Plant Uptake and Soil Retention of Phthalic Acid Applied to Norfolk Sandy Loam

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Plant uptake and soil retention of ^{14}C carboxyl-labeled phthalic acid were studied at application rates of 0.6, 6.0, 60.0, and 600.0 ppm (soil dry weight) to Norfolk sandy loam (Typic Paleudult, fine loamy, kaolinitic, thermic). Height and dry weight of corn (*Zea mays* L. "Pioneer 3368A") (21 day), tall fescue (*Festuca arundinacea* Schreb. "Kentucky 31") (45 day), immature soybean (*Glycine max* (L.) Merr. "Altoona") (21 day) plant, mature soybean plant, and mature wheat (*Triticum aestivum* L. "Butte") straw were not affected ($p \leq 0.05$) by phthalic acid applied to soil. In addition, soybean seed and wheat seed dry weight were unaffected. Immature wheat (40 day) height decreased at the 600 ppm rate. Plant uptake of phthalic acid ranged from 0 to 23 ppm and was significantly above background ($p \leq 0.05$) for all plants and plant materials except soybean pods. Fescue and immature plants exhibited the highest concentration of phthalic acid while mature wheat plants and wheat seeds exhibited the least. Most of the phthalic acid volatilized or was decomposed from the soil by the end of the study; an average of only 5.7% of the originally applied chemical was recovered in both soil or plants. An average of 0.02% of the originally applied phthalic acid leached out of the treated zone. Considering the low toxicity of phthalic acid and its relatively rapid disappearance from soil, it is unlikely to become a health hazard from contaminated plants. However, plant uptake of other toxic organics could potentially become a hazard on soils treated with sludge containing significant quantities of these substances.

Application of municipal and industrial sludge to agricultural land has become common practice. Past research has examined the effect of heavy metals in sludge on crop plant growth and uptake. Recently, concern has been expressed about crop uptake of toxic organic compounds from sludge (Gonzalez-Villa et al., 1982). For instance, Shea et al. (1982) studied corn uptake of di-*n*-butyl phthalate (DnBP). They found 0.32 ppm of DnBP in corn from soil treated with 200 ppm. The behavior of some organic compounds not on the U. S. Environmental Protection Agency's Priority Pollutant list has been discussed in soil/plant systems (Overcash, 1981; Overcash and Pal, 1979).

Phthalic acid (1,2-benzenedicarboxylic acid) consists of a benzene ring with two adjacent (ortho) carboxylic acid side groups. It has a density of 1.593 g/mL and relatively high water solubilities of 0.54 g/100 mL at 14 °C and 1.01 g/100 mL at 35 °C (Seidell, 1928). Phthalic acid has a low toxicity in mammals with a LD_{50} of 12.6 g/kg in rats (Leah, 1977). For comparison, rat acute oral toxicities (LD_{50}) of some phthalic acid esters are either similar (di-*n*-butyl phthalate, 12.5 g/kg), less toxic (bis(2-ethylhexyl) phthalate, 30.6 g/kg), or more toxic (dimethyl phthalate, 6.8 g/kg) (Leah, 1977) than the acid itself.

Phthalic acid is used to tan hides and remove Cr from tannery wastes (Balberova et al., 1984). It is also a catalyst in resorcinol–formaldehyde resin manufacturing (Gamidov et al., 1982) and or a corrosion inhibitor (Nakajima et al, 1974). Phthalic acid forms Fe chelates and can be used to remove pyritic Fe from coal (Burk et al., 1979). It is found in waste from the polyester fiber industry (Leenheer et al., 1976). Jolley et al. (1976) found 200 ppb of phthalic acid in primary domestic sewage effluent. Raikina et al. (1981) reported phthalic acid concentrations of 0.027–1.34 g/L in waste water from a dialkyl phthalate manufacturing plant in Russia. The USSR has set a limit of 0.5 mg/L to prevent inhibition of self-purification processes in receiving water (Verschueren, 1977).

The phthalic acid esters (particularly bis(2-ethylhexyl), diisodecyl, diethyl, and di-*n*-butyl phthalates) are industrial plasticizers commonly found as low level environmental pollutants (Peakall, 1975). Strachen (1979) found phthalic acid concentrations ranging from 0.2 to 1.6% oven dry weight of three municipal sludges from Indiana.

Phthalic acid is a common microbial breakdown product of phthalates in freshwater (Sodergren, 1982; Saeger and Tucker, 1976), activated sludge (Saeger and Tucker, 1976; Shelton et al., 1984), and under anaerobic conditions (Benckiser and Ottow, 1982, Shelton et al., 1984). Shelton et al. (1984) found that anaerobic breakdown of butyl benzyl phthalate resulted in a peak accumulation of phthalic acid at 10 days with a gradual decline to zero by 24 days. Phthalic acid is also present in low levels in precipitation. Likens et al. (1983) detected a variety of organic acids in precipitation in Ithaca (IT), NY, and the Hubbard Brook (HB) Experimental Forest, NH. They found phthalic acid in samples from HB at concentrations less than 0.1 μmol. Other organic acids (e.g., acetic acid at 2.8 μmol at HB and 1.1 μmol at IT, formic acid at 0.2 μmol at HB and 2.4 μmol at IT) were present in higher concentrations. Matsumoto and Hanya (1980) also found phthalic acid in atmospheric fallout near Tokyo, Japan.

Degradation of phthalic acid is rapid in soil. Conversion to benzoic acid by *Bacillus* sp. and *Pseudomonas testosteroni* was reported by Taylor and Ribbons (1983). Aftring et al. (1981), Harada and Koiwa (1977), and Englehardt et al. (1976) isolated various soil bacteria which rapidly degraded phthalic acid to protocatechuate. El-Shinnawi and Shalabi (1981) studied soil degradation of 16 organic

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acids and reported that citric and phthalic acid degraded more slowly than other organic acids.

The input of phthalic acid to agricultural land from the atmosphere is probably minor. More important inputs are directly from sewage sludge and from phthalate ester breakdown in the soil. Purposes of our research were to determine (1) the effect of phthalic acid on plant growth, (2) the amount of uptake in crops, (3) the amount of phthalic acid remaining in the soil at plant maturity and (4) the extent of leaching.

MATERIALS AND METHODS

Apparatus. Two gallon plastic containers (21 cm diameter, 24 cm tall) were lined with aluminum foil, filled with 7.9 kg (oven dry weight) of Norfolk sandy loam (*Typic Paleudult*, fine loamy, kaolinitic, thermic) which had been air-dried, sieved through a 6.4 mm mesh screen, and placed on aluminum pans. Pots were watered to approximately 80% of field capacity and placed in a greenhouse for two weeks to initiate microbial growth. The soil was not sterilized and was a structureless sand (87% sand, 10% silt, 3% clay), low in organic matter (1.3% by the chromic acid method), moderate in acidity (pH 6.1), and with low cation exchange capacity (1.78 mequiv/100).

Reagents. ^{14}C-labeled phthalic acid (Amersham CFA 766 Batch 5, Specific Activity 60 mCi/mmol) was added to unlabeled phthalic acid (Fisher certified grade), dissolved in ethanol, and thoroughly mixed into the upper 15 cm (5.8 kg) of the soil in each pot (6400 dpm/g of dry weight soil). Three replicates of a randomized, complete block design were treated with application rates of 0.6, 6.0, 60.0, and 600.0 ppm and two crop combinations. Phthalic acid rates were based on three assumptions: (1) Municipal sludge is normally applied at rates of from 10 to 60 ton/acre (dry weight), (2) phthalic acid in sludges may range from 0.1 to 1.0% (dry weight), and (3) sludges are normally tilled into the plow layer of the soil (0–6 in. depth, weighing approximately 1000 ton/acre). Six control (background) pots were also included for a total of 30 pots. A time release fertilizer (Osmocote 14-14-14, Sierra Chemical Co., Miltites, CA) was added to each pot at a rate of 30 g/pot at planting.

Procedure. Four days after chemical application was made, three pots from each treatment were planted with 25 wheat (*Triticum aestivum* L. "Butte") and 10 corn (*Zea mays* L. "Pioneer 3368A") seeds. Three other pots were planted with 15 soybean (*Glycine max* (L.) Merr. "Altoona") and 2.5 cm^3 of tall fescue (*Festuca arundinacea* Schreb. "Kentucky 31") seeds. Seeds were planted on September 26, 1980; poor germination necessitated replanting on October 20, 1980. Height measurements were made and recorded weekly. Corn and fescue plants were harvested at 21 and 45 days after the final planting, respectively. Immature soybean plants were thinned and retained for analysis at 21 days and the remaining plants were harvested when mature at 74 days. Wheat plants were transplanted from control pots at thinning (40 days old) to make six wheat plants available for mature growth measurements in the 60 and 600 ppm pots. Immature wheat plants were thinned at 40 days and remaining mature plants were harvested at 74 days. Wheat and soybean seeds were separated by hand from nonedible plant portions. Volunteer weeds were also harvested and retained for analysis when the final crop was sampled.

The aboveground portion of each crop was cut into 1–2 cm pieces, weighed, and divided into separate portions for chemical analysis and moisture determination. Portions for moisture determination were weighed, oven-dried at 70 °C for 7 days, and reweighed. Average dry weight percentages for plants were corn (9.8%), fescue (16.5%), immature wheat (18.6%), immature soybean (15.6%), mature wheat (67.6%), and mature soybean (31.8%). Seeds were placed on greenhouse benches for a week until air dry. Portions for chemical analysis were placed in paper bags and frozen until extracted. Soil samples (approximately 150 g) were taken to a depth of 11 cm with a cork borer (1.5-cm diameter) from each pot 80 days after planting. The bottom soil layer from each pot was sampled by inverting the pot, removing the foil, and sectioning the soil. Samples were taken for dry weight determination and chemical analysis. Soil moisture determination was made by weighing, drying overnight at 100 °C, and reweighing. Samples were frozen until extracted.

Insect pests were controlled with oxamyl (methyl N^1,N^1-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxaminidate) and diazinon (*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and diseases were controlled with benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate). Greenhouse temperatures were established at 29 °C for days and 21 °C for nights with relative humidity varying inversely with temperature from 50 to 70%. Supplemental lighting was supplied by four mercury vapor lights which provided a day length of 16 h.

Chemical Analysis. Plant samples were homogenized for 1 min with a "45" Virtis homogenizer at about 39 000 rpm. Hexane–acetone (50 mL, 1:1 Fisher H-300 pesticide grade and Fisher A-18 certified grade, respectively) was used to extract each sample. After homogenization, plant material was washed from the grinding flask and flask contents were filtered through a Whatman GF/A 5.5-mm glass microfiber filter under suction into a side-armed Ehrlenmeyer flask. This flask was washed with hexane–acetone into a 150-mL beaker and the filtrate evaporated to dryness under a hood. The filtrate was then redissolved in 5 mL of methanol and ultrasonicated for 1 min, and 2 mL were removed and retained for layer analysis. Preliminary extractions were redissoved in hexane with a low efficiency of recovery (18%); methanol was more effective (75%). Several drops of hypochlorite bleach (The Chlorox Co., Oakland, CA) were added to the vials to bleach out the chlorophyll in the remaining 3 mL of filtrate. Vials were placed under fluorescent lighting for 15 min to 2 days to aid in chlorophyll bleaching.

Scintillation cocktail [16.5 g of 2,5-diphenyloxazole (PPO), 0.5 g of 1,4-bis[2-(4-methyl-5-phenyloxazoyl)]-benzene (POPOP), 1000 mL of Triton X-100, and 2000 mL of toluene] was added to each vial. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to twice assay each vial (20-min counts). Observed radioactivity was corrected for counting efficiency which ranged from 77 to 89%.

In soil extractions, a 50-g soil sample was weighed, placed in a plastic beaker with 50 mL of hexane–acetone (1:1), and ground for 2 min with a tissue homogenizer (Tek-Mar Homogenizer). After settling for 30 min, the samples was filtered under suction through Whatman GF/B Glass Microfiber filter paper into a side-armed Ehrlenmeyer flask. The filtrate was allowed to evaporate until nearly dry; contents were then transferred to a scintillation vial with 1:1 hexane–acetone washes and then allowed to dry overnight. Methanol (4 mL) was added to each vial, allowed to stand overnight, and ultrasonicated for 1 min. Two milliliters were retained for later analysis and 2 mL were radioassayed by using the same methods as described previously.

Extraction Efficiencies. A mixture of ^{14}C-labeled and unlabeled phthalic acid was injected into mature corn and soybean stems with a microliter syringe. After 24 h, the plants were harvested and analyzed to determine chemical extraction efficiencies. To calculate the extraction efficiency from soil, the [^{14}C]-phthalic acid mixture was mixed into soil in an Ehrlenmeyer flask, extracted, and analyzed after 2 h. For both plant and soil samples, ^{14}C chemical application levels were calculated to be similar to those found in extracted samples. Chemical extraction efficiencies averaged 75% for corn and soybean plants and 87% for soil.

Determination of unextractable (bound) ^{14}C was done for previously extracted plant and soil samples by standard oxidation in a Biological Oxidizer (OX 300) for 4 min under O_2 followed by flushing with N_2. Released $^{14}CO_2$ was captured in a Harvey Cocktail and samples were counted as before.

Plant Uptake and Soil Concentration. Calculations. Concentrations of phthalic acid were calculated by subtracting background counts (from control samples), multiplying by 1.5 or 2.0 (to account for sample splitting for plant and soil samples, respectively), and dividing by sample dry weight. A conversion factor was calculated based on the original ratio of labeled and unlabeled phthalic acid and multiplied by dpm/gdw to yield ppm of extractable ^{14}C in the sample. The ppm value was divided by chemical extraction efficiency (75% for plants and 87% for soil) and then divided by percent dry weight to yield ppm of ^{14}C (dry weight). This value was multiplied by 1000 and by the percentage of radiation identified as phthalic acid by TLC analysis to yield ppb of phthalic acid.

To determine a ^{14}C balance for each pot, total harvest fresh weight for each crop was multiplied by crop dpm/gfw for each pot and divided by ^{14}C extraction efficiency determined from oxidation. These values were summed for all plants in each pot to determine total aboveground ^{14}C in each pot. Since insufficient samples were available, ^{14}C extraction efficiency values were not obtainable for wheat and soybean seed; the values for mature wheat and mature soybean plants were used. Total radiation in bottom (6 cm) and top (15 cm) soil samples were determined in the same manner as plants. To estimate root biomass, all roots were removed from the pots by sieving the soil through two soil sieves (4.0 mm and 1.7 mm). Roots were weighed, a subsample dried for dry weight determination and the remainder frozen for later chemical analysis. Root:shoot ratios were calculated. Root ^{14}C extraction efficiency was assumed to be the same as the surrounding soil. Total ^{14}C activity found in aboveground plants, roots, and soil was summed and chemical extraction efficiencies were included. Total ^{14}C activity accounted for was divided by the originally applied ^{14}C activity to calculate percent recovery.

Leaching. Since the phthalic acid was added to the top 15 cm of each pot, the amount of chemical found in the bottom 6 cm of soil layer was an indication of chemical mobility through leaching. Extractable ^{14}C concentrations of bottom soil were multiplied by weight of the bottom soil, converted to phthalic acid with the TLC data, and divided by total phthalic acid applied to yield percent leaching.

Thin-Layer Chromatography. To determine the percentage of extractable ^{14}C which was phthalic acid, a 2-mL aliquot of duplicate extractions was streaked onto 1000 μm silica gel plates (Type GF, Sigma Chemical Company). Data were aggregated for plants and soil from all application rates. Plates were developed in benzene–acetic acid (8:2). The phthalic acid R_f (0.23) value was determined by using standards mixed with control sample extract and developed in a spark chamber (Birchover Inst. Co., 9.84% methane in argon). Plates were sectioned, scraped, and counted. The percent of the total ^{14}C on the plate which was located at the phthalic acid R_f was determined. Mayer (1976), Metcalf et al. (1973), and Stalling et al. (1973) used TLC to identify ^{14}C-labeled phthalates and their metabolites (including phthalic acid) in aquatic insects, fish, and aquatic plants.

Statistics. Data were analyzed with the General Linear Model (GLM) program from SAS (Barr et al., 1979). Plant uptake and soil retention data were transformed to $\log_{10}$ because of the logarithmic application rates, after converting all data to ppb + 1, to allow for comparison with the 0 rate and the GLM program used to calculate analyses of variance (Snedecor and Cochran, 1967). Independent variables used in the analysis of variance model were replicate and rate. Since fescue grew poorly, only rate was used in order to retain sufficient degrees of freedom for analysis. Regressions were done to predict phthalic acid uptake based on original soil application rate; the SAS STEPWISE program was used.

RESULTS AND DISCUSSION

Plant Germination and Growth. Wheat germination was reduced significantly ($p \leq 0.05$) from the 60 and 600 ppm application rates of phthalic acid by 21 and 13%, respectively. Corn and soybean germination was not affected. Average germination percentages were 63% (corn), 76% (soybean), and 51% (wheat). There were no significant differences ($p \leq 0.05$) attributable to phthalic acid rate on plant dry weight or height except for immature wheat height which was decreased by 24% at the 600 ppm rate. Average heights and dry weights per pot: corn (40 cm, 2.9 g); fescue (20 cm, 1.3 g); immature soybean (23 cm, 1.4 g); immature wheat (45 cm, 0.7 g); mature wheat (52 cm, 23.6 g); mature soybean (45 cm, 7.8 g); soybean seed (10.2 g); wheat seed (13.6 g).

Plant Uptake. Total plant uptake of extractable ^{14}C ranged from 0.3 to 449.3 ppm. From the TLC analysis, only a small portion of the extractable ^{14}C was identified as phthalic acid 1–4 cm from the origin (Table I). The percent of extractable ^{14}C identified as phthalic acid was 4.5% for corn, 5.2% for fescue, 9.2% for mature wheat, 46.7% for wheat seed, and 15.3% for mature soybean plants. Most of the ^{14}C extracted from plants was associated with chlorophyll at the plate origin or solvent front. Sufficient samples were not available for TLC analysis on immature wheat and soybean plants or soybean seed. Average percentages for similarly aged plants were used in the conversion of extractable ^{14}C to extractable phthalic acid. The percentage of ^{14}C identified as phthalic acid were assigned as follows: immature wheat and soybean plants at 4.8% and soybean seed at 46.7%.

Average extractable phthalic acid content of plants increased with increasing rates of application and ranged from 0 to 23.4 ppm (Table II). Uptake was significant ($p \leq 0.01$) for all plants and plant parts except soybean pods. Fescue and immature wheat plants (at the 600 ppm application rate) had the greatest quantity (23.4 and 15.0 ppm, respectively). Uptake was significant only at the 600 ppm rate for all plants. Phthalic acid concentration in fescue was significant at the 6.0, 60.0, and 600.0 ppm application rates (Table II). The bioaccumulation factor (BF) [ppm in plant tissue/ppm initial soil application (dry weight basis)] of phthalic acid averaged 0.013 for all plants. The BF averaged 0.0046 for seeds, indicating a low accumulation potential for both plants and seeds.

Table I. Distribution of ^{14}C Extracted and Recovered on TLC Plates from Plant and Soil Samples

cm from origin[a]	plant, %					soil, %			
						top, 0–15 cm		bottom, 15–21 cm	
	corn	fescue	mature wheat	wheat seed	mature soybean	corn and wheat pots	soybean and fescue pots	corn and wheat pots	soybean and fescue pots
16–18	1.3	2.0	3.6	0.0	2.7	0.9	0.0	3.1	2.8
14–16[b]	1.2	40.1	23.0	4.2	5.3	2.1	0.1	3.9	4.5
12–14[c]	2.3	14.3	12.3	4.2	12.0	13.8	2.3	9.1	9.5
10–12[c]	52.0	16.2	13.7	0.4	24.6	14.9	13.4	23.5	13.2
8–10	6.8	2.5	9.0	5.0	14.0	13.0	11.6	8.8	9.2
6–8	3.5	7.4	5.3	3.8	10.0	11.8	12.5	7.9	11.7
4–6	3.7	4.5	6.7	22.9	10.3	14.8	13.0	9.5	10.7
1–4[d]	4.5	5.2	9.2	46.7	15.0	19.7	20.2	13.1	15.7
−1 to 1[e]	24.6	7.0	15.7	9.6	5.6	8.6	26.7	20.1	19.8
−2.5 to −1	0.0	0.7	1.4	3.3	0.7	0.6	0.0	1.1	1.6
av dpm/sample[f]	167	251	119	80	100	158	239	158	208

[a] TLC plate origin = 0 cm. [b] Solvent front. [c] Chlorophyll location (plants only). [d] Phthalic acid location. [e] Initial sample location (origin). [f] Average dpm per sample after substracting background from control samples (ranged from 24 to 48 dpm/sample).

Table II. Effect of Application Rate on Plant Content of Extractable Phthalic Acid in Greenhouse-Grown Plants

applicatn rate, ppm	phthalic acid content, ppb dry weight[a]								
	corn	fescue	immature soybean plants	mature soybean plants	soybean pods	soybean seeds	immature wheat plants	mature wheat plants	wheat seeds
0	0	0	0	0	0	0	0	0	0
0.6	2 (1)	23 (19)	4 (3)	3 (1)	0 (0)	6 (3)	14 (4)	1 (1)	0 (0)
6.0	56 (11)	97 (17)[c]	35 (7)	47 (18)	0 (0)	42 (36)	149 (34)	12 (6)	3 (2)
60.0	532 (233)	1359[c]	1362 (2)[c]	307 (210)	0 (0)	485 (601)	[b]	7 (4)	9 (2)
600.0	6554 (1069)[c]	23365[c]	2842 (534)[c]	4151 (1306)[c]	0 (0)	6119 (2166)[c]	14976 (7583)[c]	3407 (622)[c]	1711 (917)[c]
LSD (0.05)	1281	72	392	898	0.1	1900	12581	436	820

[a] Numbers in parentheses represent standard deviations. [b] No data. [c] Statistically significant from control at $p \leq 0.05$. Data $\log_{10}$ transformed after changing all data to ppb + 1.

Immature plants (corn, fescue, soybean, and wheat) generally contained higher concentrations of phthalic acid than mature soybean and wheat plants, which probably reflected the higher soil levels of the chemical while plants were young (Table II). Shea et al. (1982) reported a concentration of di-*n*-butyl phthalate in immature corn plants of 0.32 ppm wet weight at a 200 ppm soil application rate. This is similar to corn uptake of phthalic acid on a dry weight basis as reported here when differences in application rate are taken into account.

Mature wheat plants and wheat seeds had low levels of phthalic acid except at the 600 ppm loading rate, where uptake was substantially higher than at lower rates (Table II). Low levels of phthalic acid in mature wheat plants and seeds might have been influenced by transplanting control plants to replace dead plants. Phthalic acid in mature wheat plants and seed grown continuously in soil with phthalic acid would probably have been higher. Mature soybean plants and seeds contained relatively similar quantities of phthalic acid. Plants and seeds from the 600 ppm application rate contained 4.2 and 6.1 ppm phthalic acid, respectively.

Predictions of plant uptake of phthalic acid were made from linear regression models (Table III). Regressions were based on soil application rates and had correlation coefficients (r) ranging from 0.89 (immature wheat plants) to 1.00 (fescue). When data from all immature plants, all plants, or all seeds were analyzed together, regression accuracy dropped considerably to r values of 0.77, 0.71, and 0.79, respectively. A regression with the two mature plants (wheat and soybean) was still quite accurate ($r = 0.96$). These models can be used to predict phthalic acid uptake (ppb dry weight) based on soil application levels of phthalic acid (ppm dry weight).

Extraction efficiencies for phthalic acid from plants

Table III. Regressions of Plant Content and Soil Retention of Phthalic Acid over a 80-Day Period[a]

parameter measured	applicatn rate (A), ppm	constant (B)	correln coeff (r)	F^b
Direct Plant Measurements				
corn plants	10.9	−30.8	0.99	393.5
fescue plants	38.9	−146.7	1.00	4089.8
immature soybean plants	4.7	15.4	0.99	489.7
mature soybean plants	6.9	−33.6	0.96	180.6
soybean seeds	10.2	−34.7	0.94	110.8
immature wheat plants	24.8	−0.3	0.89	27.0
mature wheat plants	5.7	−67.5	0.98	441.0
wheat seeds	2.9	−44.9	0.89	47.0
Means of Plant Measurements				
all immature plants	17.3	−62.4	0.77	60.7
all mature plants	6.3	−48.5	0.96	422.8
all plants	12.9	−82.3	0.71	81.5
all seeds	6.5	−38.6	0.79	46.0
Soil Measurements				
top soil (0–15 cm)	0.39	12.6	0.93	88.6
bottom soil (15–21 cm)	0.19	−3.4	0.76	18.1

[a] Model: plant content or soil retention [dry weight phthalic acid, ppb] = A [application rate of phthalic acid, ppm] + B [constant]. [b] All $p \leq 0.01$.

(based on ^{14}C from oxidation measurements) were 38.1% (corn), 19.2% (mature wheat), 24.9% (fescue), 31.4% (immature soybean plants), 39.5% (mature soybean plants), and 43.4% (wheat seed). These values were used in ^{14}C balance sheet calculations (below). No values were obtained for immature wheat or soybean seed. They were assumed to have 43.4% and 39.5% ^{14}C extraction efficiencies, respectively. Average bioaccumulation ratios for total ^{14}C were 0.003 for plants and 0.0005 for seeds, again demonstrating the relatively low potential for accumulation

Table IV. Effect of Application Rate on Extractable Phthalic Acid Remaining in Soil after Final Harvest (80 Days)

applicatn rate, ppm	top soil, 0–15 cm, ppb[b]		bottom soil,[a] 15–21 cm, ppb[b]	
	corn and wheat pots	soybean and fescue pots	corn and wheat pots	soybean and fescue pots
0	0	0	0	0
0.6	0.2 (0.1)	0.5 (0.1)	0.1 (0.1)	0.2 (0.0)
6.0	3.0 (0.7)[c]	6.0 (1.0)[c]	1.8 (1.2)[c]	1.3 (0.5)[c]
60.0	33.5 (4.6)[c]	91.0 (24.5)[c]	9.9 (0.6)[c]	4.9 (2.3)[c]
600.0	262.2 (64.0)[c]	397.6 (147.7)[c]	58.8 (43.0)[c]	185.8 (169.9)[c]
LSD (0.05)	0.42	0.70	0.23	0.11

[a] No phthalic acid was applied to bottom soil. [b] Numbers in parentheses represent standard deviations. [c] Statistically significant from control at $p \leq 0.05$. Data $\log_{10}$ transformed after changing all data to ppb + 1.

Table V. Effect of Application Rate on the Percent of Applied ^{14}C Recovered from Soils and Plants at the End of the Experiment and Average Percent of ^{14}C Found in Aboveground Plant Tissue[a]

applicatn rate, ppm	^{14}C recovered from soil and plant, % of applied			^{14}C in aboveground plant tissue, % of applied		
	corn and wheat pots	soybean and fescue pots	mean	corn and wheat pots	soybean and fescue pots	mean
0.6	6.9	5.6	6.2	0.2	0.3	0.2
6.0	6.9	5.4	6.1	0.2	0.2	0.2
60.0	6.1	5.1	5.6	0.1	0.3	0.2
600.0	5.4	4.4	4.9	0.8	0.7	0.7
av	6.3	5.1	5.7	0.3	0.3	0.3

[a] ^{14}C extraction efficiencies included.

of phthalic acid in these crops.

Soil Retention. The amount of extractable ^{14}C which was identified by TLC as phthalic acid averaged 20.0% for top soil and 14.4% for bottom soil, respectively (1–4 cm from origin, Table I). These values were used to convert extractable ^{14}C to extractable phthalic acid values (Table IV). Large percentages of the ^{14}C remained at the sample origin and at bands from 4 to 14 cm from the origin (Table I).

Statistically significant amounts of phthalic acid remained in the soil for both sets of pots for all chemical rates (Table IV). Amounts present were significant at all treatments with the exception of the 0.6 ppm rate, which had significant differences only in the top soil of the corn and wheat pots. The average half-life of phthalic acid (assuming a linear decay) was 37 days for all rates and pots.

A small but significant portion of the originally applied phthalic acid was leached into the bottom of the pots by the final harvest at application rates of from 6 to 600 ppm (Table IV). An average of 0.02% of the originally applied chemical was recovered in the bottom. Some of the chemical may have leached out of the bottom of the pot but this was minor since pots were never over watered and very little ever appeared in the aluminum pans under each pot.

^{14}C extraction efficiencies were low for both sets of pots for top (10.2%) and bottom soils (5.7%). Soxhlet extraction (24-h) in methanol increased the ^{14}C extraction efficiency for the top soil to only 13.6%.

Regression equations were produced to predict soil concentrations of phthalic acid at plant harvest (80 days Table III). Equations for both top and bottom soil measurements were statistically significant ($p \leq 0.05$), but the top soil equation had higher r and F values than the bottom soil equation.

Based on analysis of the ^{14}C balance sheets (Table V), an average of 5.7% of the originally applied ^{14}C was recovered from soil and plants at the end of the experiment (80 days). Most of the phthalic acid (94.3%) was lost through volatilization or decomposition. A small portion (0.3%) of the recovered ^{14}C was found in aboveground plant tissue.

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Effect of $FeCl_3$ on Heat Denaturation of β-Lactoglobulin A in Acid Media

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Effects of $FeCl_3$ on the heat denaturation of β-lactoglobulin A (β-Lg A) in acid media were investigated by circular dichroism (CD) and immunochemical techniques. β-Lg A (0.1% solutions at pH 1.5, 2.0, 2.5, and 3.0) heated (90 °C, 15 min) with $FeCl_3$ ($FeCl_3$ (mole)/β-Lg A (mole) = 5 or 10) was partially and irreversibly unfolded as indicated in the CD spectra, but its partially disordered structure retained some β-Lg A antigenecities. Heating (90 °C, 15 min) of β-Lg A (0.1% at pH 1.5) with $FeCl_3$ ($FeCl_3$ (mole)/β-Lg A (mole) = 10) decreased the maximum immnoprecipitation to about 83% of those by native β-Lg A and heated β-Lg A without $FeCl_3$. Thus, $FeCl_3$ promoted the irreversible denaturation of β-Lg A in combined effect with heating.

Proteins occurring in cheese whey are recovered as precipitate by heating whey solution below the isoelectric point, then cooling it, and adjusting the pH to 4.5. The recovered proteins which were neutralized and spray-dried possessed superior solubility and functional properties (Harwalkar, 1979; Harwalkar and Modler, 1981). It was also demonstrated that the protein fraction denatured by heating at pH 2.5, rendered insoluble at pH 4.5, was unfolded partially and irreversibly (Harwalkar, 1980a). When $FeCl_3$ was added to whole whey, the protein recovery as precipitate was increased whereas the solubility of the protein was reduced (Modler and Emmons, 1977). Amantea et al. (1974) produced an iron-fortified whey protein concentrate by heating concentrated whey at pH 2.5–3.5. The resulting product had an excellent amino acid profile and good functional properties, although high solubility was limited to the neutral pH range.

β-Lg A normally exists at neutral pH as oligomers of the 18400 dalton monomer and the degree of association varies as the pH changes (McKenzie, 1971). At pH 2.0 approximately 90% of the protein has been reported to be present as the monomer (McKenzie and Ralston, 1973; Townend et al., 1960). Because such a low pH would prevent interchange reaction of the protein, heat denaturation of β-Lg at low pH has been studied by several workers (Ananthanarayanan et al., 1977; Ananthanarayanan and Ahmad, 1977; Harwalkar, 1980ab). But none of the earlier investigations was performed on β-Lg A in the presence of $FeCl_3$.

In the present investigation, a comparison has been made of the heat denaturation of β-Lg A with and without $FeCl_3$ in acid media by means of circular dichroism (CD) and immunochemical techniques. The results obtained are expected to provide basic information on the methods of whey protein preparation.

MATERIALS AND METHODS

Preparation of β-Lg A. β-Lg A was prepared from the milk of homozygous cows by using the procedure of Armstrong et al. (1967). The prepared protein which was electrophoretically pure was dialyzed exhaustively in distilled water and lyophilized.

Heat Treatment. The lyophilized β-Lg A was dissolved in aqueous solution up to a final concentration of about 1 mg/mL, and the pH was adjusted to the desired values (pH 1.5–3.0) by addition of 1 N HCl. Heat treatment of β-Lg A was conducted by placing 3 mL of β-Lg A solution in test tubes (1.2 × 14 cm), which were positioned in a rack and immersed in a controlled temperature water bath. The test tubes were shaken gently for 30 s and then kept at 90 °C for 15 min. Strict corrections for temperature lag were not made. Each sample was cooled immediately after heat treatment by placing the tubes in ice water.

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