PCP with subsequent further degradation of PCP (Murthy and Kaufman, 1978) would seem to be the only pathway by which PCNB is more completely degraded. Further work with PCA and PCTA is in progress, however, to verify this conclusion.

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Physicochemical Characteristics of Bound Dinitroaniline Herbicides in Soils

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Soil bound residues of six [*phenyl*-^{14}C]dinitroaniline herbicides constituted 7–21% of the original ^{14}C added to aerobically incubated Matapeake soil. Bound residues of butralin [4-(1,1-dimethylethyl)-*N*-(1-methylpropyl)-2,6-dinitrobenzeneamine] from Chillum soil were 3 and 13% after aerobic and anaerobic incubation, respectively. Prolonged benzene–methanol extraction gave stable residue values. The extracts contained unidentified radioactive components, chromatographically separable from the parent compounds. Ultrasonic dispersion in certain alkaline solvents increased extractability of anaerobic butralin residues, probably because organic matter was solubilized. Distribution of residues in fulvic acid, humic acid, and humin was (a) butralin (aerobic Chillum soil), 51, 7, and 42%; (b) butralin (anerobic Chillum), 14, 40, and 46%; and (c) 6 herbicides (aerobic Matapeake soil), 50, 15–20, and 25–35%. When we used a milder extraction procedure, anaerobic bound butralin was widely distributed among soil organic and organo/mineral fractions, especially in humic acid and humin. The six dinitroaniline residues were thermally degraded between ca. 250 and 500 °C: anaerobic butralin was lost at 300–375 °C. This corresponds to oxidation of phenolic hydroxyl (and perhaps carboxyl) groups in soil organic matter, but tends to negate the occurrence of bound ^{14}C in clay interlayers or as carbonate.

Using radioisotopes as tracers within pesticide molecules has been of inestimable value in understanding their behavior in living systems, including the soil. It has also made us aware that unextractable residues may accumulate, since combustion of the extracted soil (when ^{14}C was used) yields $^{14}CO_2$. These residues are now termed "soil bound residues", defined as "that unextractable and chemically unidentifiable pesticide residue remaining in fulvic acid, humic acid, and humin fractions after exhaustive sequential extraction with nonpolar organic and polar solvents" (U.S. Environmental Protection Agency, 1975). Bound residues have been addressed as a possible concern for pesticide registration.

Bound and conjugated residues in soils and plants were the focal point of a recent conference (Kaufman et al., 1976). Kearney's (1976) final summary reflected the uncertainties of the bound residue question. Chemical identification of the bound entities is rare (Booth et al., 1976; Hill, 1976), although this is a primary research objective. Usually, measurement is of the total residue present and its distribution among three rather arbitrarily

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defined soil organic matter (OM) fractions.

Chloroanilines are metabolites common to many herbicides. They seem especially prone to bound residue formation (Bartha, 1971; Chisaka and Kearney, 1970). Hsu and Bartha (1974) found that ~55% of 3,4-dichloroaniline was converted to nonhydrolyzable residues which, they suggested, may be integrated into the soil OM nuclei. Bonding to isolated humic acid was much firmer than to whole soil. More recent evidence of Bandal et al. (1976) supported the role of free $-NH_2$ groups in strong bonding to soil.

The dinitroaniline herbicides may form metabolites that contain up to three such amino groups and, thus, they also have potential for forming a significant pool of bound residues. The purpose of this paper (previously summarized by Helling, 1976a) is to describe our attempts to characterize bound dinitroaniline residues. Another publication (Helling and Krivonak, 1978) deals with their significance in terms of biological availability.

MATERIALS AND METHODS

Pesticides and Soils. All dinitroanilines were U-phenyl-^{14}C labeled. The residue-containing soils came from two previous metabolism studies (Kearney et al., 1974, 1976). Studies conducted with bound butralin alone used Chillum silt loam soil in which 10 ppm butralin (1 μCi/mg) had been incubated for 6 months, either at 70% field moisture capacity (aerobic) or flooded (anaerobic). These soils were then extracted with benzene (Bz), methanol (MeOH), and Bz–MeOH (Kearney et al., 1974) and were stored dry for ~20 months before we began our analyses of the bound residue.

In a second series of investigations, we used six dinitroanilines: butralin, 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). Matapeake silt loam had been treated with 10 ppm (1 μCi/mg) of each herbicide, then incubated aerobically for 5 months (dinitramine) or 7 months, as described by Kearney et al. (1976). At this time, the soils had been extracted with Bz–ethyl acetate (EtOAc) for 1 min in a Waring blender, filtered, and then similarly reextracted with MeOH. After air-drying, the soils were stored at 4 °C for 10 months until we began bound residue studies.

Extraction of Residues. The first studies, with bound butralin in Chillum soil, concentrated on the use of ultrasonification with various solvents. These were normally reagent grade or redistilled; 2,4-lutidine was technical grade. Ultrasonic generating instruments used were: Lab-Line Ultratip Labsonic System No. 9100 (170 W acoustical power) and Bronwill Biosonik IV Ultrasonic System (300 watts acoustical power). For comparison, samples were sometimes shaken for 16 h, rather than sonified. After centrifugation, radioactivity in 1-mL aliquots was measured by liquid scintillation. One replicate of duplicate samples was spiked with an internal ^{14}C standard to verify applicability of the quench curve.

Later, the effect of pulverization of soil on extractability was also examined. Thirty grams of soil (from both Chillum and Matapeake experiments) was pulverized for 2 min in a Spex Industries "Shatterbox", then was shaken 24 h at 25 °C with 90 mL of Bz–MeOH (1:1, v/v). The extract was filtered through Whatman GF/A glass fiber filter paper, concentrated to 10 mL, and counted in a toluene-based scintillation cocktail. Nonpulverized soil was similarly extracted. Residual ^{14}C in the soil was measured by combustion.

Because extraction of the six dinitroanilines in Matapeake soil led to reduction in remaining bound ^{14}C, these shake-extracted samples were Soxhlet-extracted with Bz–MeOH for 24 h. Aliquots of the concentrated extract were counted (with and without prior centrifuging); the remaining solution was saved for TLC analysis. A second Soxhlet extraction for 24 h was made on the basis of solution and soil analyses.

Thin-Layer Chromatography. Precoated silica gel GF-254 plates were used, generally 2 mm thick. Benzene–ethyl acetate (1:1) was the usual elutant. Developed TLC plates were examined by visible and UV light and by autoradiography. For the anaerobically bound butralin, the total extract applied to a plate, after concentration under N_2, represented 1 g of soil. Extracts of the six bound dinitroanilines were centrifuged and then concentrated, and each streak represented 20 g of soil.

Fractionation of Residues. *"Classical" Procedure.* Five grams of soil was shaken with 25 mL of 0.5 N NaOH for ca. 21 h at 25.0 °C. The suspension was centrifuged, and the sediment was reextracted twice with 10 mL of NaOH. The sediment was then washed with 3 × 10 mL of H_2O, with prolonged centrifugation necessary as soil dispersion increased. The combined NaOH and H_2O extract, containing humic + fulvic acids, was brought to volume with water, and aliquots were removed for scintillation counting. Humic acid was precipitated in the remaining extract by adding ca. 250 μL of concentrated HCl, which brought the pH to ca. 1.15. After 30 min, this solution was centrifuged and the remaining humic acid was washed two or three times with 0.01 N HCl, totalling 10 mL. The combined solutions were brought to 25-mL volume with 0.01 N HCl and 1-mL aliquots were counted for distribution of ^{14}C in fulvic acid. The residual humic acid was brought to 10-mL volume with 0.05 N NaOH, and aliquots were counted.

Gascho/Stevenson Procedure. This method essentially follows that of Gascho and Stevenson (1968) and is outlined in Figure 1. Anaerobic butralin-treated Chillum soil (1 kg) in dialysis tubing was extracted with 7 × 14 L of 0.3 M HF. The first extract was extracted with diethyl ether; the water phase, which remained yellow, was brought to pH 7.5 with Na_2CO_3, then reextracted with ether. The additional HF extracts contained no ^{14}C and were discarded. The soil was next dialyzed against H_2O until pH 3 was attained, discarding the extracts. Floating undecomposed OM within the dialysis bags was collected.

Dialysis/extraction was continued with 0.02 M $Na_4P_2O_7$. The reddish-brown dialysates were concentrated by vacuum evaporation at 50 °C, neutralized with HCl, and freeze-dried. The nondialyzable extract was neutralized and dialyzed sequentially against H_2O, 0.3 M HF, 0.1 N HCl, and H_2O. The first H_2O extract (colored) was concentrated and freeze-dried. Some precipitation had occurred after neutralization; this material was resuspended in 0.03 N NaOH and added to the residual soil.

The $Na_4P_2O_7$-soluble extract was acidified to pH 1.5, precipitating "$Na_4P_2O_7$ humic acid"; this was washed with HCl (pH 1.5) before recovery. The remaining solution was concentrated and "$Na_4P_2O_7$ fulvic acid" was isolated.

The soil previously extracted with $Na_4P_2O_7$ was extracted in the same manner with 0.03 N NaOH; comparable fractions were recovered.

The residual soil was dialyzed against H_2O to pH 7. Suspended material (thought to contain much OM) was

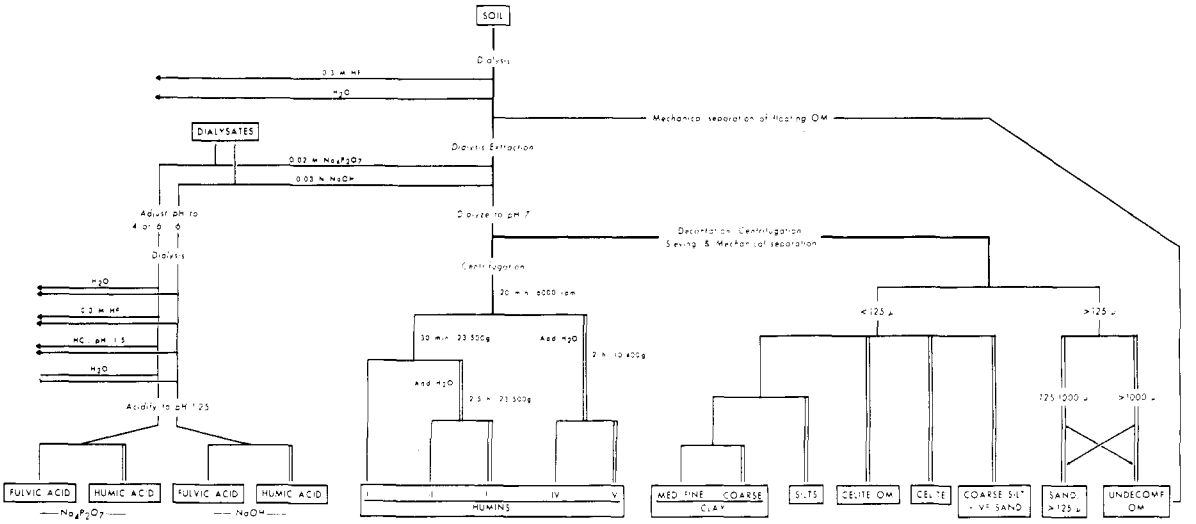

Figure 1. Fractionation of anaerobic bound butralin, based on the procedure of Gascho and Stevenson (1968).

separated by decanting from the bulk of the soil (thought to be predominately inorganic). A series of humin fractions were isolated, based on their sedimentation characteristics (Figure 1). A lighter colored "mineral" zone was removed from "humin V" and brought into the inorganic fractionation scheme. Celite (a siliceous filtering aid used during the original incubated soil extraction) was separable into white and gray portions, the latter called Celite/OM because we thought it included OM. During sieving of sand, some additional "heavy" undecomposed OM was recovered.

All fractions were freeze-dried and saved for further analyses.

Thermal Stability. Soil samples containing anaerobically bound butralin or the six bound dinitroanilines (as received) were heated in an O_2 stream at various temperatures for a fixed time, collecting the evolved $^{14}CO_2$. The samples (300 mg) were covered by 7–9 mm of a combustion catalyst (CuO–Al_2O_3, 1:5, v/v) in a ceramic combustion vessel, then placed into the heated combustion tube. Oxygen sweeping the system passed through a Drierite trap, then into the CO_2 trapping solution, 10 mL of 2-aminoethanol–2-methoxyethanol (1:7.3). After collecting CO_2 for 20 min, 5 mL of the trapping solution was added to 10 mL of scintillation cocktail for analysis. Combustion tubes were flushed for ≥15 min between samples. In some cases, collections were made at 2-min intervals to ascertain the rate of $^{14}CO_2$ release.

Routine residue analysis of soils was conducted by this technique; 15 min at 1000 °C, without intervening blank samples, was sufficient.

RESULTS

Our research on bound dinitroanilines was divided into earlier investigations of bound butralin remaining in aerobic or anaerobically incubated Chillum soil (Kearney et al., 1974), and later work with six dinitroaniline from aerobic Matapeake soil (Kearney et al., 1976). Here we have combined the results for comparison.

Bound Residue Content. Butralin-treated soils, after incubation, had been relatively exhaustively extracted. Recovery of only 43% of added ^{14}C after 6 months (Kearney et al., 1974) suggested that much aerobically incubated butralin remained bound. (The anaerobic incubation, conducted at the same time, was not described in their publication). Combustion analysis revealed only 3.0 and 12.8% residual ^{14}C in the aerobic and anaerobic Chillum soil (Table I). Thus, we devoted most further effort to the anaerobic sample.

Table I. Bound Residue Content from Six Dinitroanilines

	Chillum silt loam			
		residue after Bz-MeOH extr., %		
herbicide	bound residue, %[a]	24-h shake	+ 24-h Soxhlet	+ 24-h Soxhlet
butralin (aerobic)	3.0 ± 0.1			
butralin (anaerobic)	12.8 ± 0.4	12.4		

	Matapeake silt loam (aerobic only)			
	partially bound[b]	24-h shake	+ 24-h Soxhlet	bound
butralin	(25 ± 0)	18	18	17 ± 0
chlornidine	(28 ± 0)	20	18	17 ± 1
dinitramine	(19 ± 0)	11	10	8 ± 0
fluchloralin	(37 ± 1)	27	24	21 ± 3
profluralin	(21 ± 0)	14	12	11 ± 0
trifluralin	(14 ± 0)	8	8	7 ± 1

[a] Residues are expressed as percent of applied radioactivity (±SD). Since 10 ppm was originally applied, 25% residue corresponds to 2.5 ppm herbicide (assumes parent). [b] "Partially bound" residues in Matapeake were as received after incubation and metabolite extraction; they are distinguished from "bound" residues left after more exhaustive solvent extraction.

Apparent "bound residues" from the six dinitroanilines in Matapeake were much higher (Table I), although the only direct comparison is with aerobic butralin (3 vs. 25%). Since we assumed the six samples had been exhaustively extracted, further extraction was not attempted until plant uptake studies (Helling and Krivonak, 1978) indicated both uptake and phytotoxicity.

We chose Bz–MeOH as a moderately polar solvent system, since it had effectively removed extractable residues from butralin-treated Chillum (Kearney et al., 1974), although it had not been used for Matapeake. We compared pulverization, which greatly increases soil surface area and potentially improves residue extractability, with nonpulverized samples. Finally, we centrifuged a portion of each extract before scintillation counting.

Apparently (Table I) much of the residue in Matapeake soil could be extracted. The originally received soils were, therefore, redesignated as containing "partially bound" residues. A second extraction (Soxhlet) slightly reduced the remaining residue. A final Soxhlet extraction indicated that further extraction was unwarranted and so we termed

Table II. Ultrasonic Extraction of ^{14}C from Bound Butralin in Chillum Soil

solvent[a]	soil/soln ratio, g/mL	sonification conditions: time, min	power, W	temp, °C	extr. effic.,[b] % of residual ^{14}C
		aerobic soil			
EtOAc	1:4	5	50	~30	2
EtOAc	1:4	5	100	~30	3
EtOAc-H_2O (19:1)	1:4	5	50	~30	5
EtOAc-H_2O (19:1)	1:4	5	100	~30	6
		anaerobic soil			
EtOAc	1:4	5	100	~30	1 ± 0
EtOAc-H_2O (19:1)	1:4	5	100	~30	3 ± 0
MeOH	1:4	5	100	~30	2
MeOH-NH_4OH (9:1)	1:5	5	80	~30	6
MeOH-NH_4OH (3:1)	1:5	5	80	~30	11 ± 1
MeOH-NH_4OH (1:1)	1:5	5	80	~30	17
DMF	1:5	5	80	~30	3 ± 0
DMF	1:6	5	80	~80	7
Me_2SO	1:5	5	80	~30	5 ± 1
Me_2SO	1:5	5	80	~90	9 ± 0
Me_2SO-NH_4OH (3:1)	1:5	5	80	~30	6
Me_2SO-NH_4OH (1:1)	1:5	5	80	~30	4
Me_2SO-γ-Bu-NH_4OH (1:1:1)	1:5	5	80	~30	7
γ-Bu-NH_4OH (1:1)	1:5	5	80	~30	16
γ-Bu	1:4	5	100	~30	3
2-aminoethanol	1:5	5	80	~30	14
pyridine	1:5	5	80	~30	4 ± 0
pyridine	1:4	7	100	~30	5

[a] Abbreviations are EtOAc = ethyl acetate; MeOH = methanol; DMF = dimethylformamide; Me_2SO = dimethyl sulfoxide; and γ-Bu = γ-butyrolactone. Solvent proportions are vol/vol. [b] Ranges shown are for duplicated samples. Most values are unreplicated, because many changes were made in experimental conditions during testing.

the remaining ^{14}C "bound residue".

Data in Table I are averages of both nonpulverized and pulverized soil. Only ca. 2% more ^{14}C, of that remaining, was extractable if soils were pulverized before the first Bz–MeOH extraction. After three extractions, the difference represented only ca. 1% of the residual ^{14}C. Centrifugation did not significantly affect the analyses.

Extraction of Anaerobic Bound Butralin. Extraction of the Chillum with Bz–MeOH, in contrast to the Matapeake soils, was relatively ineffective in removing bound butralin (Table I). In this experiment, pulverization seemed to increase extractable ^{14}C, but the additional radioactivity may indicate contamination by finely suspended solids.

Table II shows results of some ultrasonic extraction experiments. Limited work with aerobically bound butralin demonstrated that EtOAc was not effective in removing bound ^{14}C, although some advantage is gained when water is introduced or (to a minor extent) power to the probe tip is increased. The same results occurred with anaerobic soil.

Extraction efficiency remained low with MeOH, DMF, or Me_2SO, but increased when DMF and Me_2SO were maintained at 80–90 °C. The most effective single solvent tested was 2-aminoethanol (14% of residual ^{14}C was solubilized), an ingredient used to trap CO_2 from our combustion technique. However, extractability was highest (17%) when NH_4OH was introduced with MeOH (1:1) or γ-butyrolactone (1:1) (16%).

Other solvents not shown in Table II extracted <5% of residual ^{14}C. These included water, MeOH–H_2O, Me_2SO–H_2O, DMF–H_2O, formamide, 2,4-lutidine, and acetonitrile. Aniline produced a dark suspension and quenched the scintillation cocktail too strongly for analysis.

Thin-Layer Chromatography of Extracts. Concentrated Bz–MeOH extracts from anaerobic bound butralin (pulverized and nonpulverized) were chromatographed with either Bz or Bz–EtOAc as solvent. Very little radioactivity had been extracted (Table I) and this remained at the origin, which was yellow-brown. Nonradioactive bands, appearing in UV illumination, were at R_f 0.77 (major), 0.15, and 0.04 in the Bz system. Butralin itself moved to R_f 0.77. In Bz–EtOAc, bands were at R_f 0.87, 0.77, and 0.63. Bands seemed to be slightly stronger from pulverized soil, perhaps a consequence of greater extraction.

Unlike our results with anaerobic bound butralin, TLC of six dinitroanilines (the first Bz–MeOH extract) produced many radioactive bands. These extracts contained a major band at a R_f similar to the corresponding parent: butralin, chlornidine, and fluchloralin (traces). No clear difference emerged between pulverized and nonpulverized soil extracts. The extract from untreated Matapeake soil gave visible origin and R_f 0.70 zones, while bands at 0.67 and 0.53 were visualized by UV light. This TLC plate showed no zones by autoradiography.

The second extracts (by Soxhlet) of the six herbicide residues gave a radioactive origin streak and 1–3 very faint slightly mobile bands from dinitramine and profluralin. By the final extraction, only the origin streak remained. A fairly consistent unlabeled band at R_f 0.75–0.85 began to disappear by the third extraction.

Because the amounts of residue were very low, we made no further investigations of the TLC-separated materials.

Fractionation of Residues. *"Classical" Procedure.* Chillum and Matapeake soils were fractionated by a simple procedure involving alkali extraction of humic and fulvic acids, then acid precipitation of the humic acid. Humin is the unextractable OM.

The proportion of total bound ^{14}C isolated from each fraction (Table III) may be affected by prior incubation conditions, based on Chillum soil-bound butralin, in which 51 vs. 14% (fulvic acid) and 7 vs. 40% (humic acid) of ^{14}C was bound to aerobic and anaerobic soils, respectively. The six "partially bound" dinitroanilines in aerobic Matapeake soil were distributed generally similarly, the results tending to resemble those from the aerobic Chillum experiment. Average content of the six Matapeake residues was 52 ±

Table III. Fractionation of Bound Dinitroaniline Residues

herbicide	% bound residue in fulvic acid	humic acid	humin
Chillum silt loam			
butralin (aerobic)	51 ± 10	7 ± 1	42 ± 9
butralin (anaerobic)	14 ± 4	40 ± 11	46 ± 10
Matapeake silt loam (aerobic only)[a]			
butralin	53 ± 0	21 ± 2	26 ± 2
chlornidine	54 ± 2	16 ± 1	30 ± 2
dinitramine	47 ± 1	15 ± 1	38 ± 0
fluchloralin	60 ± 1	25 ± 0	15 ± 0
profluralin	53 ± 0	16 ± 1	31 ± 1
trifluralin	47 ± 1	15 ± 1	38 ± 2

[a] Fractionation of "partially bound" soils. Both soils extracted by using a "classical" soil organic matter procedure. Data are averages ±SD.

Table IV. Distribution of ^{14}C from Bound Butralin in Anaerobic Chillum Soil

fraction	unpurified fraction wt, g	residue ppm[a]	residue % of recovered ^{14}C
organic fractions			
undecomposed OM	(9.27)	(7.43)	(8.28)
light	0.54	7.35	0.48
heavy	8.73	7.43	7.78
dialysates	(41.05)	(0.51)	(2.52)
$Na_4P_2O_7$	30.49	0.35	1.27
-H_2O (1st)	6.53	0.54	0.42
-HCl (1st)	1.69	0.31	0.06
NaOH	2.34	2.66	0.75
fulvic acid	(19.21)	(0.71)	(1.68)
$Na_4P_2O_7$	15.87	0.56	1.08
NaOH	3.34	1.42	0.57
humic acid	(13.9)	(15.8)	(26.3)
$Na_4P_2O_7$	6.61	13.17	10.44
NaOH	7.3[b]	18.18	15.9[b]
humin	(12.05)	(8.65)	(12.48)
I	2.17	9.87	2.57
II	0.01	7.22	0.01
III	0.77	11.47	1.06
IV	2.52	8.68	2.62
V	6.58	7.92	6.25
mineral fractions			
clay	(8.95)	(5.46)	(5.87)
med./fine	1.28	8.05	1.23
coarse	7.67	5.04	4.64
silt (med./fine)	103.0	2.32	28.62
silt (coarse) + vfs	257.7	0.01	0.44
sand (>125 μm)	160.9	0.09	1.75
Celite/OM	80.4	1.23	11.83
Celite	104.7	0.02	0.20

[a] Calculated as parent butralin. [b] Amounts estimated because mechanical loss (~7%) occurred during extraction.

5% in fulvic acid, 18 ± 4% in humic acid, and 30 ± 9% in humin.

Gascho/Stevenson Procedure. The method developed by Gascho and Stevenson (1968) to extract and fractionate soil OM is potentially advantageous for bound residue research. The reagents used are relatively mild, so both the bound species and the OM fractions are more likely to survive intact than is the case when the classical procedure is used.

Table IV summarizes distribution of bound radioactivity among soil fractions. Only anaerobic butralin was fractionated by this complex procedure. By broad groups, 51% of the recovered ^{14}C was found among organic fractions, 49% among mineral fractions. The largest individual reservoirs of bound residue were silt (medium + fine) (28.6%) and humic acid (26.3%). Other significant carriers included humin (12.5%), Celite (12.0%), and undecomposed OM (8.3%). Celite was the filtering aid used during organic solvent extraction of the original incubated soil. Nearly all of its residue was actually associated with a grayish form, designated Celite/OM.

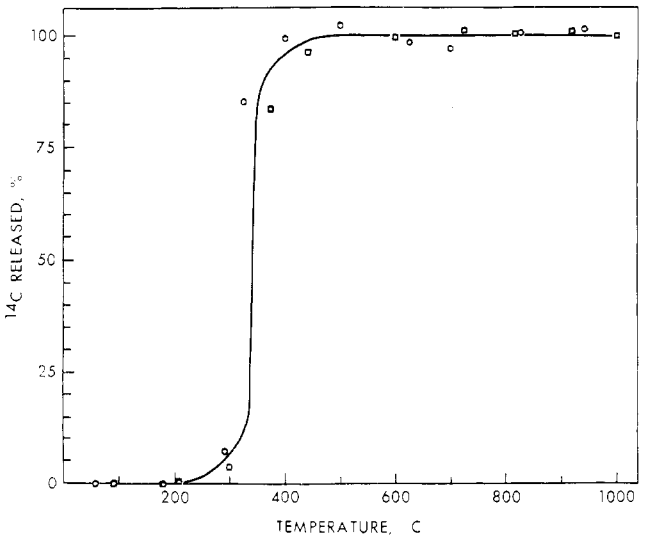

Figure 2. Thermal profile of ^{14}C release from anaerobic bound butralin.

Concentration of the residue, expressed as ppm of parent herbicide, was in the order humic acid > humin > undecomposed OM > clay. Silt was significant as a reservoir only because of its large volume. Subfractions such as humins I–V contained similar concentrations of ^{14}C; the difference between humins II (7.22 ppm) and III (11.47 ppm) would have disappeared had centrifuging been conducted for a shorter time. The two subfractions of undecomposed OM also had the same proportion of radioactivity. "Light", the floating OM, and "heavy", the OM recovered with sand, are apparently distinguishable physically but not chemically.

Some results are not shown in Table IV. For example, the initial HF dialysate contained no ^{14}C. Its yellow color was unextractable by diethyl ether; neutralization of the water phase gave a nonradioactive gelatinous precipitate (probably aluminum and/or iron hydroxides). Overall recovery of ^{14}C, from summing the fractions in Table IV, was 79.1% of that remaining after metabolite extraction, or 10.1% of the original butralin. The weight of all fractions was 811 g, or ca. 80% of the starting soil weight. Recovery becomes ca. 77% by subtracting the contaminating salts from the dialysates' weights. The difference then reflects dissolution of organic and inorganic soil constituents and mechanical losses.

Thermal Stability. The release of ^{14}C as a function of temperature was considered potentially informative of the nature and location of bound residues. This was measured in the same combustion furnace apparatus used for routine analyses.

The thermal profile of anaerobic bound butralin (Figure 2) shows no ^{14}C lost at temperatures below 200 °C, with extensive loss between 300–375 °C. By 450 °C, recovery of ^{14}C was quantitative in the 20-min collection period.

The isothermal rate of ^{14}C release from aerobic bound butralin from Matapeake soil is shown in Figure 3A. Complete $^{14}CO_2$ evolution occurred within 10–12 min at 1000 °C, verifying 20 min as ample time for quantitative collection of the ^{14}C. Cumulative loss at 300 °C was 23%

Table V. Bound Residue Levels of Various Dinitroanilines

herbicide	location	time, months	bound residue, % of applied ^{14}C	reference
[$^{14}CF_3$]benefin[a]	lab	12	14	Golab et al. (1970)
[$^{14}CF_3$]dinitramine	field	3.5	45	Smith et al. (1973)
	field	8	55	Smith et al. (1973)
fluchloralin	lab	5	10	Otto and Dresher (1973)
	?	18	85	Booth et al. (1976)
[*ring*-^{14}C]isopropalin[b]	field	6	17	Golab and Althaus (1975)
	field	12	15	Golab and Althaus (1975)
	field	36	27	Golab and Althaus (1975)
[*ring*-^{14}C]oryzalin[c]	field	6	30	Golab et al. (1975)
	field	12	30	Golab et al. (1975)
	field	36	35	Golab et al. (1975)
[$^{14}CF_3$]trifluralin[a]	field	12	8	Probst et al. (1967)

[a] Benefin is *N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine. Label position is actually 85% $^{14}CF_3$ and 15% U-ring-^{14}C. [b] Isopropalin is 2,6-dinitro-*N*,*N*-dipropylcumidine. [c] Oryzalin is 3,5-dinitro-N^4,N^4-dipropylsulfanilamide.

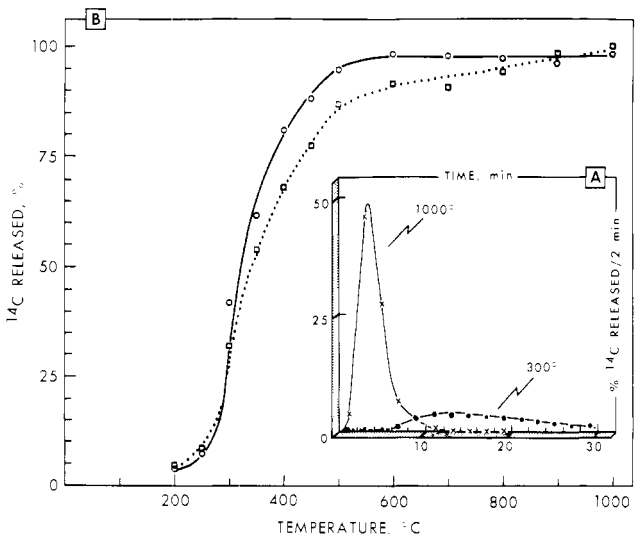

Figure 3. Thermal profile of ^{14}C release from six aerobic "partially bound" dinitroanilines: (A) rate of release of bound butralin vs. time; (B) extent of release vs. temperature [(—) average of five compounds; (····) profluralin].

by 20 min; the rate declined thereafter and totaled 31% at 30 min. Extrapolation of this curve suggests only 3–4% more ^{14}C would be lost with time.

The thermal profile of profluralin (Figure 3B) differed enough from those of the remaining five dinitroanilines (which were essentially superimposable) so as to be plotted separately. Heating began at 200 °C, at which temperature 2–5% of the radioactivity was released. The aerobic Matapeake residues released $^{14}CO_2$ over a broader temperature range, ca. 250–500 °C, than did the butralin residue in Chillum soil (Figure 2). Profluralin had the lowest release, reaching 100% loss only at 600 °C or higher.

DISCUSSION

Bound Residue Content and Extraction. The U.S. Environmental Protection Agency (1975) has described a significant quantity of bound residue as being "... any amount of unextractable residue, greater than 10% expressed as the parent compound, remaining 1 year after a single treatment". Since bound residues, by definition, represent an intractable research problem, the lower limit of 10% is also a useful limit for research activities.

Combustion of 12 replicates established the ^{14}C contents of aerobic and anaerobic Chillum soil (corrected to a Celite-free basis) as equivalent to 3.0 and 12.8%, respectively, of the originally added butralin. They can be reexpressed as concentration of the parent herbicide, 0.30 and 1.28 ppm. The lower activity in aerobically incubated soil precluded much further attention in these bound residue studies. However, the results suggest that butralin bound residue accumulation may be greater in anaerobic, than aerobic, soil. Dinitroanilines seem to degrade faster in anaerobic soil (Helling, 1976b), and there is evidence that benefin may accumulate relatively more bound residue in flooded soil (60% by 16 days) than in normal soil (14% after 1 year) (Golab et al., 1970). This evidence indirectly suggests that the dinitroaniline bound residues are a consequence of degradation and not strong adsorption of the parent per se. As with the nitroanilines, parathion's (*O*,*O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) nitro group is anaerobically reduced. Thus, further support for the role of degradation comes from the observation that 35% of [*ring*-^{14}C]parathion was bound after 2 weeks in aerobic soil, but 65% in flooded soil (Lichtenstein et al., 1977).

The results of Table I show the importance of extraction before arriving at a "bound residue" content. The Chillum treatment had been Bz, MeOH, and Bz–MeOH in sequence. Our additional extraction only slightly reduced the residue. In contrast, brief extraction of Matapeake soils by Bz–EtOAc, then MeOH was useful for identifying metabolites but inadequate for removing all nonbound residues. Two compounds (dinitramine, 8% and trifluralin, 7%) fell below the 10% EPA recommendation, but we still studied their plant uptake characteristics (Helling and Krivonak, 1978).

For comparison, Table V summarizes other reports showing bound dinitroaniline content in aerobic soils. Each herbicide is represented by a single investigation, except fluchloralin (note the wide variation in bound residue, 10 vs. 85%). Our results for trifluralin (Table I) were similar to those in Table V; however, dinitramine and fluchloralin differed substantially.

The anaerobic bound butralin sample was selected for exploratory studies with novel solvent combinations because this soil had already been thoroughly extracted by conventional methods. Extraction was conducted in conjunction with ultrasonic dispersion. This method has been useful for soil mechanical analysis (Genrich and Bremner, 1974; Watson, 1971) and is sometimes used with organic solvents for extracting soil OM (Ford et al., 1969; Halstead et al., 1966) or pesticides (Johnsen and Starr, 1972).

By trituration of soil aggregates, ultrasonication should speed the extraction process. However, pulverization

increased only slightly the extractable ^{14}C from shake- and Soxhlet-extracted samples (as previously reported), suggesting that our extraction steps were conducted for adequate periods. The ultrasonic process not only has a strong physical effect, due to cavitation, but also may lead to a variety of chemical reactions (El'piner, 1964). Ideally, one such reaction may be cleavage of the bonds linking bound residues to soil or perhaps depolymerizing these residues if polymerization has occurred. The choice of solvent may influence reactivity.

Except for EtOAc, the solvents chosen were relatively polar, especially N-containing solvents (Table II). However, lutidine, pyridine, and DMF were ineffective. Aniline may have removed much organic matter and ^{14}C, but its solution could not be counted due to quenching. We surmise that the most efficient solvents for bound butralin, i.e., NH_4OH + MeOH (1:1) or NH_4OH + γ-Bu (1:1), extracted ^{14}C-contaminated soil OM just as any alkaline reagent would do. The only effective nonaqueous system, 2-aminoethanol, is also a strong base. When tested, higher temperature led to more extractable ^{14}C. The best system removed only 17% of available ^{14}C (or ca. 2% of added butralin), so qualitative investigations were not conducted on the ultrasonic extracts.

However, TLC was carried out on the Bz–MeOH extracts of anaerobic bound butralin and six compounds from aerobic Matapeake soil. It confirmed that the traces of ^{14}C removable from Chillum soil were not butralin but rather some highly polar product(s) or ^{14}C bound to contaminating colloidal material. Evaluation of all the dinitroanilines was hindered by the low amount of extractable radioactivity. However, it was clear that Bz–MeOH did remove several ^{14}C-labeled components from each "partially bound" (Table I) herbicide and that only an origin streak remained by the third extraction. Extractable soil OM, itself, was separated, in these TLC systems, into three mobile bands plus the origin zone. It was not clear whether these organic fractions were carrying bound dinitroaniline residues.

Bound Residue Fractionation. The distribution of bound ^{14}C among soil components may offer some clue as to the chemical nature of the bound residue and, perhaps, its bioavailability. The U.S. Environmental Protection Agency (1975) suggested that pesticide registrants provide such data, the method of fractionation being the classical alkaline extraction–acid precipitation, such as described by Stevenson (1965).

Table III provides bound residue distribution for the dinitroanilines, according to the "classical" humus fractionation, after ca. 6 months incubation. In aerobic soil, distribution of residues from the six compounds was roughly the same, i.e., ca. 50% in fulvic acid, 15–20% in humic acid, and 25–35% in humin. Anaerobic incubation decreased the proportion of bound butralin in fulvic acid, although additional studies are needed to verify this phenomenon. It may indicate that more rapid degradation to polar amino and/or phenolic compounds has led to extensive polymerization and incorporation of ^{14}C-containing moieties into the higher molecular weight humic acid and humin.

Interpretation of bound residue distribution into fulvic acid, humic acid, and humin must be made cautiously, since the fractionation itself can be executed by many variations in reagents and technique. Alkaline extraction, although widely used, presents a medium favorable to hydrolysis, oxidation, and condensation reactions. Furthermore, it dissolves silica, which increases the proportion of inorganic material in the organic preparations. Yet another disadvantage may be that it artificially creates fulvic acid: 95–97% of extractable OM was "humic acid", when 0.1–0.2 M $Na_4P_2O_7$ was used; with the commonly used 0.5 N NaOH, only 37% was "humic acid" (Gascho and Stevenson, 1968). Since the quantity and characteristics of the extracts vary with procedure (Parsons and Tinsley, 1975), it is likely that the bound residue would vary as well.

This *is* what we found when we compared anaerobically bound butralin distribution in the "classical" fractions (Table III) with the analogous fractions isolated by the procedure of Gascho and Stevenson (1968) (Table IV). After extraction with 0.5 N NaOH, 14 ± 4% of bound butralin was in fulvic acid; after Gascho/Stevenson extraction, only 1.7% of recovered ^{14}C was in this fraction. Perhaps, much of the pesticide residue is associated with intermediate size colloids that are themselves associated with larger colloids by bonds hydrolyzable by strong alkali. If individual fractions of Table IV were consolidated into groups comparable to the classic fractionation, the distribution of anaerobic bound butralin would be fulvic acid, 4.2% (added dialysates); humic acid, 26.3%; and humin, 69.4% (added undecomposed OM + mineral fractions). This shift of bound ^{14}C from higher molecular weight fractions to lower fractions was also reported by Meikle et al. (1976), who compared extraction by a chelating resin with that from hot 1 N NaOH.

From the Gascho/Stevenson fractionation, we concluded that anaerobic bound butralin becomes very broadly distributed among soil fractions. Residue was concentrated in humic acid and humin (15.8 and 8.7 ppm, respectively), which suggests that "mineral" fractions such as silt and clay contained much associated unextracted OM. We found an unexpected amount (8.3%) of radioactivity in undecomposed OM (sand also had 1.8% of the ^{14}C, which was almost certainly the same material). Very little ^{14}C residue was dialyzable, which means that its average molecular weight (or that of its associated complex) exceeded 12 000–14 000.

The Gascho/Stevenson procedure gave a more descriptive profile of bound residue distribution, but was experimentally much more cumbersome, than the classical fractionation. Some of the Gascho/Stevenson fractions were subsequently used (Helling and Krivonak, 1978) to measure ^{14}C turnover and plant uptake under biological conditions.

Thermal Stability. Thermoanalytical investigations have been used to characterize soil OM and related macromolecular substances (Flaig et al., 1975; Schnitzer and Khan, 1972). For example, carboxyl and phenolic groups were eliminated at 250–400 °C, when fulvic and humic acids were heated in air, with carboxyl being more labile. Differential thermal gravimetry showed weight loss at ~200–350 °C, ascribed to loss of these groups, and much greater loss peaking at ~450 °C (fulvic acid) or ~550 °C (humic acid), thought to be decomposition of the "nuclei". Isothermal heating showed most fulvic acid carboxyl and phenolic hydroxyl groups were eliminated by 350 °C.

Figure 2 confirmed that if any parent butralin remains in the soil, it must be chemically bound, since it would have survived both exhaustive solvent extraction and heating to nearly 300 °C. Because most ^{14}C was trapped in the 300–350 °C region, the bound butralin stability may be linked with phenolic hydroxyl or carboxyl groups. Chemically, stabilizing reactions between phenolic hydroxyls and free aromatic amino groups from the metabolized herbicide are easiest to postulate. It seems unlikely, from the thermal profile data, that bound ^{14}C has

become part of a highly condensed "nucleus" of soil OM since this oxidizes mainly in a higher region, 450–550 °C.

Other possibilities for the bound dinitroanilines might have included becoming part of interlayered OM within expanding clay mineral lattices. Schnitzer and Khan (1972), summarizing research on such complexes, indicated that the OM becomes somewhat more thermally stable. Differential thermal analysis of a fulvic acid–montmorillonite complex showed a 670 °C exotherm characteristic of the interlayer complex. One of the dinitroanilines (Figures 2 and 3) showed evidence of high-temperature ^{14}C loss expected for interlayered material. Apparently no ^{14}C was transformed to inorganic carbonate since ^{14}C recovery (relative to that at 1000 °C) was quantitative at temperatures less than the 580–970 °C range at which CO_2 is lost from carbonates (Jackson, 1956).

The profile of anaerobically bound butralin (Figure 2) suggests that it is thermally more "homogeneous" than the aerobically incubated, partially bound dinitroanilines (Figure 3), which have a wider temperature range for ^{14}C loss. A much higher proportion of bound radioactivity appears in fulvic acid (Table III), which may account for some increased stability near 400 °C. Since the samples were later shown to be only "partially bound" (Table I), the slightly greater loss between 200 and 250 °C could be indicative of more loosely held metabolites. There is no obvious explanation for profluralin's behavior (Figure 3).

Where material is sufficient and levels of radioactivity are adequate, thermoanalytical methods may contribute useful information about pesticide bound residues. In particular, analysis of the effluent gases, combustion of both intact soil and soil fractions, and use of diverse chemicals with several patterns of ^{14}C labeling would be interesting.

SUMMARY

Prolonged benzene–methanol Soxhlet extraction led to soil bound residue values of 7–21% for six dinitroaniline herbicides. Residues probably depend on the soil used and were higher, in the one case tested (butralin), in anaerobic soil. Anaerobic bound residues are associated with more "humified" organic matter fractions than are residues formed during aerobic incubation. However, a milder fractionation procedure suggests that distribution of bound ^{14}C is very broad in soil components and that the classical fulvic acid/humic acid fractionation distorts this picture. Thermal breakdown of bound dinitroaniline residues tended to corroborate molecular association with carboxyl and/or phenolic hydroxyl groups, but negated incorporation of bound residues into the organic or clay matrices. Efforts to dislodge the residues were unsuccessful.

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