ppm rate for the second and third crops of soybean, respectively (Table I), can be compared to the 15% reduction in height and 22% reduction in top fresh weight reported in the first study (Weber and Mrozek, 1979). Inhibitions of water use at the 1000-ppm rate of PCB for the second and third crops reached 47 and 32%, respectively, also comparing to the 52% reduction reported in the first study. However, significant reductions in water use, which occurred at all PCB rates in the first study, were noted only at the highest rate in either later study. Significant top fresh weight reductions were not observed at the 100-ppm rate as they were during the first study. No malformations of leaves at the 1000-ppm rate in the no carbon treatment were noted for the second and third crops of soybean, as was previously reported for the first crop. These trends indicate that soil-applied PCB's diminish in toxicity to soybean with increasing time and subjection to cropping. The activated carbon treatment eliminated all significant differnces in height, top fresh weight, and cumulative water use caused by PCB in the no carbon treatment. This protective effect continued through three crops of soybean, illustrating the protectiveness, over time, given by the activated carbon treatment.

Beets were chosen as experimental plants to determine the effectiveness of activated carbon in protecting a root crop from PCB's. All PCB, carbon, and PCB X carbon effects were significant for heights and top fresh weights at all sampling dates and for water use, with one exception (Table II). The PCB X carbon interaction effect of beet

Table III. Height Inhibition at 5 Days and Recovery by 18 Days for Corn by PCB (Aroclor 1254) Applied to to Lakeland Sand

carbon, t/ha	PCB, ppm	height at 5 days, cm	5 days, % I[a]	height at 18 days, cm	18 days, % I
0	0	4.7	0	46.7	0
	1	5.6	2	48.7	−4
	10	5.8	3	49.3	−5
	100	4.0[c]	30	48.7	−4
	1000	2.9[c]	49	45.7	2
3.7	0	5.4	0	46.0	0
	1	5.8[c]	−7	42.7	7
	10	5.8[c]	−7	43.3	6
	100	5.9[c]	−9	43.7	5
	1000	5.9[c]	−9	43.7	5
	LSD 0.05	0.3	5	NS[b]	NS

[a] Percent inhibition compared to respective controls; negative inhibition indicates stimulation. [b] PCB *X* carbon effect not significant at 5% level of probability. [c] PCB effect within carbon treatment is significant, and particular mean is different from control at 5% level of probability.

top fresh weights taken at 28 days was significant only at the 10% level. When heights, top fresh weights, and water use were analyzed within blocks, effects due to PCB treatment were significant only at the 1000-ppm rate in the no carbon treatment.

By 14 days, beet foliage height was inhibited by 63% at the 1000-ppm rate, increasing to 73% by 28 days and finally to 100% with the death of the plants between 28 and 56 days (Table II). Top fresh weight was reduced to nearly 100% after 28 days of growth in the 1000-ppm rate of PCB (only one plant out of six survived). Cumulative water use reductions exhibited a progressive reduction with increasing PCB treatment, becoming significant at only the highest PCB rate (however, approaching significance at the 100-ppm rate). Beets are apparently extremely susceptible to soil-applied Aroclor 1254 at the 1000-ppm rate, yet activated carbon protected the beets from the PCB's. In fact, significant growth stimulation resulted from the carbon treatment. Possible explanations for the improved plant growth from the carbon addition include the possibility that the activated carbon adsorbed nonspecific toxic substances normally found in soils or that the activated carbon acted to buffer the availability of applied nutrients.

There was no significant effect of PCB or PCB *X* carbon interaction on corn height and top fresh weight taken at 26 days or cumulative water use taken between 12 and 26 days of growth (data not shown). Similarly, there was no significant effect of PCB on sorghum height and top fresh weight, taken at 69 days, and cumulative water used taken between 65 and 68 days (data not shown). At harvest the crops appeared healthy despite PCB treatment; even differences between carbon and no carbon treatments also appeared minimal. The only effect observed was a significant reduction in corn height at the 100- and 1000-ppm rates after 5 days of growth (Table III). Corn plants appeared to recover and no differences from the controls could be seen after 18 days. These data suggest that corn and sorghum can withstand relatively high rates of PCB in the soil without permanent effects on growth.

In order to determine the influence of activated carbon on the uptake of soil-applied [^{14}C]PCB by plants, a nontoxic rate of 20 ppm was chosen. Amounts in the plants were <1 ppm (fresh weight basis) (Table IV), which concurs with the low values reported in the literature. Reductions in [^{14}C]PCB uptake due to activated carbon treatment ranged from 81 to 100%. The lowest reduction (81%) for corn probably resulted from the high amount of variability in [^{14}C]PCB content at extremely low levels of uptake. Reductions at relatively higher amounts of PCB uptake are much more meaningful than at lower amounts of PCB uptake. These data indicate activated carbon was highly effective in reducing [^{14}C]PCB uptake by these crops.

Bioaccumulation factors are included in Table IV. Bioaccumulation is generally taken to be the factor, expressed as a ratio, at which level a chemical is present in an organism relative to the level found in its environment, either soil or water. A factor greater than 1 means the organism contains a greater concentration of the chemical than the soil or water it inhabits. The bioaccumulation factors for the crops are much lower than 1; none were greater than 0.041. This signifies that amounts in the plants were very low relative to the concentration in the soil. The connotation implied by the terms bioaccumulation factor or biomagnification factor is often misleading. In aquatic systems bioaccumulation factors are ofter large, which is predictable for chemicals of such low water solubility. The amount of chemical actually dissolved in the water is so low that any amount taken in by an organism becomes substantially larger than that found in its surroundings. In soil systems, bioaccumulation factors do not account for the water solubility of a chemical or its adsorption to a particular soil constituent, which may greatly decrease the amount of chemical in the soil solution and influence the availability of chemicals to organisms (Weber, 1972; Weber and Weed, 1974). Bioaccumulation factors are generally reported on a weight basis. Most soil weights are reported as dry weights while plant weights may be reported as either dry or wet weights, further allowing for difficulty in interpretation. The calculated PCB content reported in Table IV represents a theoretical amount of PCB in the plant which is extractable. By combustion of a sample of redroot pigweed plants (*Amaranthus retroflexus* L.) grown in [^{14}C]PCB-treated soil and entrapment of the ^{14}C activity, an estimate of unextractable residue

Table IV. ^{14}C, Estimated PCB Content of Plant Tops, and Bioaccumulation Factors of Various Crops Grown in Lakeland Sand Treated with [^{14}C]PCB[a] (Aroclor 1254) and Activated Carbon

crop	growth period, days	^{14}C and PCB content: no carbon, dpm/gfw[b]	no carbon, ppb[c]	no carbon, b.f.[d]	carbon, dpm/gfw	carbon, ppb	carbon, b.f.	reduction in uptake by carbon, %
beet[e]	39	149.1	815	0.041	18.6	102	0.005	88
sorghum[e]	39	12.5	68	0.003	0	0	0	100
peanut[f]	78	86.5	473	0.024	6.8	37	0.002	92
corn[e]	13	0.32	2	0.001	0.06	0.3	0.00002	81

[a] Applied 0.5 μCi to 300 g of soil; total [^{14}C]- and [^{12}C]PCB concentration was 20 ppm. [b] Disintegrations per minute per gram fresh weight. [c] Based on fresh weight. [d] Bioaccumulation factor based on fresh weight. [e] Mean of two replications. [f] Mean of three replications.

Table V. ^{14}C Extracted from Fescue Grown for Three Different Periods on Lakeland Sand Treated with [^{14}C]PCB[a] (Aroclor 1254) and Activated Carbon

sampling date[b]	^{14}C content: no carbon, dpm/gfw[c]	^{14}C content: carbon, dpm/gfw	reduction in uptake by carbon, %
Nov 3, 1978[d]	309.1	0	100
July 5, 1979	73.5	0.2	100
Feb 6, 1980	28.6	2.2	93

[a] Applied 0.5 μCi to 300 g of soil; total [^{12}C]- and [^{14}C]-PCB concentration was 20 ppm. [b] After 50, 25, and 48 days of growth, respectively. [c] Disintegrations per minute per gram fresh weight. [d] Originally reported in Weber and Mrozek (1979).

was obtained (Mrozek, 1980). The total residue was determined to be 500 dpm/g dry weight, whereas the level of extracted activity was 234 dpm/g dry weight. This represents an underestimation of ~50%, which is comparable to the value of unextractable residues reported in the literature (Moza et al., 1976, 1979a,c). Furthermore, the form of the PCB in the plant may not be in the parent isotype but in the form of conjugates and phenols, as reported by Moza et al. (1976, 1979a–c).

Three crops of fescue were grown in one set of treated pots at various periods to determine the long-term effectiveness of the activated carbon treatment in reducing [^{14}C]PCB uptake (Table V). Though the growing periods of the three crops of fescue varied in length, the fresh weight production of each crop was comparable, averaging 2.25, 2.26, and 3.50 g, respectively, for the three crops. ^{14}C content of fescue in the no carbon treatment decreased with each sampling over a 15-month period. Of 3667 dpm/g ^{14}C originally applied to the soil, after 1.5 years of cropping, 1939 and 3248 dpm/g could be extracted from the no carbon and carbon treatments, respectively. This corresponds to a 53% recovery in the no carbon treatment and a 89% recovery in the carbon treatment of applied ^{14}C activity after 1.5 years. The majority of loss of PCB's can probably be explained as resulting from volatilization. Discounting losses due to uptake by plants in each treatment (only a total of 0.36% of applied activity was taken up in the no carbon treatment), the activated carbon apparently inhibited the disappearance of PCB from the soil. However, the possibility remains that upon volatilization or microbial breakdown of the PCB's, the PCB or $^{14}CO_2$ may have adsorbed to the activated carbon surface and provided an underestimate of the disappearance of the parent molecule (Helweg, 1975). Another possibility is that with increasing time in the soil, PCB's may become less extractable but may remain extractable from activated carbon. Recovery of ^{14}C-labeled PCB from a forest soil averaged 80% after extraction with hexane and centrifugation, 6 months after application, whereas an acetone Soxhlet extraction was able to recover 95% of that applied (Seidl and Ballschmiter, 1976). It has been our experience that centrifugation of PCB's in hexane or water greatly reduces the amount in solution, which may explain this discrepancy in recoveries. No activity was detected in the collected leachate of carbon-amended pots; very little (less than 0.1% of applied activity) was detected from the no carbon pots. Also, the decrease in ^{14}C activity extracted from fescue tops was greater than could be explained by the decrease in ^{14}C content in the soil. This suggests that with continued residence in soil, less PCB is available to plants, possibly due to complexing by organic soil colloids. PCB's are reported to have a high affinity for the organic component of soils (Haque et al., 1974; Scharpenseel et al., 1977a,b). Lower chlorinated biphenyls are reportedly taken up in greater amounts than higher chlorinated biphenyls (Iwata et al., 1974; Wallnöfer et al., 1975; Suzuki et al., 1977; Moza et al., 1979a). Perhaps continued cropping of these pots caused a depletion of the more mobile, lower chlorinated PCB's, leaving behind less mobile, higher chlorinated PCB's. Depite the fact that the absolute amount of ^{14}C activity (either PCB or metabolites) remaining in the soil was greater and decreased less rapidly in the carbon treatment than in the no carbon treatment, the activated carbon still reduced [^{14}C]PCB uptake by 93–100% over a 15-month period.

CONCLUSIONS

Soil-applied PCB (Aroclor 1254) caused reductions of height, top fresh weight, and cumulative water use for soybean and beet at the 1000-ppm rate. Corn and sorghum appeared tolerant to high rates of PCB in the soil (1000 ppm in these studies). Little phytotoxic damage is likely to result from application of sludge containing moderate levels of PCB (1–50 ppm) applied at reasonable rates to soil (11 200–44 800 kg ha^{-1} $year^{-1}$). Measurable growth effects due to soil-applied PCB were eliminated by activated carbon.

[^{14}C]PCB studies showed a reduction in uptake of soil-applied [^{14}C]PCB by beet, corn, sorghum, and peanut due to soil treatment with activated carbon. Reduction of ^{14}C uptake by fescue in the no carbon treatment continued over a 15-month period. Calculated estimates of PCB levels in the crop foliage were low (<1 ppm fresh weight) when applied to soil at 20 ppm. Activated carbon appeared to inhibit the disappearance of [^{14}C]PCB or metabolites from soil over a 1.5-year period. These studies demonstrate that activated carbon could be effectively used to adequately reduce PCB uptake, should PCB levels in crops grown on sludge-amended soil reach high levels.

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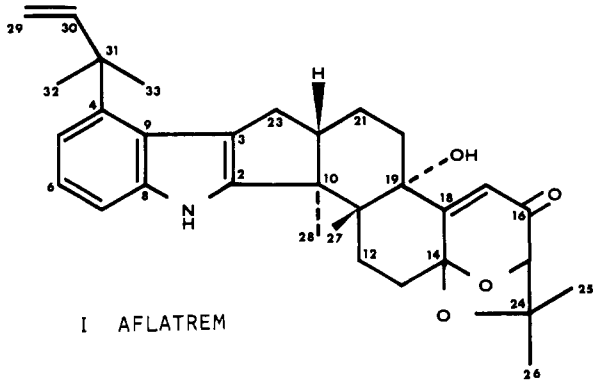
Indole Metabolites from a Strain of *Aspergillus flavus*

Richard J. Cole,* Joe W. Dorner, James P. Springer, and Richard H. Cox

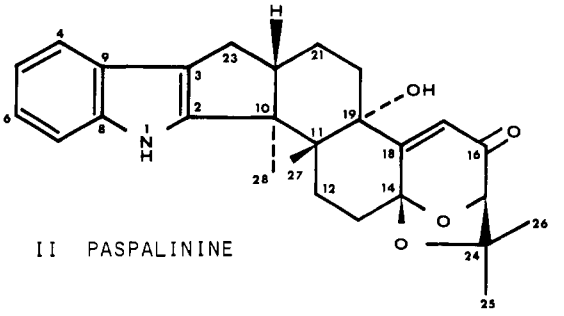
The X-ray structure of dihydroxyaflavinine, a new diterpene indole metabolite from *Aspergillus flavus*, is reported. The fungal tremorgens aflatrem and paspalinine were also isolated from the *A. flavus* isolate. Previously, paspalinine has only been reported from sclerotia of *Claviceps paspali*.

The tremorgenic indole metabolites of *Claviceps paspali* are closely related chemically to aflatrem (I), which is



I AFLATREM

produced by some strains of *Aspergillus flavus* (Cole et al., 1977; Gallagher et al., 1980a). In addition, aflatrem represents the most logical distal product in the biosynthesis of this group of fungal metabolites (Cole, 1980). Because some *A. flavus* isolates can produce this distal product, some of the *C. paspali* type indoles may be present in *A. flavus* cultures. These relationships prompted us to critically examine the indole metabolites of an aflatrem-producing strain of *A. flavus* to determine if the *C. paspali* type metabolites are also metabolites of *A. flavus*. We now report the isolation and identification of the *C. paspali* type tremorgen paspalinine (II) and a new



II PASPALININE

unrelated nontremorgenic indole diterpene metabolite, 20,26-dihydroxyaflavinine (III), from *A. flavus*.

MATERIALS AND METHODS

The *A. flavus* (NRRL 3251) isolate was obtained from the Northern Regional Research Center Culture Collection. The fungus was mass cultured in 2.8-L Fernback flasks,

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