

one of choice for estimation of recalcitrant 4-CA and possibly other xenobiotics in soil humic acids.

**Methodology Conclusions.** Figure 2 summarized results pertaining to extractability of tightly complexed HA-4-CA residues. Hydrolysis with base or acid was shown to be the best procedure to release large amounts of 4-CA from soil humic acids (90%) with approximately 40-50% analyzable as intact 4-CA.

The quantitative method reported in this study is very sensitive. A 75-fg ( $75 \times 10^{-15}$  g) injection of 4-CA, as its heptafluorobutyryl derivative, into an EC-GLC produced a standing current decrease equivalent to a 10% full-scale recorder deflection at an attenuation of 1 and signal:noise ratio of 3:1. Such high sensitivity should permit preconcentration of HA extracts to detect much lower levels of 4-CA.

Hydrolysis and quantitation techniques were reproducible and free from coextraction interferences; high sensitivity may permit one to dilute out interferences from other substrates, or if more elaborate cleanups are required or extensive subsampling employed, such sensitivity will facilitate, if not make possible, quantitation of very low concentrations. Material balances using hydrolysis were excellent; 94-100% radioactivity was accounted for. Figure 2 shows operational definitions of 4-CA interaction complexes with soil humic acid. The same scheme may be

applicable to whole soil as well as other xenobiotics.

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## Pyrolytic Release of Tightly Complexed 4-Chloroaniline from Soils and Soil Humic Acids

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The release of tightly complexed 4-chloroaniline (4-CA) from treated soil humic acids and whole soils by using pyrolysis in an atmosphere of helium was studied. Pyrolysis of soil humic acids containing tightly complexed 4-chloroaniline resulted in the release of up to 54% of the radioactivity with approximately 5% detected via radioassay or gas-liquid chromatography as the intact 4-CA. Three soils of varying organic matter and clay concentrations complexed 100% of applied 4-chloroaniline; extractable radioactivity increased and tightly complexed 4-chloroaniline decreased as organic matter concentration decreased and clay concentration increased. The quantity of radioactivity released by pyrolysis was greatest for soils with low organic matter and high clay contents; 22-73% of the radioactivity could be released with 3-16% extractable into benzene from a basified pyrolysate, the amount depending on the nature of the soil, the type of pyrolysis probe, and the concentration of the applied 4-chloroaniline.

Pesticides have the capability to complex abundantly and tenaciously to soils and especially to soil humic acids. Analytical techniques for the determination of tightly complexed ("bound") pesticides and/or their degradation products are not yet readily available. Worobey and Webster (1982) recently reported a sensitive quantitative method for the determination of 4-chloroaniline tightly complexed to a soil humic acid. Several methods were investigated, and alkaline hydrolysis under reflux yielded the greatest amounts of intact 4-chloroaniline quantified by electron-capture gas-liquid chromatography (EC-GLC)

as its heptafluorobutyryl derivative.

During these studies, anaerobic pyrolysis was intensively investigated as an analytical technique to release tightly complexed 4-chloroaniline from soil and soil humic acids, since it has proven useful in the release of tightly complexed 3-chloroaniline and 3,4-dichloroaniline from plant lignins (Balba et al., 1979). While our studies were in progress, Khan and Hamilton (1980) reported the successful release of tightly complexed prometryn from an organic soil using a similar pyrolytic technique to that of Balba et al. (1979). Our work examines the effect of soil type, that is, of varying organic matter and clay content, on the amount of tightly complexed 4-chloroaniline formed and its subsequent release using various pyrolysis conditions and pyroprobes.

#### MATERIALS AND METHODS

**Reagents.** All solvents were pesticide grade (Caledon Co.); [<sup>14</sup>C]-4-chloroaniline (4-CA) was obtained from the

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Table I. Physicochemical Properties of the Soils Used in This Study

| soil                          | % sand | % silt | % clay | % or-<br>ganic<br>car-<br>bon | % or-<br>ganic<br>matter | pH  | H <sup>+</sup> | mequiv/100 g exchangeable |                  |                 |                | CEC  | % ash | OM <sup>a</sup> |
|-------------------------------|--------|--------|--------|-------------------------------|--------------------------|-----|----------------|---------------------------|------------------|-----------------|----------------|------|-------|-----------------|
|                               |        |        |        |                               |                          |     |                | Ca <sup>2+</sup>          | Mg <sup>2+</sup> | Na <sup>+</sup> | K <sup>+</sup> |      |       |                 |
| organic (Piney)               | 0.0    | 0.0    | 0.0    | 43.2                          | 74.5                     | 5.8 | 23.6           | 135.2                     | 28.4             | 0.5             | 0.9            | 200  | 18.2  | 0.43            |
| clay-loam<br>(Brandon Carrol) | 38     | 30     | 32     | 5.4                           | 9.3                      | 5.7 | 2.8            | 27.2                      | 6.5              | 0.3             | 1.5            | 36.5 | 82.2  | 0.47            |
| silt-loam<br>(Soignies)       | 15     | 72     | 13     | 8.7                           | 15.0                     | 3.2 |                | 1.4                       | 0.2              | 0.1             | 0.4            | 28.3 | 85.5  | 0.17            |

<sup>a</sup> Using 0.025 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and absorption at 550 nm.

Aldrich Chemical Co. and recrystallized 3 times from hexane (mp 71–72 °C). The purity of the [<sup>14</sup>C]-4-chloroaniline (California Bionuclear Corp.) was confirmed by TLC and autoradiography. "Omnifluor" and "Aquasol-2" (New England Corp.) were used as scintillators for liquid scintillation counting. Soils used included Piney organic peat, Brandon Carrol clay-loam, and Soignies silt-loam; the first two are Manitoba soils and the last is a Belgian soil. All soils were air-dried and ground to pass through a 3-mm sieve. The physicochemical properties of these soils (Table I) were determined as follows: textural classification by the "standard pipet" method (Toogood and Peters, 1953), pH at a 1:1 (w/v) ratio in 0.1 M CaCl<sub>2</sub> (Peech, 1965); percent organic carbon by oxidation and titration (Walkley and Black, 1934); cation analysis of (a) Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup> by extraction into ammonium acetate and quantitation using atomic absorption spectrophotometry, and (b) H<sup>+</sup> by extraction with BaCl<sub>2</sub>-triethanolamine (Peech et al., 1962); CEC by the method of Chapman (1965); percent ash by gravimetric analysis (400 °C overnight in a muffle furnace); degree of humification of soil using 0.025 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and absorption at 550 nm (Schnitzer and Desjardins, 1965).

**Radioassay.** A Searle Model 6880 Mark III liquid scintillation spectrometer was used to assay radioactivity with a low level activity program (E<sup>2</sup>/B) for dpm computation. Radioactivity in solid residues was determined as <sup>14</sup>CO<sub>2</sub> by combustion in a Packard-Tricarb Model 306 biological oxidizer, followed by liquid scintillation counting of the trapped combustate (Carbosorb, 5 mL; Permafluor, 10 mL). Combustion of a radioactive standard assayed at greater than 98%.

**Pyrolyzer.** The pyrolyzer used was a Chemical Data System Corp. 18580A pyroprobe solids analyzer with various pyroprobes. Conditions were as follows: probe temperatures (°C), macro quartz 900, macro and micro platinum and macro stainless steel 450; pyrolysis times, macro quartz and micro platinum 20 s, macro platinum and macro stainless steel 15 min; flow rate (He), 20 mL/min; sample size, 40–80 mg. Micro platinum and macro quartz probes were used for sample sizes of 1–10 mg/experiment.

**Spiked Soil Humic Acids.** Humic acids were isolated from Carroll clay-loam soil (Manitoba) and treated at the 477-ppm level (specific activity = 2432 dpm/μg of 4-CA) as described in the preceding paper (Worobey and Webster, 1982). Both nonhydrolyzed and hydrolyzed (acid and base) tightly complexed 4-chloroanilines were pyrolyzed, and the released 4-chloroaniline was assayed as its heptafluorobutyl derivative by using EC-GLC (Worobey and Webster, 1982).

**Pyrolysis, Cleanup, and Analysis of Pyrolysates.** Pyrolysis was investigated in detail, as it was anticipated that it would release intact tightly complexed residues to a greater extent than previously available methods. Studies with filament-type probes were begun by varying

the temperature of the probe filament in increments of 100 °C, from 100 to 900 °C.

**Type of Probe.** (1) *Micro Platinum.* Dried unground or ground (agate mortar and pestle) samples of humic acid (ca. 50 mg) were weighed into a 1-mL volumetric flask, diluted to 1 mL with *N,N*-dimethylformamide (DMF), shaken thoroughly, and allowed to stand at least 3 days prior to usage. Immediately prior to use, the solution was again shaken thoroughly to resuspend any undissolved humic acid. By use of a 50-μL syringe, 2 × 10 μL (approximately 1 mg) was withdrawn and applied carefully to a Pt ribbon probe and placed in an inert atmosphere (N<sub>2</sub>), and the DMF was flashed off at 75 °C with a 2-s interval.

(2) *Macro Quartz.* Dried unground or ground samples (approximately 10 mg) were weighed into a quartz tube containing a small pledget of quartz wool at one end. Quartz wool was placed into the open end after weighing, and the quartz tube was inserted carefully into the platinum coil of the probe.

(3) *Macro Platinum.* A stainless steel probe was designed with a boat opened at one end. This was lined with platinum foil, and the dried and unground or ground samples were weighed into it (ca. 80 mg).

(4) *Macro Stainless Steel.* This was as above without the platinum foil covering.

**Treatment of Three Soil Types with 4-CA.** One-hundred grams of each soil (Table I) was adjusted to 80% of its water holding capacity with distilled water, shaken thoroughly, and allowed to equilibrate for 1 h. Soils were then treated with 4-CA at the 5- and 500-ppm levels (specific activities = 232 000 and 2320 dpm/μg of 4-CA, respectively), added dropwise in a total volume of 5 mL of 95% ethanol. The soils were shaken thoroughly and left standing in stoppered Erlenmeyer flasks for 4 h, after which they were opened and air-dried in a fume hood for 40 h.

**Extraction of Loosely Complexed Residues.** Soils were extracted with 5 × 50 mL of acetone and filtered (through No. 41 Whatman paper) under vacuum. The soil was redried in the fume hood, and 2-g subsamples were reextracted under reflux (15 min) by using 50 mL of methanol-water (1:1) and extracted again by shaking (1 min) with 2 × 15 mL of methanol-water (1:1). The dried soil was used for pyrolysis and combustion analysis; all extracts were counted, and controls for each soil were included for the entire procedure. All analyses were performed in duplicate.

**Pyrolysis and Release of Tightly Complexed 4-CA Radioactivity.** Figure 1 shows the apparatus used for pyrolysis. The pyroprobe was inserted into the inlet of the quartz-lined pyrolyzer interface (helium flow rate preset at 200 mL/min), and the collection tube was inserted into the outlet with the "U" tube immersed in the acetone-dry ice cooling mixture. The end of the collection tube was immersed in 5 mL of 25% methanolic HCl contained in

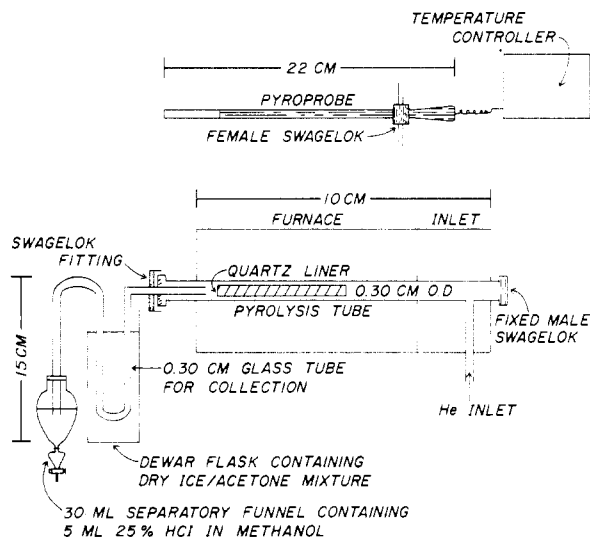


Figure 1. Apparatus used for pyrolysis of tightly complexed 4-CA.

Table II. Extractability of [ $^{14}\text{C}$ ]-4-CA from Several Soils

| soil      | ppm | % of total radioactivity applied |                                       |                 |                  |
|-----------|-----|----------------------------------|---------------------------------------|-----------------|------------------|
|           |     | acetone extract                  | water-methanol                        | total extracted | bound in residue |
| organic   | 5   | 6.3                              | 16.2 <sup>a</sup><br>0.6 <sup>b</sup> | 23.1            | 76.9             |
|           | 500 | 12.6                             | 14.1 <sup>a</sup><br>0.8 <sup>b</sup> | 27.5            | 72.4             |
| silt-loam | 5   | 6.2                              | 15.2 <sup>a</sup><br>1.0 <sup>b</sup> | 22.4            | 77.6             |
|           | 500 | 24.9                             | 23.1 <sup>a</sup><br>1.4 <sup>b</sup> | 50.4            | 52.1             |
| clay-loam | 5   | 20.6                             | 31.7 <sup>a</sup><br>1.7 <sup>b</sup> | 54.1            | 45.9             |
|           | 500 | 65.2                             | 19.9 <sup>a</sup><br>0.5 <sup>b</sup> | 85.6            | 14.4             |

<sup>a</sup> Reflux. <sup>b</sup> Shaking.

a 25-mL glass-stoppered separatory funnel. Soil humic acid pyrolysis conditions are given in Table II. The collection tube was removed from the pyrolyzer interface and washed with 10 mL of 25% methanolic HCl into the separatory funnel. The pyrolysate was concentrated to ca. 5 mL under  $\text{N}_2$  on a 60 °C water bath, 10 mL of 5 N NaOH was added, and the pyrolysate was extracted with  $4 \times 10$  mL of benzene. The benzene extract and the remaining basic solution (adjusted to pH 6.0 with concentrated HCl) were counted in a liquid scintillation spectrometer. Alternately the benzene extract was derivatized with heptafluorobutyric anhydride by the procedure of Worobey and Webster (1982) for quantitation from a hexane solution using EC-GLC.

## RESULTS AND DISCUSSION

**Pyrolysis Methodology.** *Pyrolysis of the Base-Hydrolyzed Humic Acid-4-CA Complex.* For the macro quartz (approximately 5–10-mg sample size) and micro platinum (approximately 0.5–1-mg sample size) probes, the amount of radioactivity released was similar, viz., 21.2 and 20.0%, respectively, to that from base-hydrolyzed humic acid at 550 °C (optimum temperature for radioactivity release). Five and eight percent, respectively, of the radioactivity in the pyrolysate was present in the hexane solution used for quantitation by EC-GLC. Quantitation of the 4-CA as its heptafluorobutyl (HFB) derivative did not yield reproducible results, and recoveries varied according to the particle size of the humic acid. Humic acid ground in a agate mortar and pestle resulted in 18% higher

recoveries, indicating 4-CA may have been trapped in the matrix and that exposing a larger surface area facilitates release of radiocarbon. EC-GLC chromatograms were characterized by a plethora of peaks, making quantitation difficult. No 4-CA-HFB was detected.

There was a greater variability in the radioactivity remaining in residues when the macro quartz probe was used as observed by Balba (1977) with lignin-bound chloroaniline. The macro stainless steel probe gave reproducible release of radioactivity (39.6 and 39.1%) but very low radioactivity in the EC-GLC solution (approximately 2% in hexane), and no 4-CA-HFB was detected. Pyrolysis of the original 4-CA alone using the macroprobe led to 20% of the radioactivity being trapped in the Carbosorb solution, presumably indicating thermolysis of 4-CA to volatile products which passed through the dry ice-acetone trap. When the hexane solutions were cleaned up by extraction with distilled water which had previously been washed with hexane, virtually all the radioactivity stayed in the water, indicating the presence of polar thermolysis products.

*Pyrolysis of the Nonhydrolyzed HA-4-CA Complex.* For the nonhydrolyzed HA-4-CA complex pyrolyzed by using the macro quartz probe, only  $27.1 \pm 0.5\%$  of the radioactivity was collected in the cold trap, although most of this radioactivity had been released at only 200 °C compared with 550 °C for the hydrolyzed HA-4-CA complex. Material balances were excellent for nonhydrolyzed samples: 26% trapped + 66% remaining in the residue + 8% trapped as  $^{14}\text{CO}_2 = 100\%$ . More radioactivity was released when the micro platinum probe was used than when the macro quartz probe was used, viz., 41.4% (900 °C) or 53% (550 °C) vs. 26.8% (900 °C), respectively. The same amount of radioactivity was trapped in Carbosorb (approximately 8%) whether macro quartz or micro platinum probes were used.

Use of ultrashort pyrolysis intervals, 20 ms compared with 10 s to prevent secondary reactions, resulted in zero radioactivity being released when using the micro platinum probe and  $47 \pm 7\%$  radioactivity being released and collected in the trap for the macro quartz probe; however, of this released radioactivity, only 5.5% was in the hexane solution; 4-CA-HFB was not detected. Incorporation of a 60% benzene-hexane wash cleanup prior to derivatization [as used by Balba et al. (1979) for their lignin-chloroaniline pyrolysis studies] was investigated to test for increased recoveries of 4-CA but resulted in 0% radioactivity in the solution used for quantitation of 4-CA-HFB by EC-GLC.

Pyrolysis using the macro stainless steel probe (40–50 mg), for 15 min at 400 °C, released only 1% radioactivity to the trap; 60% radioactivity remained in the residue and no 4-CA-HFB was detected; the macro or micro platinum probe released  $4.5 \pm 0.1\%$  radioactivity with 74% remaining in the residue.

*Other Pyrolysis Results.* Pyrolysis of heptafluorobutylated nonhydrolyzed humic acid-4-CA complexes [prepared by the method of Worobey and Webster (1982)] using the macro platinum probe gave similar results to those of non-HFB-HA; i.e., 4.4% radioactivity was trapped and approximately 79% radioactivity was left in the residue.

Pyrolysis of whole soil (477 ppm of 4-CA treated, aged 9 months) using the macro platinum probe (400 °C for 30 min) resulted in only 6.5% of the original radioactivity being extractable into benzene from the basified trapped pyrolysate. About 21% radioactivity in total was released.

Pyrolyzed control humic acid gave variable results and often showed the presence of an interfering EC-GLC peak

at the same retention time as 4-CA-HFB; however, varying GLC conditions could prevent coelution of interferences. Pyrolysis of 4-CA (700 °C; 5 s; macro quartz probe; 20 µg) resulted in approximately 20% recoveries, probably due to thermolysis of 4-CA to unidentified degradation products. Controls were run for 4-CA through the entire procedure, from the extraction from 25% HCl in methanol trapping solution to derivatization; recoveries averaged 98%.

**Methodology Conclusions.** The low recoveries, reported herein, of 4-CA tightly complexed with humic acid may be due both to the thermolysis of 4-CA and the effects of pyrolysis on the HA. Chemiko (1978) and Kimber and Searle (1970a) isolated humic acids from soils using various extraction techniques. They reported that the activation energy and kinetic parameters of thermal destruction reactions (pyrolysis) of humic acids depended on the method of extraction.

It has been shown by other workers that pyrolysis of humic acid under nitrogen at 700 °C results in aromatic groups remaining virtually intact while considerable breakdown of nonaromatic groups and substituents occurs (Kimber and Searle, 1970a; Wolf and Rosie, 1967; Farre-Puis and Guiochon, 1968; Doz et al., 1965). In addition, coextracted inorganic soil components might be expected to catalyze the following reactions: C-C fission, oxidation (auto), and ether formation (Kimber and Searle, 1970b; Martin, 1975).

As humic acid particle size decreased, the position of a differential thermal gravimetric 420 °C peak shifted to a lower temperature (Levesque and Dinel, 1978). In the present study there was 18% greater accountability by decreasing particle size variations in pyrolysis conditions. The effects of particle size on pyrolysate characteristics of biopolymers have also been discussed (Meuzelaar, 1978). In contrast to our work with 4-chloroaniline, Khan and Hamilton (1980) recently reported the pyrolytic release of "bound" prometryn residues from a treated organic soil using a method similar to that of Balba et al. (1979). Khan and Hamilton (1980) use the term "high-temperature distillation" in lieu of pyrolysis, indicating that the prometryn was not covalently bonded. Recoveries of bound residues from treated (12.4 ppm) soil were 88.8–62.1% (0–150 days) of the related radioactivity determined mainly as the parent compound. Recoveries of prometryn or hydroxypropazine (a metabolite) by pyrolysis were 70–82%.

Recovery of hydroxypropazine from treated soil by pyrolysis was considerably lower than that for prometryn; 80 vs. 53%. Thus, the more polar metabolite was much more recalcitrant to pyrolytic release (less volatile and, therefore, removed less rapidly from the pyrolysis tube, thus allowing greater thermal decomposition; this may be analogous to a reactive compound such as 4-CA). The authors cautioned that the data reported for residues of hydroxypropazine should only be regarded as qualitative. Moreale and Van Bladel (1979a,b) showed that adsorption of 4-CA increased with increasing temperature while Weber et al. (1965) found that prometryn adsorption decreased at higher temperatures.

Soils high in clay content (e.g., the mineral soil used in this study) may give rise to predominantly clay and clay-humic organic matter complexation whose residues are recalcitrant to pyrolytic release; complexes remain intact, even at high pyrolysis temperatures (900 °C).

Pyrolysis temperatures of up to 900 °C in this study failed to release more radioactivity than at 200 °C from nonhydrolyzed HA-4-CA complexes. No additional ra-

Table III. Pyrolytic Results for Bound [<sup>14</sup>C]-4-CA Complexes in Various Soils<sup>a</sup>

| soil      | ppm | % <sup>14</sup> C released <sup>b</sup> |    | % [ <sup>14</sup> C]C <sub>6</sub> H <sub>6</sub> extractable |    |
|-----------|-----|---|----|---|----|
|           |     | quartz                                  | Pt | quartz  | Pt |
| organic   | 5   | 36                                      | 43 | 13  | 9  |
|           | 500 | 43                                      | 51 | 15  | 8  |
| silt-loam | 5   | 53                                      | 35 | 15  | 10 |
|           | 500 | 50                                      | 34 | 13  | 9  |
| clay-loam | 5   | 28                                      | 22 | 16  | 7  |
|           | 500 | 73                                      | 67 | 4   | 3  |

<sup>a</sup> Actual dpm used; determined from combustion of a known soil air-dried weight. <sup>b</sup> HCl-CH<sub>3</sub>OH.

dioactivity was released when the sample fired at 200 °C was refired at 900 °C. Unstable products may be forming in the pyrolysis chamber which were swept out and/or removed but contained no intact 4-CA as evidenced also by the low recovery (20%) of 4-CA standard alone from the pyrolyzer. Haider (1979) attempted to release 4-CA from soil by pyrolysis in a mass spectrometer and detected mainly chlorobenzenes.

In conclusion, the pyrolysis techniques investigated herein were not as efficient compared with hydrolysis for release of nonextractable HA-4-CA complexed residues. The low recoveries observed could be explained by thermolysis of 4-CA in a noncovalently bound state (Balba, 1977). Attempts to recover 4-CA alone from the pyrolyzer led to only a 20% recovery of 4-CA. The pyrolytic rearrangements of arylamines have been reported by Bode et al. (1973).

The abundance of clays, oxides, and metal cations present in HA-4-CA may facilitate the formation of pyrolytic degradation products, whereas, in the case of lignin-bound residues, few of these components would be present.

The pyrolysis results and discussion from the current study may be useful for other pesticides or xenobiotics since knowledge regarding the influence of varying pyrolysis parameters may thus facilitate attempts to release other compounds which are tightly complexed.

**4-Chloroaniline Complexation with Soils and Its Pyrolytic Release.** Figures 2 and 3 and Tables II and III show the evidence upon which the following conclusions may be formulated in regard to the tight complexation and release of 4-chloroaniline from soil as influenced by (1) the concentration of applied 4-CA, (2) varying soil properties such as organic matter and clay, and (3) two different pyrolysis probes.

For all soils studied, binding (tight complexation; percent of applied dose) was always greatest at 5-ppm compared with 500-ppm treatment levels. This suggests that a fixed number of binding sites are available per unit of adsorbent for complexation of 4-CA and that these sites may be saturated at some concentration less than 500 ppm or that the system was disequilibrated at the higher level. Usually a greater percentage of bound radioactivity was released by pyrolysis at the 500-ppm level. This may be due to the occurrence of less strongly complexed 4-CA in the form of multilayered or inclusion and adsorbed molecules at the 500-ppm level. The percentage radioactivity extractable into benzene from a basified pyrolysate, however, was similar for all soils at both concentrations with slightly more radioactivity extractable from 5-ppm pyrolysates. Differences were too small to draw definite conclusions. Figures 2 and 3 show that the percentage of binding increased with increasing organic matter and decreasing clay, especially at the 500-ppm treatment level. At 5 ppm of 4-CA, 78% radioactivity was bound by the

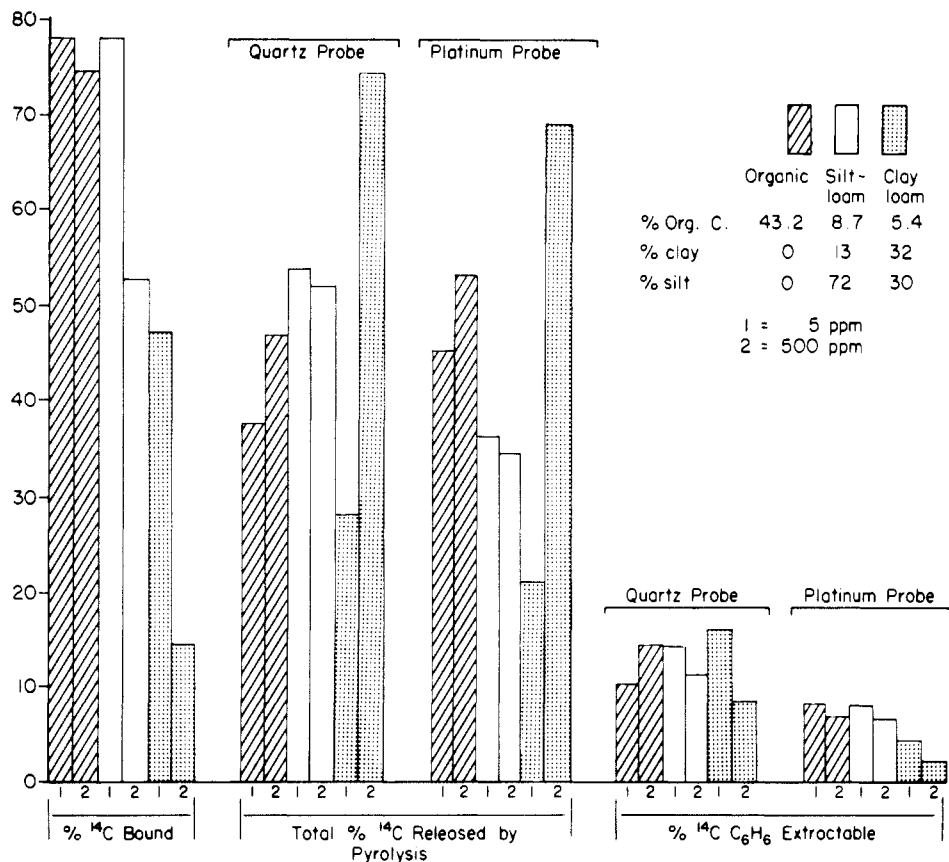


Figure 2. Binding, pyrolytic release, and extractable 4-CA from three different soils.

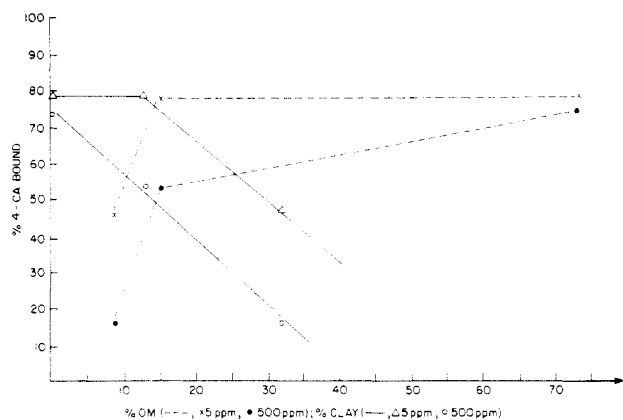


Figure 3. Effect of varying OM and clay concentrations on percent 4-CA bound.

organic and silt-loam soils. This may be because enough organic matter is available for interaction, whereas for the clay-loam soil much of the organic matter interacts with the high clay, thus reducing the amount of organic matter available for interaction with 4-CA directly. This may also explain why the organic soil bound about the same fraction of radioactivity of 5 and 500 ppm of 4-CA (i.e., 78 and 74%, respectively), while the other two soils exhibited less binding on a percentage basis at 500 ppm than at 5 ppm. That is, more "free" organic matter is available to interact with 4-CA in the organic soil. This illustrates the importance of soil organic matter in the binding of 4-CA to soil. Clay seems to have little direct effect on binding at the range of organic matter it is combined with in this study. This is demonstrated by the fact that the percent organic matter vs. percent bound and the percent clay vs. percent bound plots (Figure 3) exhibit the same kind of curve, so that consideration of clay does not alter the

of the curve or in fact the position of the curves on the X axis.

Pyrolysis released between 22 and 73% of the tightly complexed radioactivity depending on the type of probe and soil involved (Table III). For the organic soil, more bound radioactivity was released at either concentration when the platinum probe was used; the silt-loam and clay-loam released greater radioactivity when the quartz probe was used. This may occur because the less humidified OM of the organic soil was more reactive on the quartz surface, thus reacting with the 4-CA to yield thermally stable products which are nonvolatile or trapped within the OM matrix. Balba (1977) obtained lower recoveries of 3-CA and 3,4-DCA complexed to plant lignin cellulose when using a quartz probe and increased recoveries with the platinum probe. The genetic relationship of plant lignin cellulose to soil organic matter has been well documented.

In conclusion, the results of this study show the dominant role of soil organic matter in tightly complexing 4-CA and the fact that between 9 and 15% organic matter, organic matter has a greater effect than clay on the extent of tight complexation. For example, a 15% organic matter soil tightly complexed as high a percentage of 4-CA as a 73% organic matter soil, even though part of the binding capacity of the former was lowered by interaction with 13% clay. Even at 9% organic matter and 32% clay, a high percentage of binding occurs, viz., 46% at 5 ppm, so that the organic matter has been masked by the clay but a sufficient amount remains for considerable binding of 4-CA. This masking effect is demonstrated by the fact that, as the organic matter content of the soil decreased with concomitant increase in clay, the percent 4-CA bound decreased at the 500-ppm level. However, at 5 ppm the organic and silt-loam soil binding was identical (78% of applied dose), showing that at the low concentration level

of 4-CA applied, the organic matter is available for binding as if no clay were present; but at 500 ppm it is evident that the number of sites has decreased, because of clay interaction with the organic matter, and insufficient sites are available for binding (78% at 5 ppm compared with 53% at 500 ppm). This effect is magnified with the clay-loam soil (5% organic matter-32% clay) where the total amount bound decreased to 46 and 15% at 5 and 500 ppm due to masking of organic matter activity by a greater clay content. Figure 2 shows that as clay concentration increases and OM concentration decreases, total amount of 4-CA bound decreases, thus supporting the above conclusion.

Since the amount of radioactivity released by pyrolysis and extractable into benzene (4-CA) for all soils was very similar (approximately 10%), this observation might be taken to be indicative of a binding mechanism related to organic matter inclusion phenomena; low recoveries of 4-CA indicate thermolysis of "free" 4-CA in the substrate. For geographically different soils of varying organic matter contents, organic matter-4-CA interactions would be expected to vary such that greater differences in release result. From these results it appears that organic matter concentration is not important for release (except below a limiting concentration).

Low recoveries of benzene-extractable radioactivity from the pyrolysate may be due to inclusion phenomena also, whereby application of heat results in thermolysis of 4-CA itself rather than of a 4-CA organic matter covalent bond. It appears that for a reactive compound such as 4-chloroaniline tightly complexed to soil or soil humic acids, pyrolysis may be useful if the released residue is stabilized prior to trapping; recoveries are low but reasonably reproducible.

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## Mineral Composition of Small-Grain Cultivars from a Uniform Test Plot in South Dakota

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Seventy-five cultivated varieties (cultivars) of hard red spring wheat (HRS), hard red winter wheat (HRW), durum wheat, oats, and barley were harvested in 1974 from a small-grain trial plot in Harding County, SD, just north of Buffalo. Analysis of the grains reported here includes crude protein for only the wheat cultivars, ash yield, and 17 chemical elements, many of which are not commonly given in the literature (such as B, Cd, Mo, Ni, and Se). Differences in composition between the two classes of hard red wheat indicate that HRS is significantly higher ( $p < 0.05$ ) than HRW in protein content, ash yield, Ca, K, Mg, Na, P, total S, Sr, and Zn; Cd is significantly higher in the HRW cultivars. For the most part, concentrations were quite uniform within all grain types. Only two cultivars were anomalous: cv. Hi Plains in HRW wheats and cv. Astro in the oat group.

With respect to the mineral composition of wheat grains, probably the most thorough review of the literature prior

to 1944 is the monographic compilation by Bailey (1944). We are not aware of any recent report that is comparable, although Czerniejewski et al. (1964) present data on the nutritionally important elements in hard wheat from the United States, and Lorenz and Loewe (1977) have reported the mineral compositions of U.S. and Canadian wheats. Studies of the composition of oats and barley are less

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