Enantiomeric Ratios as Tracers of Soil-Air Exchange for Organochlorine Pesticides

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ABSTRACT

Past research has shown that selective degradation of enantiomers by microorganisms does occur. This work was done to determine concentrations and enantiomeric ratios of several chiral pesticides above an agricultural soil to determine if volatilization from the soil to the overlying air occurs. Quantitative analysis was done using gas chromatography with electron capture detection on a DB-5 capillary column. Concentrations were determined for five compounds (trans-Chlordane, cis-Chlordane, trans-Nonachlor, α-hexachlorocyclohexane, and γ-hexachlorocyclohexane) at varying heights above an agricultural soil for the week of June 21-26, 1998. Trans-Nonachlor was found to be the highest in concentration for the five pesticides while α-hexachlorocyclohexane was the lowest. Concentration gradients were determined for all five compounds.

Enantiomeric analysis was done for trans-Chlordane, cis-Chlordane, and MC-5 (another chlordane component) using gas chromatography-mass spectrometry on a chiral phase capillary column. Enantiomeric excesses were found for all three pesticides. The enantiomeric ratios of the pesticides in overlying air were constant at all four heights above the soil and agreed well with the soil ERs determined previously. This suggests the soil as the primary source of these pesticides to the overlying air.

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BACKGROUND

Pesticides

Pesticides are a group of chemicals that are used to control the various pests that can effect agricultural or ornamental crops. There are three major classes of pesticides: insecticides (insect control), fungicides (fungi control), and herbicides (plant control).

Pesticides in the environment are a major concern due to their widespread usage. They are applied yearly to approximately 220 million acres in the United States for such major crops such as corn, soybean, wheat and cotton (Manahan, 1994). For home and minor use such as ornamental plants, orchards, fruits, and vegetable gardens, they are applied to approximately 8 million acres yearly (Manahan, 1994).

Pesticide residues and their metabolites have been detected in soil, air, and water all over the world since their use began. Residues have been found in a great diversity of aquatic and terrestrial animals such as porpoises, fish, and deer (Granby and Kinze, 1991; Mandenjian et al., 1998; Pfaffenburger et al., 1994). Oftentimes, pesticide levels in animals are greater than levels in the surrounding environment. When concentrations in biota reach toxic amounts, they can cause adverse health effects. Pesticides have been attributed to the deaths of many types of wildlife such as waterfowl and fish and, in rare cases, humans (PHSa, 1992; PHSb, 1992; PHSc, 1992; Valigura et al., 1994).

Organochlorine Pesticides

One specific group of pesticides has received a lot of attention, namely the organochlorine (OC) pesticides. OC pesticides are organic compounds containing one or

more chlorine atoms and have been the subject of much study due to their high toxicity and persistence. These properties make OCs excellent pesticides, but also increase their negative impact on the environment. Many OC pesticides have been banned in the U.S. and Canada due to their adverse affects (PHSa, 1992; PHSb, 1992; PHSc, 1992; PHSd, 1992). However, they are still used in underdeveloped countries across the globe due to their effectiveness and low cost (PHSa, 1992; Manahan, 1994).

The range of adverse health effects caused by OC pesticides is very broad. Disruption of the nervous system is one effect shared by several OC pesticides including DDT and chlordane. The common symptoms include headaches, dizziness, nausea, and, in high levels, tremors and seizures (PHSa, 1992; PHSb, 1992; PHSc, 1992; PHSd, 1992). OC pesticides have also been known to effect liver enzyme production as was seen in animal studies using Chlordane and Heptachlor (PHSa, 1992; PHSb, 1992). Aldrin, Dieldrin, and Chlordane can effect the digestive system causing problems such as upset stomach, vomiting, and diarrhea (PHSd, 1992; PHSa, 1992). Chloracne is a skin affliction associated with exposure to chlorinated compounds like OC pesticides (DOVA, 1997). Most OC pesticides are also confirmed or suspected carcinogens (PHSa, 1992; PHSb, 1992; PHSc, 1992; PHSd, 1992; DOVA, 1997).

Chlordane

Chlordane (1,2,4,5,6,7,8,8-Octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene) (Figure 1) was used in the United States from 1948 to 1988 (PHSa, 1992). Other common names include Octachlor and Velsicol 1068. Technical chlordane is a mixture of approximately 180 compounds. The major constituents are trans-chlordane (TC), cis-

chlordane (CC), heptachlor (HEPT), and trans-nonachlor (TN) (Figure 2) (PHSa, 1992). Chlordane's primary use was for agricultural crops until it's ban in 1983 (PHSa, 1992). The only approved use of chlordane from 1983-1988 was for termite control in homes, where it was applied to soils around the house or into the foundation of the home (PHSa, 1992). Chlordane was also used as a general pesticide on lawns, and gardens prior to 1983. Chlordane is still manufactured in the U.S. for export (PHSa, 1992).

Chlordane has been found to have several adverse health effects. The major effected areas are the nervous system, digestive system, and the liver. One study found animals given high levels of chlordane (0.7 mg/day) for short periods of time died or exhibited convulsions (Dearth and Hites, 1991) and mice subjected to long-term chlordane exposure contracted liver cancer (PHSa, 1992). Chlordane is a suspected human carcinogen (PHSa, 1992).

Chlordane is a persistent chemical which has been reported to have a half-life in soil of more than 20 years (PHSa, 1992). It's lifetime in the atmosphere (approximately 8 days) allows it to be involved in long range mass transport (Valigura et al., 1994). Chlordane has been detected in remote areas such as the Canadian Arctic, where it was found in fish and plankton at concentrations as high as 107 ng/g and 20 ng/g, respectively (Hargrave et al., 1992). A study in British Columbia found concentrations in soils as high as 979 ng/g for TC and 367 ng/g for CC (Falconer et al., 1997). Chlordane was detected in soils in Nara, Japan at a concentration of 15 ng/g (Nagami, 1997). Chlordane is thought to metabolize in the environment primarily to Oxy-chlordane (OXY) (Figure 2) (PHSa, 1992). OXY is also toxic and persistent and is found regularly in environmental samples (PHSa, 1992).

Hexachlorocyclohexane

Hexachlorocyclohexane (HCH), also known as benzene hexachloride (BHC), is an OC pesticide used until the late 1970's. (Note: the name benzene hexachloride (BHC) is chemically incorrect). HCH was first synthesized in 1825, but it's insecticidal properties were not discovered until 1940 (Fed. Regist., 1978). The production of HCH yields five isomers: alpha (α) (Figure 3), beta (β), gamma (γ) (Figure 3), delta (δ), and epsilon (ε) (Fed. Regist., 1978). The technical mixture of HCH contains all five isomers and each are present in approximately the following percentages: α -70%, β -6%, γ -13%, δ -6%, and ε- trace amounts (Cremlyn, 1978). HCH was used to battle insects such as flea beetles and mushroom flies on agricultural crops (Fed. Regist., 1978). Technical HCH was used in the U.S. from it's introduction in 1940 until 1978 when it was banned (Fed. Regist., 1978).

The only insecticidally active isomer of HCH is the gamma isomer. γ-HCH, also known as Lindane, is the least persistent isomer in soil, partly due to it's high volatility (Ware, 1983) which gives it a relatively long atmospheric lifetime (about 15 days) (Valigura et al., 1994). Due to the high toxicity and non-activity of the other isomers, γ-HCH has been manufactured alone (99% pure) (Cremlyn, 1978). Lindane has been used against ticks, mosquitoes and pests in agricultural storage facilities as well as in the home as a fumigant for insects. This last use was stopped when it's toxicological effects on humans were discovered (Cremlyn, 1978). Lindane is still currently used in Canada and the U.S. as a seed dressing for corn (PHSb, 1992).

As with most OC pesticides, HCH has been found in a wide variety of biota. Seawater plankton have been found to contain HCH, with one study collected over the Canadian Arctic continental shelf detecting α-HCH, β-HCH, and γ-HCH at concentrations of 120, 10, and 30 ng/g respectively (Hargrave et al., 1992). A study of Roe-Deer in Germany found liver concentrations as high as 300 ng/g (Pfaffenberger et al., 1994). Northern fur seals in Alaska were reported to have HCH concentrations in brain as high as 138, 10, and 3.9 ng/g for α-HCH, β-HCH, and γ-HCH, respectively (Mossner et al., 1992).

Pesticide Enantiomers

Isomers are compounds that share the same molecular formula but not the same structure. Enantiomers (which come from chiral molecules) are non-superimposable mirror image isomers. Molecules that are enantiomers differ only by the arrangement of their atoms in space; their physical properties are identical as well as their chemical reactions with achiral molecules. The only difference between enantiomers is their rotation of plane polarized light and their reactions with enzymes or other chiral molecules. Enantioselectivity (different reactions and/or different reaction rates for the two enantiomers) is observed regularly in biological reactions with enzymes and proteins which are also chiral. Approximately 25% of past and current-use pesticides are chiral molecules. These pesticides are manufactured as racemic mixtures; in other words, as a 1:1 ratio of the enantiomers.

A useful way to discuss enantiomeric compounds is by using the enantiomer ratio (ER). The ER has been defined as the concentration or area of the (+) enantiomer

divided by that of the (-) enantiomer. The (+) and (-) designations refer to the enantiomers rotation of plane polarized light ((+) = right-handed rotation, (-) = left-handed rotation).

Pesticides can be degraded in the environment by chemical processes (photolysis, hydrolysis) and biological (microbial) breakdown. The latter is the only known mechanism that can result in enantioselective breakdown (Buser and Müller, 1992a). It has been proposed that chiral pesticides which have spent a relatively short amount of time in the environment (recently applied) will exhibit a racemic ER (ER = 1), the same as the original mixture (Buser and Müller, 1992a). However, chiral compounds that have been slowly degrading for years in soil or water may show non-racemic ERs (ER $\neq$1) due to enantioselective biological decomposition.

Enantiomeric ratios may be useful for distinguishing the source of past-use pesticides to the atmosphere. Differentiating "old" sources of pesticides (volatilization from soils sprayed many years ago) from "new" sources (recent applications in countries still using these chemicals) is important for controlling and understanding current levels of these pesticides in the environment.

Chiral-Phase Analysis

Historically, quantitative analysis of OC pesticides has been done using gas chromatography equipped with standard non-polar capillary columns (DB-5, OV1701, etc.). However, these columns separate compounds primarily based on vapor pressure and are not able to separate enantiomers because the vapor pressures are the same for both enantiomers (Buser and Müller, 1992a,b).

In order to successfully characterize enantiomers, a column with a chiral probe must be used. Past research has shown that modified cyclodextrins perform well for this type of separation due to the fact that cyclodextrin is chiral (Buser and Müller, 1994, 1995). Cyclodextrins have been used in LC and GC for years, primarily for the separation of chiral drugs and flavors (Dietrich et al., 1995).

Cyclodextrins are cyclic oligosaccharides containing 6-8 α-$_D$-glucose units linked by α-1,4 glycoside bonds (Figure 4). Cyclodextrin columns are usually prepared by coating fused silica capillary columns with amorphous cyclodextrin derivatives. Researchers have found that alkylated, acylated and/or silyated cyclodextrins are the most suitable stationary phases for separating chiral organochlorine compounds (Vetter and Schurig, 1997).

There has been considerable speculation on the mechanism of enantioselective retention by chiral stationary phases. However, few studies have been done. One study suggests there are at least two different enantioselective mechanisms for GC cyclodextrin stationary phases: inclusion complex formations, and loose, external associations. Compounds have been found that follow each of the models, and several have exhibited behavior intermediate of the two (Shurig, 1988; Berthod et al., 1992). Cyclodextrins columns have been used to separate a number of chiral pesticides including α-HCH, TC, CC, o,p'-DDT, Heptachlor, Heptachlor Epoxide, Oxychlordane, mecoprop and others (Buser and Müller, 1992a, 1992b, 1994, 1995; Falconer et al, 1997).

Soil Volatilization Models

Long range transport of pesticides has made many pollutants ubiquitous over the globe. In an attempt to understand the first step of this transport (volatilization from soils), researchers have developed various volatilization models. Some of the most sophisticated models involve evaporation, water flow, pesticide movement in soil, application, degradation, volatilization, leaf and root growth, temperature, and uptake by plants (Valigura et al., 1994). These models require advanced computer technology. Other, less complex models have been made which take into account mainly volatilization, degradation, soil properties, and sorption of pesticides to the soil. Some of these models yield only qualitative information (Valigura et al., 1994).

For all of the models, one of the most important processes to understand is volatilization: the exchange of pesticides between the air and soil or water. Air exchange with soil or water is one of the major sources of pesticides entering the environment especially on a global scale (Valigura et al., 1994). Temperature and atmospheric turbulence are the two most important factors effecting volatilization.

Finizio et al. (1998) studied chiral pesticides in soil and air above a soil in British Columbia known to contain non-racemic pesticide residues and found enantiomeric differences for several of the pesticides studied. The (-) enantiomer of α-HCH was depleted by 38% and the (+) enantiomer of Heptachlorepoxide was enriched by about 39% indicating enantioselective degradation. The ER of o,p'-DDT was found to be close to one. The authors also examined the relationship between the relative concentrations of OCs in air to those in soil by using a soil-air exchange model. The study provided

evidence that the pesticide residues detected in the air were from soil volatilization for all the compounds studied except α-HCH (Finizio et al., 1998).

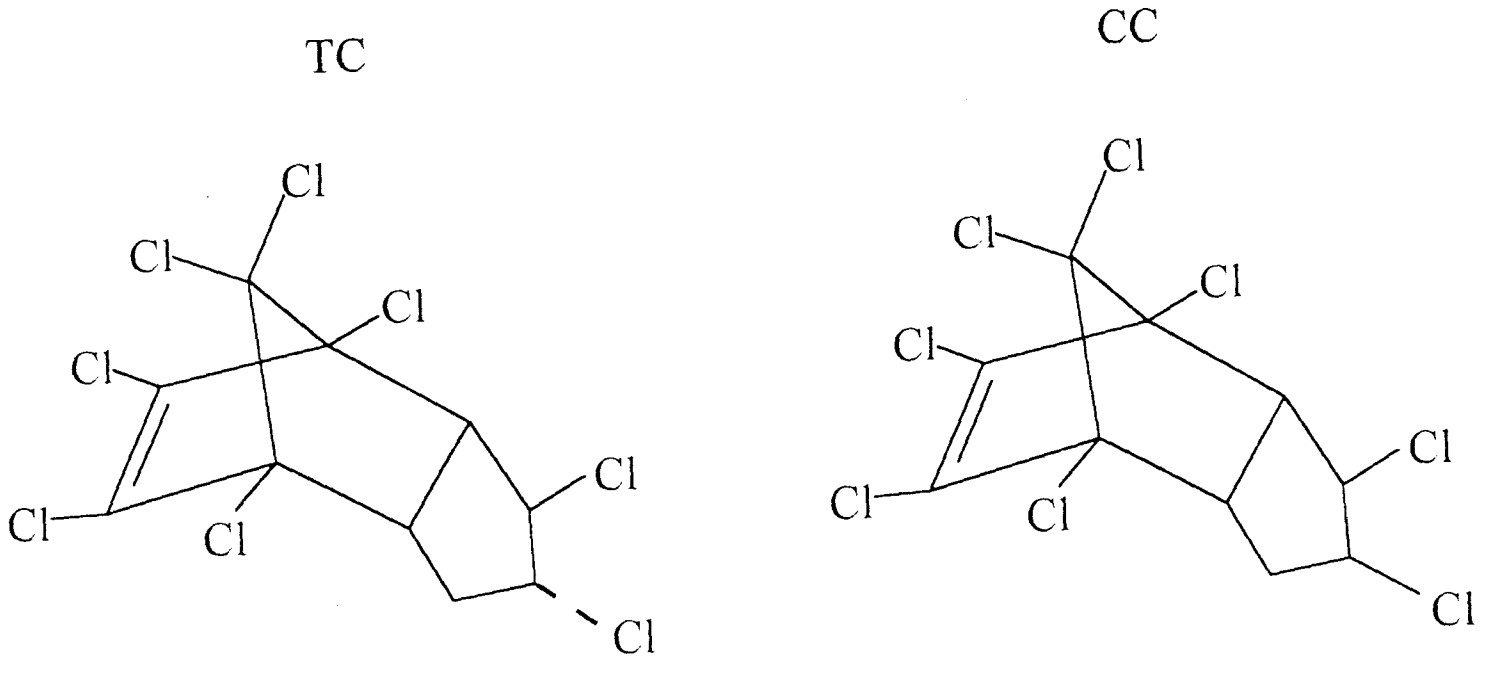


Figure 1. Structures of cis-Chlordane and trans-Chlordane

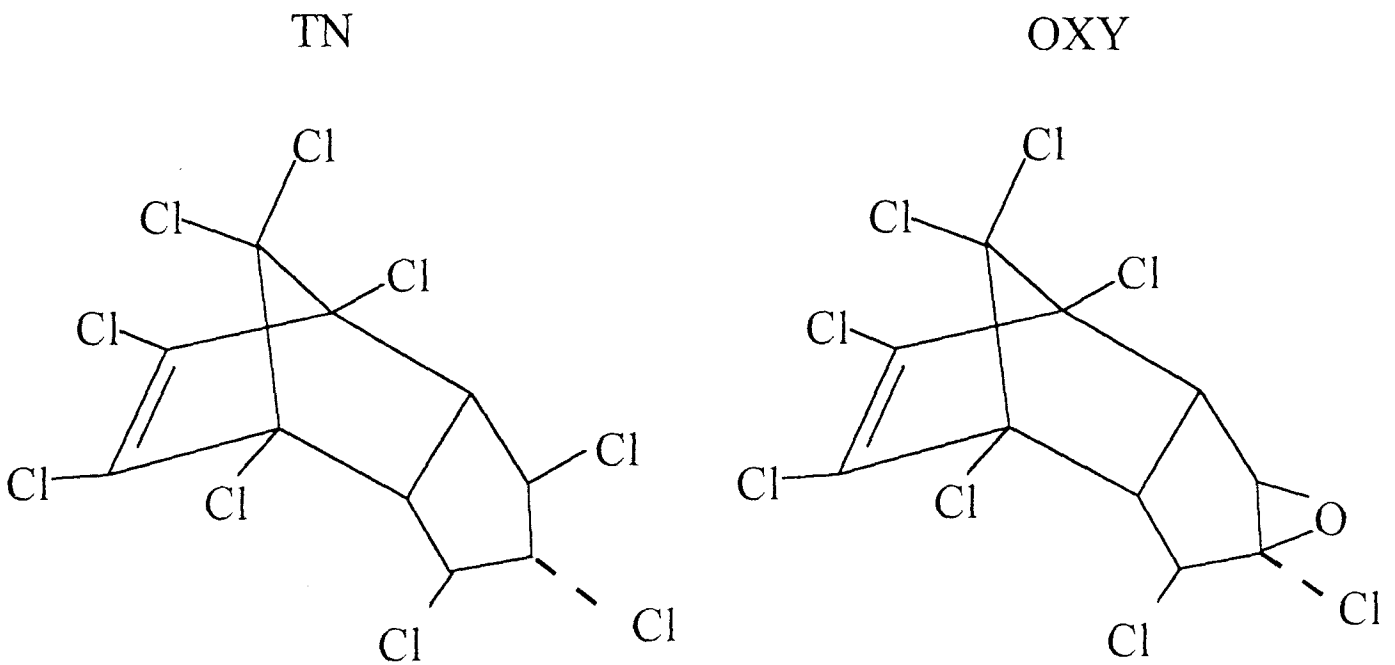


Figure 2. Structure of trans-Nonachlor and Oxy-Chlordane

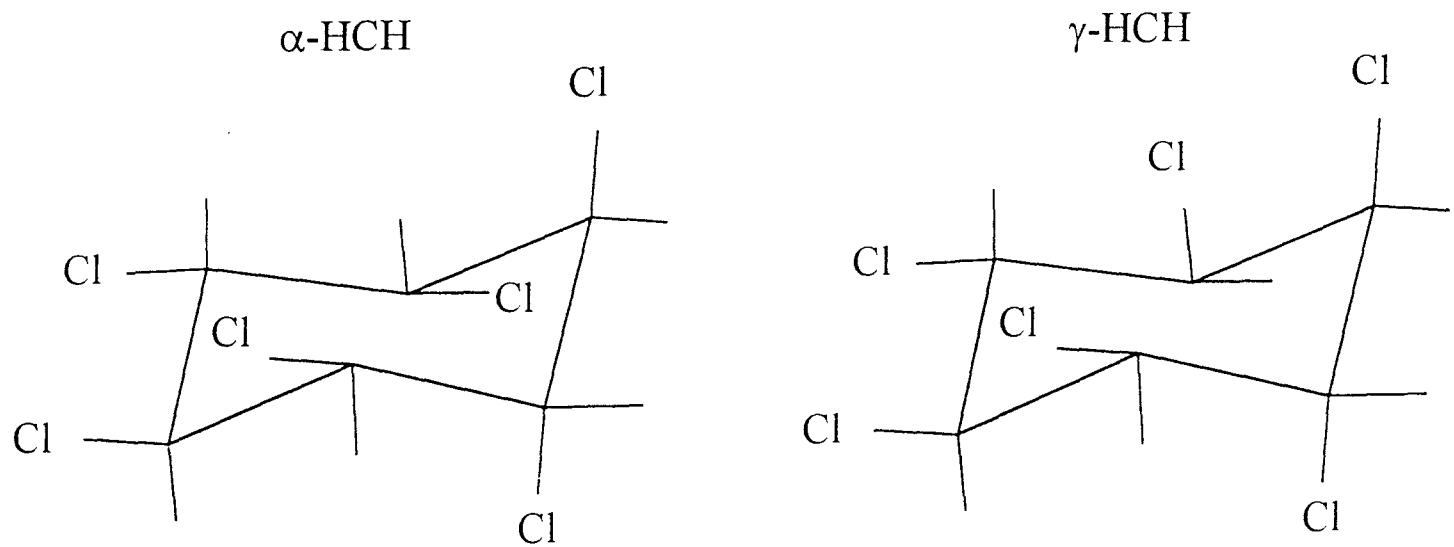


Figure 3. Structures of α and γ hexachlorocyclohexane

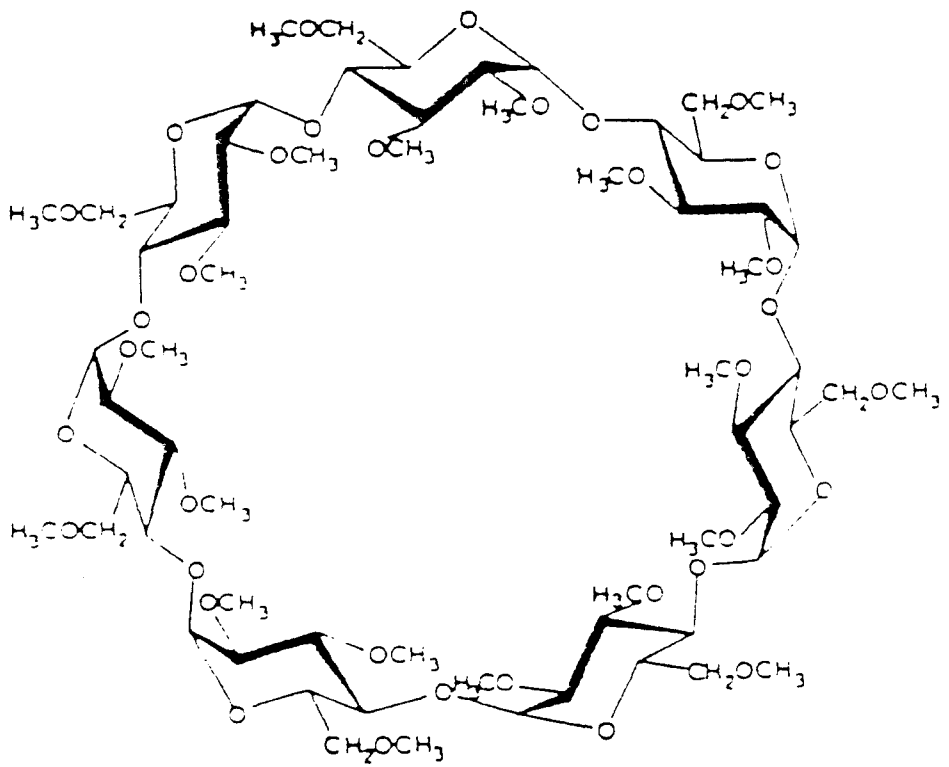


Figure 4. Structure of typical cyclodextrin molecule

STATEMENT OF PURPOSE

Due to the persistence and heavy usage of organochlorine pesticides in the U.S., past usage may be a continuing source of these pollutants to the Great Lakes. Many third-world countries continue to use these toxic pesticides because of their effectiveness, low cost, and ease of production. These countries may be another potential source of these compounds to the Great Lakes environment through long-range transport. Differentiating past and present sources of pesticides to the Great Lakes is essential for understanding and controlling present levels. OC pesticides in soils can be degraded by two different processes; chemical breakdown and microbial attack. Microbial degradation is the only process known to result in enantioselective degradation of chiral pesticides. All of the chiral pesticides explored in the present study were manufactured as racemic mixtures. If chemical degradation of the pesticides is occurring, the enantiomer ratio (ER) should be 1.00. This would be expected for new releases from current sources which have not been subjected to long periods of microbial action. ERs different from 1.00 suggest biological breakdown has occurred and may be used to differentiate "old" pesticide releases from "new" releases.

Past research has shown that microorganisms can selectively degrade enantiomeric compounds in soil, water, and biota. This study was done to determine if volatilization from the soil to the overlying air occurs and can be tracked using ERs.

Concentrations and ERs of OC pesticides have been determined previously in an agricultural soil from eastern Ohio with average soil TC, CC, and TN concentrations found to be 13.0, 23.0, and 33.0 ng/g respectively (Aigner et al., 1998). In a follow-up study, concentrations and ERs were determined in air directly above the soil (10 cm) with

ERs of 0.79 and 1.10 for TC and CC, respectively (Leone, 1998). In the present study, the concentrations and ERs for several OC pesticides were determined at several heights above the soil to determine concentration gradients and ER profiles at varying heights.

MATERIALS

Pesticide grade solvents (acetone, hexanes, iso-octane, dichloromethane, and petroleum ether) as well as concentrated sulfuric acid were purchased from Fisher Scientific (Pittsburgh, PA). Analytical standards were purchased from ULTRA Scientific North Kingstown, RI and CDN Isotopes (Pointe-Clair, Quebec). Single enantiomer standards of chiral pesticides used to determine elution orders on chiral columns were purchased from AXACT Standards (Commack, NY). The nitrogen used to concentrate samples (dry grade) was purchased from Praxair (Danbury, CT). Ultra-high grade purity helium, hydrogen, methane, and nitrogen used in chromatographic instruments were purchased from Praxair (Danbury, CT) or Air Products Canada, Ltd. (Brampton, ON). Sodium sulfate (anhydrous-granular) was purchased from Fisher Scientific and neutral alumina (Al_2O_3, 70-230 mesh) was purchased from Sigma (St. Louis, MO). Silicic acid (SA, 100-mesh) was purchased from Mallinckrodt Chemical Works (Chesterfield, MO). All solids were baked overnight (18-24 hours) in a drying oven at 250 ^{o}C and stored in clean glass jars with Teflon-lined lids.

Boiling chips were cleaned overnight by soxhlet extraction with petroleum ether (PE) in a cellulose extraction thimble, dried at 250 ^{o}C, and stored in a clean glass jar with a Teflon-lined lid. Polyurethane foam plugs (PUF) were purchased from Graseby Anderson (Cleves, OH). The PUF were extracted overnight with PE, dried overnight in a dry seal vacuum desicator with low heat and stored in clean glass jars with Teflon-lined lids.

The air sampler consisted of polytetrafluroethylene filter holder sampling heads, connected to a mass flow meter (Sierra Instruments Incorporated, Monterey, CA) followed by a vacuum pump (Gast, Benton Harbor, MI).

EXPERIMENTAL

Sample Collection

Air samples were collected at a farm in Northeast Ohio from July 21-26, 1998. Samples were taken for 12 hour increments (day and night), at four heights above the soil. Samples are hereafter referred to as 1 (12.7 cm above ground), 2 (50.8 cm above ground), 3 (106.7 cm above ground), and 4 (177.8 cm above ground). Air was collected on 3.5 cm length x 5 cm diameter polyurethane foam plugs (PUF) which were precleaned by soxhlet extraction with petroleum ether (PE). Air was pulled through the PUF using a vacuum pump and flow rates were determined using a digital flow meter. Ambient temperature was recorded at the beginning and end of each sampling period using a standard mercury thermometer. Immediately following collection, samples were spiked with 50 μL of deuterated α-HCH and placed separately in clean glass jars with Teflon-lined lids. Samples were transported on ice to the lab where they were stored at 4 °C until further workup. Sample collection data is given in Table 1.

Extraction and Clean-Up

Sample PUF plugs were soxhlet extracted in PE for 18-20 hours. Extracts were reduced and solvent exchanged into hexanes by rotary evaporation and further concentrated to 1 mL with a gentle stream of nitrogen. Samples were fractionated using a silicic acid (SA) cleanup column consisting of three layers (top to bottom): 0.5 g Na_2SO_4, 2 g Al_2O_3 (6% H_2O added), and 3 g SA (3% H_2O added). The columns were dry packed and cleaned with 30 mL dichloromethane (DCM) followed by 30 mL PE. Solvents were

pushed through the column using nitrogen. The sample was added and eluted in two fractions. Fraction 1 (F1) was eluted with 30 mL PE and contained HEPT, Aldrin and small amounts of TN, o,p'-DDT, p,p'-DDE, p,p'-DDD, and p,p'-DDT. Fraction 2 (F2) was eluted with 30 mL DCM and contained HCHs, HEPX, TC, CC, Dieldrin and the remaining o,p'-DDT, p,p'-DDE, p,p'-DDD, and p,p'-DDT. Both fractions were concentrated to 1.5-2 mL and solvent exchanged into iso-octane using nitrogen. A 200 μL portion of F2 was removed for analysis of dieldrin and HEPX. The remaining F2 and F1 were further cleaned using 1 mL of concentrated sulfuric acid (H_2SO_4) and adjusted to 2 mL for analysis. Immediately before analysis, 1385 ng of Mirex was added to samples to act as an internal standard. For this study, data will be reported for TC, CC, TN, α-HCH, and γ-HCH.

Analysis

Quantitative analysis was done on a Hewlett-Packard 5890 gas chromatograph equipped with an electron capture detector (GC-ECD) using a DB-5 column (60 m, 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific). Samples were injected splitless (split opened after 1.0 min) at an initial temperature of 90 °C. After a 1-min hold, the oven was ramped at 10 °C min^{-1} to 160 °C, 2 °C min^{-1} to 240 °C, 20 °C min^{-1} to 270 °C, and held for 10 min. Injector and detector temperatures were 250 °C and 300 °C, respectively. The carrier gas was hydrogen at 60 cm s^{-1}. Samples were quantified versus five standards that spanned a 1000 fold concentration range. Examples of calibration curves are shown in the Appendix (Figures A-1 to A-5). Chromatographic data was processed and collected using HP Chemstation software.

Enantiomeric analysis was done for TC, CC, and MC-5 with a Hewlett- Packard 5890 GC-5989B MS Engine mass spectrometer (GC-MS) operated in the negative chemical ionization mode (NIMS). Separations were carried out using a Betadex-120 column (20% permethylated β-cyclodextrin in SPB-35, 30 m, 0.25 mm i.d., 0.25 μm film thickness; Supelco Corp). Samples (2 μL) were injected splitless (split opened after 1.0 min) at an oven temperature of 90 °C. After a 1-min hold, the following oven program was used: Betadex, 15 °C min^{-1} to 140 °C, 1 °C min^{-1} to 190 °C, hold 10 min, 20 °C min^{-1} to 230 °C, hold 10 min. Carrier gas was helium at 50 cm s^{-1}; injector and transfer line temperatures were 250 °C. The ion source and quadrupole temperatures were 150 °C and 100 °C, respectively. The chemical ionization gas used was methane (1.0 Torr). The instrument was operated in the selected ion monitoring mode using the 410 m/z and 412 m/z ions. The elution orders for the enantiomers of CC, TC, and MC-5 are listed in Table A-6.

Quality Control samples were quantated using a Varian 3800 gas chromatograph-Saturn 2000 mass spectrometer operated in positive ion mode. Separations were done using a DB-5 column (60 m, 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific). Samples were injected splitless (split open after 0.80 min) at an initial temperature of 90 °C. After a 1-min hold, the oven was ramped at 10 °C min^{-1} to 160 °C, 2 °C min^{-1} to 240 °C, 20 °C min^{-1} to 270 °C, and held for 10 min. Injector temperature was 260 °C and the transferline was 170 °C. The carrier gas was helium at 60 cm s^{-1}. Data was collected and processed using Varian Saturn Workstation software.

Table 1. Sampling Parameters

	Temp Initial (Celsius)	Start Time	Stop Time	Initial Flow (L/min)	Final Flow (L/min)	Total Time (min)	Ave. Flow (L/min)	L of air (L)	m3 air
21-D-1	30.5	12:00pm	8:05 am	35.60	34.00	485	34.80	16878	16.9
21-D-2				32.40	31.00	485	31.70	15375	15.4
21-D-3				40.80	39.80	485	40.30	19546	19.5
21-D-4				35.40	34.60	485	35.00	16975	17.0
21-N-1	†N/A	8:22 am	8:14 pm	35.60	34.00	712	34.80	24778	24.8
21-N-2				42.68	42.00	712	42.34	30146	30.1
21-N-3				37.80	35.40	712	36.60	26059	26.1
21-N-4				36.60	34.60	712	35.60	25347	25.3
22-D-1	N/A	9:02 am	8:10 pm	38.80	36.40	668	37.60	25117	25.1
22-D-2				40.80	37.20	668	39.00	26052	26.1
22-D-3				39.40	35.40	668	37.40	24983	25.0
22-D-4				36.40	34.40	668	35.40	23647	23.6
22-N-1	26	8:30 am	7:35 pm	37.80	36.40	665	37.10	24672	24.7
22-N-2				39.80	38.00	665	38.90	25869	25.9
22-N-3				36.40	35.00	665	35.70	23741	23.7
22-N-4				34.20	33.00	665	33.60	22344	22.3
23-D-1	23	8:05 am	7:25 pm	43.00	39.20	680	41.10	27948	27.9
23-D-2				35.60	29.60	680	32.60	22168	22.2
23-D-3				35.00	30.20	680	32.60	22168	22.2
23-D-4				34.20	30.40	680	32.30	21964	22.0
23-N-1	23.3	7:58 am	8:05 pm	30.60	21.20	727	25.90	18829	18.8
23-N-2				29.00	26.60	727	27.80	20211	20.2
23-N-3				38.80	15.00	727	26.90	19556	19.6
23-N-4				29.40	26.60	727	28.00	20356	20.4
24-D-1	25	8:45 am	7:27 pm	34.00	29.60	642	31.80	20416	20.4
24-D-2				35.00	29.80	642	32.40	20801	20.8
24-D-3				34.40	30.40	642	32.40	20801	20.8
24-D-4				38.20	34.60	642	36.40	23369	23.4
24-N-1	27.8	8:02 am	7:25 pm	36.80	36.60	683	36.70	25066	25.1
24-N-2				37.20	38.20	683	37.70	25749	25.7
24-N-3				41.60	40.00	683	40.80	27866	27.9
24-N-4				31.60	34.20	683	32.90	22471	22.5
25-D-1	25	7:55 am	7:30 pm	33.60	27.80	695	30.70	21337	21.3
25-D-2				37.40	31.60	695	34.50	23978	24.0
25-D-3				39.20	35.60	695	37.40	25993	26.0
25-D-4				36.20	32.40	695	34.30	23839	23.8
26-D-1	N/A	8:40 am	7:10 pm	36.60	31.00	630	33.80	21294	21.3
26-D-2				38.80	32.40	630	35.60	22428	22.4
26-D-3				37.00	31.60	630	34.30	21609	21.6
26-D-4				33.20	28.20	630	30.70	19341	19.3

†N/A = Not determined

*25-N was not analyzed due to mechanical failure during this sampling period

QUALITY CONTROL

Quantitative

Four blanks were processed by extracting and analyzing clean PUF plugs using the same procedure as for samples. None of the blanks analyzed contained measurable levels of the compounds of interest so no blank corrections were made.

Four recovery experiments were done by spiking 0.8 ng of each pesticide onto a clean PUF and extracting and analyzing them as for samples. Average spike recoveries ranged from 83.1-89.7% for all compounds analyzed (Table 2).

Enantiomeric

The enantiomeric ratio is defined as the ratio of the area of the (+)/(-) peak eluting from the cyclodextrin column. Single enantiomer standards and previosly published elution orders were used. Injections of analytical standards reflected racemic compositions with standard deviations ranging from 0.00 to 0.03 for all compounds. This demonstrates that chiral-phase GC-MS is capable of precise enantioselective analysis. The following limits were set as a quality control protocol: (a) agreement of ER values at each of the two monitored ions within 5%; (b) agreement of area ratios of the two monitored ions for samples and standards within 5%. Enatiomeric data not meeting both criteria were not reported.

Silicic Acid Experiments

Fractionation of eluents was done using silicic acid (SA) deactivated with 3% H_2O. For the five compounds studied here, the SA fractionates these compounds into F2,

and removes interferences from PCBs (which fraction into F1). Separations using SA are dependent on the number of active sites, which can be controlled with the amount of H_2O added. The SA used for fractionating air samples was allowed to equilibrate with the H_2O for at least one hour after addition. The amount of time required for the H_2O to equilibrate with the SA and the stability of that equilibrium was explored as part of this study. Time duplicate experiments were conducted to determine how the compounds of interest fractionated as a function of the time the SA is allowed to equilibrate with H_2O.

Experimental parameters are shown in Table 3. For this experiment, the SA and water were equilibrated at various times from 1 min to 120 hours. Regardless of equilibration time, α-HCH and γ-HCH were recovered completely in F2. CC was fractionated into F2 in all samples with equilibration time less than 18 hours. Samples that equilibrated for 18, 25, and 45 hours showed a small portion of CC eluting in F1 (average ≈ 5%). For various equilibration times, a small portion of TC eluted in F1 (less than 3%) which is likely insignificant statistically.

These experiments show that for the five compounds explored, equilibration time was not a major factor (less than 5%) in the effectiveness of SA to fractionate certain pesticides into the appropriate fraction. Further studies are necessary to determine if equilibration time has an effect on the fractionation of other OC pesticides as well as PCBs and polycyclic aromatic hydrocarbons.

Table 2. Spike Recoveries of CC, TC, Alpha-HCH, and Gamma-HCH (ave. % recoveries)

CC	TC	Alpha-HCH	Gamma-HCH
89.7	87.7	81.2	83.1

Table 3. Experimental parameters for SA equilibrium time experiments (% in each fraction)

Time (hours)		alpha-HCH	gamma-HCH	CC	TC
0.02 trial 1	F1	0	0	0	0
	F2	100	100	100	100
trial 2	F1	0	0	0	0
	F2	100	100	100	100
0.25 trial 1	F1	0	0	2.7	0
	F2	100	100	97.3	100
trial 2	F1	0	0	0	0
	F2	100	100	100	100
0.75 trial 1	F1	0	0	0	0
	F2	100	100	100	100
trial 2	F1	0	0	0	0
	F2	100	100	100	100
1 trial 1	F1	0	0	5.5	0.5
	F2	100	100	94.5	99.5
trial 2	F1	0	0	6.6	0.8
	F2	100	100	93.4	99.2
3 trial 1	F1	0	0	0	0
	F2	100	100	100	100
trial 2	F1	0	0	0	0
	F2	100	100	100	100
18 trial 1	F1	0	0	4.2	2.1
	F2	100	100	95.8	97.9
trial 2	F1	0	0	3.9	2.7
	F2	100	100	96.1	97.3
25 trial 1	F1	0	0	16.5	4.8
	F2	100	100	83.5	95.2
trial 2	F1	0	0	3.3	0.6
	F2	100	100	96.7	99.4
45 trial 1	F1	0	0	0.9	0
	F2	100	100	99.1	100
trial 2	F1	0	0	4.6	0
	F2	100	100	95.4	100
120 trial 1	F1	0	0	0	0
	F2	100	100	100	100
trial 2	F1	0	0	0	0
	F2	100	100	100	100

RESULTS AND DISCUSSION

Concentrations

Concentrations were determined for all sampling periods except the night of June 25 (25-N) which was lost due to mechanical failure. Samples are designated by the day (21-26) followed by Day (D) or Night (N), followed by height (1 = 12.7 cm, 2 = 50.8 cm, 3 = 106.7 cm, 4 = 177.8 cm). Concentrations are listed in Table 4 and air volumes are listed in Table1. Plots of distance above soil vs. concentration for each pesticide are presented in Figures 5-9 followed by the same plots using average concentrations for the entire sampling period (week of June 21-26, 1998). Figures10 and 11 show typical chromatograms for a pesticide standard and an air sample from this study.

Trans-Chlordane

TC was detected in all samples analyzed. The concentrations ranged from 0.033 to 0.424 ng/m^3 for position 1 closest to the soil (12.7 cm). Aigner et al. (1998) found the average soil concentration at the same farm to be 13.0 ng/g. The highest concentration in air was for the sampling period 24-N, which also had one of the highest temperatures for the sampling period. Figure 5 (and Table 3) show a concentration gradient for TC which decreases from position 1 to position 4 (closest to farthest). This pattern was found for all samples except the 23-N sample. The 23-N-3 sample was higher in concentration than the 23-N-2 sample for all pesticides analyzed. It is not understood why this occurred but may be due to analytical error. Graphs of concentration vs. height for TC show a trend of decreasing concentration with increasing height above soil. This trend suggests

the primary source of TC to the air above the soil was the soil and not atmospheric transport from another location.

A similar profile volatilization study by Finizio et al. (1998) in the Frazer Valley, British Columbia found TC concentration profiles similar to the present study. In that study, the soil TC concentration was found to be 773 ng/g and the highest concentration (at 5 cm above the soil) was 1.6 ng/m^3.

Cis-Chlordane

CC was detected in all samples with concentrations ranging from 0.280 to 0.676 ng/m^3 for position 1 (closest to soil). Aigner et al. (1998) found a soil concentration of 23.0 ng/g for this soil. The CC concentration profile shown in Figure 6 shows decreasing concentration with increasing sampling height. In general, the concentrations tended to be higher during the periods with the highest average temperatures. The concentration gradients for each sample are consistent throughout the week with the exception of the 23-N period as was seen for TC. The pattern of the concentration gradients for both individual daily samples as well as the week long average suggests the soil as the primary source of CC to the air above the soil.

A study in British Columbia (Finizio et al., 1998) found a CC concentration profile similar to the present study (concentration decreased as height increased). The CC soil concentration in that study was 133 ng/g and the highest air concentration (at 5 cm) was 0.3 ng/m^3.

Trans-Nonachlor

Trans-Nonachlor, a constituent of technical chlordane, was detected in all samples. The concentrations of TN ranged from 0.630 to 1.430 ng/m^3 (position 1) for the week sampled. All plots of height above soil vs. concentration (Figure 7) yielded a consistent gradient of decreasing concentration from the closest position to the farthest (except for 23-N as was seen for TC and CC) again suggesting soil as the source of TN to the air. Aigner et al. (1998) detected an average TN soil concentration of 33.0 ng/g. The sample containing the highest TN concentration also had one of the highest average temperatures.

In the British Columbia study (Finizio et al., 1998), the authors found TN concentration gradients in air similar to the present study. The soil TN concentration in that study was 69 ng/g and the highest air concentration (at 5 cm) was 0.27 ng/m^3.

Alpha-Hexachlorocyclohexane

α-HCH was detected in very low concentrations (non-detectable at position 1 to 0.284 ng/m^3 at position 4) in all samples. However, unlike the chlordanes, no consistent pattern was found in gradient plots of height vs. concentration (Figure 8). Aigner et al. (1998) reported α-HCH as below the detection limit in the soil. This, along with a lack of a gradient, suggests the α-HCH in air above this soil is most likely coming from transport from other locations and not from volatilization from this soil.

Finizio et al. (1998) found an α-HCH profile in British Columbia similar to the other pesticides in the study (lower concentrations with increasing height). In that study, α-HCH was found in the soil at a concentration of 42 ng/g and the highest air

concentration (at 5 cm) was 1.4 ng/m^3. The lowest α-HCH concentration in British Columbia was approximately two times higher than the highest concentration at the Ohio site. Finizio et al. (1998) examined the relationship between relative concentrations of OCs in air to those in soil (by modifying a model by Jury et al., 1984 and found for α-HCH, concentrations in air were higher than accounted for by volatilization from local soil. The authors suggested atmospheric transport might be contributing to α-HCH concentrations in overlying air.

Gamma-Hexachlorocyclohexane

γ-HCH was detected in all samples in concentrations ranging from 0.324 to 0.759 ng/m^3 (position 1) for the week studied. γ-HCH was not analyzed in the soil study by Aigner et al., 1998. The highest concentration in the present study was found for a sampling period with a high average temperature. Height above soil vs. concentration plots show the same gradients as for chlordanes (decreasing concentration with increasing height) suggesting soil as the source of this compound (Figure 9). Finizio et al. (1998) found a soil γ-HCH concentration of 108 ng/g and an air concentration profile similar to the present study with a high concentration (at 5 cm) of 0.45 ng/m^3.

Table 4. Concentrations of OC Pesticides in Air (ng/m^3) at varying Heights

Sample Name*	TC	CC	TN	α-HCH	γ-HCH
21-D-1	0.151	0.521	0.974	0.083	0.625
21-D-2	0.100	0.372	0.644	0.103	0.441
21-D-3	0.042	0.115	0.369	0.029	0.152
21-D-4	0.001	0.038	0.200	0.129	0.080
21-N-1	0.326	0.494	1.130	0.030	0.388
21-N-2	0.173	0.275	0.650	0.079	0.272
21-N-3	0.055	0.098	0.281	0.019	0.081
21-N-4	0.027	0.032	0.131	0.095	0.044
22-D-1	0.318	0.533	1.060	0.093	0.680
22-D-2	0.229	0.507	0.518	0.057	0.578
22-D-3	0.129	0.334	0.473	0.020	0.257
22-D-4	0.068	0.218	0.421	0.028	0.238
22-N-1	†N/A	N/A	N/A	N/A	N/A
22-N-2	0.230	0.349	0.782	0.037	0.305
22-N-3	0.150	0.216	0.488	0.020	0.175
22-N-4	0.032	0.059	0.189	0.083	0.050
23-D-1	0.164	0.280	0.630	0.021	0.324
23-D-2	0.084	0.141	0.317	0.052	0.251
23-D-3	0.067	0.107	0.293	0.250	0.259
23-D-4	0.024	0.087	0.153	0.019	0.167
23-N-1	0.033	0.067	0.082	0.000	0.067
23-N-2	0.093	0.247	0.352	0.117	0.430
23-N-3	0.132	0.307	0.190	0.072	0.235
23-N-4	0.022	0.087	0.102	0.019	0.150
24-D-1	0.221	0.652	1.008	0.026	0.759
24-D-2	0.109	0.352	0.635	0.026	0.490
24-D-3	0.076	0.139	0.359	0.106	0.183
24-D-4	0.068	0.103	0.216	0.284	0.116
24-N-1	0.424	0.629	1.430	0.091	0.581
24-N-2	0.366	0.470	0.831	0.103	0.340
24-N-3	0.090	0.234	0.299	0.023	0.289
24-N-4	0.119	0.214	0.317	0.009	0.094
25-D-1	0.237	0.676	1.134	0.025	0.758
25-D-2	N/A	N/A	N/A	N/A	N/A
25-D-3	0.058	0.224	0.492	‡INT	0.132
25-D-4	0.038	0.148	0.311	0.025	0.138
26-D-1	0.186	0.331	0.790	0.065	0.334
26-D-2	0.126	0.217	0.539	0.038	0.247
26-D-3	0.081	0.134	0.300	0.133	0.104
26-D-4	0.039	0.065	0.219	0.033	0.054

*Sample Name gives day taken - day/night - height above soil
†N/A = not analyzed
‡INT = interference which did not allow for quantitation

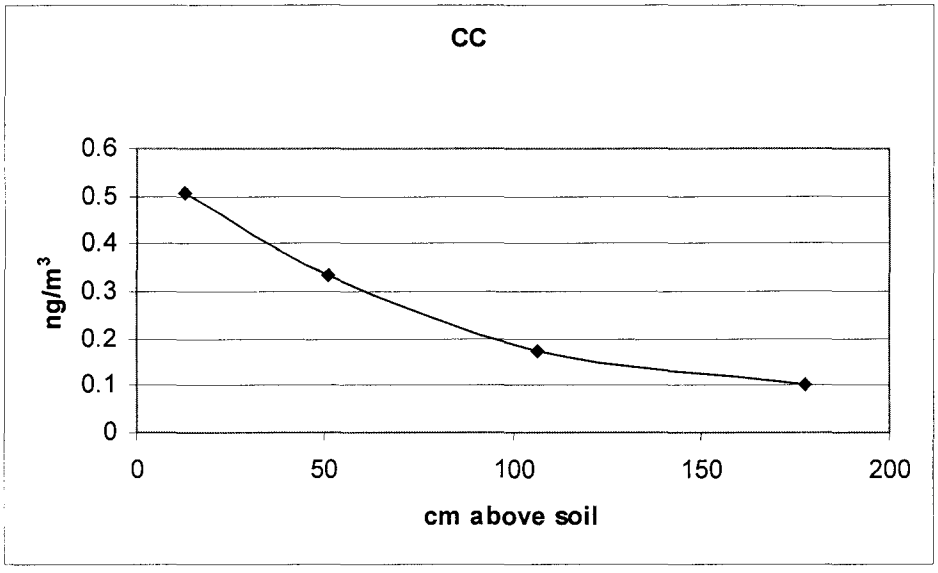


Figure 5. Plot of average concentration (ng/m^3) for week of June 21-26 vs. height above soil (cm) for cis-Chlordane

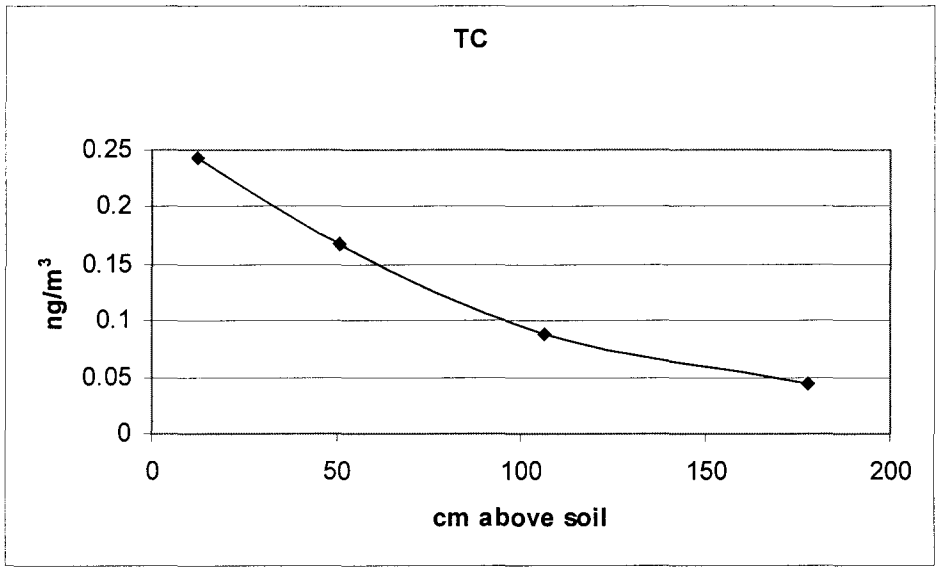


Figure 6. Plot of average concentration (ng/m^3) for week of June 21-26 vs. height above soil (cm) for trans-Chlordane

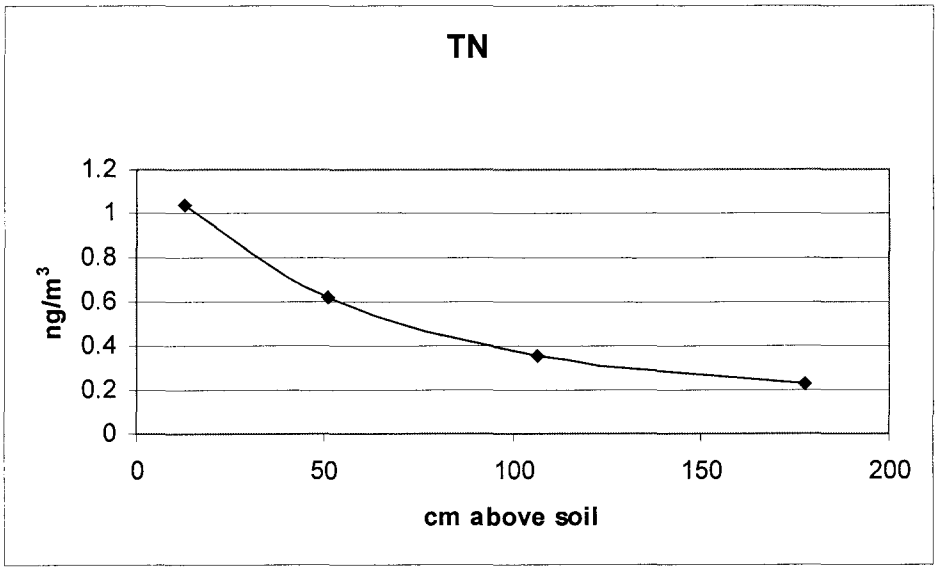


Figure 7. Plot of average concentration (ng/m^3) for week of June 21-26 vs. height above soil (cm) for trans-Nonachlor

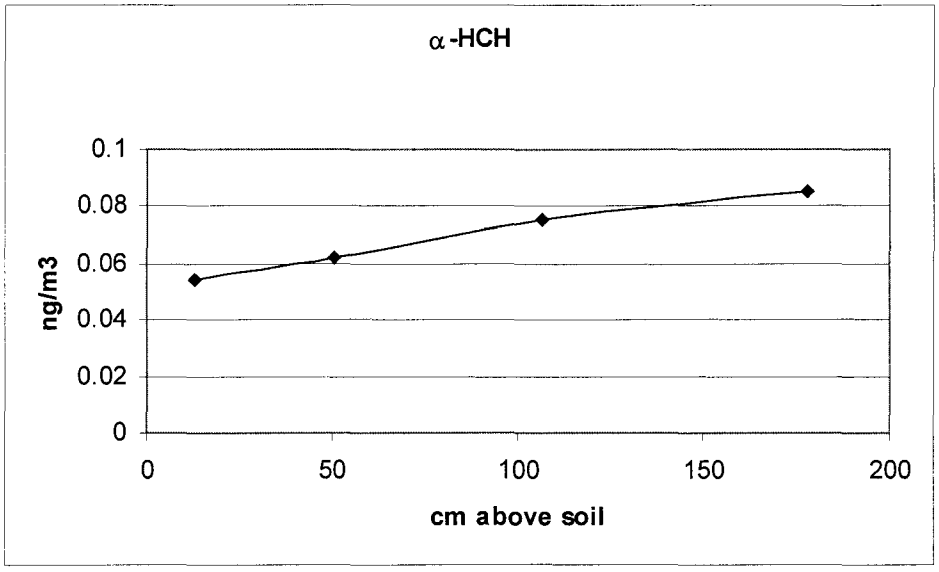


Figure 8. Plot of average concentration (ng/m^3) for week of June 21-26 vs. height above soil (cm) for α-HCH

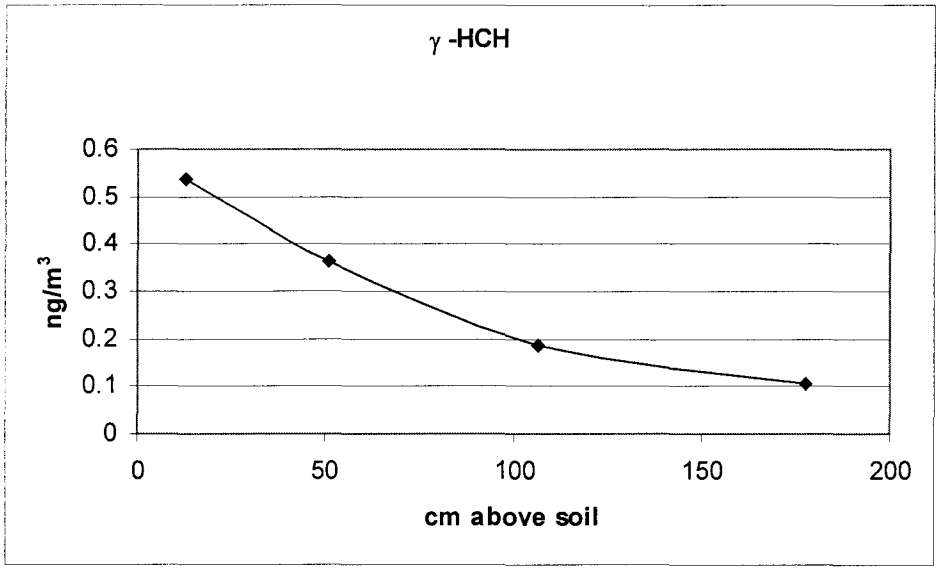


Figure 9. Plot of average concentration (ng/m^3) for week of June 21-26 vs. height above soil (cm) for γ-HCH

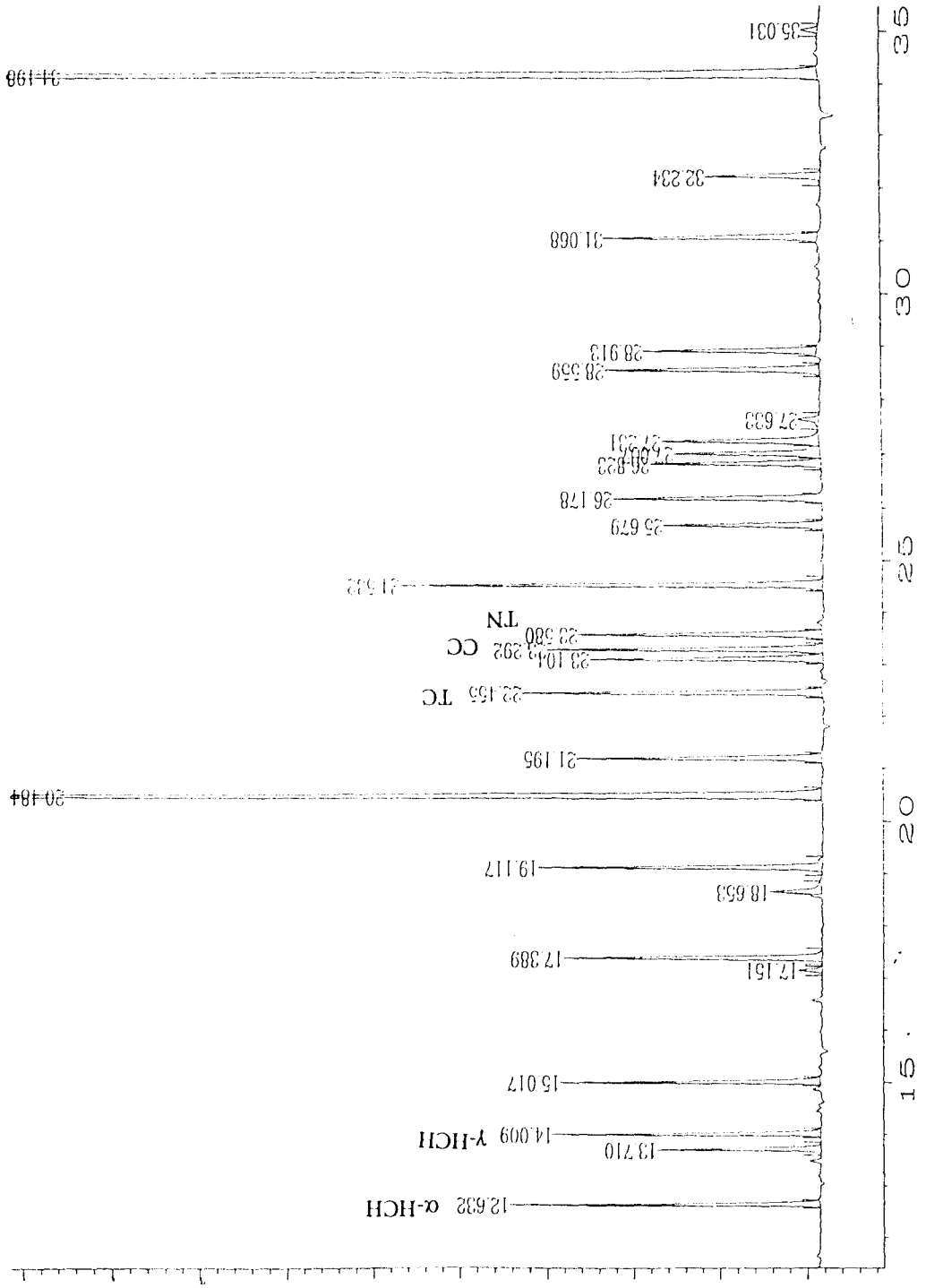


Figure 10. Chromatogram of typical pesticide standard used in quantitation

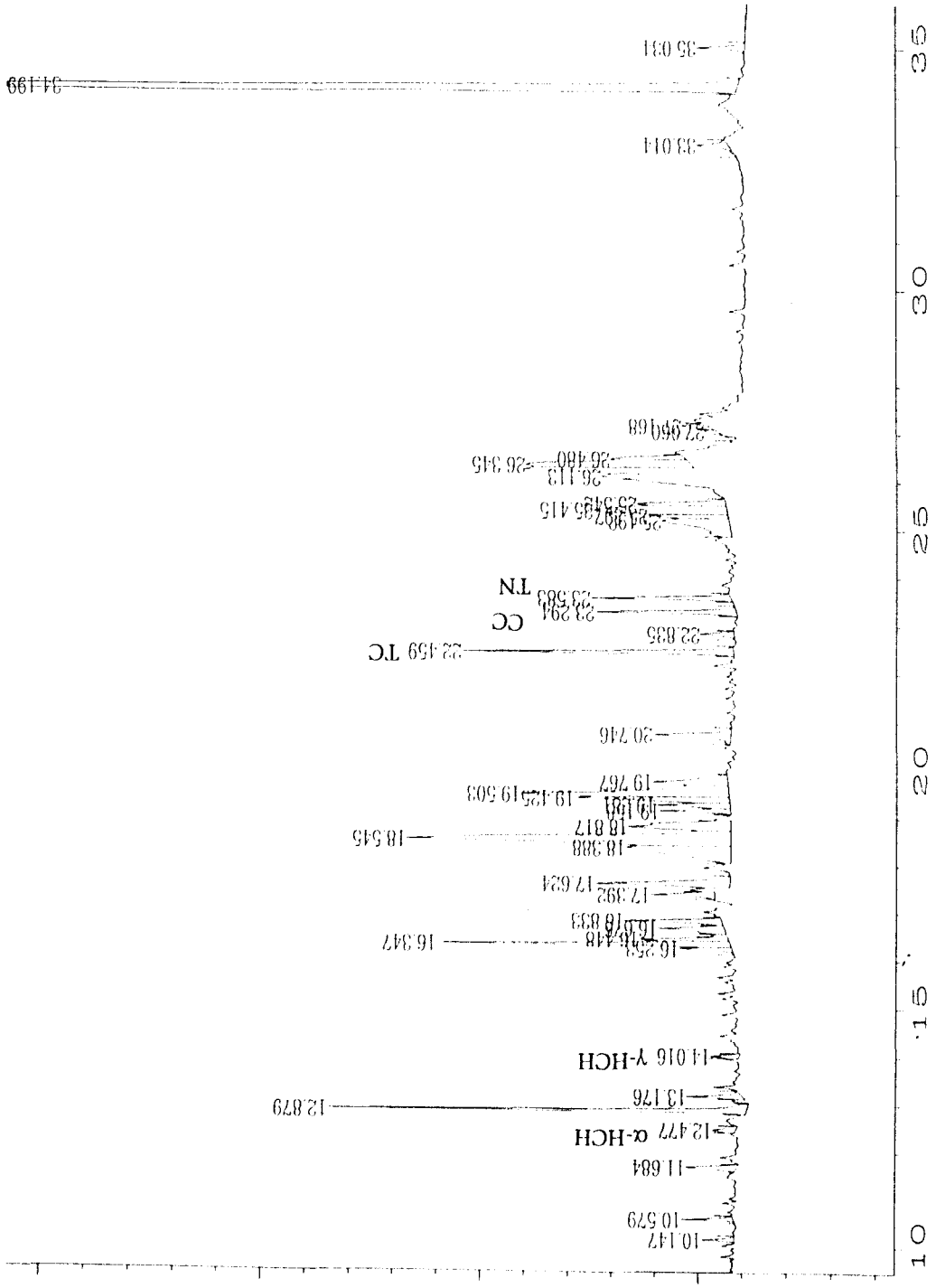


Figure 11. Chromatogram of typical air sample showing compounds of interest

Enantiomeric Ratios

ERs were determined for CC, TC, and MC-5 (another chiral component of technical chlordane). Due to time constraints and instrument problems, the ERs of other compounds quantified were not explored. Standard deviations for the ERs ranged from 0.02 – 0.04 with an average value of 0.03 for all three compounds. The requirements discussed in the Quality Control section were used to eliminate questionable data and those not passing both requirements were reported as not determined (ND) in Table 5. Figures 12 and 13 show typical chromatograms of TC and CC enantiomers in a standard and a typical air sample from this study.

Trans-Chlordane

Enantioselective breakdown occurred in all samples containing TC. In all cases the (+) enantiomer was preferentially degraded with ERs ranging from 0.72 – 0.84. The average for the week for all heights was 0.74. This is very close to the soil ER value (average = 0.79) and to that of an overlying air sample taken 15 cm above the soil (ER = 0.70) determined in a previous study (Leone, 1998). The similarity between ERs for soil and overlying air at the four different heights suggest that the TC detected in overlying air was from soil volatilization. This finding is consistent with what was found for concentration gradients for this compound.

A similar study was done by Finizio et al. (1998). The authors of that work were unable to determine ERs for chlordanes but did do enantiomeric analysis of several other OC pesticides. They found enantioselective degradation of the (-) enantiomer of Heptachlor Epoxide (HEPX). The ERs of HEPX at different heights in the overlying air

had an average of 1.43 and were similar to what was detected in the soil (1.39). The authors showed that several pesticides detected in air above the soil were coming from volatilization from the soil by using a soil-air exchange model. α-HCH differed from the HEPX however. The ER of α-HCH varied over the heights studied (1.35 closest to 1.19 farthest). The authors suggest the differences with α-HCH were probably due to atmospheric contributions.

Cis-Chlordane

Enantioselective breakdown was found for CC in all air samples with (-) CC enantiomer being preferentially degraded. The CC ERs for the week studied ranged from 1.08 – 1.19 with an average of 1.13. The standard deviation over the week was 0.03. The study by Leone (1998) at the same site found an average soil CC ER of 1.10 and an ER for air 15 cm above the soil of 1.16. The close agreement with the soil ER and the lack of a gradient suggests that the CC detected in the overlying air in the present study is coming from soil volatilization. This finding was consistent with what was found with the concentration gradients.

MC-5

MC-5, another chiral chlordane isomer, was detected in all samples with the first eluting enantiomer being preferentially degraded in all samples (the exact elution order is not known as there are no single enantiomer standards available for this compound). The MC-5 ERs for the study ranged from 0.82 – 0.91 with an average of 0.87. The standard deviation for the week was 0.02. MC-5 was not determined in soils in the previous work

by Leone (1998). The non-racemic ER for MC-5 suggests microbial breakdown is occurring in, probably in the soil as for TC and CC. The lack of a gradient with height suggests the soil as the primary sources of MC-5 to overlying air.

Table 5 ERs for TC, CC, and MC-5 for all samples

Sample	TC	m/z 410 CC	MC-5
21-D-1	0.74	1.15	‡N/D
21-D-2	0.73	N/D	N/D
21-D-3	0.72	1.15	0.86
21-D-4	0.73	1.19	0.83
21-N-1	0.74	1.16	0.86
21-N-2	0.74	1.15	0.84
21-N-3	0.74	1.11	0.84
21-N-4	0.75	1.15	0.88
22-D-1	0.76	1.12	0.82
22-D-2	0.84	N/D	0.88
22-D-3	0.83	N/D	0.87
22-D-4	0.77	N/D	0.88
22-N-1	0.74	1.12	0.88
22-N-2	0.75	1.15	0.88
22-N-3	0.79	1.16	0.88
22-N-4	0.77	1.13	0.9
23-D-1	0.74	N/D	0.89
23-D-2	0.77	N/D	N/D
23-D-3	0.77	N/D	0.87
23-D-4	0.79	N/D	N/D
23-N-1	N/D	N/D	N/D
23-N-2	N/D	N/D	N/D
23-N-3	N/D	N/D	N/D
23-N-4	0.77	1.04	0.91
24-D-1	0.74	1.14	0.86
24-D-2	0.73	N/R	0.88
24-D-3	0.73	1.14	0.87
24-D-4	0.8	1.09	0.89
24-N-1	0.74	1.15	0.89
24-N-2	0.81	1.13	0.88
24-N-3	0.74	N/R	0.88
24-N-4	0.9	1.08	0.82
25-D-1	N/D	N/D	N/D
25-D-2	N/D	N/D	N/D
25-D-3	N/D	N/D	N/D
25-D-4	N/D	N/D	N/D
26-D-1	0.74	N/D	0.87
26-D-2	0.75	N/D	0.88
26-D-3	0.79	1.1	0.87
26-D-4	0.77	N/D	0.86
Ave.	0.76	1.13	0.87
Stand. Dev.	0.04	0.03	0.02
Standards			
Ave.*	0.98	0.97	0.98
Stand. Dev.	0.01	0.01	0.02

Ave* = average for 5 standard injections
‡N/D = not determined due to interferences or failure to meet QC requirements

Figure 12. Chromatogram of Chlordane enantiomers in standard showing elution order for TC and CC

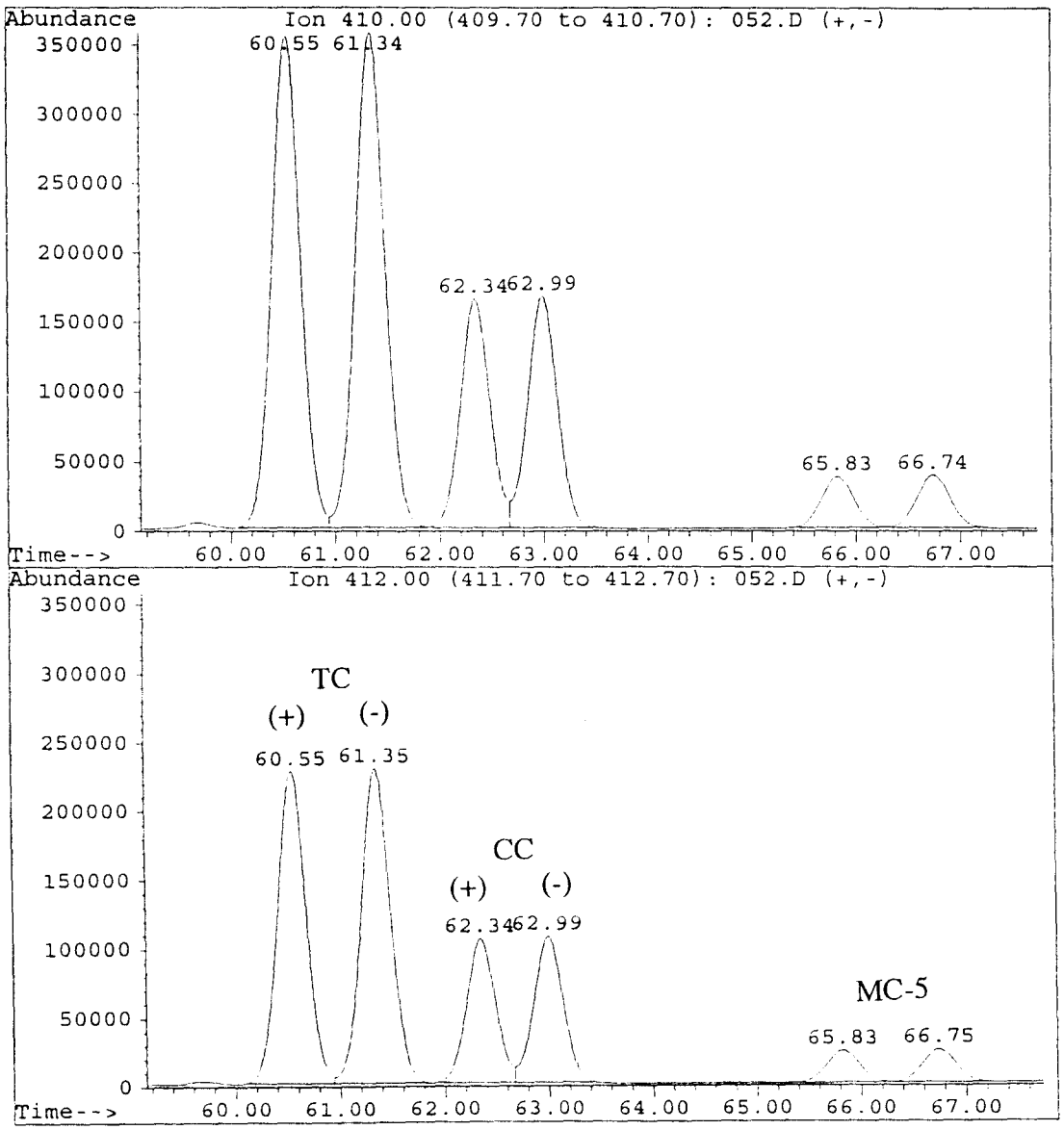
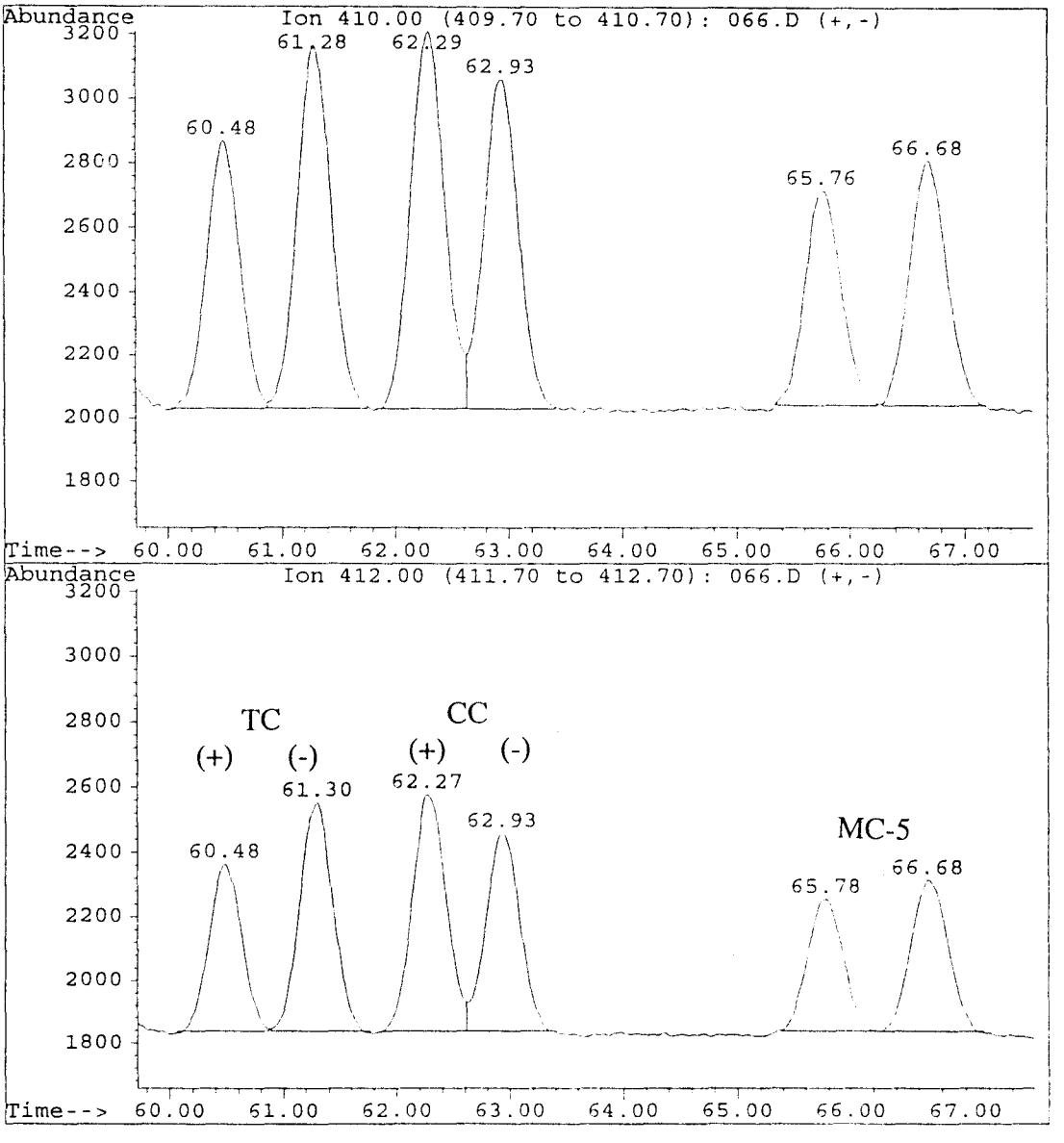


Figure 13. Chromatogram of Chlordane enantiomers in typical air sample



CONCLUSIONS

The purpose of this work was to determine concentrations and ERs of several OC pesticides in the air above an agricultural soil in Northeast Ohio. Air concentrations and Vertical concentration gradients were determined for five compounds (CC, TC, TN, α-HCH, and γ-HCH) at several heights above the soil (12.7, 50.8, 106.7, and 177.8 cm). TN had the highest average concentration for the week studied (1.0 ng/m^3 at position 1). Concentrations for the pesticides decreased with distance from the soil for the week studied for all compounds except α-HCH. α-HCH concentrations were found not to be statistically different at the varying heights and concentrations were much lower than the other pesticides. These findings suggest the soil as the primary source for TC, CC, TN and γ-HCH; while transport from other regions may be important for α-HCH for samples from this study.

Enantiomeric ratios were determined for three compounds (TC, CC, and MC-5). Samples at all heights showed enantioselective degradation of the (+) enantiomer of TC (average TC ER = 0.76). CC residues were also found to be degraded enantioselectively with the (-) enantiomer being preferentially degraded (average CC ER = 1.13). For MC-5 the first eluting enantiomer is preferentially decomposed. The ER data agreed with soil and air directly above soil (15 cm) data collected in a previous study at the same site (Leone, 1998). The ER and concentration data collected in the present study suggest the pesticide residues in overlying air were coming primarily from soil volatilization with the exception of α-HCH which is likely coming from atmospheric transport.

By measuring the ERs of OC pesticides in soil and air it may be possible to distinguish between 'old' and 'new' atmospheric sources. Freshly applied pesticides that

volatilize into the atmosphere and are only subject to non-biological degradation should remain racemic. Pesticide residues that volatilize from soils years after application and are subjected to microbial degradation often show enantioselective breakdown. Residues in air may show an 'old' signature which could be used to track releases into the atmosphere and differentiate 'old' from 'new' sources.

The use of ERs to support soil volatilization studies is promising. By doing more extensive studies similar to the present one to gather regional data, source determination and apportionment could be done based on ERs as well as traditional data.

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APPENDIX

Figure A-1. Standard calibration plot (linear) of CC. Amt Ratio equals concentration (ng) x 10. Rsp ratio equals response factor used for calculation of sample concentrations. r^2 values for curve are given.

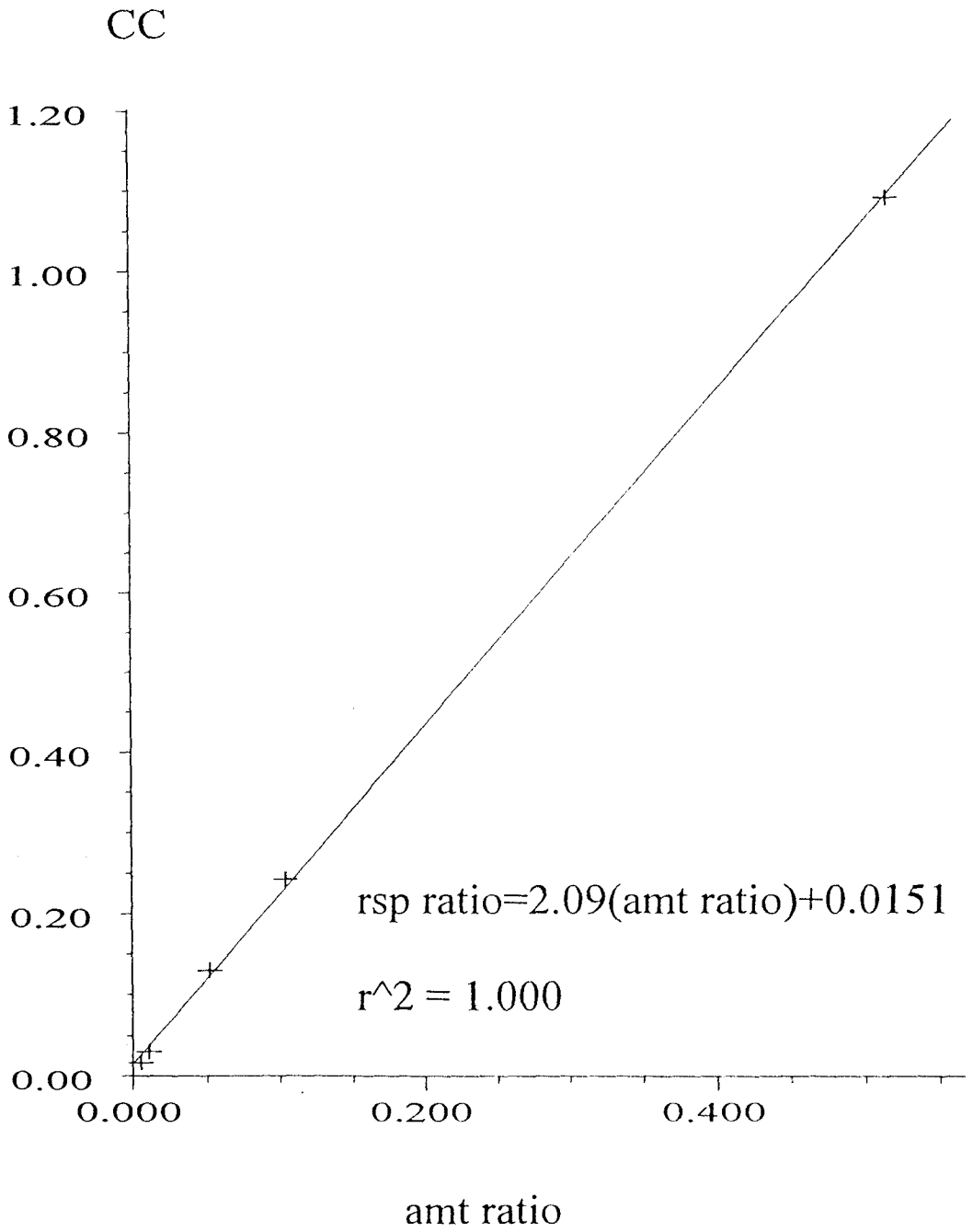

Figure A-2. Standard calibration plot (linear) of TC. Amt Ratio equals concentration (ng) x 10. Rsp ratio equals response factor used for calculation of sample concentrations. r^2 values for curve are given.

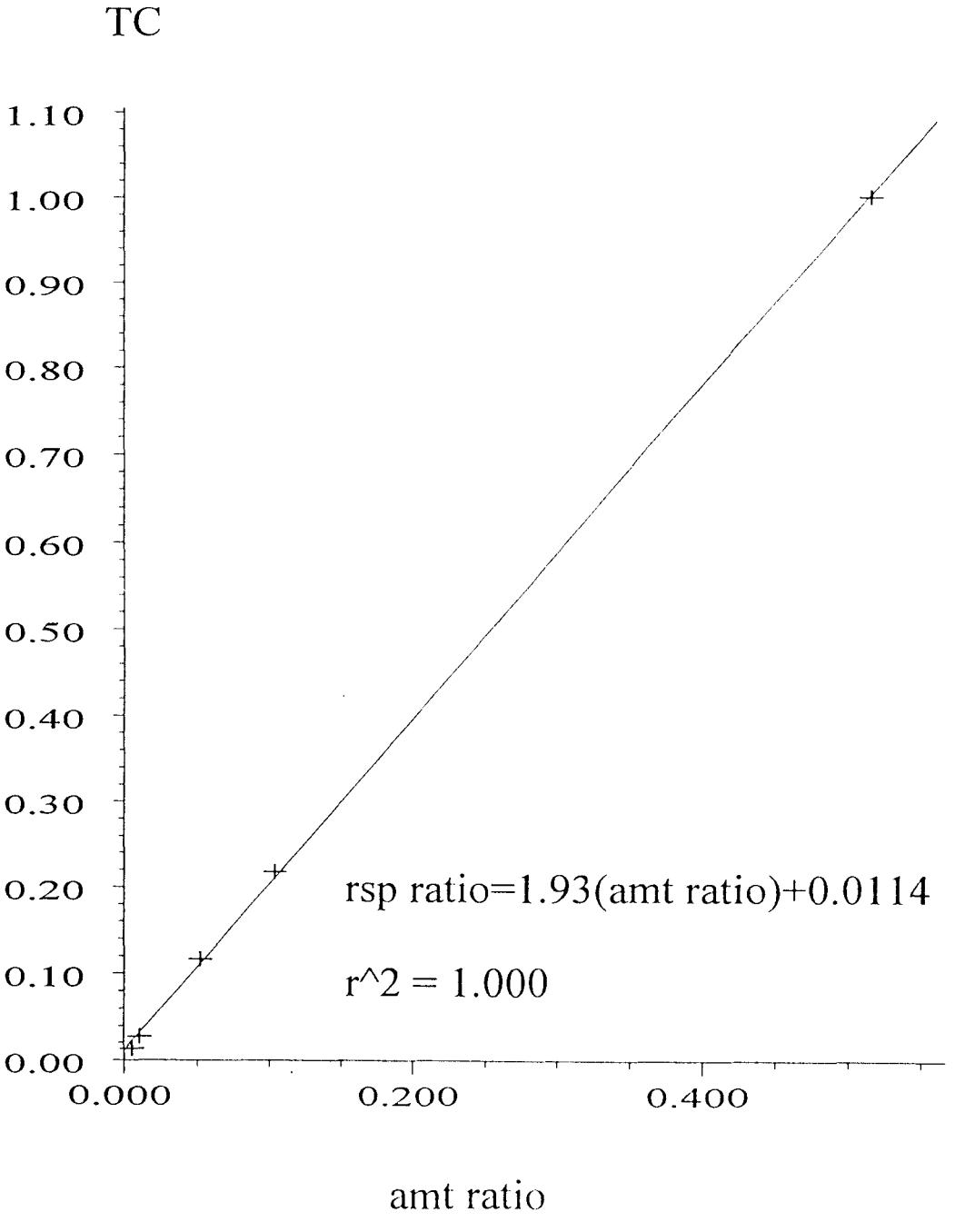

Figure A-3. Standard calibration plot (linear) of TN. Amt Ratio equals concentration (ng) x 10. Rsp ratio equals response factor used for calculation of sample concentrations. r^2 values for curve are given.

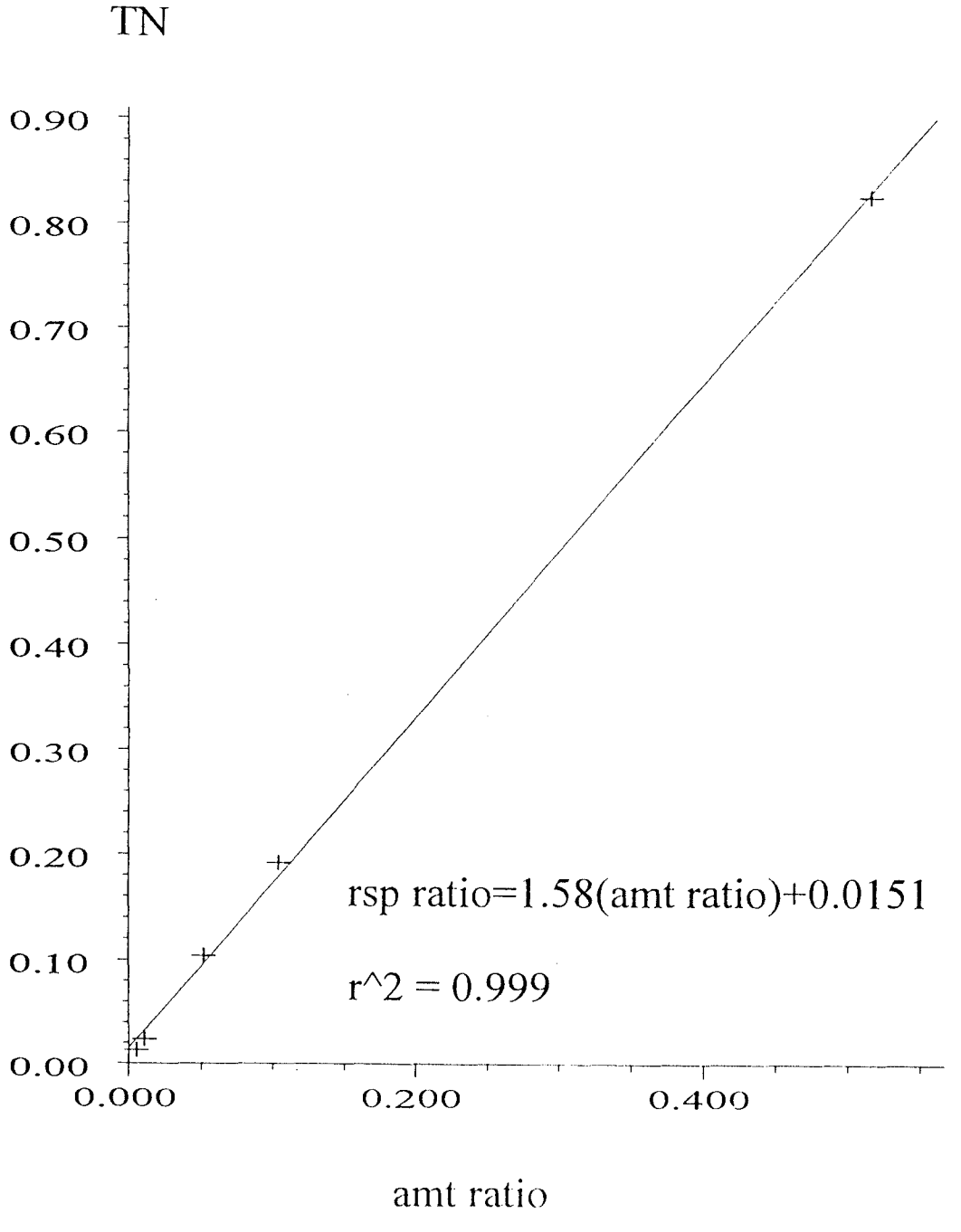

Figure A-4. Standard calibration plot (linear) of α-HCH. Amt Ratio equals concentration (ng) x 10. Rsp ratio equals response factor used for calculation of sample concentrations. r^2 values for curve are given.

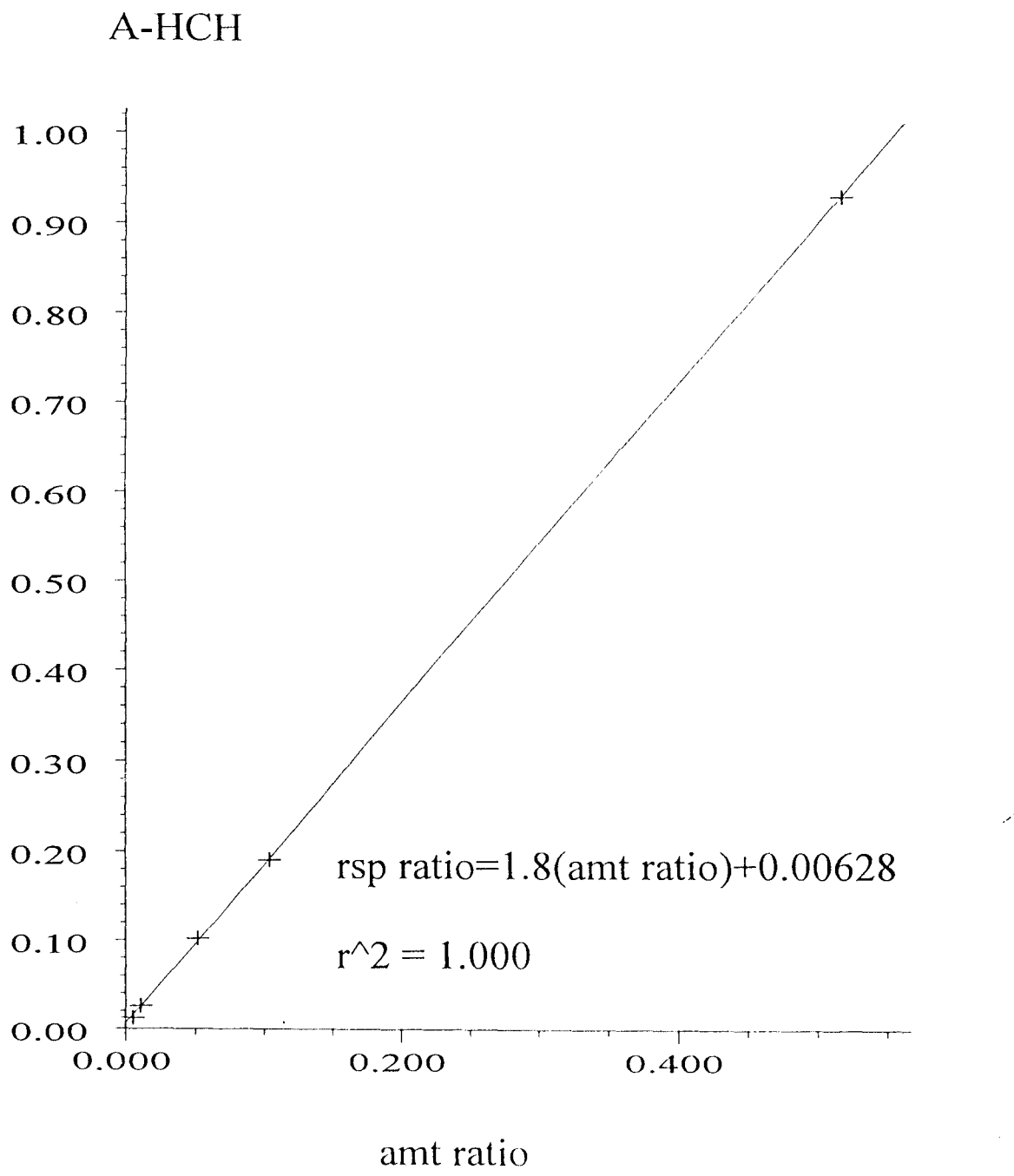

Figure A-5. Standard calibration plot (linear) of α-HCH. Amt Ratio equals concentration (ng) x 10. Rsp ratio equals response factor used for calculation of sample concentrations. r^2 values for curve are given.

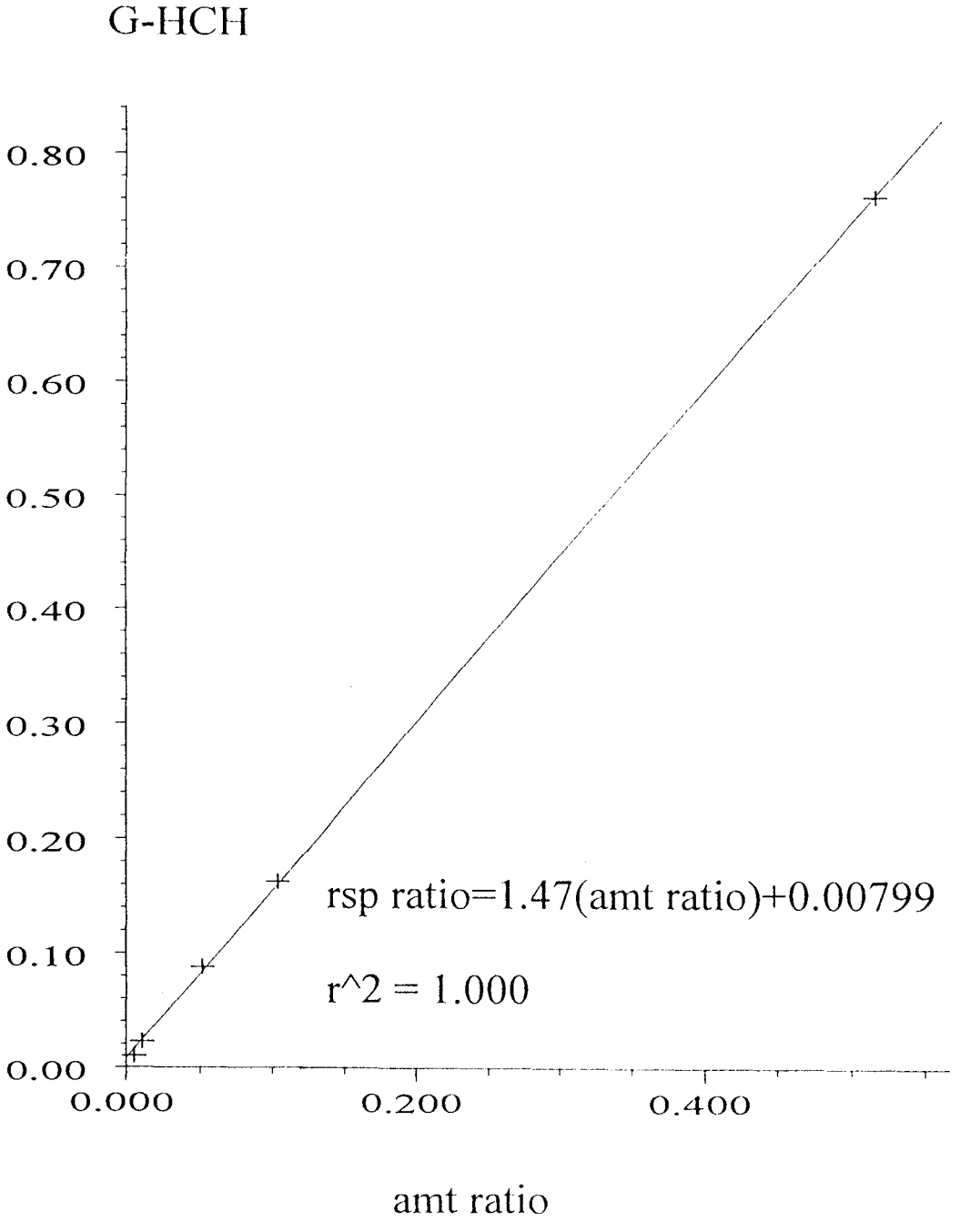

Table A-6. Elution Orders for CC, TC, and MC-5

Ions Monitored = 410 and 412 m/z

Compound	**Elution Order**
CC	(+), (-)
TC	(+), (-)
MC-5	unknown