Determinations of Clopyralid, Picloram, and Silvex at Low Concentration in Soils by Calcium Hydroxide−Water Extraction and Gas Chromatography Measurement

Liang K. Tan,* David Humphries, Paul Y. P. Yeung, and L. Zack Florence

Alberta Environmental Centre, P.O. Bag 4000, Vegreville, Alberta, Canada T9C 1T4

Clopyralid (3,6-dichloropicolinic acid), picloram (4-amino-3,5,6-trichloropicolinic acid), and silvex (2-(2,4,5-trichlorophenoxy)propionic acid) at concentrations of 0.0100 μg/g in 14 fortified Alberta soils were determined by calcium hydroxide−water extraction and gas chromatography measurement. Precision of analyses was 1−13%. The herbicide recoveries from the soils with two different fortification procedures were compared. The relationships between recoveries and soil components were examined and discussed. Results from fortified soils, which were extracted immediately following spiking of herbicides, indicated clopyralid recovery of 95.2 ± 6.7% and was independent of the organic matter (0.4−5.3%), clay (3.6−44.2%), sand (16.5−94.1%), or iron (3908−22 455 μg/g) content in the soil. However, picloram and silvex recoveries (58.0−97.8%) were dependent on soil properties with a significant negative trend for being affected by the organic matter content of the soil. Their recoveries decreased with increasing organic matter content of the soil. Detection limits of 0.0025−0.0500 μg/g were herbicide and soil dependent. Results from fortified soil slurries which were extracted after 2−14 days of drying indicated lower herbicide recoveries. The different recoveries from two fortification procedures were discussed in relation to herbicide solubilities, soil-to-water partition coefficients, sorption/desorption, and possible degradation.

Keywords: *Clopyralid; picloram; silvex; soil components; organic matter content; extraction; calcium hydroxide−water; low concentration; gas chromatography; determination*

INTRODUCTION

Picloram (4-amino-3,5,6-trichloropicolinic acid) is a potent herbicide that effectively controls growth of woody plants (Hamaker et al., 1963; Laning, 1963). However, it is rather persistent in the environment. Residue to a depth of 2.4 m was detected after 1 year following the application of 1.12 kg/ha picloram (Bauer et al., 1972). Since certain crops are susceptible to picloram even at 0.0010−0.0500 μg/g (Leisure, 1964), a reliable analytical method is essential to accurately determine low concentrations of picloram in soil.

Gas chromatography with electron capture detection (GC/ECD) is extremely sensitive in detecting pure picloram, but quantitative extraction of low concentrations of picloram from soil has been a major problem. Among chlorinated acid herbicides, picloram is unique due to the presence of the amino group. Picloram has been extracted from soil either using acetone under acidic conditions (Lee and Chau, 1983; Merkle et al., 1966; Saha and Gadallah, 1967) or using water under basic conditions (Bauer et al., 1972; Bruns et al., 1991; Hance and Mckone, 1971; Leahy and Taylor, 1967; McKone and Cotterill, 1974). In the former extraction method, although the addition of acid is essential to ensure that any basic salt of picloram is converted to the undissociated acid, however, an excess amount of acid would conceivably convert its amino group to a quarternary salt, thus making the picloram molecule again insoluble in acetone (Merkle et al., 1966). Further, the detection limit using this method is only 0.0250 μg/g (Lee and Chau, 1983). The latter extraction method with aqueous potassium hydroxide (KOH) solution is often used. It is followed by partitioning of the picloram from the basic aqueous phase to an organic phase. This method is also inadequate because the excessive strength of dilute KOH solution causes the coextraction of dark-colored humic matter (Bohn et al., 1985). The emulsion that forms during the next partitioning step (Leahy and Taylor, 1967) then prevents picloram partitioning to the organic phase. Smith and Milward (1983) have shown that a mixture of acetonitrile, water, and ammonium hydroxide is the best medium to extract picloram from two out of three weathered field soils. However, the picloram concentration found in one of the soils (0.934 μg/g) is 93 times larger than the anticipated detection limit (0.010 μg/g) for environmental analysis. Matrix effects are more observable at very low herbicide concentration. Their study compared the extraction efficiencies of various media from three different field soils but did not investigate the dependency of extraction recovery on the contents of various soil components.

An extraction method with the addition of calcium hydroxide ($Ca(OH)_2$) powder to the soil followed by extraction of picloram with dilute potassium chloride (KCl) solution has been introduced by McKone and Cotterill (1974). In this method, the liquid−liquid partition of picloram from the basic extract to the organic phase gives better recovery than does a cleanup process using XAD-2 polystyrene resins (Cotterill, 1982). The $Ca(OH)_2$ bears divalent Ca^{2+} ion that can precipitate humic acid (Bohn et al., 1985). This leads to a cleaner extract from soil, and therefore emulsion is not formed during the liquid−liquid partition step. Although 0.0060 μg/g picloram has been measured from field-treated samples (McKone and Cotterill, 1974), this method has not been tested for a wide range of soil properties, and only two sandy soils with 1.9% and 4.1% organic matter were tested.

The purpose of the present study is to evaluate the determination of picloram, particularly at 0.0100 μg/g, in Alberta soils of various properties, using the Ca-

0021-8561/96/1444-1135$12.00/0

Table 1. Retention Time of Herbicide Methyl Ester and Internal Reference[a]

compound	retention time, min: 5% phenyl methyl polysiloxane	retention time, min: 50% phenyl methyl polysiloxane
1,2,3-trichlorobenzene[b]	10.56	10.39
clopyralid methyl ester	19.22	22.74
silvex methyl ester	29.48	30.09
picloram methyl ester	34.89	39.89
2,2′,4,4′,6-pentachlorobiphenyl[b]	44.82	44.96

[a] Oven temperatures were the same for the two columns, but chromatograms were obtained on separate days due to only one ECD present in the GC instrument. [b] Internal reference compound.

$(OH)_2$−water extraction method followed by measurement with GC/ECD. This evaluation at the anticipated detection limit level is important because environmental samples are generally collected from a wide range of soils, yet a thorough recovery study at low concentration from soils with various properties does not exist in the published literature, particularly the relationships between recoveries and soil components. For comparison, determinations of clopyralid (3,6-dichloropicolinic acid), a herbicide similar to picloram but without an amino group, and silvex (2-(2,4,5-trichlorophenoxy)propionic acid), a chlorinated acid herbicide that is not used in Alberta, are also included in this study.

EXPERIMENTAL PROCEDURES

Apparatus. *Gas Chromatograph.* A Perkin-Elmer Model 610N instrument equipped with an autosampler and a Turbochrom computerized data system was used, with a splitless injector maintained at 285 °C. The bottom of the injector liner was plugged with silanized glass wool. Before sample measurement, the cleanliness of the liner was determined by injecting the standard. When the picloram methyl ester peak from the standard was greater than 6 s at half-height, the injector liner was replaced. This experimental condition was important and has not been noted previously. The picloram methyl ester contains an amino group that could interact with acid active sites present on the liner, causing a broad peak. However, the picloram methyl ester peak from a control (defined below) was normally sharp. The acid active sites could be deactivated by other compounds present in the soil extract which were more basic than the picloram methyl ester. This phenomenon was not observed in the case of clopyralid or silvex. The analytical column used was primarily 5% phenyl methyl polysiloxane-fused capillary (DB-5 J&W) of 30 m × 0.32 mm × 0.25 μm. A 50% phenyl methyl polysiloxane-fused capillary (DB-17 J&W) was also used in some cases (see Results and Discussion). The oven was heated from 60 to 200 °C at a rate of 3.5 °C/min and then at 15 °C/min to 280 °C and held for 15 min. The retention times of herbicide methyl esters and internal references are listed in Table 1. A ^{63}Ni electron capture detector (ECD) was used and kept clean by normal maintenance procedures, such as hydrogenation while heating. The ECD background noise was maintained below 20 mV. An unclean ECD enhanced the peak heights (or areas) of clopyralid and picloram methyl esters nonproportionally with increasing concentrations. This effect was not observed in the case of silvex.

Sonifier Cell Disruptor. A Model 350V instrument with microtip was used.

Mechanical Shaker. A Burrell Model 75 wrist action shaker was used with a degree of shaking at setting 10.

Centrifuge. A Damon/EIC B-20A centrifuge was set to a relative centrifugal force of 794*g*.

Concentrator Apparatus. A Turbo-Vap Model II evaporator (Zymark) was equipped with a 250 mL turbo-vap tube having a sloped wall and a 1 mL reservoir in the bottom. Six tubes were immersed in a water bath at 35 °C. A stream of nitrogen was directed at an angle from the top of each tube which created spiral movement of the liquid while the volume was reduced. The process was programmed to concentrate to 0.5 mL, to add 5 mL of hexane, and to concentrate further to 0.5 mL. This apparatus was used under a fume hood and only for experiments with limited number of samples. A similar Turbo-Vap Model 500 instrument (Zymark) would be better used since it is a closed cell concentrator with capability to condense the evaporated solvent. A Reacti-Vap evaporator (Pierce 18780) was also used to concentrate the solutions in the vials or centrifuge tubes using a gentle stream of nitrogen.

Reagents. *Chemicals.* Clopyralid (Lontrel), picloram (Tordon), and silvex (Fenoprop) were of standard grade (98% purity) obtained from Chem Service (West Chester, PA). The 2,2′,4,4′,6-pentachlorobiphenyl and 1,2,3-trichlorobenzene were obtained from Ultra Scientific (North Kingstown, RI). All organic solvents were of distilled-in-glass and pesticide grade. All hexane mentioned in this paper was *n*-hexane. Other chemicals were Fisher certified or analytical reagent grade.

Acidified Anhydrous Sodium Sulfate. Two and one-half kilograms of anhydrous sodium sulfate (Na_2SO_4) was added to a mixture of 1 L of dichloromethane, 125 mL of diethyl ether, and 5 mL of concentrated sulfuric acid (H_2SO_4) (Alberta Environmental Centre, 1992). After mixing, the acidified Na_2SO_4 was filtered through a Büchner funnel. Suction was continued until the acidified Na_2SO_4 was dried.

Diazomethane Reagent. Caution: *N*-Nitroso-*N*-methylurea (Sigma, St. Louis, MO; preserved with 15% acetic acid) is a suspected carcinogen. It must be handled in a fume hood and with laboratory protective apparels, such as safety glasses, gloves, and coat. Because diazomethane is extremely poisonous and explosive, preparation and application with this reagent must be done in a fume hood. Ground glass equipment or boiling chips should not be used at any time. Ten grams of *N*-nitroso-*N*-methylurea was slowly added to a mixture of 30 mL of 40% KOH in distilled deionized water and 200 mL of diethyl ether upon stirring in an ice bath (Alberta Environmental Centre, 1992). Stirring continued until all reagent had reacted. The yellow diethyl ether layer containing dissolved diazomethane was pipetted into 14 test tubes in an ice bath. These tubes were then sealed with Teflon-lined screw caps and stored at −20 °C for no longer than 1 week.

Internal References. Stock solutions of 1,2,3-trichlorobenzene and 2,2′,4,4′,6-pentachlorobiphenyl at 1000 μg/mL each were prepared in hexane. Working solutions of 40 μg/mL 1,2,3-trichlorobenzene and 4.0 μg/mL 2,2′,4,4′,6-pentachlorobiphenyl were prepared from the stock solutions in hexane. Two internal references were used because the coextractive compounds from soil could mask one of the internal references on the GC/ECD chromatogram.

Herbicide Standard. Stock solutions containing 1000 μg/mL of each herbicide were prepared in methanol. An intermediate solution at 20 μg/mL was prepared by mixing aliquots of stock solutions and then diluting with methanol. A working solution at 1 μg/mL was obtained by diluting the intermediate solution in methanol. The methyl ester standard was prepared by placing a known volume of working solution into a vial followed by the addition of 1 mL of diazomethane reagent. After the mixture was allowed to react for 15 min at room temperature, 0.5−1 mL of isooctane was added. Excess diazomethane was removed under a gentle stream of nitrogen. Ten microliters of trichlorobenzene and 25 μL of pentachlorobiphenyl internal reference working solutions were then added. The volume in the microvial was adjusted to 2 mL with isooctane.

Soils. One kilogram each of 14 Alberta soils of various properties was air-dried and ground to pass through a 2 mm sieve. Then samples were taken from each homogenized soil for testing of soil properties, blanks, and fortifications. The soil properties are listed in Table 2. Organic carbon was determined by the Walkley−Black (1934) wet oxidation method using a correction factor of 1.2 and converted to organic matter content by multiplying by 1.724. Particle size distribution was analyzed by the hydrometer method (Bouyoucos, 1962). Cation exchange capacity was determined by the sodium saturation

Table 2. Properties of Soil

soil series	organic matter, %	clay, %	sand, %	silt, %	[iron], μg/g	CEC,[a] mequiv/100 g	pH
Cooking Lake A	0.4	9.4	37.0	53.6	12 469	9.7	6.2
Cooking Lake B	0.7	36.8	23.6	39.6	22 455	34.8	6.0
Torlea C	0.9	31.2	45.9	22.9	12 700	23.7	8.5
Heisler C	1.0	30.7	37.5	31.8	15 706	21.6	8.3
Halkirk C	1.0	30.4	33.2	36.4	14 527	34.4	8.7
Dune Sand A	1.2	3.6	94.1	2.3	3908	9.3	7.0
Daysland A	1.7	21.8	25.5	52.7	14 562	23.9	6.7
Halkirk B	1.8	34.5	29.8	35.7	16 309	30.6	8.6
Torlea B	2.9	33.5	37.6	28.9	13 707	33.2	9.1
Halkirk A	3.0	20.5	44.7	34.8	11 780	33.4	8.6
Daugh A	3.6	44.2	16.5	39.3	11 824	46.0	9.0
Vegreville[b] A	5.1	36.7	18.1	45.2	16 722	42.1	8.1
Wetaskiwin A	5.3	27.8	21.0	51.2	14 775	36.7	5.9
Malmo A	10.1	32.8	16.7	50.5	18 088	58.7	7.9

[a] Cation exchange capacity. [b] The series is unknown.

method described in Chapman (1965). Soil pH was measured in a saturated paste by a combination electrode and pH meter calibrated with buffer solutions of known pH. Metals were recovered from 1 g of soil sample by refluxing for 30 min at 85 °C using 4 mL of water/nitric acid (1:1) and 10 mL of water/hydrochloric acid (1:4) in a beaker covered with a watch glass (Martin et al., 1991). After dilution with deionized water in a volumetric flask, the dilute extract was centrifuged and then analyzed by inductively coupled plasma mass spectrometry. Total Fe (not total Fe as iron oxide) is reported in this manuscript.

Procedures. *Fortification of Soil.* Two fortification procedures were carried out for each soil. Fortification A: An aliquot (50−200 μL) of working solution containing a mixture of herbicides at 1.0 ng/μL each was added to 20 g of soil in a mortar, which was then mixed by grinding. The resulting fortified soil (contained 0.0025−0.0100 μg/g of each herbicide) was extracted immediately. For each soil, four fortified replicates were prepared for four separate determinations of the herbicide recoveries. Fortification B: An aliquot (50−250 μL) of intermediate solution containing a mixture of herbicides at 20 ng/μL each was added to 25−50 mL of distilled deionized water in a flask. This solution was poured into 100 g of soil in a glass jar. The flask was rinsed three times with distilled deionized water, and the rinsing solutions were combined with the soil slurry in the jar. The slurry was mixed using a spatula and allowed to dry at room temperature to 100 ± 0.2 g. Depending on the soil type, drying the soil to its original weight took 2 (for sandy soil) to 14 (for high-clay soil) days. The dried soil theoretically contained 0.0100−0.0500 μg/g of each herbicide. The recoveries of herbicides were determined in four replicates of analysis using an aliquot of 20 g of soil at each separate determination. The remaining 20 g soil portion was retained as a contingency measure.

Extraction of Herbicides from Soil. Twenty grams of fortified soil was mixed with 2 g of $Ca(OH)_2$ powder in a mortar. A small volume (0.5 mL) of distilled deionized water was added to moisten the mixture, and a pestle was used to further mix and crush the soil mixture for 2 min. This procedure was laborious but might be useful for initiating contact of picloram with the base, which could affect the interaction of picloram in the soil. The resulting homogeneous moist mixture was transferred to a 150 mL glass bottle. The mortar, pestle, and scoopula were rinsed three times with distilled deionized water, and the rinsing solutions were added to the bottle. Water was then added to yield a total volume of 100 mL. The bottle was capped using a Teflon-lined stopper and allowed to stand at room temperature overnight. After sonification (3−6 min) followed by shaking for 1 h, the soil was separated from the basic aqueous extract by centrifugation at a relative centrifugal force of 794*g* for 20 min. The resulting extract was immediately used for the liquid−liquid partition process outlined below.

Liquid−Liquid Partition. Fifty milliliters of the basic aqueous extract in a 500 mL separatory funnel was acidified with 0.25 mL of concentrated phosphoric acid (H_3PO_4). Ten grams of sodium chloride (NaCl) was then dissolved in the mixture. The pH was typically ≤1. No dilution was performed at this stage. The herbicides were then partitioned into dichloromethane by three serial extractions using 50 mL of dichloromethane each time. Emulsions were not formed. Following each extraction, the dichloromethane phase was dried by passing it through prerinsed (with 20 mL of dichloromethane) 50 g of acidified anhydrous Na_2SO_4 in a coarse sintered glass funnel. At the end of the extraction process, the Na_2SO_4 was rinsed with 30 mL of dichloromethane. The combined dichloromethane solutions were collected in a 250 mL turbo-vap tube and concentrated to 0.5 mL (see the above section). An aliquot of 5 mL of hexane was used to displace the dichloromethane. The resulting 0.5 mL of concentrate in hexane was not homogeneous, and there was often a slight precipitate marked by a light-colored ring adhering to the sloped wall at the bottom of the tube. Therefore, this concentrate was not transferred, and derivatization was done directly in this tube.

Derivatization. Caution: Derivatization using diazomethane must be done in a fume hood with safety glasses and gloves. The turbo-vap tube containing 0.5 mL of concentrate in hexane was removed from the turbo-vap water bath; 1.5−2.0 mL of diazomethane reagent was added to the concentrate using a Pasteur pipet while also rinsing the sloped wall at the tube bottom. The diethyl ether in the reagent dissolved all insoluble substances in the tube. The mouth of the tube was then covered with a piece of aluminum foil and allowed to stand at room temperature for 15 min. The yellowish liquid was quantitatively transferred using a Pasteur pipet either to a 2 mL vial or to a centrifuge tube (when further dilution was necessary) containing 1 mL of isooctane. Excess diazomethane was removed under a gentle stream of nitrogen while the transfer was completed. Rinsing of the turbo-vap tube was done three times with isooctane. The rinsing solutions were combined into the microvial or the centrifuge tube while reducing the volume. Internal reference solutions were added, and the volume was adjusted to 2 mL with isooctane.

Blank. A blank for each type of soil was prepared in the same manner using 20 g of nonfortified soil throughout the entire procedures of extraction, liquid−liquid partition, and derivatization as mentioned above.

Controls. Three controls for each type of soil were prepared in the same manner as for the blank, except that known quantities of herbicides were added prior to derivatization. The concentrations of the herbicides in the controls were the same as in the standards.

Quantitation. Sample measurements were done by injecting 2 μL of test solutions into the GC/ECD instrument. The linear range was tested at 10−200 pg with three different concentrations of herbicide methyl esters. Samples with higher concentrations were diluted with isooctane to a concentration within this working range. The instrument performance was maintained so that the peak height of the herbicide methyl esters in the three standards agreed to within 10% of those in the three controls containing the same concentrations of herbicide methyl esters. Also, the heights of the internal references were reproduced within 10%.

Statistical Analyses. Pearson correlations and regression analyses were done using SAS, version 6.10 (SAS Institute Inc., Cary, NC). Mean and standard deviation were determined using Microsoft Excel, version 5.0 (Microsoft Corp.). All samples, with one exception (at 10.1% organic matter; Table 2), contained 5.3% or less organic matter, and there were no samples between organic matter content 5.3−10.1%. Therefore, linear regression analysis did not include the one sample at 10.1% organic matter. This approach was taken based on the following considerations: (1) no *a priori* model was assumed regarding the relationship between recovery and organic matter content; (2) preliminary analyses indicated the one sample at 10.1% organic matter consistently contributed a disproportionate amount to the overall error variance; and (3) better comparisons among the three herbicides were enabled, since there were no data at 10.1% organic matter for silvex due to the unresolved peaks of silvex and the coeluting compound. However, the one sample at 10.1% organic matter

Table 3. Effect of pH on Herbicide Partition from Aqueous Extract to 5% Methanol−Dichloromethane[a]

	recovery, %						
	Daugh A			Malmo A		Vegreville A	
herbicide[b]	pH 0.5	pH 1.5	pH 2.5	pH 0.5	pH 2.2	pH 0.5	pH 2.5
clopyralid	88.3 ± 9.3	97.9	7.1	100 ± 8.5	34.2	94.5 ± 15.0	*c*
picloram	68.7 ± 6.6	59.6	*c*	69.8 ± 4.1	12.4	74.7 ± 1.9	*c*
silvex	58.0 ± 3.1	57.5	52.0	*d*	*d*	67.0 ± 6.4	63.5 ± 6.4

[a] Data with ± sign are mean percent recovery and standard deviation based on four replicates of separate determinations. [b] Herbicide concentration was 0.0100 μg/g in soil. [c] Cannot be detected due to very low recovery values. [d] Cannot be measured due to interference from coextractant.

for clopyralid and picloram was included in Figures 1 and 2 and marked with arrows for comparisons. This conservative approach to trend analysis was deemed appropriate because the shape of the response is likely to be data dependent over varying soil properties.

RESULTS AND DISCUSSION

Importance of pH in Liquid−Liquid Partition of Herbicide. Following extraction of herbicides from the soil, partition was carried out as a cleanup process. In the extraction process, the herbicides are extracted from soil into a basic aqueous phase in their dissociated anionic form, whereas in the partitioning process, the herbicides are partitioned from basic aqueous extract to an organic phase in their undissociated acid forms. Therefore, the pH of the basic aqueous extract must be adjusted prior to partitioning in order to shift the acid−base equilibrium to favor the presence of the undissociated acid.

The undissociated acid fraction of picloram in the aqueous phase is difficult to determine theoretically because of the uncertainty of its acid dissociation constant (Osteryoung and Whittaker, 1980). Literature data for pK_a of picloram vary from 1.97 to 4.1. When a pK_a of 4.1 (Hamaker et al., 1966) is assumed, estimates of the undissociated acid fraction at pH 3.5 and 2.0 are 0.80 and 0.99, respectively. Either one of these pH conditions will lead to satisfactory partition recovery of picloram. However, when a pK_a of 1.97 (Osteryoung and Whittaker, 1980) is considered, only fractions of 0.03 and 0.48 undissociated acid are present in the aqueous phase at pH 3.5 and 2.0, respectively. These amounts of undissociated acid will not lead to a successful partition to the organic phase.

To ascertain maximum herbicide recovery, obviously, the optimum pH needs to be determined experimentally. For this purpose, Daugh A, Malmo A, and Vegreville A soils were selected from those listed in Table 2. The picloram was more difficult to extract from these soils than from other soils. Thus, these soils led to greater differences in picloram recoveries when the experimental conditions (i.e., the pH in partition) were changed than might be expected for other soils. Each soil was fortified with the herbicides at 0.0100 μg/g (based on fortification A) and then used immediately in the experiment. When partition was carried out at pH 2.5, over 50% silvex was recovered but only negligible amounts of clopyralid and picloram were recovered (Table 3). Partition at pH 2.2 increased clopyralid (34.2%) and picloram (12.4%) recoveries, but the results were still far from satisfactory. The greatest recoveries of these herbicides were obtained from partitions at pH 1.5 or 0.5. Thus, further experiments in this study were carried out with partitions at pH ≤1.

Selection of Organic Solvent for Partition Process. The solubility of picloram in various solvents has been determined previously (Saha and Gadallah, 1967). The best solvent for the partition process has been reported to be diethyl ether or 5% ethanol−chloroform (Cheng, 1971). However, diethyl ether is not practical (due to safety concerns) for routine sample analysis, and 5% ethanol−chloroform is not volatile enough for rapid concentration. In experiments to determine optimum pH (see above), 5% methanol−dichloromethane was used (Table 3), whereas subsquent experiments (Table 4) were done with dichloromethane. The recoveries (at pH ≤1.5) from Daugh A, Malmo A, and Vegreville A soils from these two tables indicate that there is no advantage to using the former solvent. The presence of 5% methanol actually enhances the extraction of H_3PO_4, making the final sample solution acidic (effective pH 5.5). The resulting acid that is deposited on the injector liner of GC/ECD causes a broad peak of picloram methyl ester (see the Experimental Procedures). Therefore, dichloromethane was selected for further experiments.

Recovery of Herbicide from Soil Extracted Immediately after Fortification. Recovery values of herbicides are important to evaluate the accuracy of an analytical method. Recoveries are usually assessed by analyzing soil samples fortified with known quantities of herbicides. Although the interaction of herbicides in weathered field soils may not be identical with that in fortified soils, the experiments with fortified soils are fundamentally needed because (1) the quantities of herbicides in the fortified soils are known, and therefore the actual recovery values can be assessed and related to the soil matrices; and (2) the same soils without fortification can be used as blanks to determine the presence or absence of interferences from coeluting compounds.

Table 4 lists the mean percent recoveries of herbicides and the standard deviations resulting from soils fortified at 0.0100 μg/g by fortification A. Extraction was carried out immediately after fortification. Each datum listed was based on four separate determinations that were carried out through the entire analytical procedures.

The relationships between soil components were tested by Pearson correlation analysis, and the results are given in Table 5. Although correlation coefficients (r) between clay vs sand contents (−0.74), clay vs iron contents (0.66), and sand vs iron contents (−0.79) appear to be strong ($p \leq 0.0001$), however, when data from Dune Sand A (3.6% clay, 94.1% sand, and 3908 μg/g iron) are omitted, these relationships are much reduced. Thus, the relationships between each soil component and herbicide recoveries at 0.0100 μg/g fortification can be examined (Table 5).

Clopyralid is recovered almost totally from all soils (Table 4). Previous investigators who used a limited number of soils also reported high recoveries of clopyralid (Cotterill, 1978; Pik and Hodgson, 1976). Some loss in the recovery of clopyralid due to the volatility of its methyl ester has been previously mentioned (Cotterill, 1978), and derivatization to its 1-butyl ester was

Table 4. Percent Recovery of Herbicide from Fortified Soil When Analyzed Immediately[a]

	concentration of herbicide in soil, μg/g									
	0.0025			0.0050			0.0100			GC
soil series	clopyralid	picloram	silvex	clopyralid	picloram	silvex	clopyralid	picloram	silvex	column
Cooking Lake A							97.9 ± 1.4	90.8 ± 3.5	95.0 ± 2.8	*d*
Cooking Lake B							98.1 ± 1.9	96.4 ± 4.8	94.3 ± 2.1	*d*
Torlea C	79.9 ± 2.0	*b*	*b*	90.3 ± 8.2	74.6 ± 10.7	90.2 ± 2.7	97.9 ± 2.1	96.0 ± 0.8	88.4 ± 2.3	*d*
Heisler C							88.6 ± 1.1	74.3 ± 3.4	75.3 ± 1.0	*d*
Halkirk C							99.5 ± 7.7	91.4 ± 2.1	84.5 ± 4.0	*d*
Dune Sand A							98.9 ± 2.8	92.6 ± 6.2	89.3 ± 4.9	*d*
Daysland A							94.6 ± 0.9	74.8 ± 4.3	85.1 ± 4.7	*d*
Halkirk B							98.1 ± 12.7	86.9 ± 8.4	85.3 ± 10.7	*d*
Torlea B							97.8 ± 6.4	97.8 ± 4.6	95.0 ± 5.9	*d*
Halkirk A							85.9 ± 9.8	82.1 ± 4.6	73.4 ± 3.0	*d*
							89.3 ± 3.2	83.4 ± 6.0	*c*	*e*
Daugh A	*c*	*b*	*c*	94.1 ± 3.6	*b*	*c*	99.6 ± 3.7	71.9 ± 2.2	60.7 ± 2.3	*d*
				c	*b*	*c*	100 ± 2.2	63.3 ± 2.7	*c*	*e*
Vegreville A							89.3 ± 6.2	58.0 ± 7.4	61.9 ± 4.7	*d*
Wetaskiwin A	*c*	*b*	*c*	*c*	87.4 ± 3.5	*c*	94.6 ± 3.0	88.3 ± 1.8	87.6 ± 3.9	*d*
				c	85.4 ± 9.2	*c*	*c*	96.2 ± 6.8	89.3 ± 2.1	*e*
Malmo A	*c*	*b*	*c*	*c*	*b*	*c*	*c*	59.7 ± 2.1	*c*	*d*
				b	*b*	*c*	91.7 ± 5.9	55.8 ± 6.0	*c*	*e*

[a] Data listed are mean and standard deviation based on four replicates of separate determinations. Solvent used for partition process was dichloromethane. [b] Cannot be measured due to the height being <3 times the background. [c] Cannot be measured due to the presence of coeluting compound. [d] GC column with 5% phenyl methyl polysiloxane. [e] GC column with 50% phenyl methyl polysiloxane.

Table 5. Pearson Correlation Matrix

	correlation coefficient and probability value[a]									
					recovery, %					
					clopyralid		picloram		Silvex	
soil component	organic matter, %	clay, %	sand, %	[iron], μg/g	fortn A[b]	fortn B[c]	fortn A[b]	fortn B[c]	fortn A[b]	fortn B[c]
organic matter, %	1.0000				−0.2388	−0.6756	−0.4825	−0.5508	−0.5075	−0.6811
	(0.0)				(0.0883)	(0.0001)	(0.0003)	(0.0001)	(0.0001)	(0.0001)
clay, %	0.3100	1.0000			−0.0152	0.1190	−0.2643	0.1063	−0.4066	−0.0723
	(0.0201)	(0.0)			(0.9148)	(0.4007)	(0.0583)	(0.4533)	(0.0028)	(0.6105)
sand, %	−0.4435	−0.7424	1.0000		0.0735	−0.1241	0.3847	−0.1088	0.3096	0.0159
	(0.0006)	(0.0001)	(0.0)		(0.6047)	(0.3809)	(0.0049)	(0.4428)	(0.0255)	(0.9107)
[iron], μg/g	0.2378	0.6551	−0.7872	1.0000	−0.0808	0.3421	−0.0950	0.3543	0.0223	0.2907
	(0.0776)	(0.0001)	(0.0001)	(0.0)	(0.5691)	(0.0130)	(0.5027)	(0.0100)	(0.8754)	(0.0365)

[a] Probability value is listed in parentheses. [b] Fortification A at 0.0100 μg/g. [c] Fortification B at 0.0100 μg/g.

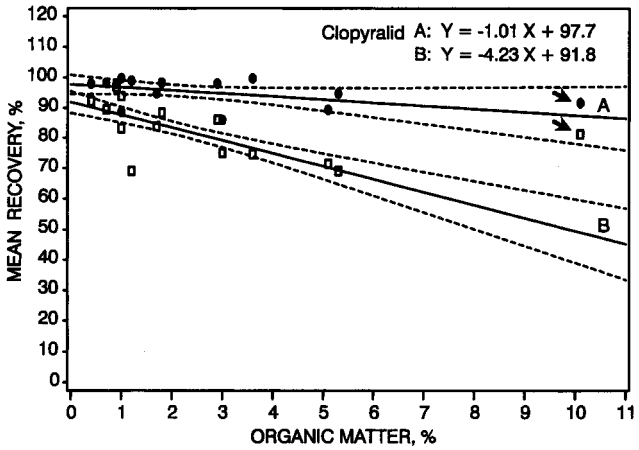

Figure 1. Clopyralid mean recovery as a function of organic matter content. The dashed lines are ±95% confidence limits about the mean. Data marked by arrows are excluded from the linear regression analysis (see Experimental Procedures). Curves A and B represent the results from soils at 0.0100 μg/g with fortifications A and B, respectively.

suggested; however, no such phenomenon was found in the current study. Clopyralid recovery does not correlate with organic matter, clay, sand, or iron content in soils (Table 5). Curve A in Figure 1 shows the regression line between clopyralid recovery and organic matter content in soil. Regression analysis indicates no significant change in the recovery of clopyralid when organic matter increases from 0.4% to 5.3%, where recovery_a (%) = −1.01 × organic matter (%) + 97.6, r^2 = 0.06 and p = 0.09. Therefore, with a slope not different from zero, the best estimate is the overall mean percent recovery (95.2 ± 6.7). The dashed lines above and below curve A are the ±95% confidence limits about the predicted mean, given percent organic matter.

Picloram recoveries varied from 58.0% to 97.8% depending on soil properties (Table 4). Despite the limited number of soils with higher organic matter contents, there is a trend of decreasing picloram recovery with an 0.4−5.3% increase in organic matter content (r = −0.48, p = 0.0003; Table 5). Curve A in Figure 2 illustrates this relationship where recovery_a (%) = −3.69 × organic matter (%) + 92.8, r^2 = 0.23 and p = 0.0003. The dashed lines are the ±95% confidence limits about the predicted mean, given percent organic matter.

Previous literature supports the interaction of picloram with organic matter in soil. Hamaker et al. (1966) observed the greatest sorption of picloram in high-organic matter content soils and red acidic soils. Cheng (1969) discussed the incomplete recovery of picloram from soils containing >3% organic matter and with pH below 6 when extracted with 2 M KCl solution. Treating the soil with lime and incubating it for 1 month to adjust the soil pH to 7 led to complete picloram recovery. Grover (1968), in a study on the influence of soil properties on the phytotoxicity of picloram, found there was adsorption of picloram on the organic matter.

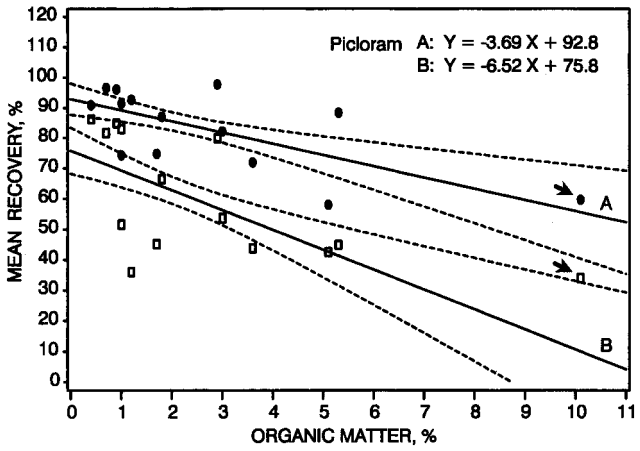

Figure 2. Picloram mean recovery as a function of organic matter content. The dashed lines are ±95% confidence limits about the mean. Data marked by arrows are excluded from the linear regression analysis (see Experimental Procedures). Curves A and B represent the results from soils at 0.0100 μg/g with fortifications A and B, respectively.

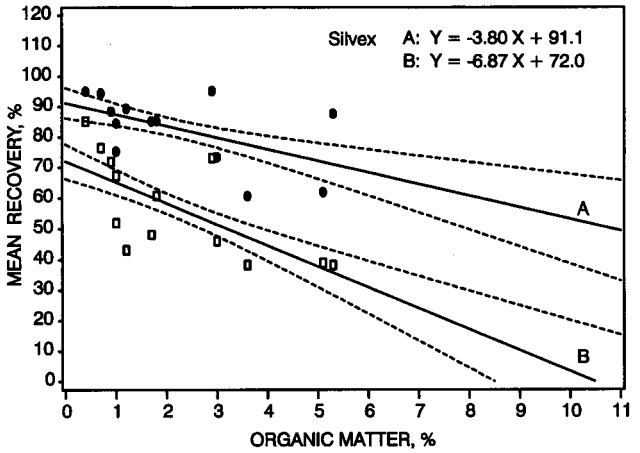

Figure 3. Silvex mean recovery as a function of organic matter content. The dashed lines are ±95% confidence limits about the mean. Curves A and B represent the results from soils at 0.0100 μg/g with fortifications A and B, respectively.

Biggar et al. (1978), in a study on the equilibrium and kinetics of adsorption of picloram with soils, found that the picloram in Palouse soil interacted predominantly with the organic matter. They suggested that the interaction of picloram with soil organic matter might require conformational changes of functional groups. Increasing organic matter content in soils reduced the movement (by leaching) of picloram to lower soil depths (Herr et al., 1966; Keys and Friesen, 1968).

Clay has been known to play a minor role in the adsorption of picloram (Biggar et al., 1978). Picloram, being an anion in Palouse soil, was found highly unfavorable to interact with the negatively charged clay surface. In the present study, picloram recoveries among clay contents are not consistent. However, picloram recovery is relatively consistent with increasing sand content, except for soils having sand <20% (Daugh A, Malmo A, and Vegreville A). The relationship between picloram recovery and iron content is less clear. Biggar and Cheung (1973) concluded that picloram interacted in soil by chelation with metal ions in which its pyridinium nitrogen and the carboxyl group formed a five-membered ring.

Silvex recovery is moderately dependent on organic matter content ($r = -0.51$, $p = 0.0001$; Table 5). The trend is very similar to the case of picloram, i.e., decreasing recovery of about 4% per 1% increase in organic matter content, or recovery_A (%) = −3.80 × organic matter (%) + 91.1, $r^2 = 0.26$ and $p = 0.0001$ (curve A in Figure 3). The ±95% confidence limits about the predicted mean are indicated by the dashed lines, given percent organic matter. Recovery of silvex is fairly consistent with an increase in clay content, except for Daugh A and Vegreville A soils, which both yielded low silvex recoveries for high clay content. Variation in sand contents does not affect the silvex recoveries, except for Daugh A and Malmo A soils, which have the lowest sand content.

Precision of the analyses for soils with fortification A at 0.0100 μg/g can be deduced from the data listed in Table 4. The relative standard deviations are in the 1−13% range. However, detection limits are dependent on herbicide and soil properties. Analyses were also carried out at lower fortification concentrations in several soils. For example, Torlea C, which has relatively low organic matter content (0.9%), allowed determinations of clopyralid, picloram, and silvex at 0.0025, 0.0050, and 0.0050 μg/g concentrations, respectively (Table 4). Daugh A, Wetaskiwin A, and Malmo A, which contain higher amounts of organic matter (3.6%, 5.3% and 10.1%, respectively), were also analyzed at those concentrations. All herbicides could not be successfully measured at 0.0025 μg/g. In an extreme case (Malmo A), clopyralid and picloram could only be measured at 0.0100 μg/g (with 91.7 ± 5.9% and 59.7 ± 2.1% recoveries, respectively) and silvex could not be measured at all.

Reliability of Herbicide Peak Identification. Figure 4 illustrates typical GC/ECD chromatograms of methyl ester standards and test solutions from the extraction of 20 g of soil with fortification A at 0.0100 μg/g. Two examples are presented, Torlea A and Vegreville A soils that contain 0.9% and 5.1% organic matter, respectively.

The presence of compounds extracted from soil matrices (coextractants) defines the background and the good separation of the herbicide methyl ester peaks on the GC/ECD chromatograms, as can be observed from Figure 4b,c. Increasing the sample size will not increase the detection limit of the herbicide because the detection limit is affected by this background rather than by the amount of the herbicide. This phenomenon has also been observed in both extractions using KOH solution (Bauer et al., 1972) or phosphoric acid in acetone (Saha and Gadallah, 1967).

In soil with a high organic matter content, compounds extracted from soil matrices (coextractants) can have the same retention times as that of the herbicide methyl esters in the GC/ECD chromatogram. An attempt has been made to resolve the herbicide methyl ester peaks from interference peaks by using two different types of columns: a nonpolar 5% phenyl methyl polysiloxane column and a midpolar 50% phenyl methyl polysiloxane column (marked with labels d and e, respectively, on Table 4). With this change, the retention times of clopyralid and picloram methyl esters shifted as much as 3.5 and 5.0 min, respectively (Table 1). In some cases, results from both columns are complementary. For example, the peak due to clopyralid at 0.0100 μg/g from Wetaskiwin A (Table 4) is resolved by the former column but not by the latter, whereas from Malmo A, it is resolved by the latter column but not by the former. However, cases where a coextracted compound is present in the new vicinity, changing the column does not

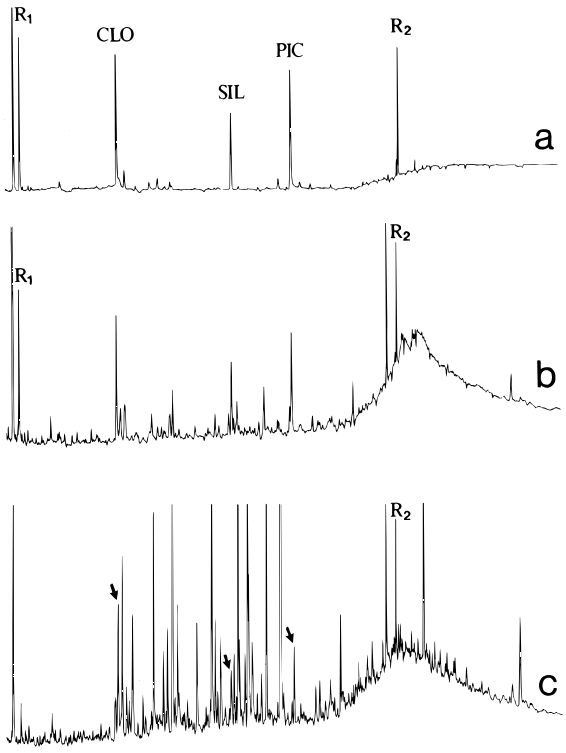

Figure 4. Typical GC/ECD chromatograms using 5% phenyl methyl polysiloxane column of (a) methyl ester standards of clopyralid (CLO), picloram (PIC), and silvex (SIL) at 100 pg each, (b) test solution from the extraction of 20 g of Torlea C with fortification A at 0.0100 μg/g and (c) test solution from the extraction of 20 g of Vegreville A with fortification A at 0.0100 μg/g, where the methyl ester peaks of herbicides are marked by arrows. Based on 100% recovery, the expected amount of each herbicide methyl ester in chromatograms b and c is 100 pg. The internal references are labeled as R_1 and R_2. Amplitude is kept constant in all chromatograms. Retention times are listed in Table 1.

always provide good peak resolution. For example, both columns fail to resolve the silvex peak at 0.0100 μg/g from Malmo A.

A thorough study using gas chromatography with mass spectrometry detection (GC/MS) is worthwile. Previous investigators have attempted various cleanup alternatives, such as the use of an alumina column followed by oxidation with potassium permanganate (Bjerke et al., 1967), deactivated silica gel column (Lee and Chau, 1983), Florisil column (Leahy and Taylor, 1967), and XAD-2 polystyrene column (Cotterill, 1982). The detection limit of picloram using these cleanup procedures was still not better than 0.0100−0.0500 μg/g, and the coextractive compounds were not completely removed. Recently, Lee et al. (1991) introduced gel permeation chromatography using a Bio-Beads SX3 column to remove fatty acids with 12 carbons or more, but the shorter carbon chain and benzoic acids could not be separated and still interfered.

Recovery of Herbicide from Soil Slurry after Drying. Table 6 lists the recovery data of herbicides from soils with fortification B at 0.0100 and 0.0500 μg/g. Mean data at these two concentrations are not statistically different ($p > 0.05$) for picloram or silvex but are different at the 0.05 significance level for clopyralid.

When the herbicide recovery data at 0.0100 μg/g from soils with fortification A (Table 4) are compared to those with fortification B (Table 4), the following trends are observed. For clopyralid: (1) Fortification A (95.2 ± 6.7) has a significantly greater recovery ($p = 0.0001$) than with fortification B (82.4 ± 9.8). (2) Results from fortification B are more dependent on soil properties, i.e., those having organic matter of 3−5% exhibit smaller recoveries as can be seen in curve B of Figure 1, where recovery_B (%) = −4.23 × organic matter (%) + 91.8, $r^2 = 0.46$ and $p = 0.0001$; dashed lines are the ±95% confidence limits about the predicted mean, given percent organic matter. (3) The results from fortifications A and B are not correlated, as would be expected if both yielded the same information. For picloram: (1) The data show a much greater difference at $p = 0.0001$ between results with fortification A (82.9 ± 13.5) and fortification B (59.5 ± 19.6) as can be seen in Figure 2. (2) Results with fortification B, while highly variable, exhibit either an interaction with soil properties and/or a threshold between 4% and 6% organic matter, i.e., recovery drops greatly for all soils having organic matter >5%. Regression equation for curve B in Figure 2 is recovery_B (%) = −6.52 × organic matter (%) + 75.8, $r^2 = 0.30$ and $p = 0.0001$. (3) The results from fortifications A and B are correlated with $r = 0.68$ and $p = 0.0001$. For silvex: (1) It behaves very similarly to picloram in that the difference of recovery is significant between fortification A (82.8 ± 12.0) and fortification B (56.9 ± 16.1) with $p = 0.0001$. (2) Similar to the case with fortification A, recovery with fortification B is dependent on organic matter content in the soil ($r = -0.68$, $p = 0.0001$). Regression equation for the bottom curve in Figure 3 is recovery_B (%) = −6.87 × organic matter (%) + 72.0, $r^2 = 0.46$ and $p = 0.0001$. (3) The results from fortifications A and B are correlated, with $r = 0.65$ and $p = 0.0001$.

Correlations between the recoveries of herbicides from fortification B and iron content in soils are all unclear due to a few extreme data, such as those of Dune Sand and Malmo.

Explanation for the Lower Recoveries of Herbicides from Fortification B. Clopyralid is the more water soluble herbicide (solubility at 20 °C is 1000 mg/L; Worthing and Hance, 1991), and it has a lower soil-to-water partition coefficient (K_{oc} is 98; Kenaga, 1980). Therefore in soil slurry, clopyralid is more readily available to microbes for degradation during the drying period. In this case, organic matter should give much less influence in the recovery of clopyralid as indicated by the smaller slope (−4.23) of the regression line between recovery and organic matter content in soil (Figure 1B). On the other hand, picloram and silvex are less soluble in water with solubilities of 430 and 140 mg/L at 25 °C, respectively (Worthing and Hance, 1991). They also have higher K_{oc} values, i.e., 160 and 290, respectively (Kenaga, 1980), and thus are sorbed strongly into the organic matter matrixes in soil. They are less likely to biodegrade but are more difficult to extract. Therefore, their recoveries from soils with fortification B are much lower than those with fortification A (Figures 2 and 3). Also, the dependency of their recoveries from fortification B on the organic matter content in soil is larger than that of clopyralid, as indicated by the values of the slopes in the regression lines (−6.52 and −6.89, respectively, for picloram and silvex).

In addition to the above explanation, the sorption and desorption processes of organic molecules into soil organic matter are rate limited which are governed by slow kinetics (Khan, 1973). Longer time is allowed for sorption of the herbicides in fortification B but not in

Table 6. Percent Recovery of Herbicide from Soil Fortified in Aqueous Slurry and Analyzed after the Soil Is Dry[a]

	concentration of herbicide in soil, μg/g[b]						
	0.0100			0.0500			
soil series	clopyralid	picloram	silvex	clopyralid	picloram	silvex	GC column
Cooking Lake A	92.2 ± 6.5	86.2 ± 5.9	85.2 ± 1.7	94.8 ± 13.5	89.3 ± 11.9	91.3 ± 5.2	*d*
Cooking Lake B	89.4 ± 0.9	81.7 ± 3.2	76.4 ± 1.3	100 ± 11.3	84.2 ± 8.2	78.7 ± 4.9	*d*
Torlea C	96.7 ± 4.9	84.8 ± 1.8	71.9 ± 2.7	100 ± 5.4	80.6 ± 3.0	77.9 ± 2.7	*d*
Heisler C	83.1 ± 4.6	51.6 ± 6.3	52.0 ± 2.6	84.8 ± 6.5	64.1 ± 4.1	60.0 ± 0.3	*d*
Halkirk C	93.7 ± 1.8	82.9 ± 5.1	67.3 ± 1.0	112 ± 11.6	87.5 ± 4.5	73.2 ± 7.3	*d*
Dune Sand A	69.0 ± 4.7	36.0 ± 2.4	43.1 ± 2.6	56.2 ± 6.3	35.6 ± 1.9	39.6 ± 2.0	*d*
Daysland A	83.8 ± 4.5	45.1 ± 2.1	48.2 ± 1.2	75.2 ± 10.2	37.1 ± 2.3	46.8 ± 2.5	*d*
Halkirk B	88.3 ± 3.4	66.4 ± 0.9	60.8 ± 4.6	85.9 ± 8.0	55.6 ± 5.4	61.9 ± 4.5	*d*
Torlea B	86.0 ± 6.8	79.8 ± 7.5	73.2 ± 3.7	92.2 ± 8.4	77.4 ± 6.2	71.5 ± 3.7	*d*
Halkirk A	75.0 ± 1.9	53.6 ± 1.9	46.1 ± 2.5	90.6 ± 5.5	50.1 ± 3.4	59.5 ± 2.4	*d*
	74.9 ± 5.3	46.8 ± 0.8	*c*	90.7 ± 7.5	59.2 ± 2.2	46.4 ± 3.4	*e*
Daugh A	74.6 ± 3.4	43.7 ± 3.6	38.3 ± 3.1	76.9 ± 1.9	47.8 ± 1.6	39.0 ± 0.5	*d*
	74.9 ± 5.6	48.7 ± 1.2	*c*	73.9 ± 3.7	51.5 ± 2.4	45.7 ± 1.7	*e*
Vegreville A	71.5 ± 2.7	42.4 ± 1.2	38.9 ± 1.0	71.9 ± 4.6	36.8 ± 1.0	37.1 ± 1.9	*d*
Wetaskiwin A	69.1 ± 1.9	44.8 ± 1.0	38.1 ± 5.4	70.5 ± 4.2	46.5 ± 2.3	48.6 ± 1.5	*d*
	c	43.8 ± 3.4	47.6 ± 2.3	64.0 ± 3.6	48.4 ± 3.5	47.1 ± 2.9	*e*
Malmo A	*c*	34.0 ± 1.3	*c*	80.5 ± 8.4	38.5 ± 3.2	27.2 ± 1.2	*d*
	81.4 ± 8.4	33.0 ± 3.0	*c*	82.7 ± 2.7	40.6 ± 0.9	38.9 ± 1.1	*e*

[a] Data listed are mean and standard deviation based on four replicates of separate determinations. Solvent used for partition process was dichloromethane. [b] Concentration based on dry soil. [c] Cannot be measured due to the presence of coeluting compound. [d] GC column with 5% phenyl methyl polysiloxane. [e] GC column with 50% phenyl methyl polysiloxane.

fortification A. Alternately wetting and drying the soil increases the sorption of herbicides by soil colloids (Adams, 1973), and time is an important factor that influences the efficiencies of extraction (Chiba, 1969). Therefore, for herbicides that sorped strongly into soil organic matter, fortification B may be better for assessing the true extraction efficiency of an analytical method.

CONCLUSIONS

The important findings in this study are the differences in the recoveries of clopyralid as compared to those of picloram and silvex from soils fortified at 0.0100 μg/g. Clopyralid recovery is independent of organic matter, clay, sand, or iron content in soils, and its overall mean recovery is 95.2 ± 6.7%. However, picloram and silvex recoveries (58.0−97.8%) are more highly dependent on variability of soil properties, with a significant trend to decreasing picloram and silvex recoveries with an increase in 0.4−5.3% organic matter. The trend is very similar for both picloram and silvex, i.e., the decrease of 4% recovery per 1% increase in organic matter content.

There are advantages in using $Ca(OH)_2$ and water to extract these herbicides from soils. The divalent Ca^{2+} ion precipitates humic acid, and hence an emulsion is not formed during the next partition process. This leads to a successful partitioning of the herbicides. However, this analytical method is not reliable for determinations of these herbicides at 0.0100 μg/g in soils that contain ≥5% organic matter. Although extraction of clopyralid is successful from all soils (including the Malmo A sample with 10.1% organic matter), coextractants from soils with high organic matter content can interfere in the GC/ECD measurements. In this study, blanks are available from the nonfortified soils. Therefore, the coeluting compounds can be identified, and concentrations of herbicides can be measured using two columns with different polarity. However, blanks are usually not available for the analyses of routine environmental samples. In this case, confirmation of the herbicides is necessary, e.g., using a GC/MS technique.

Drying the fortified soil slurries prior to extraction yielded lower herbicide recoveries than those extractions which were carried out immediately after fortification. Clopyralid mean recovery decreased by 13%. Greater decreases were observed for picloram (23%) and silvex (26%). On the basis of these results, wet soil samples should be extracted as soon as they are received rather than being dried many days or weeks at room temperature. If the results are to be reported on a dry basis, another portion of the sample can be dried to determine the water content in the soil, and concentration in the dry soil can then be obtained. In this manner, herbicides will be easier to recover and will not undergo degradation prior to extraction.

ACKNOWLEDGMENT

We thank the reviewer for suggesting valuable ideas in the explanation of low herbicide recoveries after drying soil slurries, R. D. Smillie for proposing this research topic, S. Wu and A. Wharmby for the analysis of metals from soils, R. N. Coleman, L. Dosdall, and J. Xu for meaningful suggestions, and M. Herbut for assistance with the computer graphics.

LITERATURE CITED

Adams, R. S., Jr. Factors influencing soil adsorption and bioactivity of pesticides. In *Residue Reviews*; Gunther, F. A., Gunther, J. D., Eds.; Springer-Verlag: New York, 1973; Vol. 47, pp 1−54.

Alberta Environmental Centre. *Methods Manual for the Chemical Analysis of Trace Organics and Pesticides in Environmental Samples*; Vegreville, Alberta, Canada, 1992; AEC V92-M2.

Bauer, J. R.; Baker, R. D.; Bovey, R. W.; Smith, J. D. Concentration of picloram in the soil profile. *Weed Sci.* **1972**, *20* (4), 305−309.

Biggar, J. W.; Cheung, M. W. Adsorption of picloram (4-amino-3,5,6-trichloropicolinic acid) on Panoche, Ephrata and Palouse soils: a thermodynamic approach to the adsorption mechanism. *Soil Sci. Soc. Am. Proc.* **1973**, *37*, 863−868.

Biggar, J. W.; Mingelgrin, U.; Cheung, M. W. Equilibrium and kinetics of adsorption of picloram and parathion with soils. *J. Agric. Food Chem.* **1978**, *26* (6), 1306−1312.

Bjerke, E. L.; Kutschinski, A. H.; Ramsey, J. C. Determination of residues of 4-amino-3,5,6-trichloropicolinic acid in cereal grains by gas chromatography. *J. Agric. Food Chem.* **1967**, *15* (3), 469−473.

Bohn, H. L.; McNeal, B. L.; O'Connor, G. A. Soil organic matter. In *Soil Chemistry*, 2nd ed.; Wiley: New York, 1985; Chapter 5, pp 135−152.

Bouyoucos, G. J. Hydrometer method improved for making particle size analyses of soils. *Agron. J.* **1962**, *5*, 464−465.

Bruns, G. W.; Nelson, S.; Erickson, D. G. Determination of MCPA, bromoxynil, 2,4-D, trifluralin, triallate, picloram and diclofop-methyl in soil by GC-MS using selected ion monitoring. *J. Assoc. Off. Anal. Chem.* **1991**, *74* (3), 550−553.

Chapman, H. D. Cation exchange capacity. In *Methods of Soil Analysis*; Black, C. A., Eds.; American Society of Agronomy: Madison, WI, 1965; Agronomy No. 9, Part 2, pp 891−901.

Cheng, H. H. Extraction and colorimetric determination of picloram in soil. *J. Agric. Food Chem.* **1969**, *17* (6), 1174−1177.

Cheng, H. H. Picloram in soil: extraction and mechanism of adsorption. *Bull. Environ. Contam. Toxicol.* **1971**, *6* (1), 28−33.

Chiba, M. Factors affecting the extraction of organochlorine insectisides from soil. In *Residue Reviews*; Gunther, F. A., Gunther, J. D., Eds.; Springer-Verlag: New York, 1969; Vol. 30, pp 63−113.

Cotterill, E. G. Determination of 3,6-dichloropicolinic acid residues in soil by gas chromatography of the 1-butyl ester. *Bull. Environ. Contam. Toxicol.* **1978**, *19*, 471−474.

Cotterill, E. G. Determination of acid and hydroxybenzonitrile herbicide residues in soil by gas-liquid chromatography after ion-pair alkylation. *Analyst* **1982**, *107*, 76−81.

Grover, R. Influence of soil properties on the phytotoxicity of picloram. *Weed Res.* **1968**, *8*, 226−232.

Hamaker, J. W.; Johnson, H.; Martin, R. T.; Redemann, C. T. A picolinic acid derivative: a plant growth regulator. *Science* **1963**, *141*, 363.

Hamaker, J. W.; Goring, C. A. I.; Youngson, C. R. Sorption and leaching of 4-amino-3,5,6-trichloropicolinic acid in soils. *Adv. Chem. Ser.* **1966**, *No. 60*, 23−27.

Herr, D. E.; Stroube, E. W.; Ray, D. A. The movement and persistence of picloram in soil. *Weeds* **1966**, *14*, 248−250.

Kenaga, E. E. Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. *Ecotoxicol. Environ. Saf.* **1980**, *4*, 26−38.

Keys, C. H.; Friesen, H. A. Persistence of picloram activity in soil. *Weed Sci.* **1968**, *16*, 341−343.

Khan, S. U. Equilibrium and kinetic studies of the adsorption of 2,4-D and picloram on humic acid. *Can. J. Soil Sci.* **1973**, *53*, 429−434.

Laning, E. R., Jr. Tordon controls deep-rooted perennial herbaceous weeds. *Down Earth* **1963**, *19* (1), 3−5.

Leahy, J. S.; Taylor, T. A gas-chromatographic determination of residues of picloram. *Analyst* **1967**, *92*, 371−374.

Lee, H. B.; Chau, A. S. Y. Analysis of pesticide residues by chemical derivatization VI. Analysis of ten acid herbicides in sediment. *J. Assoc. Off. Anal. Chem.* **1983**, *66* (4), 1023−1028.

Lee, H. B.; Peart, T. E.; Carron, J. M.; Tse, H. Chemical derivatization analysis of pesticide residues. Part XI. An improved method for the determination and confirmation of the acidic herbicides in water. *J. Assoc. Off. Anal. Chem.* **1991**, *74* (5), 835−842.

Leisure, J. K. Bioassay methods for 4-amino-3,5,6-trichloropicolinic acid. *Weeds* **1964**, *12*, 232−233.

Martin, T. D.; Creed, J. T.; Long, S. E. *Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements. Method 200.2 (Revision 2.3)*; U.S. Environmental Protection Agency, Office of Research and Development Environmental Monitoring Systems Laboratory: Cincinnati, OH, 1991.

McKone, C. E.; Cotterill, E. G. Extraction of picloram residues from a sandy loam soil. *Bull. Environ. Contam. Toxicol.* **1974**, *11* (3), 233−237.

Merkle, M. G.; Bovey, R. W.; Hall, R. The determination of picloram residues in soil using gas chromatography. *Weeds* **1966**, *14*, 161−164.

Osteryoung, J.; Whittaker, J. W. Picloram: solubility and acid-base equilibria determined by normal pulse polarography. *J. Agric. Food Chem.* **1980**, *28*, 95−97.

Pik, A. J.; Hodgson, G. W. Gas liquid chromatographic determination of 3,6-dichloropicolinic acid in soils. *J. Assoc. Off. Anal. Chem.* **1976**, *59* (2), 264−268.

Saha, J. G.; Gadallah, L. A. Determination of the herbicide Tordon (4-amino-3,5,6-trichloropicolinic Acid) in soil by electron capture gas chromatography. *J. Assoc. Off. Anal. Chem.* **1967**, *50* (3), 637−641.

Smith, A. E.; Milward, L. J. Comparison of solvent systems for the extraction of diclofop acid, picloram, simazine, and triallate from weathered field soils. *J. Agric. Food Chem.* **1983**, *31*, 633−637.

Walkley, A.; Black, I. A. An Examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29−38.

Worthing, C. R.; Hance, R. J. *The Pesticide Manual: A World Compendium*; The British Crop Protection Council; Unwin Brothers Ltd.: Old Working, Surrey, U.K., 1991; pp 181−182, 682−683; *Ibid.* 1983; p 262.

Received for review June 16, 1995. Accepted February 2, 1996.[⊗]

JF950365G

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.