# Novel Solvent Extraction of Lindane from Soil

Neil D. Westcott* and Brian L. Worobey[1]

Boron trifluoride–methanol, a reagent useful for esterifying acids, was used as a pretreatment for the extraction of lindane residues from soil. Boron trifluoride–methanol pretreatment resulted in an increase of lindane extracted from field-weathered soils of 20–70% compared to extraction with hexane–acetone. The lindane that was unextractable by hexane–acetone but was extractable with the boron trifluoride–methanol treatment was confirmed by chemical and mass spectral methods.

The adsorption of chemicals by soil is a complex reaction influenced by several factors including organic matter, clay, and mineral composition, pH, exchangeable cation content, moisture, temperature, and of course the nature of the chemical itself. It is recognized that some chemicals including pesticides can be strongly absorped or bound to the soil and are unextractable by normal extraction techniques (Lichtenstein et al., 1977; Khan and Hamilton, 1980). Lindane adsorption by soils has been studied by several investigators. Mills and Biggar (1969) report that the influence of temperature on solubility had a significant effect on adsorption. A correlation between clay content and lindane adsorption was observed by Swanson et al. (1954). However, the influence of organic matter on the adsorption of lindane is often of more importance than the other soil characteristics (Kay and Elrick, 1967; Adams and Li, 1971; Wahid and Sethunathan, 1979).

Studies on the adsorption and extraction of chemicals in soils have been aided by the use of radioisotopes. Many of the studies have shown that some of the radioisotope is unextractable from the soil by conventional techniques. This has given rise to concern on the ecological significance of the bound or strongly absorped residues. Attempts to release the bound residues has often involved drastic measures like combustion or hydrolysis, which prevents idenitification of the nature of the bound residue. High-temperature distillation has allowed the release of some bound residue although a portion is still thermally degraded on release (Khan and Hamilton, 1980).

Studies with several soil-applied radiolabeled herbicides have shown that some of the radioactivity was still associated with the fulvic acid, humic acid, or humin fractions after extraction (Helling and Krivonak, 1978). In studies on the occurrence of fatty acids, alkanes, and dialkyl phthalates in fulvic acid, it was determined that methylation of the fulvic acid allowed these organic compounds to be more fully extracted (Schnitzer and Onger, 1970; Onger and Schnitzer, 1970a,b). Reagents used in methylating fulvic and humic acids fractions include diazomethane, silver oxide–methyl iodide, and methyl iodide–methyl sulfinyl carbanion (Wershew and Pinckney, 1978).

The use of another methylating reagent, boron trifluoride–methanol, was interesting for several reasons. The reagent is commercially available and can be stored until required. When boron trifluoride is hydrolyzed, it will produce both boric and hydrofluoric acids. It is known that hydrochloric–hydrofluoric acid mixtures can alter the inorganic ion and mineral content associated with solubilized organic matter (Pietz and Adams 1974). Of more importance to the present study is the knowledge that boron trifluoride–methanol can methylate aromatic acids (Hallas, 1965). Boron trifluoride–methanol has been used previously to methylate suberin, a polymeric material derived from lipids (Kolattukady et al., 1975). As organic matter is composed of aromatic acids and phenols, it was thought that this reagent would at least partially methylate the organic matter and increase the amount of lindane that could be extracted.

**Table I. Extraction of Lindane from Weathered Soils**

| plot | method | lindane, application rate ppm | kg/ha |
|---|---|---|---|
| | Langham Soil | | |
| 14 | hexane–acetone | 0.23 | 1.12 |
| | boron trifluoride–methanol | 0.39 | |
| | boron trifluoride–methanol after hexane–acetone | 0.10 | |
| 11 | hexane–acetone | 0.49 | 1.12 |
| | boron trifluoride–methanol | 0.60 | |
| 15 | hexane–acetone | 1.11 | 3.36 |
| | boron trifluoride–methanol | 1.38 | |
| 4 | hexane–acetone | 1.52 | 3.36 |
| | boron trifluoride–methanol | 1.82 | |
| 6 | hexane–acetone | n.d.[a] | 0 |
| | boron trifluoride–methanol | n.d. | |
| | Bradwell Soil | | |
| 10 | hexane–acetone | 0.74 | 3.36 |
| | hexane–acetone repeat | 0.08 | |
| | boron trifluoride–methanol | 1.16 | |
| | boron trifluoride–methanol after hexane–acetone | 0.22 | |
| | methanol | 0.70 | |
| | acidified hexane–acetone | 0.98 | |

[a] Not detectable.

## EXPERIMENTAL SECTION

**Instrumentation.** A Hewlett-Packard Model 5713A gas chromatograph (GC) equipped with a $^{63}Ni$ electron capture detector was used for detecting the lindane and the dehydrohalogenation products obtained from the confirmation reaction. Lindane was separated on a glass column (6 ft by $^1/_8$ in. i.d.) packed with Chromsorb W (AW-DMCS) coated with either 5% SE-30 (column temperature 200 °C) or 4% SE-30–6% QF-1 (column temperature 190 °C). The dehydrohalogenation products obtained from the confirmation reaction were separated on a glass column (6 ft by $^1/_8$ in. i.d.) packed with 15% OV-101 on Chromosorb W at a column temperature of 130 °C. Carrier gas was argon–methane (95:5) at a flow rate of 40 mL/min for both lindane and the dehydrohalogenation products obtained from the confirmation reaction.

Research Station, Research Branch, Agriculture Canada, Saskatoon, Saskatchewan S7N 0X2, Canada.

[1] Present address: Health and Welfare Canada, Bureau of Chemical Safety, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada.

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The gas chromatograph–mass spectrometer (GC–MS) was a Varian GC Model 1400 interfaced with a Finnigan 3000 quadrapole mass spectrometer (MS). A stainless steel column (6 ft by $^1/_8$ in. o.d.) packed with 5% OV-101 on Chromosorb W(HP) with a helium flow rate of 25 mL/min at a column temperature of 205 °C was used for GC–MS investigations.

**Materials.** All solvents (excluding boron trifluoride–methanol) were distilled in glass grade. Boron trifluoride–methanol was 10–14% $BF_3$ in methanol and was used as received.

The plots (10 ft × 25 ft) were treated with a lindane emulsion at the rates indicated in Table I. The plots were immediately cultivated to a depth of 4–6 in. to incorporate the lindane. Composite soil samples were collected 16 months after treatment by combining 20 soil cores of 2-in. diameter obtained from the 0–6-in. soil depth. After collection the samples were stored at −20 °C for 6–8 months before being analyzed. Soil characteristics were as follows: Langham soil, sand 41.8%, silt 35.2%, clay 19.3%, organic matter 3.6%, and pH 7.6; Bradwell soil, sand 28.8%, silt 43.1%, clay 23.4%, organic matter 4.6%, and pH 5.4.

**Methods.** *Hexane–Acetone Extraction [Adapted from Head and McKercher (1971)].* The soil moisture of 20 g of air-dried soil was adjusted to 20% (w/w), and the soil was allowed to equilibrate for 1 h. A hexane–acetone solution (1:1 v/v, 100 mL) was added to the soil, and the mixture was shaken for 1 h on a mechanical shaker. The suspension was filtered into a 1-L separatory funnel containing distilled water (500 mL) and saturated sodium chloride solution (30 mL). The aqueous layer was extracted 3 times with petroleum ether (bp 30–60 °C; 100, 50, and 50 mL). The petroleum ether solution, dried by filtering through anhydrous sodium sulfate, was evaporated to near dryness, transferred to a volumetric flask, and brought to final volume with hexane for analysis.

*Boron Trifluoride–Methanol Extraction.* Air-dried soil (20 g) containing 7–10% moisture (w/w) and 30 mL of 10–14% boron trifluoride–methanol were placed in a stoppered flask and shaken for 1 h on a mechanical shaker. After 1 h hexane–acetone (1:1 v/v, 100 mL) was added and the shaking continued for 1 h. The solution was filtered as before into a separatory funnel containing salt solution and extracted with petroleum ether 3 times (100, 50, and 50 mL). The petroleum ether solution was dried with sodium sulfate, partially evaporated, and transferred to a volumetric flask, and the final volume was adjusted with hexane prior to analysis.

*Acidified Hexane–Acetone Extraction.* The soil moisture of 20 g of air-dried soil was adjusted to 20% and the soil allowed to equilibrate for 1 h before the addition of 4 mL of 50% sulfuric acid. The soil was extracted with acetone–hexane as before.

*Methanol Extraction.* The soil moisture of 20 g of air-dried soil was adjusted to 20%, and methanol (100 mL) was added. The mixture was shaken for 1 h before the mixture was filtered and the soil washed with methanol (50 mL). The methanol solution was poured into a separatory funnel containing water (500 mL) and saturated sodium chloride (30 mL) and extracted with petroleum ether (100, 50, and 50 mL) as before.

*Chemical Confirmation of Lindane [Adapted from Cochrane and Maybury (1973)].* An aliquot (10–50 μL) of the concentrated petroleum ether extract was transferred to a 15-mL stoppered test tube and the solvent evaporated to just dryness. The residue was dissolved in 2 mL of methanol, and powdered sodium methoxide (about 100 mg) was added. The stoppered tube was heated in a water bath at 60 °C for 15 min. The tube was removed and cooled, and sodium sulfate (0.5 g), water (5 mL), and benzene (2 mL) were added. The tube was shaken, the layers were allowed to separate, and the upper layer was analyzed by injection of an aliquot onto the 15% OV-101 column.

## RESULTS AND DISCUSSION

Boron trifluoride–methanol treatment of the soil resulted in more lindane being extracted compared to a conventional hexane–acetone extraction in all cases that were studied with field-weathered soils treated with lindane (Table I). Extraction of the same soil twice by the conventional extraction procedure did not result in as much total lindane being extracted as with one boron trifluoride–methanol extraction. Extraction of the soil with methanol or acidified acetone–hexane did not extract as much lindane as the boron trifluoride–methanol treatment described (Table I, Bradwell soil). This suggests that the increased recoveries observed cannot be explained solely on the basis of methanol as an extracting solvent or the acidic nature of boron trifluoride–methanol.

It was possible to extract soil samples with boron trifluoride–methanol that had been extracted with acetone–hexane and obtain an additional amount of lindane (see Langham plot 14 and Bradwell plot 10, Table I). That the additional extractable material contained lindane was determined by several methods. Control soils that had not been treated with lindane were extracted by both conventional and boron trifluoride–methanol pretreatment procedures, and no extractives with the same retention time of lindane could be detected by electron capture GC analysis. Extraction of lindane treated soil has a peak with the same retention time as authentic lindane, i.e., the γ isomer of BHC but not of the α isomer of BHC. In addition, the extract was subjected to single ion monitoring (SIM) by combined GC–MS of the $m/e$ 181 peak of lindane ($C_6H_4Cl_3$) (Safe et al., 1973). In the samples where boron trifluoride–methanol had extracted additional material that had a gas chromatographic peak corresponding to that of lindane, SIM at $m/e$ 181 detected a substance with the same retention time as that of lindane standard. It was also possible to quantitatively determine the amount of lindane present by measuring peak heights of the SIM chromatogram. The amount of lindane detected by SIM confirmed the amount previously detected by using the electron capture detector. When an aliquot of the material that was extractable by the boron trifluoride–methanol but not extractable by the conventional hexane–acetone procedure was subjected to the confirmation reaction, the chromatogram obtained had peaks corresponding to those of trichlorobenzenes. When lindane was subjected to the same reaction conditions, the chromatogram of the reaction mixture exhibited the same pattern of peaks. On this basis it was concluded that the boron trifluoride–methanol treatment of the field-weathered treated soil released more lindane from the soil than did the usual hexane–acetone procedure.

The experiments, while not clarifing the mechanism(s) of sorption or desorption of lindane by soil, do indicate that by using boron trifluoride–methanol it is possible to extract an additional portion of the lindane in the soil that was not extractable with hexane–acetone. It is not likely that boron trifluoride–methanol is a better solvent per se, but rather it causes some disruption of the soil complex, allowing more lindane to be released.

**Registry No.** Lindane, 58-89-9; boron trifluoride–methanol, 373-57-9.

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# Nondestructive Method for Determination of Water-Soluble Oxalate in Cultured *Amaranthus tricolor* Cells

Rita A. Teutonico[1] and Dietrich Knorr*

A nondestructive method was developed for screening cultured cells of *Amaranthus tricolor* to isolate low-oxalate variants, which upon regeneration could produce plants with improved bioavailability of calcium, iron, and zinc. Cells were chilled at 7 ± 1 °C for 45 min to enhance oxalate release, and methylated extracts were spotted on thin-layer chromatographic (TLC) plates that previously had been treated with a hydroxylamine hydrochloride–ferric chloride dye. Dimethyl oxalate spots were developed in an ammonia vapor and quantified with a densitometer. TLC determinations of the oxalate content of extracts gave reproducible results with a mean coefficient of variation of 4.51%. The TLC results correlated ($P < 0.01$) with oxalate data obtained from gas chromatographic analysis. In addition, cells were found to remain 77% viable after chilling. Thus, this screening method could be used effectively to isolate low-oxalate variants from cultured cells for the generation of low-oxalate *A. tricolor* cell lines.

At present, there is much investigation into the use of plant tissue culture for synthesis of food ingredients and food production (Nickell, 1980; Teutonico and Knorr, 1984a). Current applications of plant tissue culture methods to improve food productivity include increased stress tolerance such as salt, frost, or disease resistance of plants (Chaleff, 1983; Widholm 1979), an increase of yield including an increase in protein content (Schaeffer, 1981) and modification of processing characteristics of plant foods such as flavor, color, and texture (Sharp et al., 1984). Teutonico and Knorr (1984a) recently examined the improvement of food productivity through the reduction of nutritional stress factors in plants that increases the availability of essential nutrients and consequently increases the amount of nutrients available per unit weight of plant food.

Oxalate is a naturally occurring nutritional stress factor that interferes with the absorption of the essential nutrients calcium, iron (Bothwell and Charlton, 1982), and to a certain extent zinc (Kelsay, 1981).

Oxalate is found in appreciable quantities in spinach (5.6% dry weight), rhubarb (7.8% dry weight), swiss chard (5.5% dry weight), and amaranth (7.2% dry weight), a recently rerecognized ancient food crop (NAS, 1975, 1984; Sanchez-Marroquin et al., 1980).

Our approach for reducing the oxalate content of plant foods utilizes the natural and induced variability possible among cells in tissue culture by selecting for variant cell lines with the desired quality (Teutonico and Knorr, 1984a). One of the key problems in the selection of low-oxalate cell lines is the lack of a simple nondestructive screening test for oxalate.

Traditional methods of oxalate analyses are destructive, involving extraction from the plant material, followed by a number of different quantitative procedures as reviewed by Hodgkinson (1977). Prior to 1977, the most commonly used procedures were permanganate titration, various colorimetric methods, and paper chromatography, while radioisotope and gas chromatographic methods, though more accurate, were used to a lesser extent (Hodgkinson, 1977). Since 1977, improvements in enzymatic (Potezny et al., 1983) and gas chromatographic (Sarkar and Malhotra, 1979) methods, as well as development of high-pressure liquid chromatographic analyses for oxalate (Bushway et al., 1984; Libert, 1981; Wilson et al., 1982), have made these the preferred procedures, but they all still involve sample homogenization. This makes these methods inappropriate for screening since the selected plant cell cannot be regrown on a basal medium.

Preliminary studies (Teutonico, 1984; Teutonico and Knorr, 1984a) have shown that chilling of *Amaranthus tricolor* cells results in oxalate release while Palta and Li (1980) found that while chilling down to −2.5 °C resulted in ion leakage from *Solanum tuberosum* cells the freezing

Department of Food Science and Human Nutrition, University of Delaware, Newark, Delaware 19716.
[1]Present address: Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

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