Fate of Bis(2-ethylhexyl) [^{14}C]Phthalate in Laboratory and Outdoor Soil–Plant Systems

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Fate and degradation of bis(2-ethylhexyl) [*carboxyl*-^{14}C]phthalate ([^{14}C]DEHP) were studied in various soil and soil–plant systems. In suspended soil, degradation rates to $^{14}CO_2$ were 9.5% after 9 days at room temperature under aerobic conditions; in a closed aerated laboratory soil–barley system, they were 8.2% after 7 days. In a lysimeter under outdoor conditions, total recovery of radioactivity in soil was 6.9%, in potatoes 0.11%, and in leached water 0.51% after one growing period. After two growing periods, recovery in soil was 1.7%, in barley 0.005%, and in leachate 0.01%. The radioactivity in the top soil layer (0–20-cm depth), after one growing period, consisted of DEHP (3% of applied ^{14}C), mono(2-ethylhexyl) phthalate (0.14%), phthalic acid (0.35%), unidentified soluble metabolites (1.29%), and unextractable residues (1.84%). Uptake by plants was low; most of the radioactivity absorbed was very polar or unextractable.

Esters of phthalic acid are ubiquitous contaminants in the biosphere. Global annual consumption is about 20×10^6 tons. Bis(2-ethylhexyl) phthalate (DEHP) is the phthalic acid ester most frequently used in PVC formulation. Annual production is about 0.7×10^6 tons. Due to the high production and application figures, phthalic esters occur in air (Mayer et al., 1972; Bove et al., 1978; Karasek et al., 1978; Giam et al., 1978, 1980; Hoffmann et al., 1980), in water and sediments (Hites, 1973; Morita et al., 1974; Robertson and Li, 1976; Giam et al., 1976, 1978; Brownlee and Strachan, 1977; Corcoran and Curry, 1978; Jungclaus et al., 1978; Schouten et al., 1979; Schwartz et al., 1979; Erhardt and Derenbach, 1980; Giam and Atlas, 1980; Pierce et al., 1980; Müller et al., 1980; Payne and Benner, 1981; Rhoades et al., 1981; Murray et al., 1981; Malisch et al., 1981; Peterson and Freeman, 1982; Michael et al., 1984; Thurén, 1986), and in soils (Cifrulak, 1969; Persson et al., 1978). Khan and Schnitzer (1972) isolated relatively large amounts of phthalates from a methylated humic acid extracted from a Black Chernozem soil, the largest portion being DEHP, and postulated that a biosynthetic origin in the soil cannot be excluded. Ogner and Schnitzer (1970) found phthalates complexed with fulvic acids.

Several review articles deal with the occurrence of phthalates in organisms, their biological activity, metabolism, and toxicity (Fishbein and Albro, 1972; Peakall, 1975; Daniel, 1978; Lawrence, 1978; Thomas et al., 1978; Lawrence and Tuell, 1979; Melancon, 1979; Thomas and Northup, 1982).

Most of the publications reporting fate, mobility, and degradation of phthalates relate to aquatic systems; only a minor part of reports deals with the fate of phthalates in terrestrial systems, especially in soils and plants. However, since phthalates have been detected in municipal sewage sludge, often used as a fertilizer in agriculture and forestry, and since they are emitted into soil also by deposition from the air, information on their mobility, conversion, and degradation in soil as well as on their uptake by plants is important. The potential uptake of phthalates from soil into food plants is of special interest since carcinogenic effects of DEHP have been observed in rats and mice (National Toxicological Program, 1982).

Inman et al. (1984) reported the decomposition of ^{14}C-labeled phthalic acid, monobutyl phthalate, and dibutyl phthalate to $^{14}CO_2$ in soil. Shanker et al. (1985) studied the biodegradation of dimethyl phthalate, dibutyl phthalate, and DEHP in soil by determining the decrease in parent compound concentrations. Fairbanks et al. (1985) monitored degradation, volatilization, and adsorption of [^{14}C]DEHP in three calcareous soils and found that evolution of $^{14}CO_2$ was the only mechanism of loss of DEHP from these soils. Soil sorption and migration in river sand was studied for three phthalate esters (Russell and McDuffie, 1986). DEHP was strongly adsorbed and relatively immobile, whereas diethyl phthalate had a low soil–water partition coefficient and was fairly mobile, and di-*n*-butylphthalate had an intermediate partition coefficient.

Plant metabolism of DEHP in cell suspension cultures of wheat was reported by Krell and Sandermann (1986). However, uptake from soil by intact plants under environmental conditions has not been studied with ^{14}C-labeled compound thus far. Likewise, for this most important phthalate ester, the identification of metabolites in soil has not been reported. A total mass balance of the compound in a complete soil–plant system after application to soil, also, has not been established.

This paper reports mass balance, mobility in soil, degradation and conversion in soil, and uptake into agricultural plants of DEHP under various laboratory and long-term outdoor conditions. Conversion products in soil are isolated and identified by combined gas chromatography/mass spectrometry.

MATERIALS AND METHODS

Apparatus. Radioactivity measurements were carried out in a liquid scintillation counter Betaszint BF 8000 from Berthold. The radioactivity left in extracted solid samples was determined by combustion in a sample oxidizer Tri-Carb B 306, Packard. For the localization of radioactive zones on thin-layer plates, a radioactive TLC scanner, LB 2722, from Berthold was used.

Gas chromatography was performed on a Hewlett-Packard unit, Model 5880 A (combined with HP integrator, Model 3388 A), equipped with a flame ionization detector. Mass spectra were taken with a gas chromatograph/mass spectrometer unit (Hewlett-Packard, Model 599A).

Reagents. DEHP, carboxyl-^{14}C-labeled (sp act. 5 μCi/mg, radiochemical purity 99%), was purchased from The Radiochemical Centre, Amersham. Before use, it was mixed with varying amounts of inactive DEHP purchased from Fluka AG, Switzerland (purity >99%), depending on the kind of experiment.

Phthalic acid was obtained from E. Merck, Darmstadt, Federal Republic of Germany (purity >99.5%). Mono(2-

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ethylhexyl) phthalate was synthesized according to Albro et al. (1973). The resulting mixture of mono(2-ethylhexyl) phthalate and phthalic acid (about 1:1) was separated by preparative layer chromatography (PSC plates ready-coated with silica gel 60 F_{254}s with concentration zone, layer thickness 1 mm, from E. Merck, Darmstadt; solvent, chloroform/methanol/acetic acid, 143:7:2). R_f values: phthalic acid, 0.05; mono(2-ethylhexyl) phthalate, 0.39.

Dimethyl phthalate was prepared by methylation of phthalic acid with diazomethane, which was freshly prepared from [(*p*-tolylsulfonyl)methyl]nitrosamide and KOH in diethyl ether and then distilled.

For liquid scintillation counting of extracts and of leached water, a scintillation liquid based on dioxane was used. $^{14}CO_2$ obtained after the combustion of extracted solid samples was trapped and counted in a toluene-based scintillation liquid containing phenethylamine.

The properties of the soils used were as follows:

1. For laboratory degradation studies in suspended soil: sand (0.063–2 mm) 8%, silt (2–63 μm) 75%, clay (<2 μm) 17%; organic matter, 2.45%; pH 7.3.

2. For laboratory soil–plant studies: sand (0.063–2 mm) 32.4%, silt (2–63 μm) 27.4%, clay (<2 μm) 33.6%, coarse matter (>2 mm) 6.6%; organic matter, 3.15%; pH 6.4.

3. For outdoor studies: sand 52.2%, silt 34.5%, clay 13.3%; organic matter, 0.3%; pH 6.8.

Procedures. *Laboratory Degradation Studies in Suspended Soil.* Degradation studies were performed according to the method of Scheunert et al. (1987). A mixture of 250 g of soil and 250 mL of water was shaken for 5 days at 22 °C in a 1-L wide-mouth bottle equipped with gas inlet and outlet and two valves. In order to maintain aerobic conditions, the suspension was kept in an oxygen atmosphere. After the equilibration time, 14.6 μg of [^{14}C]DEHP was added, and the mixture was shaken for 33 days. At appropriate time intervals, the atmosphere in the vessel was flushed with oxygen and drawn through a trapping system consisting of three 15-mL absorption tubes, the first being filled with 10 mL of ethylene glycol monomethyl ether for absorption of organic volatiles, the second with 8 mL of Carbo-Sorb (Packard) for absorption of $^{14}CO_2$, and the third with 10 mL of diluted H_2SO_4. Carbo-Sorb containing $^{14}CO_2$ was mixed with 4 mL of Permafluor V (Packard) and counted in a liquid scintillation counter.

Laboratory Soil–Plant Studies. Soil–plant studies were carried out in a closed aerated apparatus as described by Scheunert et al. (1986). Plants were grown in desiccators (300-mm high and 150–250-mm diameter) connected with a pump and a special trapping system similar to that used in soil suspension degradation studies, giving the possibility to trap organic volatiles and $^{14}CO_2$ separately. In order to determine the uptake of radioactivity from soil by plant roots and that from air by leaves separately, a Petri dish with 400 g of soil (20% moisture content) was put into each desiccator. Around this dish, 600 g of soil containing ^{14}C-labeled chemical was mixed with commercially available inactive compound, resulting in an application rate of about 1–2 μCi/test and in soil concentrations of 3.33 and 1.0 mg/kg, respectively. In order to determine foliar uptake of chemicals via the air, the soil in the dish was covered with a plate containing 10 holes for 10 barley grains. Thus, uptake of chemicals from the air could occur only by the leaves, since the soil was covered and uptake of chemicals by soil from the air and hence uptake by plants from the soil was largely suppressed. Into the treated soil were placed 10 barley seeds to determine total uptake both via roots and leaves. After the exposure time, plant roots were washed and analyzed together with plant tops. Chemical sublimated from the air on glass walls were removed with methanol and, after quantification, added to those trapped in ethylene glycol monomethyl ether. All experiments were carried out in duplicate.

For the determination of unextractable radioactivity and of conversion rates to soluble metabolites, plants were homogenized in methanol and extracted with methanol in a Soxhlet for 48 h. Aliquots of soil were also extracted with methanol in a Soxhlet. The radioactivity in the extracts was determined in a liquid scintillation counter. The radioactivity left in extracted solid matter was counted after combustion to $^{14}CO_2$.

For the determination of the ratio of DEHP and its conversion products, the individual soil and plant extracts were concentrated in a rotary evaporator. The sample extracts were chromatographed on silica gel G plates with benzene/*n*-hexane/acetone/acetic acid (65:25:25:5). The separated radioactive zones were localized with a scanner. For quantitative determinations, the silica gel layer of the plate was cut to 1-cm parts, and their radioactivity was determined by liquid scintillation counting.

Outdoor Experiments. Outdoor experiments were carried out in lysimeters as described by Scheunert et al. (1977, 1986). Plants were grown in a water-resistant plywood box (60 × 60 × 60 cm) with a perforated base. The box was placed in a metal tray to collect the leached water. The box was filled with 160 kg of soil to 1 cm from the top and was kept in a large pit with the upper surface of the soil at the same level as the surrounding ground. A 1.9-mg portion of [*carboxyl*-^{14}C]DEHP and 38.1 mg of inactive DEHP were dissolved in 50 mL of acetone, applied dropwise on the soil, and incorporated to an about 10-cm depth, corresponding to an initial concentration of about 1 mg/kg dry soil in a 10-cm depth. Four potatoes (Juliver) were planted 8–10 cm deep and at a distance of 20 cm in the first year, immediately after application of the chemical. In the following year, barley was grown as a rotation crop without further soil treatment with [^{14}C]DEHP. Fertilization was done as in agricultural practice. The leached water collected in the metal tray was analyzed for radioactivity during the experimental time.

Potatoes were harvested 111 days after application of [^{14}C]DEHP and planting; barley was harvested 446 days after application of [^{14}C]DEHP and 104 days after planting. After the harvest, the different parts of the plants were separated, weighed, and stored at −20 °C until analysis. Immediately after harvest, four representative soil samples of about 400–600 g each were taken from different depths with the help of an auger. These samples were also stored at −20 °C until analyzed. The moisture content of soil samples was determined by drying to constant weight in a vacuum desiccator at room temperature. The potato and the barley samples were homogenized by an ultra-turrax and extracted in a Soxhlet with methanol for 48 h. The soil samples were extracted likewise with acetone for 48 h in a Soxhlet.

The radioactivity in the extracts and in the leached water was determined by counting in a liquid scintillation counter. Unextracted radioactivity in plants and soil was determined by combustion. The determination of the ratio of parent compound and conversion products in the extracts was performed as in the case of laboratory soil–plant studies.

Isolation of Conversion Products. Isolation of conversion products in soil was carried out only with the top soil layer (0–20-cm depth) of the outdoor experiments of the first year, since in deeper layers as well as in all soil samples

Table I. $^{14}CO_2$ Formed from [^{14}C]DEHP in a Shaken Aerobic Soil–Water Suspension, 22 °C (Percent of ^{14}C Initially Applied)

day of measmt	time, days	$^{14}CO_2$ det	$^{14}CO_2$/day
5	5	5.56	1.11
9	4	3.95	0.99
13	4	2.83	0.71
22	9	4.31	0.48
28	6	2.65	0.44
33	5	2.58	0.52
sum	33	21.88	0.66

of the second year radioactivity was too low. The soil extract was resolved into six zones by chromatography on preparative silica gel plates (20 × 20 cm) with benzene/*n*-hexane/acetone/acetic acid, 65:25:25:5. R_f values: phthalic acid, 0.11; mono(2-ethylhexyl) phthalate, 0.55; DEHP, 0.93. The radioactivity in each zone was desorbed from silica gel with methanol and purified by repeated thin-layer chromatography. Then, the isolated metabolites were methylated with diazomethane, rechromatographed on silica gel plates, desorbed with benzene, concentrated, and subjected to gas chromatography and combined gas chromatography/mass spectrometry. In order to isolate conversion products of the plants, the potato and barley extracts were separately pooled. From these extracts neither any metabolites nor the unconverted DEHP could be isolated.

Identification of Conversion Products. Gas chromatography was performed on a fused silica column, 25 m × 0.25 mm (i.d.), 0.5-μm df (df = film thickness = column radius divided by 2 × phase ratio) with methylsilicone SP 2100, Hewlett-Packard. The carrier gas was nitrogen at a flow rate of 2 mL/min. The column was programmed from 100 to 250 °C at 5 °C/min. The injector and detector temperatures were 260 °C. The substances were identified on the basis of their retention times compared with those of standards. Retention times: dimethyl phthalate, 11.34 min (157 °C); methyl 2-ethylhexyl phthalate, 24.35 min (222 °C); DEHP, 34.39 min (250 °C).

Mass spectrometric conditions were as follows: electron energy, 70 eV; mass range, 50–400 amu; mass scan rate, 380 amu/s; source temperature, 148 °C; analyzer temperature, 180 °C. The chromatographic separation was achieved on a 25 m × 0.25 mm (i.d.) fused silica column with methylsilicone SP 2100 (Hewlett-Packard). The carrier gas was helium at a flow rate of 2 mL/min. The column was programmed from 100 to 250 °C at 5 °C/min. The injector temperature was 250 °C. The mass spectra were compared with those of reference compounds.

RESULTS AND DISCUSSION

Laboratory Degradation Studies in Suspended Soil. $^{14}CO_2$ formed from [^{14}C]DEHP in a shaken soil–water suspension at 22 °C under aerobic conditions is listed in Table I. The left column presents $^{14}CO_2$ formed during the time interval since the last measurement, whereas the right one shows $^{14}CO_2$ formed/day.

The amount of $^{14}CO_2$ evolved/day was higher during the first 2–3 weeks and reached a nearly constant level after the 22nd day. The sum of $^{14}CO_2$ after 9 days was 9.5%, and after 33 days, 21.9%. The half-life of DEHP, as derived from the data of Table I, is about 95 days. DEHP was reported to be readily biodegradable also in the aerobic semicontinuous activated sludge (SCAS) test (Saeger and Tucker, 1973). In an anaerobic soil suspension, degradation to CO_2 was lower than under aerobic conditions (Scheunert et al., 1987). In anaerobic freshwater hydrosoils (Johnson and Lulves, 1975) as well as in anaerobic digester sludge (Shelton et al., 1984), DEHP was not degraded.

Laboratory Soil–Plant Studies. The results of investigations with [^{14}C]DEHP in closed aerated laboratory soil–plant systems after 7 days are shown in Table II.

It can be seen from the table that there is no difference in mineralization between the two soil concentrations. The value of 8.2% CO_2 after 7 days corresponds well to the value of 9.5% after 9 days in the soil suspension. The main portion of radioactivity was recovered in soil, and most of it was extractable by methanol. Uptake of ^{14}C by plants was low; more than half of the absorbed radioactivity is due to uptake by leaves from the air. Since the volatilization of organic substances from soil into the air was very low, it is assumed that the radioactivity taken up via leaves was preferably $^{14}CO_2$ and, therefore, does not represent uptake of a xenobiotic compound. The bioaccumulation factor of radioactivity taken up by roots, i.e., concentration of radioactivity in plants taken up by roots divided by concentration of radioactivity in treated soil, was 0.10 in the experiment with the higher soil concentration and 0.23 in the experiment with the lower soil concentration. That means that a bioconcentration of radioactivity by plants from soil does not occur.

Thin-layer chromatography of soil and plant extracts of the 1 ppm experiment revealed that 89.8% of the radioactivity extracted from soil was the unchanged parent compound. The remaining radioactivity was polar material that was too low an amount for further investigation. Extracted radioactivity in plants grown in treated soil contained 98.7% of very polar materials, that in plants grown in untreated soil, 99.6%. This radioactivity is assumed to include natural plant constituents assimilated from $^{14}CO_2$.

Outdoor Experiments. *Balance and Residues.* One vegetation period after the application of [^{14}C]DEHP to potato soil (111 days), 6.9% of the applied radiocarbon was recovered in soil (Table III). In the potato plants, only 0.11% was detected, most of which was in the peeled tubers in an unextractable form. Residues in terms of ppm (mg/kg) were low. The water leached from the base of the box contained 0.51% of the radioactivity initially applied; this radioactivity was not investigated further. It is assumed that this radioactivity is due to phthalate complexed with fulvic acid and, thus, transformed into a more water-soluble form, as reported by Ogner and Schnitzer (1970). The remaining radioactivity—92.5%—was lost into

Table II. Mass Balance and Fate of [^{14}C]DEHP in a Laboratory Soil-Barley System (after 7 days, in Percent[a] of ^{14}C Applied)

	3.33 mg/kg dry soil			1 mg/kg dry soil		
sample	extr	unextr	total	extr	unextr	total
soil, treated	81.37	1.75	83.12	88.06	1.30	88.36
plants in treated soil	0.70	0.55	1.25	0.44	0.17	0.61
soil, untreated	0.46	0.23	0.69	0.14	0.11	0.25
plants in untreated soil	0.49	0.48	0.97	0.23	0.16	0.39
$^{14}CO_2$			8.34			8.18
organic volatiles			0.21			0.64
sum			94.58			99.43

[a] Mean value of two replicates.

Table III. Residues of [^{14}C]DEHP and Its Conversion Products in Soil, Potatoes, and Leached Water, One Vegetation Period (111 days) after Treatment of Soil (Depth 0–10 cm) under Outdoor Conditions

	extract		unextr residues		total	
sample	ppm[a]	%[b]	ppm	%	ppm	%
soil depth, cm						
0–20	0.024	4.78	0.009	1.84	0.033	6.62
20–30	<0.001	0.04	<0.001	0.02	<0.001	0.06
30–40	<0.001	0.01	<0.001	0.05	<0.001	0.06
40–50	<0.001	0.02	0.001	0.12	0.001	0.14
total	0.010	4.85	0.004	2.03	0.014	6.88
potatoes, peeled	0.010	0.009	0.067	0.060	0.077	0.069
peel	0.004	0.001	0.028	0.009	0.032	0.010
shoots	0.009	0.002	0.110	0.020	0.119	0.022
roots	0.030	0.002	0.130	0.007	0.160	0.009
plants, total	0.009	0.014	0.067	0.096	0.076	0.110
leached water					0.01	0.51
total rec						7.50

[a] In ppm equivalent to [^{14}C]DEHP, based on dry weight for soil and fresh weight for plant. [b] Percent of total radioactivity applied.

the atmosphere. The chemical nature of this evaporated radioactivity cannot be determined in an open natural system; according to the results of the laboratory experiments, most of this radioactivity is carbon dioxide derived from total degradation of the chemical. Volatilization probably plays only a minor role in loss of radioactive residues since Henry's law constant for DEHP at 25 °C, as estimated from saturation vapor pressure and solubility in water, is low (4×10^{-3} Pa·L·g^{-1}; Klöpffer et al., 1982), and only a small portion of the substance is in the soil water phase and, thus, available for ready volatilization. This assumption was confirmed by Fairbanks et al. (1985) who found that degradation to CO_2 was the only mechanism of loss of [^{14}C]DEHP from calcareous soils; no volatilization of parent compound or organic metabolites was detected. If we assume that, in these outdoor experiments, the loss of radioactivity is completely due to degradation to $^{14}CO_2$, the degradation rate is higher than that extrapolated from laboratory data. This difference might be due to the differences in soil properties and to better growing conditions for soil microflora under outdoor than under laboratory conditions.

In the second vegetation period, barley was grown in the same soil without retreatment with radiochemical. Total recovery of radioactivity in soil after two vegetation periods (446 days) was only 1.7%, preferably in a soil-bound form. Uptake by barley plants amounted to only 0.005%, and leaching into water within the second vegetation period (104 days), to 0.01% (Table IV).

Identification and Quantification of Conversion Products. The extracted radioactivity of the top *soil* layer of the first year was separated into parent compound and various conversion products by thin-layer chromatography. Conversion products were quantified and identified by combined gas chromatography/mass spectrometry after methylation with diazomethane. The results are shown in Table V.

The mass spectrum of TLC fraction 6 (Table V) showed the characteristic fragments of the parent compound DEHP. These were the fragments m/e 279 and 167, formed by the loss of both carbon chains in a double McLafferty rearrangement, and the base peak m/e 149 typical for the diesters of phthalic acid (except for dimethyl ester). The mole peak $M^{\bullet+}$ 390 was not present. The parent compound represents the major radioactive product in soil (3% of radioactivity applied).

In fraction 4 (Table V), mono(2-ethylhexyl) phthalate was identified after methylation to methyl 2-ethylhexyl phthalate (0.14% of radioactivity applied). The mass spectrum showed the typical base peak m/e 163 as well as the characteristic fragments m/e 181 and 149, but no mole peak $M^{\bullet+}$ 292.

In fraction 2 (Table V), phthalic acid was identified after methylation to dimethyl phthalate (0.35% of radioactivity applied). In the mass spectrum, the mole peak $M^{\bullet+}$ 194 as well as the base peak m/e 163 and the typical fragments m/e 135 and 133 were seen.

A very polar group of conversion products (Table V, fraction 1) as well as two further conversion products (Table V, fractions 3 and 5) could not be identified (1.29% of radioactivity applied). Similarly, the chemical identity of a small fraction of unextractable residues (1.84%) was not established.

Figure 1 presents the pathway of conversion of DEHP in soil. First, the monoester is formed by hydrolysis and then free phthalic acid. Since abiotic hydrolysis of DEHP is very slow (half-life time at pH 6 and 30 °C of 100 years; Wolfe et al., 1980), it is concluded that the rapid hydrolysis occurring in soil is an enzymatic process. In laboratory experiments with bacteria, carried out by several authors, the formation of mono(2-ethylhexyl) phthalate and of phthalic acid was observed (Pierce et al., 1980).

The radioactivity in *plants* consisted, like that in the plants of laboratory experiments, of very polar materials

Table IV. Residues of [^{14}C]DEHP and Its Conversion Products in Soil, Barley Plants, and Leached Water, Two Vegetation Periods (446 days) after Treatment of Soil (Depth 0–10 cm) under Outdoor Conditions

	extract		unextr residues		total	
sample	ppm[a]	%[b]	ppm	%	ppm	%
soil depth, cm						
0–20	<0.001	0.01	0.007	1.33	0.007	1.34
20–30	<0.001	<0.01	<0.001	0.09	<0.001	0.09
30–40	<0.001	<0.01	<0.001	0.09	<0.001	0.09
40–50	<0.001	<0.01	0.001	0.19	0.001	0.19
total	<0.001	0.01	0.003	1.70	0.003	1.71
barley						
husks	0.003	<0.001	0.006	<0.001	0.009	<0.001
grain	0.010	0.002	0.006	0.001	0.016	0.003
straw			0.006	<0.001	0.006	<0.001
roots, stubbles	0.005	<0.001	0.019	0.002	0.024	0.002
plants, total	0.006	0.002	0.009	0.003	0.015	0.005
leached water					<0.01	0.01
plants, 1st veg period						0.11
leached water, 1st veg period						0.51
total rec						2.34

[a] In ppm equivalent to [^{14}C]DEHP, based on dry weight for soil and fresh weight for plant. [b] Percent of total radioactivity applied.

Table V. Separation of Radioactivity Derived from [^{14}C]DEHP in the Top Soil Layer (0–20-cm Depth) by Thin-Layer Chromatography and Identification of Conversion Products

fraction	R_f[a]	radioact,[b] %	subst ident	R_f[a]
1	0–0.05	0.42		
2	0.05–0.20	0.35	PA[c]	0.15
3	0.20–0.40	0.17		
4	0.40–0.60	0.14	MEHP[d]	0.54
5	0.60–0.80	0.70		
6	0.80–0.95	3.00	DEHP	0.89

[a] TLC, solvent system benzene/*n*-hexane/acetone/acetic acid, 65:25:25:5. [b] Percent of radioactivity applied. [c] Phthalic acid. [d] Mono(2-ethylhexyl) phthalate.

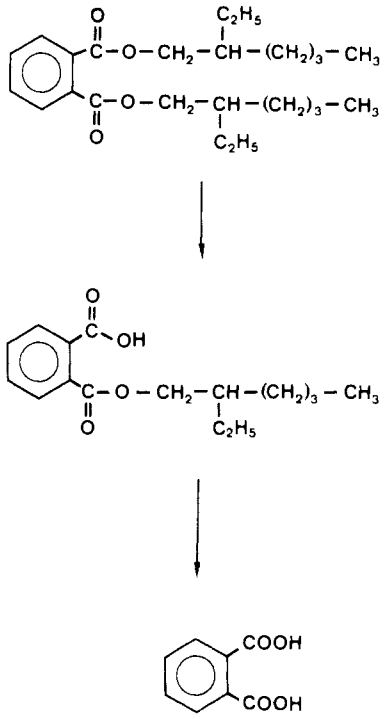

Figure 1. Conversion of DEHP in soil.

that could not be identified. Neither the parent compound DEHP nor the monoester nor phthalic acid was detected in the plants. This is in line with observations of Kato et al. (1980) who did not find any DEHP in several plant species (*Chrysanthemum coronarium*, *Brassica rapa*, spinach) grown in DEHP-contaminated soil. Krell and Sandermann (1986) found that DEHP was not taken up into wheat cells when applied to wheat leaves but was partitioned or adsorbed to cuticles, triglyceride droplets, and cell wall components. When DEHP was taken up into plant cells, e.g. in cell culture experiments, it was metabolized rapidly, predominantly to polar β-D-glucosyl conjugates. The same occurred with mono(2-ethylhexyl) phthalate (Krell and Sandermann, 1986). For phthalic acid, which may be formed from DEHP in soil, it was shown that a significant uptake of ^{14}C from the ^{14}C-labeled compound by plants occurred (Dorney et al., 1985). However, only a small portion of this ^{14}C was due to phthalic acid; most of the ^{14}C was polar and associated with chlorophyll.

All of these observations give an explanation for the fact that in this study neither DEHP nor one of its soil metabolites was detected in plants.

CONCLUSION

It may be concluded that DEHP is mineralized readily in soil to carbon dioxide. As conversion products, mono-(2-ethylhexyl) phthalate and phthalic acid are formed; after two vegetation periods, however, these also are either mineralized or converted into soil-bound residues. Excessive mobility in soil and leaching are prevented by the low persistence of the residues. Uptake by plants is of minor importance.

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LITERATURE CITED

Albro, P. W.; Thomas, R.; Fishbein, L. "Metabolism of Di(2-ethylhexyl)phthalate by Rats: Isolation and Characterization of Urinary Metabolites." *J. Chromatogr.* **1973**, *76*, 321–330.

Bove, J. L.; Dalven, P.; Kukreja, V. P. "Airborne Dibutyl and Di(2-ethylhexyl)phthalate at Three New York City Air Sampling Stations." *Int. J. Environ. Anal. Chem.* **1978**, *5*, 189–194.

Brownlee, B.; Strachan, W. M. J. "Distribution of Some Organic Compounds in the Receiving Waters of a Kraft Pulp and Paper Mill." *J. Fish. Res. Board Can.* **1977**, *34*, 830–837.

Cifrulak, S. D. "Spectroscopic Evidence of Phthalates in Soil Organic Matter." *Soil Sci.* **1969**, *107*, 63–69.

Corcoran, E. F.; Curry, R. W. "Phthalic Acid Esters in the Marine Environment." *Rev. Biol. Trop.* **1978**, *26* (Suppl. 1), 125–133.

Daniel, J. W. "Toxicity and Metabolism of Phthalate Esters." *Clin. Toxicol.* **1978**, *13*, 257–268.

Dorney, J. R.; Weber, J. B.; Overcash, M. R.; Strek, H. J. "Plant Uptake and Soil Retention of Phthalic Acid Applied to Norfolk Sandy Loam." *J. Agric. Food Chem.* **1985**, *33*, 398–403.

Erhardt, M.; Derenbach, J. "Phthalate Esters in the Kiel Bight." *Mar. Chem.* **1980**, *8*, 339–346.

Fairbanks, B. C.; O'Connor, G. A.; Smith, S. E. "Fate of Di-2-(ethylhexyl)phthalate in Three Sludge-Amended New Mexico Soils." *J. Environ. Qual.* **1985**, *14*, 479–483.

Fishbein, L.; Albro, P. W. "Chromatographic and Biological Aspects of the Phthalate Esters." *J. Chromatogr.* **1972**, *70*, 365–412.

Giam, C. S.; Atlas, E. "Accumulation of Phthalate Ester Plasticizers in Lake Constance Sediments." *Naturwissenschaften* **1980**, *67*, 508–510.

Giam, C. S.; Chan, H. S.; Neff, G. S. "Concentrations and Fluxes of Phthalates, DDTs and PCBs to the Gulf of Mexico." In *Marine Pollutant Transfer*; Windom, H. L., Duce, R. A., Eds.; Lexington Books, D. C. Heath Co.: Lexington, MA, 1976.

Giam, C. S.; Chan, H. S.; Neff, G. S.; Atlas, E. L. "Phthalate Ester Plasticizers: A New Class of Marine Pollutant." *Science (Washington, D.C.)* **1978**, *199*, 419–421.

Giam, C. S.; Atlas, E.; Chan, H. S.; Neff, G. S. "Phthalate Esters, PCB and DDT Residues in the Gulf of Mexico Atmosphere." *Atmos. Environ.* **1980**, *14*, 65–69.

Hites, R. A. "Analysis of Trace Organic Compounds in New England Rivers." *J. Chromatogr. Sci.* **1973**, *11*, 570–574.

Hoffman, W. A., Jr.; Lindberg, S. E.; Turner, R. R. "Some Observations of Organic Constituents in Rain above and below a Forest Canopy." *Environ. Sci. Technol.* **1980**, *14*, 999–1002.

Inman, J. C.; Strachan, S. D.; Sommers, L. E.; Nelson, D. W. "The Decomposition of Phthalate Esters in Soil." *J. Environ. Sci. Health, Part B* **1984**, *B19*, 245–257.

Johnson, B. T.; Lulves, W. "Biodegradation of Di-(n-butyl)-phthalate and Di-(2-ethylhexyl)phthalate in Fresh Water Hydrosoil." *J. Fish. Res. Board Can.* **1975**, *32*, 333–339.

Jungclaus, G. A.; Lopez-Avila, V.; Hites, R. A. "Organic Compounds in an Industrial Wastewater: A Case Study of Their Environmental Impact." *Environ. Sci. Technol.* **1978**, *12*, 88–96.

Karasek, F. W.; Denney, D. W.; Chan, K. W.; Clement, R. E. "Analysis of Complex Organic Mixtures on Airborne Particulate Matter." *Anal. Chem.* **1978**, *50*, 82–87.

Kato, K.; Nakaoka, T.; Ikeda, H. "Contamination of Phthalic Acid Esters in Vegetables." *Kanagawa-ken Eisei Kenkyusho Kenkyu Hokoku* **1980**, *67* (Japan); *Chem. Abstr.* **1981**, *95*, 60034k.

Khan, S. U.; Schnitzer, M. "The Retention of Hydrophobic Organic Compounds by Humic Acid." *Geochim. Cosmochim. Acta* **1972**, *36*, 745–754.

Klöpffer, W.; Kaufmann, G.; Rippen, G.; Poremski, H.-J. "A Laboratory Method for Testing the Volatility from Aqueous Solution: First Results and Comparison with Theory." *Ecotoxicol. Environ. Saf.* **1982**, *6*, 545–559.

Krell, H.-W.; Sandermann, H., Jr. "Metabolism of the Persistent Plasticizer Chemical Bis(2-ethylhexyl) phthalate in Cell Suspension Cultures of Wheat (*Triticum aestivum* L.). Discrepancy from the Intact Plant." *J. Agric. Food Chem.* **1986**, *34*, 194–198.

Lawrence, W. H. "Phthalate Esters: The Question of Safety." *Clin. Toxicol.* **1978**, *13*, 89–139.

Lawrence, W. H.; Tuell, S. F. "Phthalate Esters: The Question of Safety—an Update." *Clin. Toxicol.* **1979**, *15*, 447–466.

Malisch, R.; Schulte, E.; Acker, L. "Chlororganische Pestizide, polychlorierte Biphenyle und Phthalate in Sedimenten aus Rhein und Neckar." *Chem.-Ztg.* **1981**, *105*, 187–194.

Mayer, F. L., Jr.; Stalling, D. L.; Johnson, J. L. "Phthalate Esters as Environmental Contaminants." *Nature (London)* **1972**, *238*, 411–413.

Melancon, M. J. *Metabolism of Phthalate Esters in Aquatic Species*; Advances in Chemistry Series 99; American Chemical Society: Washington, DC. 1979; pp 77–94.

Michael, P. R.; Adams, W. J.; Werner, A. F.; Hicks, O. "Surveillance of Phthalate Esters in Surface Waters and Sediments in the United States." *Environ. Toxicol. Chem.* **1984**, *3*, 377–389.

Morita, M.; Nakamura, H.; Mimura, S. "Phthalic Acid Esters in Water." *Water Res.* **1974**, *8*, 781–788.

Müller, G.; Dominik, J.; Reuther, R. "Sedimentary Record of Environmental Pollution in the Western Baltic Sea." *Naturwissenschaften* **1980**, *67*, 595–600.

Murray, H. E.; Ray, L. E.; Giam, C. S. "Phthalic Acid Esters, Total DDTs, and Polychlorinated Biphenyls in Marine Samples from Galveston Bay, Texas." *Bull. Environ. Contam. Toxicol.* **1981**, *26*, 769–774.

National Toxicological Program "Carcinogenesis Bioassay of Di(2-ethylhexyl) Phthalate in F 344 Rats and B6C3F1 Mice (Feed Study)". Report NTP-80-37, NIH 82-1773: Research Triangle Park, NC 27709, and Bethesda, MD 20205, 1982.

Ogner, G.; Schnitzer, M. "Humic Substances: Fulvic Acid–Dialkylphthalate Complexes and Their Role in Pollution." *Science (Washington, D.C.)* **1970**, *170*, 317–318.

Payne, W. R.; Benner, J. E. "Liquid and Gas Chromatographic Analysis of Diethyl Phthalate in Water and Sediment." *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1403–1407.

Peakall, D. B. "Phthalate Esters: Occurrence and Biological Effects." *Res. Rev.* **1975**, *54*, 1–41.

Persson, P.-E.; Penttinen, H.; Nuorteva, P. "DEHP in the Vicinity of an Industrial Area in Finland." *Environ. Pollut.* **1978**, *16*, 163–166.

Peterson, J. C.; Freeman, D. H. "Phthalate Ester Concentration Variations in Dated Sediment Cores from the Chesapeake Bay." *Environ. Sci. Technol.* **1982**, *16*, 464–469.

Pierce, R. C.; Mathur, S. P.; Williams, D. T.; Boddington, M. J. "Phthalate Esters in the Aquatic Environment". Publication NRCC No. 17583; National Research Council Canada, Environmental Secretariat: Ottawa, Ontario K1A 0K9, 1980.

Rhoades, J. W.; Thomas, R. E.; Johanson, D. E. "Determination of Phthalates in Industrial and Municipal Waste Waters". EPA 600/4-81-063; NTIS: Springfield, VA, 1981.

Robertson, J. M.; Li, E. C. C. "Organic Leachate Threatens Groundwater Quality." *Water Sewage Works* **1976**, *123*, 58–59.

Russell, D. J.; McDuffie, B. "Chemodynamic Properties of Phthalate Esters: Partitioning and Soil Migration." *Chemosphere* **1986**, *15*, 1003–1021.

Saeger, V. W.; Tucker, E. S. "Phthalate Esters Undergo Ready Biodegradation." *Plastics Eng.* **1973**, *29*, 46–49.

Scheunert, I.; Kohli, J.; Kaul, R.; Klein, W. "Fate of ^{14}C-Aldrin in Crop Rotation under Outdoor Conditions." *Ecotoxicol. Environ. Saf.* **1977**, *1*, 365–385.

Scheunert, I.; Zhang, Q.; Korte, F. "Comparative Studies of the Fate of Atrazine-^{14}C and Pentachlorophenol-^{14}C in Various Laboratory and Outdoor Soil-Plant Systems." *J. Environ. Sci. Health, Part B* **1986**, *B21*, 457–485.

Scheunert, I.; Vockel, D.; Schmitzer, J.; Korte, F. "Biomineralization Rates of ^{14}C-Labelled Organic Chemicals in Aerobic and Anaerobic Suspended Soil." *Chemosphere* **1987**, *16*, 1031–1041.

Schouten, M. J.; Copius Peereboom, J. W.; Brinkman, U. A. Th. "Liquid Chromatographic Analysis of Phthalate Esters in Dutch River Water." *Int. J. Environ. Anal. Chem.* **1979**, *7*, 13–23.

Schwartz, H. E.; Anzion, C. J. M.; Van Vliet, H. P. M.; Copius Peereboom, J. W.; Brinkman, U. A. Th. "Analysis of Phthalate Esters in Sediments from Dutch Rivers by Means of High Performance Liquid Chromatography." *Int. J. Environ. Anal. Chem.* **1979**, *6*, 133–144.

Shanker, R.; Ramakrishna, C.; Seth, P. K. "Degradation of Some Phthalic Acid Esters in Soil." *Environ. Pollut., Ser. A* **1985**, *39*, 1–7.

Shelton, D. R.; Boyd, S. A.; Tiedje, J. M. "Anaerobic Biodegradation of Phthalic Acid Esters in Sludge." *Environ. Sci. Technol.* **1984**, *18*, 93–97.

Thomas, J. A.; Northup, S. J. "Toxicity and Metabolism of Monoethylhexylphthalate and Diethylhexylphthalate: A Survey of Recent Literature." *J. Toxicol. Environ. Health* **1982**, *9*, 141–152.

Thomas, J. A.; Darby, T. D.; Wallin, R. F.; Garvin, P. J.; Martis, L. "A Review of the Biological Effects of Di-(2-ethylhexyl)-phthalate." *Toxicol. Appl. Pharmacol.* **1978**, *45*, 1–27.

Thurén, A. "Determination of Phthalates in Aquatic Environments." *Bull. Environ. Contam. Toxicol.* **1986**, *36*, 33–40.

Wolfe, N. L.; Steen, W. C.; Burns, L. A. "Phthalate Ester Hydrolysis: Linear Free Energy Relationships." *Chemosphere* **1980**, *9*, 403–408.

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