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Uptake and Phytotoxicity of Di-*n*-butyl Phthalate in Corn (*Zea mays*)

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Phthalate esters (PAE's) are widely used plasticizers which impart flexibility to thermoplastic resins by weakening polymer-polymer bonds (KLAUSMIER and JONES 1960). PAE's have also been reported to occur naturally in soils and plants (FISHBEIN and ALBRO 1972), although much of the material may result from contamination *in situ* or during analysis (MATHUR 1974). PAE's are commonly found in waste waters (HITES and BIEMANN 1972; MAYER et al. 1972), and their levels vary widely. In one Indiana study, total PAE's constituted 0.9 to 5.1% of anaerobically digested sludge collected from three treatment plants. The total U.S. PAE production was estimated to be 9.98×10^8 tons in 1973 (ANONYMOUS 1973).

Di-*n*-butyl phthalate (DBP) is one of the more widely used PAE's. It has a low acute toxicity (oral LD_{50} in rats of 8.0 g/kg body weight), however the effects of chronic exposure are uncertain (FISHBEIN and ALBRO 1972). Some researchers suggest that PAE toxicity may be related in part to the specific alcohol release following ester hydrolysis (FISHBEIN and ALBRO 1972), which for DBP is *n*-butyl alcohol (ENGLEHARDT et al. 1975) [acute oral LD_{50} in rats of 4.36 g/kg (WINDHOLZ 1976)].

PAE's are generally not considered to be very persistent in soils, and although branched-chain PAE's are degraded more rapidly than *n*-chain isomers, DBP has been observed to dissipate more rapidly than other *n*-chain phthalates (KURANE et al. 1977). JOHNSON and LULVES (1975) found that 46% of ^{14}C-DBP added to hydrosoil cultures was aerobically degraded within 24 h at 22°C to mono-n-butyl phthalate (MBP), and after 5 days 98% of the radiolabel had disappeared. Thirty days were required for 98% disappearance under anaerobic conditions, and no significant degradation was observed if the soil was sterilized. Thus DBP dissipation has been largely attributed to microbial degradation. Several soil organisms commonly found in soil have been reported to degrade DBP, including several species of *Nocardia* (KURANE et al. 1977, 1978, 1979, 1980), *Pseudomonas* (KURANE et al. 1977, 1979; ENGLEHARDT and WALLNOFER 1978), *Arthrobacter* (ENGLEHARDT and WALLNOFER 1978), *Fusarium* (KLAUSMIER and JONES 1960), and *Pencilium*, *Corynebacterium*, and *Mycobacterium* (ENGLEHARDT et al. 1975). A complete degradation scheme in *Nocardia erythropolis* S-1 culture was reported by KURANE et al. (1980), in which DBP was hydrolyzed to MBP and phthalic acid, followed by hydroxylation and decarboxylation to protocatechuic acid, intradiol fission to β-ketoadipic acid, and

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finally mineralization to CO_2 and H_2O. Although mixed microbial populations can degrade PAE's, the rate of decomposition has been reported to decrease at higher phthalate concentrations and at lower pH levels (SAEGER and TUCKER 1976; KURANE et al. 1977). Less details have been reported on DBP metabolism in plants and animals, which may proceed to phthalic acid (FISHBEIN and ALBRO 1972), or result in the accumulation of MBP (ALBRO et al. 1973). Unmetabolized DBP has been reported in several species of fish at concentrations from 0.2 to 3.2 ppm (FISHBEIN and ALBRO 1972).

The application of the tripropyl ester of 1,2,3-benzene-tricarboxylic acid at 1×10^{-2}M to six-week-old tobacco (Nicotiana tabacum L.) resulted in extensive chlorosis and subsequent growth inhibition (BUTA 1975). Chlorosis was confined to meristematic portions of the treated apex, which remained bleached. Growth was inhibited by 74% for at least 14 days, then returned to normal. New leaves were normal in size and no new chlorosis occurred.

Since phthalate esters are commonly found in wastewater and municipal sewage sludge and the disposal of these waste products in agricultural land management systems is increasing annually, it was the purpose of this study to determine: (a) the effects of soil-applied DBP on the growth of corn, and (b) DBP concentrations in the plants after three weeks exposure to the ester.

MATERIALS AND METHODS

Plant Bioassay. The soil used in the assay was a Lakeland sand (Typic quartzipsamments; siliceous, thermic, pH 4.0, 1% organic matter, 5% clay, 6% silt) which was limed with $CaCO_3$ to a pH of 6.0. DBP was incorporated into 523.5 g (dry weight per pot) of soil in amounts of 0, 0.1, 1.0, and 10 ml to result in concentrations of 0, 200, 2000, and 20000 ppmw, respectively. The treated soils were adjusted to field capacity, and 10 ml full strength modified Hoagland's nutrient solution (WEBER 1977) were added. Four seeds of corn (Zea mays L. 'Pioneer 3368') were planted in each pot at a depth of 1 cm, and grown for three weeks in the greenhouse (an additional nutrient application was made after 10 days). At harvest, plant heights and shoot fresh weights were determined, and plants were frozen until DBP analysis. Corn was then replanted in all pots, grown for three weeks, and heights and shoot weights were again determined. A completely randomized design with three replications was used in the study.

Determination of DBP. The corn plants were thawed and cut into small pieces. Two gram samples were mascerated for 3 minutes in 50 ml 1:1 (v/v) hexane:acetone (solvents free from phthalate contamination) using a Virtis homogenizer. The extract was filtered from the plant tissue by suction filtration through

Whatman GFA glass microfibre filters, evaporated to near dryness under a hood, and redissolved in 2 ml of the solvent mixture by sonicating for 3 minutes. One ml of each extract was then applied with a syringe in 0.5 x 18 cm bands to 20 x 20 cm, 500 μm silicagel G thin layer plates. After drying, the plates were developed in a closed glass developing chamber using 50 ml petroleum ether:diethyl ether:acetic acid (90:10:1, v/v/v) [CERBULIS and ARD 1967]. The plates were exposed to iodine vapors to resolve the chromatographed bands. The R_f for DBP was determined to be 0.27 by spotting plates with extraction solution containing 1000 ppm hexane. This was also done for corn extracts following homogenization of 2 g of untreated plant material in 50 ml of 1000 ppm DBP solution. DBP bands were verified by scraping into 10 ml of 95% ethanol (DBP-free) and determining the U.V. absorption spectra with a scanning spectrophotometer. The spectra, with the DBP analytical peak at 273 nm, supported the presence of the ester. The ethanol was then evaporated to near dryness with a rotary evaporator, and redissolved in 2 ml hexane for glc analysis.

Standards of 0 to 100 μg/l DBP were prepared by adding DBP to hexane. These were injected in 1 μl aliquots into a Varian 3700 gas chromatograph equipped with an FID detector. A 183 cm stainless steel column packed with Ultrabond (Carbowax 20M on 100/120 chromosorb WAW) was used under the following operating conditions: nitrogen carrier gas, 20 cc/min; injector port, 250°C; column oven, 190°C; and detector, 270°C. The relative retention time from the solvent peak for DBP was 5.4 min. DBP was quantified by constructing tangents to the peak and calculating area. A standard curve was generated from which DBP concentrations in the extracted samples were determined. By spiking one of the sample extracts with DBP, the chromatogram peak corresponding to DBP was intensified, further verifying its identification. The extraction efficiency for the entire procedure from spiked extraction solutions was 86.3 $\pm$ 7.4%.

RESULTS AND DISCUSSION

Plant Bioassay. The heights, shoot fresh weights, and corresponding DBP levels in the first crop of corn plants are given in Table 1. Corn germination was not significantly affected by DBP at the concentrations used. Plant heights and shoot weights were not significantly reduced at 200 ppmw; but at 2000 ppmw DBP, plant height and shoot weight were significantly reduced by 17 and 25 percent, respectively. At 20000 ppmw DBP, corn growth was severely inhibited, as indicated by a 45 percent reduction in height and 72 percent reduction in shoot fresh weight. Other than stunting at the higher rates, the most apparent injury symptom was the appearance of transverse, bleached bands in the first leaves, as has been observed for other PAE's (BUTA 1975). This effect was also observed in some of the plants at the 200 ppmw rate, although plant growth was not significantly affected. The bleaching did not appear in later developing leaves. Localized bleaching was also observed in studies where DBP in aqueous solution

TABLE 1

Heights, fresh shoot weights and DBP concentrations in corn plants after three weeks growth on soil containing DBP.

Soil DBP conc. (ppmw)	Plant height (cm)	(%)[1]	Shoot fresh weight (cm)	(%)[1]	DBP concentration (ppm)[2]
0	50.2 ± 0.7[3]	0	18.49 ± 2.20	0	0
200	51.0 ± 6.3	-2	17.46 ± 1.43	6	0.32 ± 0.08
2000	41.9 ± 1.5	17	13.94 ± 0.25	25	1.24 ± 0.08
20000	27.7 ± 6.9	45	5.12 ± 3.28	72	13.80 ± 4.20
r[4]	-0.95		-0.95		0.99

[1]Percent reduction from controls.

[2]Parts per million fresh weight.

[3]Mean ± standard error.

[4]Correlation coefficient for parameter with DBP soil concentration.

containing Tween 20 surfactant was applied to the foliage of corn plants at the first leaf stage. In these studies, 1000 kg/ha DBP caused significant yield reductions.

After three weeks' growth following replanting, corn plant heights and fresh shoot weights were not significantly different from the controls at DBP concentrations $\leq$ 2000 ppmw (Table 2). However, at 20000 ppmw DBP, plant heights were reduced by 27 percent and weights were reduced 37%. In this bioassay, bleaching was observed only in plants grown in soil containing 20000 ppmw DBP. These observations suggest that plant-available DBP levels had decreased, either through complexing of DBP with soil compounds, or as a result of the microbial decomposition discussed previously.

DBP Analyses. Organo-soluble DBP concentrations in corn plants increased with DBP soil concentration to a maximum of 13.8 ± 4.2 ppm (fresh weight) in soil treated with 20000 ppmw of the plasticizer (Table 1). Although this level represents readily extractable or free DBP, additional phthalate may be bound within the plant which was not determined by the methods used. Metabolized derivatives may also be present, and the literature suggests these would be mainly MBP and phthalic acid hydrolysis products. The appearance of an additional peak eluting before DBP in some of

TABLE 2

Heights and fresh shoot weights of corn plants after three weeks growth following replanting in soil containing DBP.

Soil DBP conc. (ppmw)	Plant height (cm)	Plant height (%)[1]	Shoot fresh weight (g)	Shoot fresh weight (%)[1]
0	49 ± 3[2]	0	18.14 ± 2.68	0
200	50 ± 5	-2	16.83 ± 3.18	7
2000	48 ± 3	2	19.27 ± 0.77	-6
20000	36 ± 4	27	11.38 ± 2.70	37
r[3]	-0.85		-0.77	

[1]Percent reduction from controls.

[2]Mean ± standard error.

[3]Correlation coefficient for parameter with DBP concentration.

the GLC chromatograms, which occurred more frequently in extracts of plants grown at the lower DBP soil concentrations, might suggest that a monoester derivative has been formed. Further metabolism studies would be required to confirm this. The low levels (1.24 + 0.08 ppm) of DBP found in plants grown in soil containing concentrations up to 2000 ppmw suggests that very low levels of the compound would be expected to be taken up by corn in soils treated with sludge containing DBP. Replanting of the DBP-treated pots suggests that the chemical does not persist long in the soil environment. Thus it appears that corn grown on soil containing original levels of DBP less than 200 ppmw would not represent a serious hazard to animals consuming the plants. Long term studies are required to determine DBP levels in older plants and in corn grain. Studies should also be conducted to determine the metabolites of DBP in corn plants and the level of DBP causing no effects in representative animals.

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