Biological Characteristics of Bound Dinitroaniline Herbicides in Soils

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Bound [*phenyl*-^{14}C]butralin residue, recovered after anaerobic incubation, was persistent in aerobic soil. Some ^{14}C from butralin residues bound to humic acids was lost (≃20%) during a 21-week incubation, but the whole soil and other fractions were less degradable. Although no plant uptake of this ^{14}C was detectable, uptake did occur from partially bound and bound residues of six dinitroanilines previously incubated aerobically: butralin, chlornidine, dinitramine, fluchloralin, profluralin, and trifluralin. Residues in soybean tops, after 10 weeks growth, decreased from 0.7–2.3 ppm (partially bound) to 0.2–0.4 ppm (bound). Soybean top residues of ~1.4–1.8 ppm (dinitramine) to 0.5–0.8 ppm (trifluralin) were measured in soil treated with 0.29 ppm parent herbicide, but not preextracted; levels were slightly less from extracted soil. Extraction of soil with benzene–methanol (to remove nonbound residues) led to Mn toxicity in soybeans. Phytotoxicity was characterized by crinkled and spotted leaflets, branching from the cotyledon node, columnar plant shape, increased number of trifoliate leaves, reduced plant weight, and chlorosis. Mn in tops increased from 350 to 500 ppm because of extraction; leaf levels ≤1660 ppm Mn were found. Extractable Mn in soil increased because of previous benzene–methanol extraction (two–three-fold), prolonged dry storage (threefold), or autoclaving (sixfold). Preextraction with benzene–methanol may have increased plant sensitivity to some bound residues and parent herbicides. Investigation of the bioavailability of bound residues to plants may be hampered by phytotoxicity caused by organic solvent removal of nonbound pesticide residues.

Bound pesticide residues are, basically, those residues remaining in soil after exhaustive solvent extraction. Residues remaining affixed to solubilized fulvic or humic acid are still considered bound, since they are not in a discrete and chemically identifiable form.

The significance of bound residues is chiefly addressed in terms of their bioavailability, both in amount and form of uptake. These and other considerations were topics of a research conference (Kaufman et al., 1976). Microbial evolution of $^{14}CO_2$ from ^{14}C-labeled pesticides has been a measurement of ultimate degradation, but soil-bound 3,4-dichloroaniline released $^{14}CO_2$ only very slowly (Hsu and Bartha, 1974). Bound paraquat (1,1′-dimethyl-4,4′-bipyridinium ion) has been extensively studied (Riley et al., 1976): apparently, it is unavailable to microorganisms, earthworms, microarthropods, and plants. Bound residues of several insecticides also showed greatly reduced activity against fruit flies (Lichtenstein et al., 1977).

The formation and slow turnover of bound residues is a natural process. Wagner (1975) noted that ~25% of the readily decomposable glucose becomes incorporated into microbial tissue or soil organic matter and that 15–20% remained even after 3 years. Hamaker and Goring (1976) emphasized turnover as a kinetic process in which the pesticide or its metabolites coexist in "labile" and "unavailable" pools. Decomposition or plant uptake may occur from the labile pool; slow release from the bound state may account for deviation from an apparent first-order dissipation curve.

The present study investigates the biological availability of bound dinitroaniline herbicides, partially summarized earlier (Helling, 1976). All residue samples are from concurrent research on the physical aspects of these bound herbicides (Helling and Krivonak, 1978).

MATERIALS AND METHODS

The source, analyses, and fractionation of bound dinitroanilines were described by Helling and Krivonak (1978). The six [U-*phenyl*-^{14}C]dinitroanilines used were: butralin [4-(1,1-dimethylethyl)-*N*-(1-methylpropyl)-2,6-dinitrobenzeneamine], chlornidine [*N*,*N*-bis(2-chloroethyl)-4-methyl-2,6-dinitroaniline], dinitramine (N^4,N^4-diethyl-α,α,α-trifluoro-3,5-dinitrotoluene-2,4-diamine], fluchloralin [*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)aniline], profluralin [*N*-(cyclopropylmethyl)-α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine), and trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine).

Preparation of Bound Residues. Bound residues were obtained for subsequent study by first extracting herbicide-treated soil to remove soluble residues. Aerobically incubated Matapeake silt loam (pH 5.3), treated 5 months (dinitramine) or 7 months earlier with 10 ppm herbicide, was extracted for 30 min with benzene (Bz)–ethyl acetate, then methanol (MeOH) (Kearney et al., 1976) and stored at 4 °C for 10 months. We refer to residues in this soil as "partially bound". Part of the soil was Soxhlet-extracted with Bz–MeOH (1:1, v/v) for 36 h, leaving only the "bound" dinitroaniline residue. In some cases, soil was pulverized before extraction. The extract was analyzed for radioactivity by direct addition to a liquid scintillation cocktail and the extracted soil, by combustion (Helling and Krivonak, 1978). Control (fresh, untreated) soil was similarly extracted.

Persistence of Anaerobic Bound Butralin Residues. We incubated bound butralin to evaluate its breakdown and persistence in soil. Anaerobically bound butralin and seven of its fractions from a Gascho/Stevenson fractionation were used (Helling and Krivonak, 1978). Samples of moist, biologically active Matapeake silt loam (50 g, oven-dry equivalent) were treated with an amount of bound residue (whole or fractionated) containing ~10000 cpm total activity. The control was fresh untreated Matapeake. The samples were wetted to ~75% of field moisture capacity and maintained in biometer flasks (Bartha and Pramer, 1965) at 22–25 °C. The sidearm contained 10 mL of 0.1 N KOH as a CO_2 trap, vented through an external Ascarite trap.

Periodically, soil and CO_2 samples were assayed for radioactivity. The traps were replaced. Soil moisture

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content was adjusted as necessary.

Total CO_2 evolved was assayed periodically after day 16. Samples (5 mL) in 2 N $BaCl_2$ (10 mL) were titrated with ~0.05 N HCl to a colorless phenolphthalein endpoint. At 7 weeks, 1 g of air-dried ground hay (an energy source for microorganisms) was mixed with each soil sample. At 13 weeks, an additional trap was inserted after the KOH: it contained 10 mL of combustion trapping solution (methoxyethanol–ethanolamine) and would collect CO_2 (if the KOH trap became saturated) or organic volatiles.

The experiment was concluded after 21 weeks. Moist samples (equivalent to 5–10 g of dry soil) were Soxhlet-extracted with Bz–MeOH for 24 h. Methanol was boiled off by refluxing (using a Snyder column) and ^{14}C was measured in the remaining extract.

Plant Uptake of Anaerobic Bound Butralin Residues. To determine uptake of bound butralin by plants, three plant species were grown in the greenhouse in soils containing bound butralin and its fractions (with and without the prior 21-week incubation). The containers were paper cups [6.1 cm (diameter) × 6.5 cm] containing ~30 g of sand + vermiculite (lower layer, 0–1 cm), 30 g of sand + a variable amount of soil (middle layer, 1–3 cm), and vermiculite (upper layer, 3–5 cm). Six nonincubated Matapeake controls and three incubated controls, the same numbers of unfractionated bound butralin soils, and three replications of all fractionated samples were used. Total radioactivity in each pot was ~0.004 μCi for most nonincubated samples (0.022 μCi was added in one case). Incubated samples had ~0.001 μCi each.

Three seeds each of soybeans [*Glycine max* (L.) Merr., cv. Kent], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and redroot pigweed (*Amaranthus retroflexus* L.) were planted in the middle (treated) layer. After the first trifoliate leaves appeared, two soybean shoots were excised and combusted. Approximately every third watering was with a modified Hoagland's nutrient solution (Johnson et al., 1957). At 6 weeks, all plants in one replication were harvested, dried at 60–70 °C, and combusted. The remaining samples were harvested and combined at 9 weeks.

Plant Uptake of Partially Bound Dinitroanilines. We attempted to determine uptake and phytotoxicity from incompletely extracted residues of six dinitroaniline herbicides. Soybeans were grown in a sand–soil mixture containing enough soil to give 0.1 μCi of partially bound (+ bound) residue (100 μg, assuming all is parent). Thus, 30–70 g soil was mixed with 300 g of clean Ottawa sand and added to a 8.9 cm (diameter) × 8.3 cm undrained plastic pot. Triplicate pots were prepared per herbicide, and additionally, six controls containing the six different amounts (30–70 g) of untreated, unextracted soil. Two soybean seedlings (cv. Cutler) were transplanted, when at the primary leaf stage, into each pot; the pots were watered and the soil–sand mixture covered with 1 cm of sand. Periodically the pots were fertilized with a modified Hoagland's solution.

One plant per pot was harvested at 4 weeks and the remaining plant at 10 weeks. Leaves and stems were combined in the first harvest and dried at 60 °C before combustion of duplicate 50-mg samples. At 10 weeks, leaves, stems, pods, and beans were freeze-dried and combusted separately. Roots were also removed, carefully rinsing in water, then for 5 s in acetone (to remove surface ^{14}C contamination). Each root mass was xeroxed (to record morphological appearance), ground in a Wiley mill, and analyzed by combustion.

Plant Uptake of Bound Dinitroanilines. To determine uptake from bound residues only, the experiment was conducted similarly to the previous one, except that: (a) soil was pulverized before extraction; (b) this soil was Soxhlet-extracted 36 h with Bz–MeOH; (c) the control soils were also pulverized and extracted; and (d) supplemental light (Westinghouse Agro-light) was used to extend day length to 16 h during the first 8 weeks of growth.

Dinitroaniline Phytotoxicity Experiments. Experiments were designed to produce phytotoxicity symptoms from the six dinitroaniline herbicides for comparison with symptoms seen in the bound residue work. However, we recognized during the bound residue experiments and the first dinitroaniline phytotoxicity experiments that factors independent of the bound herbicide were retarding soybean growth. The following sequence of experiments represents our increasing effort to explain this.

Unless otherwise specified, 'Cutler' soybeans were grown under conditions described in the previous two experiments, the major exceptions being: (a) 60 g of freshly dug and screened Matapeake silt loam soil was used (both unextracted and Soxhlet-extracted for 36 h); (b) two-thirds of the pots per treatment were limed initially by adding 50 mL (0.103 g) of $Ca(OH)_2$ solution to each; (c) six analytical grade herbicides in 10 mL of acetone were applied to the soil, with air-drying, before mixing with sand—the rates were 0.1 ppm (36 μg) and 0.29 ppm (104 μg); and (d) only two compounds, dinitramine and trifluralin, were ^{14}C labeled.

Experiment I (Basic). The objective of experiment I was to determine whether the parent dinitroaniline herbicides, applied at rates comparable to those of their bound residues, were phytotoxic. At 4 weeks (rather than initially), two of the three replicate pots per treatment were limed by pipetting the $Ca(OH)_2$ solution into the subsurface. This was done because, by 3 weeks, many soybeans were growing poorly. We recognized that previously extracted (but untreated) soils were themselves retarding soybean growth. Manganese toxicity associated with low soil pH was suggested as the cause. Four days after liming, the average soil pH had increased from 5.75 to a more favorable 6.65.

One soybean per pot was harvested at 4 weeks, the second at 10 weeks. Yield and morphological observations were made, and the dinitramine- and trifluralin-treated plants were analyzed for ^{14}C residue.

Experiment II (Variety). To ascertain the effect of plant variety, both 'Cutler' and 'Kent' soybeans were grown; liming was done at 2 weeks. Butralin was applied at both 0.1 and 0.29 ppm, the other compounds only at 0.29 ppm.

Experimental III (Soil Only). Experiment III was performed to determine whether uptake and phytotoxicity of dinitramine and trifluralin differed when plants were grown in whole soil, rather than soil–sand. It resembled experiment II, except: (a) the potting medium was 180 g of soil only (no sand); (b) liming was done initially; (c) the herbicide rates were 0.1, 0.2, 0.29, 0.58 ppm; (d) only dinitramine and trifluralin (both ^{14}C labeled) were used; and (e) these pots contained 50% of the total amount of herbicide used in experiments I or II, when comparing the same rate. The radioactivity added per pot was 0.05–0.30 μCi for dinitramine, 0.025–0.14 μCi for trifluralin. Combustion of tissue was by means of a Packard Tri-Carb Model 306 Sample Oxidizer. Sample size was ~100 mg, burn time was 0.35 or 0.5 min, and collection of $^{14}CO_2$ was in 5 mL of Carbo-Sorb + 9 mL of Permafluor V.

Experiment IV (Large Pot). To study the effect of a larger rooting volume on uptake and phytotoxicity, soy-

beans were grown 10 weeks in large (15.2 × 15.2 cm) plastic pots containing the usual 1:5 soil–sand ratio, or 300 + 1500 g. [^{14}C]Dinitramine (0.21 μCi) and [^{14}C]trifluralin (0.29 μCi) were each used at 0.1 and 0.29 ppm.

Soil Extraction Experiments. *Experiment A (Fresh Soil).* This considered only the soil extraction process as it may affect soybean growth. The soil was: (a) fresh unextracted; (b) fresh extracted; (c) 10 g of unextracted + 50 g of extracted soil; or (d) unextracted soil to which 10 mL of Bz–MeOH had been added, with subsequent air-drying. Imposed on these treatments was the effect of lime vs. no lime. Herbicides were not used.

Experiment B (Fresh/Aged Soil). Experiment B was run to determine why prior soil extraction or aging was detrimental to soybean growth. 'Cutler' growth was compared when the aged soils used 5 months earlier for experiment A (except condition d) were compared with newly processed soil. The aged soil had been stored dry in the greenhouse. The newer soil treatments were: (a) fresh unextracted soil; (b) fresh extracted soil; (c)–(e) fresh unextracted + extracted soil in ratios of 1:1, 1:5, and 1:10 (60 g total weight); (f) fresh extracted soil + the Soxhlet extract equivalent removed from it; and (g) autoclaved (three times, at daily intervals) soil. Four replicate pots were used for the new treatments, none of which was limed.

Soil and Tissue Analyses of Manganese. Fifteen-gram samples of air-dry soil were shaken 2 h with 30 mL of DTPA–TEA reagent in plastic Erlenmeyer flasks (Lindsay and Norvell, 1969). This extractant contains 0.005 M DTPA (diethylenetriaminepentaacetic acid), 0.01 M $CaCl_2$, and 0.1 M triethanolamine, adjusted to pH 7.30. The suspension was filtered and analyzed by atomic absorption spectrophotometry.

Soybean tissue samples (100–200 mg) were ashed at 500 °C, then shaken vigorously for 2 h with 20 mL of 1 N HCl. After settling 1 h, the solutions were analyzed by atomic absorption spectrophotometry.

Soybean leaf and stem samples were combined for analysis of 15 elements (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, Si, Sr, and Zn) by emission spectrograph, done at the Ohio Agricultural Research and Development Center, Wooster, Ohio.

RESULTS

Biological availability of bound herbicides was investigated from the aspects of $^{14}CO_2$ release by soil microorganisms and ^{14}C uptake by plants. In both approaches, we used bound butralin from anaerobically incubated Chillum silt loam. Only plant uptake was examined with the six aerobically incubated dinitroanilines in Matapeake silt loam.

Persistence of Bound Butralin. Residual ^{14}C data, from bound butralin fractions incubated aerobically for 21 weeks, are presented as curves based on linear regression analysis (Figure 1). For ease of comparison, residual ^{14}C has been converted to percent of added ^{14}C. The zero-time values are based on the intercepts obtained from least-squares analysis of the original data. Correlation coefficients (r) are unaffected by this data conversion.

Three samples lost significant ($P < 0.05$) radioactivity, NaOH humic acid (H), silt (D), and $Na_4P_2O_7$ humic acid (G). At the 10% level of significance, humin I (C), humin V (F), and coarse clay (E) also showed ^{14}C loss during the 21 weeks. Decline of Celite/OM (A) or whole soil (B) residue was insignificant.

The data used in Figure 1 were also plotted as a nonlinear regression of log (% remaining) on time. Correlation coefficients were always slightly lower, except for silt (r = −0.682*).

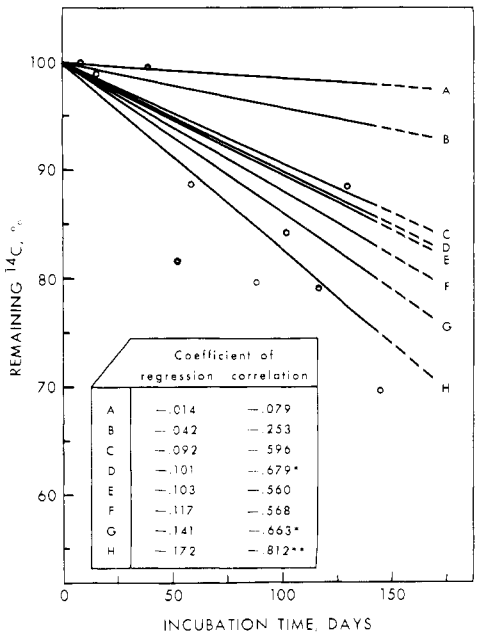

Figure 1. Persistence of anaerobic bound butralin residues incubated 21 weeks in an aerobic soil. The fractions are: (A) Celite/OM, (B) whole soil, (C) humin I, (D) silt, (E) coarse clay, (F) humin V, (G) humic acid/$Na_4P_2O_7$, (H) humic acid/NaOH. Data points shown are for fraction H.

The $^{14}CO_2$ traps were analyzed 17 times during the incubation, but observed cpm were usually near background level. The cumulative loss of $^{14}CO_2$ represented 2.8% and 2.4% of residual ^{14}C in NaOH humic acid and humin I samples, respectively. The other samples showed apparent loss of <1% radioactivity. The backup traps (organic solvent) added at day 92 collected no measurable radioactivity.

Total CO_2 released between 16 and 147 days ranged from 403 mg (aerobic $Na_4P_2O_7$ humic acid) to 635 mg (humin I). Increased CO_2 was produced after ground hay was added at day 49; the cumulative CO_2 corresponds to loss of ca. 20–30% of this carbon. Frequently the traps were saturated, which necessitated using more concentrated KOH trapping solution. Total CO_2 is, therefore, a minimum value.

Correlation coefficients were nonsignificant at the 10% level for the relationships % residual ^{14}C vs. % evolved $^{14}CO_2$ and % residual ^{14}C vs. total CO_2 (all computed for 147 days). $^{14}CO_2$ and total CO_2 were directly related ($P < 0.1$).

Plant Uptake of Anaerobic Bound Butralin Residues. Soybean seedlings harvested at 2 weeks contained no measurable radioactivity above background (counted 80 min). No phytotoxicity was observed. The 6- and 9-week harvests contained soybeans, pigweed, and crabgrass. Neither uptake nor phytotoxicity was apparent. Table I summarizes results from subsequent studies on bound residues, herbicide phytotoxicity, and soil extraction effects.

Plant Uptake of Partially Bound Dinitroanilines. At 4 weeks, some "partially bound residues" seemed clearly to be affecting plant growth. Based on average plant height and weight, soybean growth was in this order: control > butralin > fluchloralin = profluralin > dinitramine = chlornidine = trifluralin. Butralin plants appeared normal in shape and color, relative to the controls; all others were columnar, had excessive budding and branching, and were yellowish green. Residues, i.e., ^{14}C accumulation, in the tops were 0.9–1.8 ppm (based on the

Table I. Summary of Experimental Design and Effects of Bound and Nonbound Dinitroaniline Herbicides (Applied at 0.29 ppm) on Soybeans after 10 Weeks Growth

experiment	control treatment[a]	liming[b]	degree of phytotoxicity[c]	top dry wt, g: control U	control E	herbicides[d] 1, 2, 4, 5 U	1, 2, 4, 5 E	3, 6 U	3, 6 E	uptake, %[e] U	uptake, %[e] E	residue, ppm[e] U	residue, ppm[e] E
bound residues													
anaerobic butralin	U	–									ND[f]		ND[f]
six partially bound cmds	U	–	+++	2.9			1.7		1.0		1.1–2.3		0.7–2.3
six bound cmds	E	–	++		4.4		3.2		2.9		0.5–1.1		0.2–0.4
herbicide phytotoxicity													
I. basic	U/E	–/+[g]	+++	2.5	2.1	2.6	1.9	1.4	1.3	0.8–1.2	0.5–0.9	0.5–1.4	0.4–0.7
II. variety	U/E	–/+[g]	+++	3.4	2.2	3.3	2.3	2.8	1.7	2.5–2.7	0.8–1.6	0.9–1.2	0.7–0.9
III. soil only	U/E	–/+	+++	5.6	1.8			5.5	1.9	1.2–3.3	0.5–0.8	0.1–0.3	0.1–0.3
IV. large pot	U/E	–/+	+++	6.1	2.5			3.7	1.8	0.6–0.9	0.2–0.3	0.9–1.8	0.6–0.7
soil extraction													
A. (fresh soil)	U/E	–/+	+	3.3	2.8								
B. (fresh/aged soil)	U/E	–	++	4.0	2.8								

[a] Control soils were (E) or were not (U) preextracted with Bz–MeOH before use. [b] Samples were (+) or were not (–) limed initially. [c] Phytotoxicity (+ to +++ shows least to most severity) symptoms, relative to unextracted control, included: more trifoliate leaves and spindly growth; leaflet curling or cupping; and chlorosis and/or brown spots or veins. [d] Herbicides 1, 2, 4, and 5 were butralin, chlornidine, fluchloralin, and profluralin; herbicides 3 and 6 were dinitramine and trifluralin. [e] Uptake and residue in soybean tops are ranges for 6 cmds in bound residue experiments and for dinitramine and trifluralin only in the herbicide phytotoxicity experiment. [f] ND = nondetectable. For this experiment, limit of sensitivity was ~0.2% for uptake and ~0.1 ppm for residue. [g] Liming of "basic" and "variety" samples was done, respectively, 4 and 2 weeks after the experiments were begun.

Table II. Effect of Partially Bound Dinitroaniline Residues on Growth and ^{14}C Uptake in Soybeans

herbicide	initial residue[a] in soil–sand, ppm	no. of trifoliate leaves[b]	height, cm	dry wt,[c] g	residue, ppm	uptake, %
	0	9.7c	28.0a	2.92a		
butralin	0.296	15.0b	24.5a	2.14b	1.09bc	2.3a
chlornidine	0.301	21.3a	28.5a	1.74c	0.66c	1.1b
dinitramine	0.284	18.3ab	21.5ab	0.96d	2.28a	2.2a
fluchloralin	0.319	17.7ab	22.8ab	1.57c	1.28b	1.9ab
profluralin	0.288	18.7ab	21.8ab	1.43c	1.47b	2.2a
trifluralin	0.270	17.0ab	15.5b	0.99d	1.16b	1.2b

[a] Based on parent herbicide as the bound residue. [b] At 10 weeks. Values in Tables I–V are based on three replications, except six replications for the untreated controls. Values in the same column not followed by the same letter are significantly different at $P < 0.05$. [c] Weight, residue, and uptake (% of residual ^{14}C) are for 'Cutler' soybean tops.

parent herbicide). Although these levels were three–six times soil residue, the total ^{14}C uptake was only 0.2% (chlornidine) to 0.6% (butralin) of that available. Residue levels in the tissue were not unambiguously related to growth or phytotoxicity.

The plants harvested at 10 weeks confirmed earlier trends, except for chlornidine's reduced phytotoxicity. Dry weights of the aerial portion (Table II) ranged from 73% (butralin) to 33% (dinitramine) of the unextracted control. Control plant weight was not correlated with the amount of untreated soil used. Fresh weights and total (including roots) dry weights gave the same trend. Morphological effects on soybeans in this experiment were: smaller and frequently crinkled leaflets, brown spots and veins, and more trifoliate leaves (Tables I and II; Figure 2). Pods and beans were more mature in control plants.

As expected, a much greater relative amount of the ^{14}C added to the pots was taken up by soybeans at 10 weeks (Table II) as compared to 4 weeks (data not shown). At final harvest, this averaged 1.8 ± 0.6% of residual ^{14}C in the tops and 3.0 ± 1.4% in roots, i.e., ~4.5% overall uptake into plant tissue. Residue levels were ~6–12 ppm in roots, 1–2 ppm in leaves (slightly lower in stems), 0.1–0.2 ppm in pods, and 0.05–0.1 ppm in beans. Residue levels were similar at 4 and 10 weeks, seemingly showing that bound (or partially bound) ^{14}C was being released rather steadily into the rhizosphere.

Figure 2. 'Cutler' soybeans grown 10 weeks in soil/sand that contained a partially bound dinitroaniline herbicide. Treated soils (but not the control) had been extracted with benzene–methanol.

Plant Uptake of Bound Dinitroanilines. This experiment was conducted with exhaustively extracted soil, in contrast to the incompletely extracted soil used earlier. Our tests showed that the same amount of ^{14}C was removed from "partially bound butralin" soil by a 36-h Soxhlet extraction as by three 24-h shake–Soxhlet–Soxhlet

Table III. Effect of Bound Dinitroaniline Residues on Growth and ^{14}C Uptake in Soybeans

herbicide	residue[a] in extr., soil, ppm	4-week harvest[b] dry wt, g	4-week harvest[b] residue, ppm	10-week harvest no. of trifoliate leaves	10-week harvest dry wt, g	10-week harvest residue, ppm	10-week harvest uptake, %
		1.00a		11.5cd	4.37a		
butralin	1.77	1.25a	0.27bc	7.0d	4.14a	0.17a	0.74bc
chlornidine	1.57	1.00a	0.16c	17.0ab	2.78b	0.16a	0.46d
dinitramine	1.17	0.94a[c]	1.07a	14.0bc	3.94a[c]	0.37a	1.07a
fluchloralin	2.61	1.10a	0.45bc	9.0cd	3.96a	0.22a	0.90ab
profluralin	1.23	0.84b	0.58b	21.3a	1.93b	0.39a	0.70bc
trifluralin	0.80	0.40b	0.56b	20.7a	1.84b	0.34a	0.59cd

[a] Calculated as parent herbicide. Adjusted to 0.29 ppm in the potting mixture. [b] Weight, residue, and uptake are for 'Cutler' soybean tops. Values in the same column not followed by the same letter are significantly different at $P < 0.05$. [c] A dwarf plant (weight = 0.43 g) was excluded from dinitramine calculations.

cycles (Helling and Krivonak, 1978). The former procedure was more convenient for preparing the large quantities of soil needed and was therefore adopted. Subsequent analyses, however, showed that 20% more ^{14}C from "partially bound fluchloralin" were extractable by the longer method. Plants were grown in a mixture of the two (36 h and 3 × 24 h) soils. The initial residues in the soil–sand mixture were 0.24 ppm (trifluralin) to 0.30 ppm (fluchloralin). Variation occurs because although 100 μg of equivalent bound residue was added to each pot, soil weights varied.

Table III the results of both 4- and 10-week harvests. Plants were heavier than those in the "partially bound" experiment (Table II). The more complete extraction seemed to eliminate yield differences between the control and bound residues from butralin, dinitramine, and fluchloralin, although it should be noted that the control was extracted in experiment II but not in experiment I. Growth was ~60% of normal from chlornidine residues and ~50% from profluralin and trifluralin residues. Phytotoxicity observed in the preceding experiment was also seen here, but now butralin and fluchloralin were relatively unaffected. Production of extra leaves (Table III) may indicate damage or inhibition of the apical bud.

The ^{14}C residue in the plants decreased between 4 and 10 weeks. Soybean tops accumulated 79% lower residue (0.28 ppm) and had 59% less uptake (0.74%) in fully bound residue soils than in partially bound soils. The total ^{14}C in soil had been the same in both experiments. The average distribution of radioactivity in the soybean was 76% in roots, 17% in leaves, 5% in stems, 1.2% in pods, and 0.3% in beans. This also reflected residue levels, which were ~3.5, 0.5, 0.2, 0.1, and 0.03 ppm in roots, leaves, stems, pods, and beans, respectively. The highest leaf residue (0.88 ppm, in dinitramine) was associated with only a low bean residue (0.02 ppm).

Dinitroaniline Phytotoxicity Experiments. *Experiments I (Basic) and II (Variety).* Liming did not affect freeze-dried top weights of plants grown 10 weeks in experiment I. (Detailed summary tables for experiments, I, II, and IV may be obtained on request from the author.) Preextracted soil caused reduced growth (Table I), manifested in leaf and bean weights. The top weight averages were 2.54 and 2.09 g from, respectively, unextracted and extracted control soils.

The herbicides, except dinitramine at 0.29 ppm (which killed the plants by 5 weeks), produced no phytotoxicity or yield reduction in soybeans grown in unextracted soil. In extracted soil, dinitramine and trifluralin were phytotoxic at 0.1 and 0.29 ppm rates.

Besides weight reduction, extracted-soil plants were significantly taller (giving a columnar shape) and had crinkled leaflets (often with brown spots), more trifoliate leaves (8.7 vs. 5.0 for extracted vs. unextracted controls), branching from the cotyledon node, and fewer mature beans. The plants adversely affected by prior soil extraction also may be less capable of absorbing dinitramine and trifluralin (the only ^{14}C-labeled herbicides tested). Although ^{14}C uptake from these treatments was 2.3 ± 0.8 times greater in unextracted than extracted soil, the total amount was relatively small, 2.31% and 0.95%, respectively, for 0.1 ppm rates of dinitramine and trifluralin. Residues were highest in leaves and stems (bean residues averaged <0.04 ppm), in plants from unextracted soil, and at the higher herbicide rate.

The results from experiment II essentially corroborated those of experiment I. By 10 weeks, at 0.29 ppm, only dinitramine consistently reduced growth. "Cutler" soybeans grown in extracted soil–sand may be more sensitive to dinitramine, but there was no other obvious difference between the 'Cutler' and 'Kent' varieties.

Experiment III (Soil Only). This experiment was conducted to disclose any effect of soil alone as opposed to the usual soil–sand potting mixture. Also, since earlier work had not definitely indicated dinitroaniline phytotoxicity symptoms, the range of application was broadened to include 0.58 ppm. Only dinitramine and trifluralin were used.

These herbicides at rates of 0.1, 0.2, and 0.29 ppm did not affect soybean top weight (Table IV), but by 4 weeks, 0.58 ppm had retarded growth. At the high rate, dinitramine caused vine-like growth, short root-like projections on the lower stem, and, by 6 weeks, the death of most plants. Soybean varietal differences appeared in this experiment: although phytotoxic symptoms were generally typical, 'Cutler' soybeans from extracted soil lacked branching from the cotyledon node; furthermore, these plants died by 7 weeks, but 'Kent' soybeans survived for 10 weeks. The soil extraction effect reduced soybean growth by ~65%.

After 10 weeks growth in unextracted soil, soybean plants had 3–4% of added ^{14}C from dinitramine, and 1–2% from trifluralin in their tops (Table IV). Uptake from extracted soil was less, ~0.8–1.5% from dinitramine, 0.5–0.9% from trifluralin. Relative uptake decreased abruptly at the 0.58 ppm rate, reflecting the markedly inhibited growth of these plants. Residue increased in approximate proportion to treatment rate. Preextraction of soil reduced both uptake and residue comparatively more for dinitramine than for trifluralin. Residue in beans from unextracted soil averaged 0.04 ppm for dinitramine and 0.02 ppm for trifluralin. Radioactivity in beans from extracted soil was somewhat lower, but nearly all bean residues were at the limit of detection, 0.02–0.03 ppm.

Experiment IV (Large Pot). In this experiment only, plants were grown in larger containers. Herbicide rates were as used previously, 0.1 and 0.29 ppm, but five times as much total herbicide was in each pot.

Table IV. Growth and Uptake of ^{14}C by Soybeans from Dinitramine- and Trifluralin-Treated Soil (Experiment III, "Soil Only")

rate, ppm	dry wt of top,[a] g		uptake,[b] %		residue, ppm	
	unextr.	extr.	unextr.	extr.	unextr.	extr.
			dinitramine			
0.1	5.75ab	2.23c-e	4.25a	1.51cd	0.13d-f	0.12d-f
0.2	5.80ab	2.01c-f	3.29b	0.94d-f	0.21b-d	0.17c-f
0.29	5.67ab	1.62c-g	3.31b	0.81ef	0.31b	0.26bc
0.58	0.90f-i[c]	0.56g-i[c]	0.68e-g	0.12g	0.74a	0.28b
			trifluralin			
0.1	5.77ab	2.29c-e	1.70c	0.87ef	0.05f	0.08ef
0.2	5.61a	2.22cd	1.28c-e	0.52fg	0.07f	0.09d-f
0.29	5.40ab	2.23c-e	1.19c-e	0.48fg	0.12d-f	0.13d-f
0.58	0.46g-i[c]	0.30hi[c]	0.08g	0.06g	0.21b-e	0.17b-f

[a] Weight, uptake, and residue are for soybean tops at 10 weeks; data combined for 'Cutler' and 'Kent' varieties. Control weights averaged 5.60ab and 1.82c-f in unextracted and extracted soil, respectively. [b] Values not followed by the same letter are significantly different at $P < 0.05$, comparing weight, uptake, and residue data separately. [c] One or more plants died before harvest.

Table V. Effect of Soil Extraction and Aging on Growth of 'Cutler' Soybeans

soil treatment	dry wt. of top,[a] g	
	expt. A	expt. B
unextracted	3.28a	4.04a
unextr + extr (1:1)		4.10a
unextr + extr (1:5)	3.50a	3.17ab
unextr + extr (1:10)		2.57bc
unextr + solvent	3.02a	
unextr + extrn soln		2.42bc
extracted	2.80a	2.81bc
aged unextr[b]		0.73d
aged unextr + extr[b]		2.03c
aged extr[b]		0.91d
autoclaved		c

[a] At 10 weeks. Unlimed and limed samples are averaged together (experiment A). Within columns, values not followed by the same letter are significantly different at $P < 0.05$. [b] Previously used in experiment V, then stored dry 5 months in the greenhouse. [c] All plants died by 4 weeks.

Table VI. Manganese Content of 'Cutler' and 'Kent' Soybean Tissue Grown in Unextracted and Extracted Soil (Experiment II, "Variety")

sample population	Mn content of tops,[a] ppm		P[b]
	unextracted	extracted	
all samples[c]	353 ± 119	513 ± 102	<0.001
unlimed only	424 ± 105	544 ± 84	<0.01
limed only	282 ± 85	482 ± 111	<0.001

[a] At 10 weeks. Analyzed by emission spectrography; mean ± SD. [b] Level of significance for difference between groups of unextracted and extracted samples, determined by two-sided t test. [c] The 64 samples include 'Kent' and 'Cutler' varieties, unlimed and limed soil, unextracted and extracted soil, and untreated and herbicide-treated (6 cmds, 1 is at two rates) soil.

At 10 weeks, soil extraction had produced the usual growth abnormalities, as well as growth reduction (Table I). Trifluralin itself caused no obvious effect, but dinitramine plants (two of three at the 0.29 ppm rate) were somewhat columnar in shape although grown in unextracted soil–sand. Both rates of dinitramine also caused formation of small root-like projections along the lower 5 cm of stem. Dinitramine also reduced growth by ~70% in unextracted soil and by ~40% in extracted soil. Dinitramine seemed to reduce leaf, pod, and bean growth relatively more than stem growth. Liming is not separated as a treatment in Table I, but it did improve growth of control ($P < 0.02$) and trifluralin ($P < 0.01$) plants.

Although uptake increased from 4 to 10 weeks, especially for trifluralin, residues generally remained the same. Residues were highest from unextracted soil and were proportional (2.6 times vs. theoretical 2.9 times) to the rate of added chemical. Dinitramine gave higher residues, but lower percent uptake, than trifluralin. Residue level was not correlated with liming. Average residue, expressed as parent herbicide, was highest in "unextracted 0.29 dinitramine" (leaves had 3.33 ppm and beans 0.25 ppm). Most bean samples had much lower residues and were at the limit of detection.

Soil Extraction Experiments. *Experiment V (Fresh Soil).* We further explored the role of soil extraction, independent of herbicide treatment, on soybean growth. The difference between plants from unextracted and extracted soil was less pronounced than usual (Table V), perhaps because plants were subjected to somewhat less water stress (deficiency and excess) than occurred during earlier experiments. Since plants in unextracted soil + solvent (with air-drying) grew normally, we concluded that traces of residual solvent had not caused the phytotoxicity associated with extracted soil.

Experiment B (Fresh/Aged Soil). In this final experiment, watering was carefully controlled by weighing each pot. Soybeans seemed to exhibit fewer symptoms such as curled leaflets and columnar shape. When unextracted soil was mixed with extracted soil, plants grew normally in a 1:1 mixture (Table V), but dry weight progressively decreased at 1:5 and 1:10 ratios. Extracted soil alone gave the same results, as did this soil plus whatever nonvolatile material was extracted from it by Bz–MeOH. Soybeans in the three aged soils grew poorly, and some plants had died by 4–6 weeks. With autoclaving, the most severe soil treatment tested, all plants died within 4 weeks.

Manganese in Soil and Plant Tissue. Elemental analyses were obtained on all leaf + stem (10-week) samples of experiment II ("Variety"). The results from unextracted and extracted soil were separately averaged, and the degree of significance between the sample means was assessed via a t test. In the case of Mn, whose content in plants was significantly ($P < 0.001$) increased by prior soil extraction with Bz–MeOH, many additional comparisons were made.

Table VI shows that in unlimed soil, extraction increased Mn concentration in plants to 544 ppm. Liming at 2 weeks reduced Mn content by one-third ($P < 0.001$) in plants from unextracted soils, but by only ~10% ($P < 0.1$) in extracted soil plants. Varietal differences were nonsignificant, as was the comparison between control and herbicide-treated plants.

Table VII. Manganese Content of 'Cutler' Soybean Tissue Grown in Unextracted and Extracted Soil ("Basic" Experiment)

herbicide rate, ppm	soil treatment	Mn content,[a] ppm leaf unextr.	leaf extr.	stem unextr.	stem extr.
		control			
	unlimed	ND[b]	1097 ± 111[c]	ND	885 ± 404
	limed[d]	350 ± 57	1099 ± 93	253 ± 112	375 ± 87
		dinitramine			
0.1	unlimed	489	1662	ND	ND
0.1	limed	436	1033	ND	ND
0.29	unlimed	ND	ND	200	265
0.1	limed	ND	ND	608 ± 501	351 ± 64
		trifluralin			
0.1	unlimed	370	1101	ND	ND
0.1	limed	512	1284 ± 262	ND	ND
0.29	unlimed	ND	1168	ND	ND
0.1	limed	367	1411 ± 450	ND	ND

[a] At 10 weeks. Analyzed by atomic absorption spectrophotometry. [b] Not determined. [c] Mean and deviation of duplicate samples is shown; others are nonreplicates. [d] Limed at 4 weeks.

Table VIII. DTPA-Extractable[a] Manganese Content of Matapeake Silt Loam Soil

soil treatment[b]			pH[c]	Mn concn, ppm in potting mixture	Mn concn, ppm whole soil basis
fresh	unextr.	unlimed	4.87	50	50
fresh	unextr.	limed	5.32	34	34
fresh	extr.	unlimed	4.34	109	109
fresh	extr.	limed	4.51	90	90
fresh + sand	unextr.	unlimed	5.20	13	78
fresh + sand	unextr.	limed	5.14[d]	14	84
stored[e]	unextr.	unlimed	5.12	147	147
stored	unextr.	limed	5.23	134	134
stored + sand	unextr.	unlimed	5.44	24	144
stored + sand	unextr.	limed	6.97	16	96
stored + sand	extr.	unlimed	4.84	19	114
stored + sand	extr.	limed	5.92	10	60
aged[f] + sand	unextr.	unlimed	5.05	25	150
aged + sand	unextr.	limed	5.51	17	102
aged + sand	extr.	unlimed	4.99	25	150
aged + sand	extr.	limed	5.74	26	156
autoclaved + sand	unextr.	unlimed		96	576
sand	unextr.	unlimed		0	0

[a] Extracted by DTPA-TEA reagent (Lindsay and Norvell, 1969). [b] Extraction and liming were procedures used as in soybean experiments. Soil-sand ratio was 1:5, as used for soybeans. [c] Determined as 1:1 soil-water paste. [d] pH suggests that this sample was not limed. [e] Soil stored air-dry 6 months. [f] Soil had been used to grow soybeans for experiment A, then stored dry ~5 months.

Other elements whose content in soybean tops changed significantly after soil extraction included the following: Mg (0.24 to 0.32%, $P < 0.001$), K (0.98 to 1.34%, $P < 0.001$), P (0.17 to 0.20%, $P < 0.01$), Fe (117 to 140 ppm, $P < 0.01$), B (17.1 to 19.0 ppm, $P < 0.02$), Mo (1.4 to 1.9 ppm, $P < 0.05$), and Sr (34.8 to 33.8 ppm, $P < 0.05$). In every case except Sr, extraction increased the concentration. When compared with a sufficiency range for nutrient elements in soybean leaves (Small and Ohlrogge, 1973), Mn was high but other elements were near normal (K and P were slightly low).

Because Mn levels were high (Table VI) in soybean tops, we made further analyses to screen Mn concentration in leaf and stem samples from another experiment. Leaf Mn content averaged 421 ± 69 ppm from unextracted soil and 1232 ± 213 ppm from extracted soil (Table VII). Stem Mn content was lower, but more variable, than leaf Mn. One stem replicate value is questionable because the tissue weight reported for the analysis was exceptionally low; omitting this changes the 0.29 ppm dinitramine sample from 608 to 107 ppm. Liming at 4 weeks had no definite effect on Mn content, despite its having increased soil pH.

The manganese extractable from soil by DTPA–TEA reagent (Table VIII) increased in the order fresh unextracted soil < fresh extracted soil < stored extracted soil + sand < stored unextracted soil = aged soil + sand ≪ autoclaved soil + sand. Storing air-dry soils led to high extractable Mn. Liming was associated with a reduction in extractable Mn, although the increase in pH was not significantly (5% level) correlated with declining Mn.

DISCUSSION

Anaerobic Bound Butralin Experiments. Reduction of total ^{14}C in aerobically incubated soil systems indicated that breakdown of some bound butralin residues was occurring. In the most active cases, the humic acids, only 20–25% loss occurred by termination of the incubation. Thus no quantitative statement of persistence is possible, except that at the present rate, ~50% loss in 10–12 months is projected for ^{14}C in these fractions. Loss is slower in other fractions. The fractionation procedure itself may make the bound residue more susceptible to

breakdown, since residue in whole soil (curve B in Figure 1) exhibited no statistically significant decline.

The major discrepancy in assessing ^{14}C turnover from the butralin residues is that measured $^{14}CO_2$ was much less than apparent loss of ^{14}C in soil. If $^{14}CO_2$ data are used, the most degradable fraction, NaOH humic acid, would require ca. 7 years before 50% loss occurred. The principal conclusions remain, i.e., that the bound residues from butralin are very persistent and that their type or location within soil components apparently influence their biological susceptibility.

These anaerobically formed butralin residues (even after incubation) failed to release any detectable ^{14}C to soybeans, pigweed, or crabgrass. The equivalent rates, expressed as parent, were very low, however, ranging from 4×10^{-3} to 8×10^{-2} kg/ha.

Plant Uptake of Partially Bound and Bound Dinitroanilines. The phytotoxicity and ^{14}C uptake caused by six partially bound herbicides was a consequence, in part, of less exhaustive soil extraction than received by anaerobic bound butralin. Since additional extraction significantly reduced the remaining ^{14}C in soil (Helling and Krivonak, 1978), the effects seen in the "partially bound" experiment may have been due only to plant-available parent herbicide (TLC had suggested the presence of butralin, chlornidine, and fluchloralin). The same bioassay done with bound dinitroanilines showed, however, that soybeans did extract ^{14}C in some form from the bound ring-labeled herbicides. This was less available, as expected, than was the partially bound residue. Leaf residues dropped from 1–2 ppm to an average of 0.3 ppm. Beans, which represent the economically most significant plant part, had barely detectable residues.

Characteristic phytotoxicity occurred in soybeans grown in both partially extracted and fully extracted soil–sand mixtures. This was most apparent in the first case, with columnar growth, small and crinkled leaflets, more trifoliate leaves (often branching from the cotyledon node), and some leaf spotting. Bound residues from profluralin, trifluralin, chlornidine, and dinitramine seemed to cause abnormal growth. This suggests that some parent herbicide or phytotoxic metabolite *may* remain (after the original 5- or 7-month incubation) in a form resistant to solvent extraction, but slowly available to plants.

Herbicide and Extraction Effects on Phytotoxicity and ^{14}C Uptake. Some ^{14}C uptake from bound [^{14}C]dinitroanilines was not unexpected, especially since anaerobic bound butralin lost ^{14}C during aerobic incubation (Figure 1). The phytotoxicity was surprising: applied rates were lower (0.2–0.3 kg/ha, expressed as parent) than field rates, which apparently cause no problems to soybeans (Stoller and Wax, 1977). Furthermore, binding, even if all residue is parent, should reduce availability for uptake. Accordingly, a series of experiments was run (a) to identify the symptoms and relative activity of dinitroaniline herbicides and (b) to ascertain whether soil preextraction was affecting results.

Experiment I did not demonstrate any adverse effects of five herbicides applied at 0.1 or 0.29 ppm (0.07 or 0.21 kg/ha), when growing conditions were identical—*except* for soil extraction—to the partially bound and bound residues experiments. Dinitramine at 0.29 ppm killed the plants, which had average top residues of 1.4 ppm. When soil was previously Soxhlet extracted with Bz–MeOH, however, all plants showed symptoms seen earlier in the two bound residue studies. Furthermore, the soil extraction seemed to synergize phytotoxicity from trifluralin and perhaps dinitramine, despite reducing both uptake and residue levels in the soybean top. Experiment II reconfirmed most findings Experiment I and also showed that two varieties ("Cutler" and "Kent") behaved generally similarly. There is indication, here and from experiment III, that 'Cutler' soybeans may be slightly more sensitive to herbicide injury than are 'Kent'.

The potting medium for most experiments was a 1:5 soil–sand mixture, chosen primarily to maximize use and potential sensitivity of the limited supply of bound residue-containing soils and ^{14}C-labeled herbicides. The same effects on growth and uptake occurred when soil alone was used (experiment III). However, the additional soil may have reduced bioactivity, because 0.29 ppm is nontoxic (dinitramine and trifluralin); the higher rate (0.58 ppm) killed most plants. Alternatively, the effect may simply be due to the two times greater amount of herbicide in soil–sand, at equivalent rates, because of the soil and sand weight difference.

When soybeans were grown in larger pots (experiment IV), control plants achieved the highest top weight of any experiment. However, the same rate of dinitramine, 0.1 ppm, which did not injure soybeans in small pots, now reduced growth by $\simeq$70%. Presumably this is because the individual plant was now exposed to five times more herbicide. (The equivalent rates for 0.1 and 0.29 ppm, here, were 0.12 and 0.34 kg/ha.) Dinitramine-injured plants had at least one symptom in common with those from extracted soil alone, i.e., columnar shape.

The distinction between unextracted- and extracted-soil plants was unclear in only one experiment (A). Residual traces of solvent were eliminated as a cause of the usual difference, however. When special care (weighing of individual pots) was taken to avoid over- or underwatering, soybeans tended to be less affected by prior soil extraction (experiment B). Since Soxhlet extraction presumably sterilized the soil, we thought that restoration of microbial activity may alleviate the phytotoxicity. Dilution with fresh soil, a method of inoculation, was completely effective only at the highest ratio, 1:1. This suggests a chemical, not biological, factor. However, the Bz–MeOH extractable material, when added back to extracted soil, did not restore normal growth. Thus, a growth-promoting factor is unlikely to have been removed by the organic solvent. Both prolonged drying and, particularly, autoclaving were profoundly detrimental to soybean growth. These treatments have a sterilizing effect, but they also lead to chemical changes in soil. One of these is an increase in soluble Mn concentration.

Manganese and the Extraction Effect. Classical symptoms on Mn toxicity to soybeans include leaf crinkling and necrotic spotting (Carter et al., 1975; Heenan and Carter, 1976; Ohki, 1976; Parker et al., 1969), sometimes with chlorosis and leaf drop, as well. We observed these symptoms on many plants grown in extracted soil, effects seemingly exacerbated by some herbicide residues. Such plants frequently had more trifoliate leaves and were relatively more columnar in shape than normal plants. The latter characteristics were unreported in descriptions of Mn toxicity and they may, therefore, indicate some additional phytotoxic interaction.

Stronger evidence for Mn toxicity came in the elemental analyses of soybean tops from experiment II (these plants were typical in their responses to herbicide and extraction treatments). Tissue levels of Mn were very high (Table VII) and increased by 100–200 ppm in plants grown in extracted soil. These concentrations equal or exceed other soybean leaf or top values known to cause Mn toxicity (Heenan and Carter, 1976; Ohki, 1976; Parker et al., 1969;

Somers and Shive, 1942). Soybean genotypes vary greatly in their tolerance to Mn, and one of our varieties, 'Kent', was among the more susceptible tested (Carter et al., 1975); 'Cutler' was not reported.

Leaf samples from experiment I contained 350–500 ppm Mn in plants from unextracted soil, i.e., the same amount as reported from experiment II. However, soil extraction led to Mn values of ~1000–1600 ppm (Table VIII). Liming, in all experiments, was not clearly effective in improving soybean growth *in extracted soils*, and the slight reduction in averaged Mn content probably explains this.

Benzene–methanol preextraction of soil has been shown in our studies to cause both symptoms and elevated tissue concentrations typical for Mn toxicity to soybeans. Availability of Mn is related to factors affecting its soil solution concentration in the divalent state (Foy, 1973). Low soil pH (<5.5), poor aeration, and high salt concentration are all conditions favoring Mn uptake. The organic solvent extraction step was undoubtedly effective in sterilizing the Matapeake soil, including its manganese-oxidizing organisms. This change, coupled with the naturally low pH (~5–5.5) in our Matapeake soil, probably accounts for the soybean phytotoxicity in extracted soils. Soil Mn, estimated by DTPA extraction, doubled (Table VIII) when fresh unlimed soil was extracted first with Bz–MeOH. These soil analyses also explain the very poor growth of soybeans in soil given prolonged dry storage (Mn, ~145 ppm) or autoclaving (Mn, 576 ppm). These treatments, and chemical fumigation, increase Mn solubility to toxic levels in high-Mn soils (Foy, 1973); unfortunately, prediction of these effects requires detailed investigations of individual soils (Nelson, 1977).

Other factors helped to predispose our bioassay to possible Mn toxicity. To prevent loss of ^{14}C, the pots were undrained. Thus, waterlogging sometimes occurred, a condition known to favor Mn^{2+} formation. In experiment B we prevented or minimized this by weighing each pot: the soybeans at 10 weeks showed fewer symptoms of Mn toxicity. Undrained pots also increased the likelihood of salt accumulation from periodic fertilization, a factor that also has promoted Mn availability.

In summary, evaluation of the uptake and bioactivity of bound pesticide residues may be complicated by phytotoxic concentrations of Mn in the soil. This situation was created when a moderately acidic soil, Matapeake silt loam, was Soxhlet-extracted with Bz–MeOH in order to leave only bound residues of six [^{14}C]dinitroaniline herbicides. It seems likely that any common pesticide extraction technique would also kill or inhibit soil microorganisms to the degree that plants subsequently grown in the soil might be artificially affected. Although uptake of ^{14}C from the bound dinitroanilines did occur, studies with the parent herbicides indicated that the bound residues themselves, at their low concentrations, are unlikely to cause phytotoxicity unless the soybeans are unexpectedly sensitized by the toxic Mn levels.

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