ENANTIOMERIC RATIOS AND CONCENTRATIONS OF ORGANOCHLORINE PESTICIDES IN OHIO SOILS

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Enantiomeric Ratios and Concentrations of Organochlorine Pesticides in Ohio Soils

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ABSTRACT

Past research has shown that selective enzymatic degradation of enantiomers by microorganisms does occur. This work was done to determine the enantiomeric ratios and concentrations of several chiral organochlorine pesticides in soils from across Ohio. Quantitative analysis was done using gas chromatography with electron capture detection using a DB-5 column. Concentrations were determined for nine compounds (o,p'-DDT, p,p'-DDT, p,p'-DDD, p,p'-DDE, cis-chlordane, trans-chlordane, heptachlor epoxide, dieldrin and trans-nonachlor) in eleven agricultural soils and one garden soil. The components of DDT were the only compounds present in all soils analyzed. The garden soil concentration values were similar to those for the agricultural soils. Enantiomeric analysis was done using gas chromatography-mass spectrometry with chiral-phase capillary columns. Enantiomeric excesses were found for five compounds: o,p'-DDT, cis-chlordane, trans-chlordane, oxychlordane and heptachlor epoxide. The largest excesses were seen for heptachlor epoxide. The garden soil showed enantiomeric differences for trans-chlordane, cis-chlordane and heptachlor epoxide.

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CHAPTER ONE

BACKGROUND

PESTICIDES

Pesticides are chemicals designed to combat the various pests that attack agricultural or horticultural crops. The three major classes are: insecticides (insect control), fungicides (fungi control), and herbicides (plant control). As of the mid 1990's farmers in the U.S. were using approximately 365 million kg of pesticides a year, while non-agricultural applications, such as forestry, landscaping, gardening, food distribution and home pest control, consumed approximately 900 million kg.[1]

Organochlorine pesticides (OCs) are organic compounds that contain one or more chlorine atoms. The introduction of DDT in World War II to combat disease-transmitting insects marked the start of very fast growth in pesticide use. After the war DDT came into general use as an agricultural insecticide. In time, DDT began to lose its effectiveness due to build up of insect resistance. New insecticides were developed to replace DDT, the first being minor variations of DDT, such as methoxychlor. It was soon found that other OCs (including aldrin, chlordane, dieldrin, endosulfan, endrin, heptachlor, hexachlorocyclohexane, and toxaphene) share common insecticidal properties as well as the characteristics of high persistence and potential carcinogenicity.[2-5] OCs are non-polar, lipophilic and relatively inert to both oxidation and hydrolysis reactions. These properties cause OCs to accumulate in the fatty tissues of animals.[6]

Bioaccumulation and the fact that many OCs are potential carcinogens make them persistent and harmful environmental pollutants.

Current evidence suggests that some OCs can act as an estrogen mimic[7] and thus pose an additional environmental hazard. In a recent study of the synergistic activation of estrogen receptors with a combination of environmental chemicals, Arnold et al.[8] compared the transcriptional activation of human estrogen recepter in yeast in response to environmental chemicals individually and in combination. They found that dieldrin, endosulfan, and toxaphene only weakly increased β-galactosidase (β-Gal) activity when studied alone. However, when combining any two of these compounds, a synergistic increase in β-Gal activity as compared with the individual compounds was produced. Chlordane, which had no measurable activity alone, also enhanced the potency of other environmental chemicals.

The use of OC pesticides has drastically declined in the U.S. since the 1980's due to environmental concerns and build-up of insect resistance. Agrochemical companies have since turned towards more selective and less persistent compounds such as organophosphates and carbamates as well as natural insecticides, pheromones and better land usage practices for combating pests and increasing crop productivity.[9] However, many developing countries still use OC pesticides because they are inexpensive and effective. Due to their persistence in the environment, residues are still found in soil, air, water and sediments across the world.

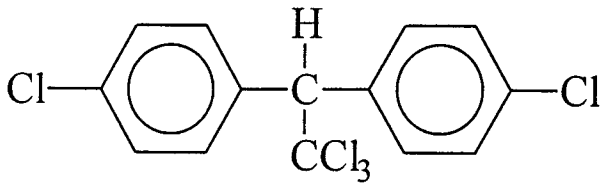
ORGANOCHLORINE PESTICIDES

DDT

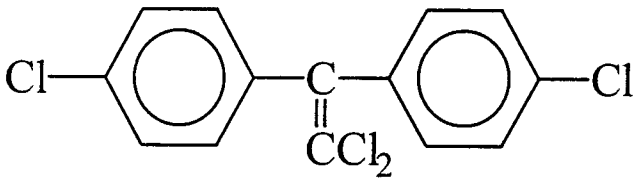
Otherwise known as dichlorodiphenyltrichloroethane (Figure 1), DDT was first synthesized by Zeidler in 1874. Its insecticidal properties, however, were not discovered until 1939. DDT was introduced during World War II to control typhus and malaria outbreaks. It was found to be extremely effective in controlling vector-transmitted diseases and, following the war, was the first OC pesticide to come into widespread agricultural use.[9] DDT was heavily used in the U.S. with peak production occurring in 1963, when 80 million kg were used.[2] DDT is technically produced as a mixture of ≈70% 4,4'-DDT (p,p'-DDT; insecticidally active isomer) and ≈25% 2,4'-DDT (o,p'-DDT).

DDT and its primary metabolites are man-made chemicals and are not known to occur naturally in the environment.[10] Once released in the environment, DDT breaks down primarily into the dichloroethene and ethane derivatives, DDD and DDE (Figure 1). The main route of metabolism for DDT in fish, birds and insects appears to be dehydrochlorination to DDE.[11] DDE is often the primary residue found in the tissues of mammals due to bioaccumulation in the food chain. Since DDE has a prolonged half-life, average levels of DDT are expected to decline slowly while the ratio of DDE to DDT is expected to increase.[2] In addition, DDT and its metabolites are continually being transformed and redistributed in the environment because of their persistance and extensive past worldwide use.[2] Soils treated in years past have been found to contain DDT, DDE and DDD residues although the relative proportions differ with climate,

DDT



DDE



DDD

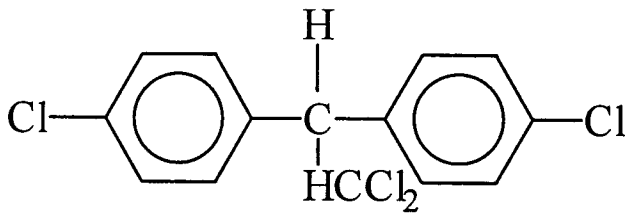


Figure 1. Structures of DDT, DDE, and DDD

location, soil type and agricultural activity.[12] DDT was banned in the U.S. in 1972,[2] but is still used in Asia, Africa and Central and South America.[13]

Chlordane

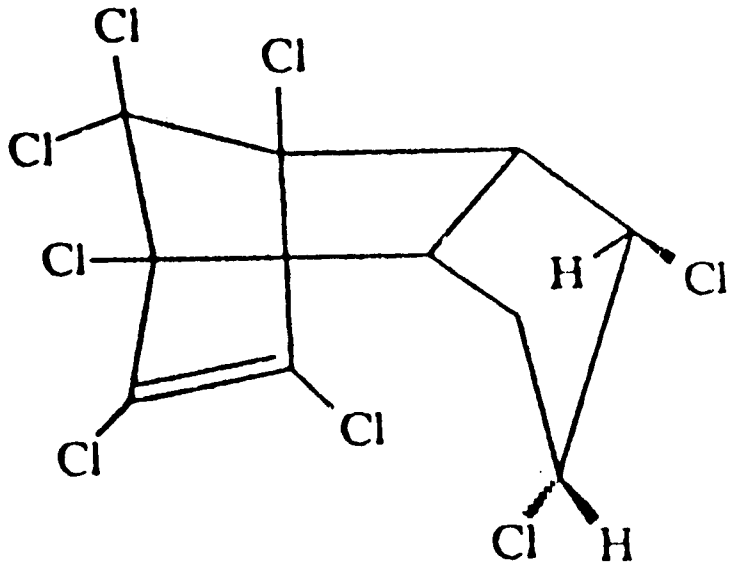
Technical chlordane is a multicomponent mixture of more than 140 compounds. It was first produced by Hyman and publicly announced in 1945 by Kears et. al.[14] The main components (Figure 2) are cis-chlordane (CC, 19%), trans-chlordane (TC, 24%), heptachlor (7%, see heptachlor section), and trans-nonachlor (TN, 7%).[15] The chlordane compounds have eight chlorine atoms, the nonachlors have nine, and heptachlor has seven. These compounds are metabolized in almost all organisms to two persistent epoxides: heptachlor epoxide (see heptachlor section) and oxychlordane (OXY).[16] OXY is the principal mammalian metabolite of CC, TC and the nonachlors[17] and is resistant to further degradation.

Chlordane was used for crops, home lawns and gardens, turfs and ornamentals,[3] with peak production occurring in the 1970's.[18] It is very persistent in soil, lasting over 20 years in some soils.[18-22] In 1979, chlordane was restricted due to its known toxicity and possible carcinogenicity.[23] From 1983 until its ban in 1988, chlordane was used exclusively for structural termite control, resulting in greatly elevated levels in home air.[3] Although standards exist in the former U.S.S.R., there are no use restrictions and chlordane is still registered for use in Asia.[24]

Hexachlorocyclohexane

Hexachlorocyclohexane (HCH, Figure 3) was synthesized many years before its insecticidal properties were discovered in 1942. HCH can theoretically exist as eight

TC



CC

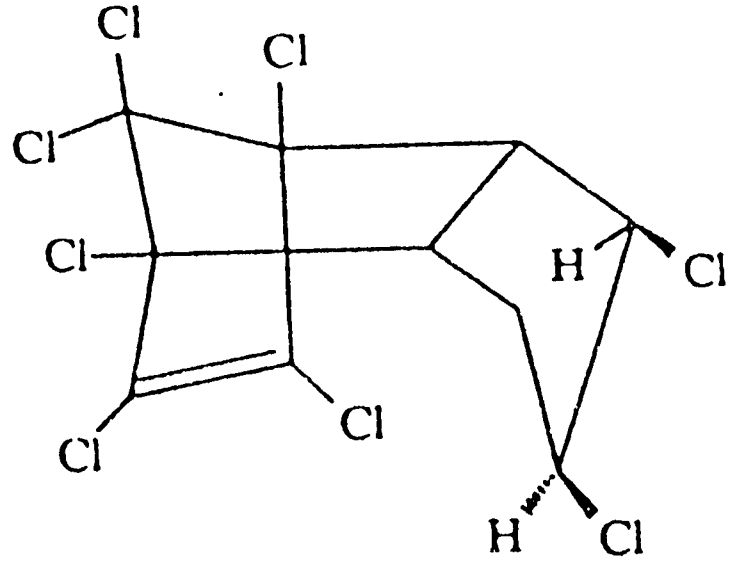


Figure 2. Structures of TC and CC[3]

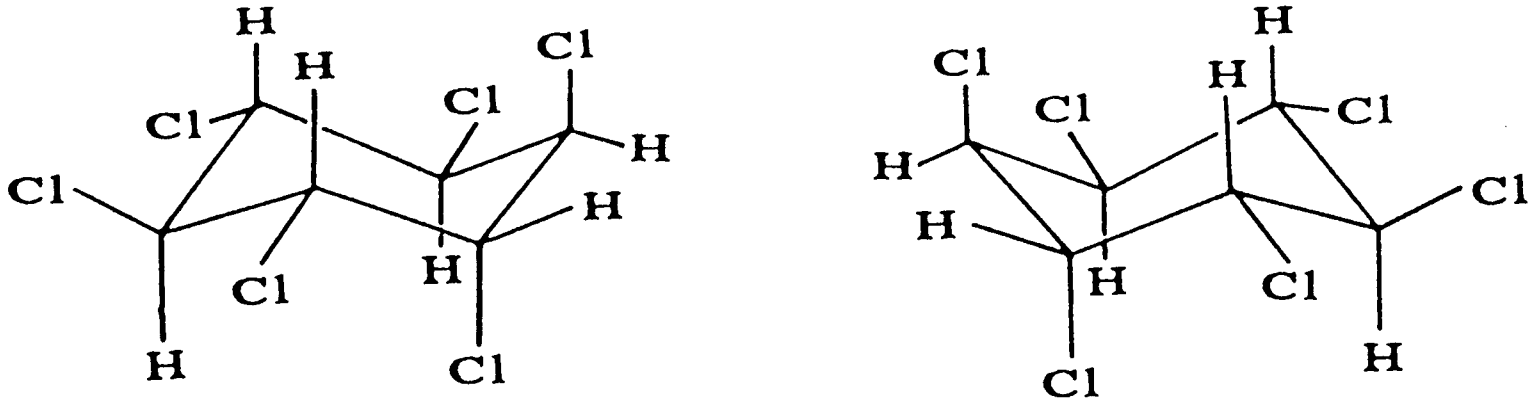


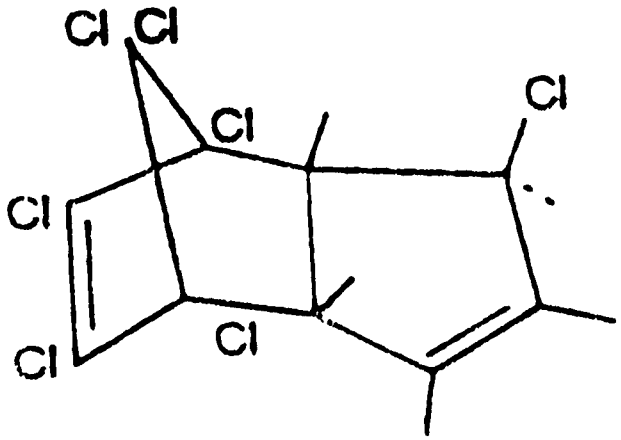
Figure 3. Enantiomers of α-HCH

different stereoisomers of which five (designated α, β, γ, δ, ε) are found in the technical mixture.[11] The technical product contains ≈80% α-HCH, 10-15% γ-HCH and minor amounts of the other isomers. Of the 5 isomers, only the γ-isomer (common name lindane) is insecticidally active. Technical HCH was used as a seed dressing as well as for controlling cockroaches and other pests. The technical mixture has been banned in most countries, including the U.S. in 1978,[23] however, lindane is still used in many countries.

Heptachlor

Heptachlor (HEP, Figure 4) is both a component of the pesticide chlordane as well as a pesticide itself. It was first registered for use in the U.S. in 1952, with commercial production beginning the following year.[25] Technical-grade HEP usually consists of ≈72% HEP and ≈28% impurities such as TC and CC and nonachlors.[26] HEP was used extensively from 1953 to 1974 for killing insects in homes, buildings, on seed grains and crops.[25,27] It was also used by exterminators and homeowners for termite eradication. HEP is transformed both metabolically and photochemically (relatively quickly for an OC compound) to the carcinogenic heptachlor epoxide.[28] Heptachlor epoxide (HEPX) degrades more slowly than HEP in the environment.[4] HEP was prohibited for agricultural use in the U.S. in 1988.[29] Use of existing stocks of HEP-containing termiticide products in the possession of homeowners, along with the commercial use of HEP products for fire ant control in power transformers, is still permitted.[29]

HEP



HEPX

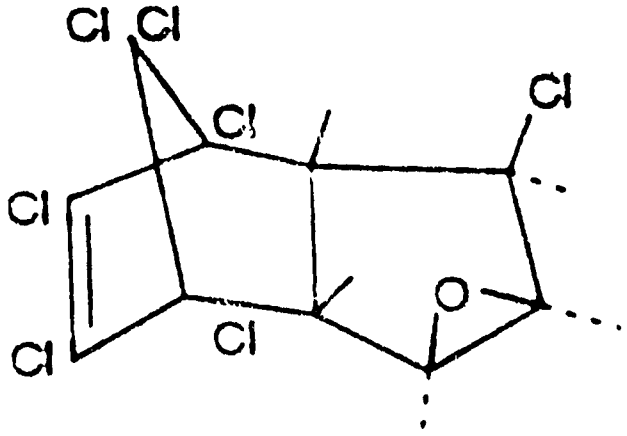


Figure 4. Structures of HEP and HEPX[4]

Aldrin and Dieldrin

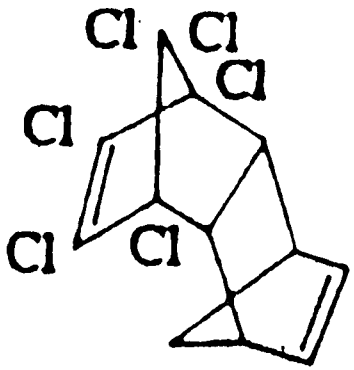
Aldrin and dieldrin (Figure 5) are the common names of two structurally similar compounds that were co-produced as insecticides. Aldrin was first synthesized in the U.S. as a pesticide in 1948.[30] It is quickly metabolized in the environment into dieldrin. Dieldrin was first described by Kearns in 1948 as Compound 497.[14] The name dieldrin was chosen because the compound is obtained from the Diels-Alder condensation reaction. It is highly toxic to mammals as well as many insects, acting as a contact and stomach poison.[14] Dieldrin, like most OC pesticides, is very persistent in the environment. It is more resistant to biotransformation and abiotic degradation than aldrin, and is found in low levels in all environmental media, with the highest levels occurring in soil and animal fat.[5] The primary uses for aldrin and dieldrin were control of corn pests and in the citrus industry.[31] The EPA has determined that aldrin and dieldrin are probable human carcinogens.[5] All uses of aldrin and dieldrin were canceled in the U.S. in 1987,[29] although countries in Asia and Central and South America continue to use these compounds.[32]

PESTICIDE ENANTIOMERS

Isomers

The complex structures of pesticide molecules result in the existence of many different types of isomers in commercial preparations. Isomers are groups of two or more molecules that have the same empirical formula but differ in the arrangement of their constituent atoms. Due to this difference, isomers have different chemical and physical

Aldrin



Dieldrin

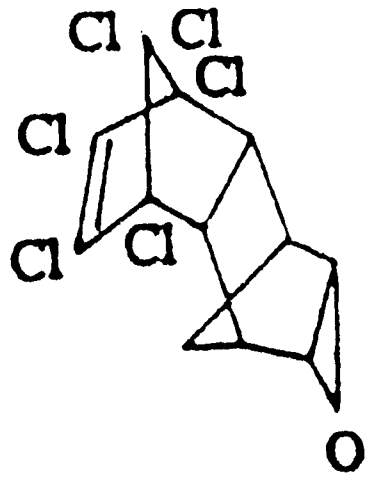


Figure 5. Structures of Aldrin and Dieldrin[5]

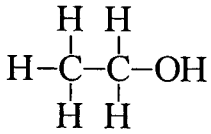
properties. Isomers can be broken into two main classes: regioisomers and stereoisomers. Regioisomers are those that have different connections among their constituent atoms, such as ethanol and dimethyl ether, both with the empirical formula C_2H_6O, but having different structures (Figure 6). Stereoisomers are those that have the same connection among atoms but a difference in the relative spatial positions of the atoms.

Stereoisomers

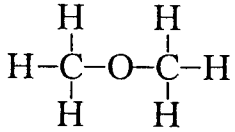
There are two kinds of stereoisomers: diastereosomers and enantiomers. Diastereosomers, also called geometric isomers, are molecules with two or more stereogenic centers that are not superimposable and not mirror images. An example of this is the α- and γ-isomers of HCH. The difference in these two isomers is in the orientation of the chlorine and hydrogen atoms on the ring (Figure 7).

Whereas diastereosomers can be separated by conventional methods such as chromatography due to the slight differences in their physical and chemical properties, enantiomers are not so easily distinguished. Enantiomers are molecules or ions that are non-superimposable mirror images of one another. These types of isomers are considered to have 'handedness' and are said to be chiral. A molecule that is chiral does not have a symmetry plane. A simple example of chirality and 'handedness' is the human hand (hence the term 'handedness'). If you place your left and right hand palm-to-palm, they are mirror images of each other. However, if you put one hand on top of the other, they are not superimposable. In the same manner as your hands are non-superimposable

C_2H_6O



Ethanol



Dimethyl ether

Figure 6. Structures of the Regeoisomers Ethanol and Dimethyl ether

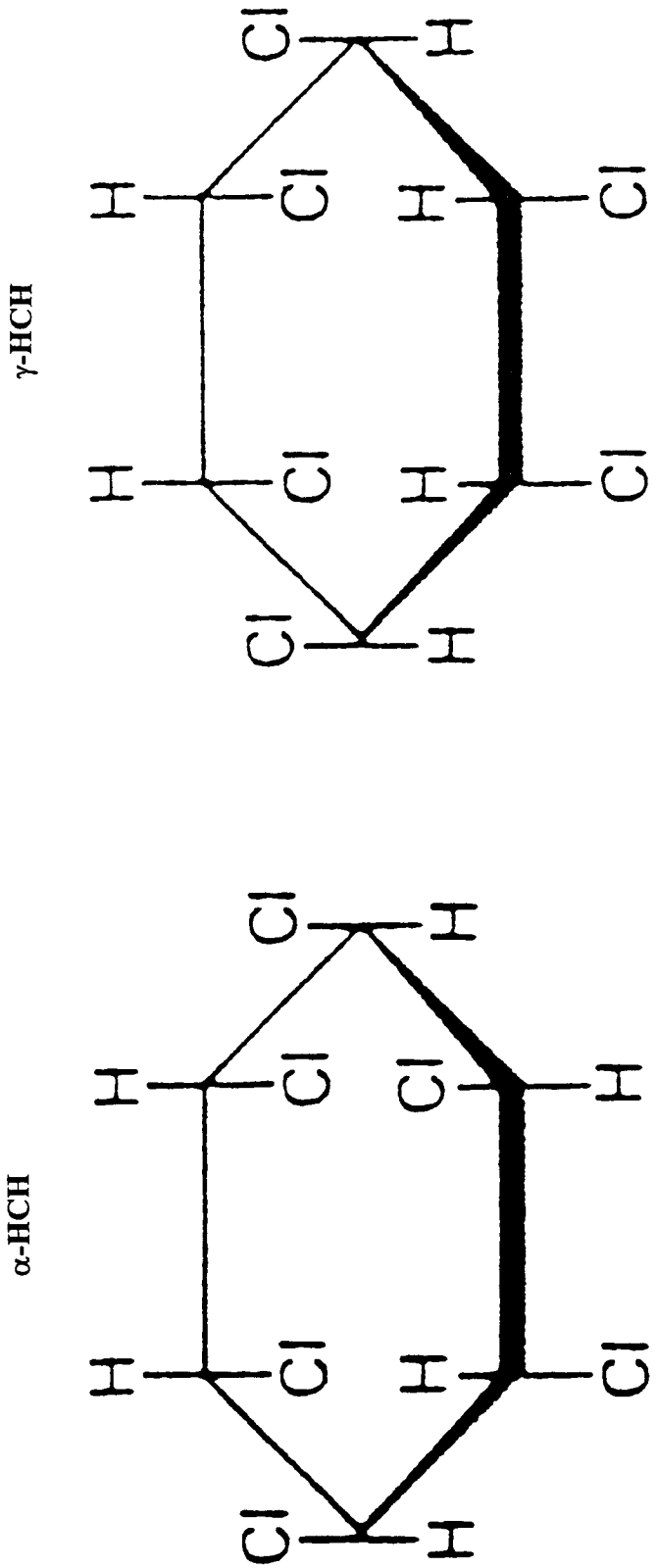


Figure 7. Structures of the Diastereomers α- and γ-HCH

mirror images of each other, certain chemicals also exist in 'left-handed' and 'right-handed' configurations.

Enantiomers are often referred to as optical isomers because of their effect on plane polarized light (ppl). One enantiomer will rotate ppl to the right and is designated by a positive sign (+), while the other enantiomer rotates ppl to the left and is designated by a negative sign (-). The (+) and (-) designations do not specify how the groups are arranged spatially but simply relate each structure to the direction of its specific rotation of ppl. An equivalent representation for designating enantiomers is to use a lower case **d** (dextrorotatory, from the Greek for 'right rotating') to indicate the (+) enantiomer and lowercase **l** (levorotatory, from the Greek for 'left rotating') to indicate the (-) enantiomer. An alternate way to refer to the two members of an enantiomeric pair is to apply the IUPAC rules for designating the absolute configuration of a chiral molecule as R (rectus, from the Latin for right) and S (sinister, from the Latin for left). This method specifies the direction of groups in space without relation to physical properties. Assignment of priority is made for each of the groups attached to the assymetric carbon. The detailed rules for assigning priorities are beyond the scope of this work but can be found in most organic chemistry texts. There is no simple relation that exists between the sign of the optical rotation (+, -) and the absolute configuration (R, S) of an enantiomer.

A 1:1 or equal mixture of the (+) and (-) enantiomers is called a racemic mixture or racemate. In a racemic mixture, the optical rotations produced by the individual enantiomers cancel each other out; therefore, a racemate is optically inactive.

The physical and chemical properties of enantiomer pairs are identical with the exception of their effect on ppl and their reactions with other chiral substances. An example of the latter difference can be illustrated using enzymes. Enzymes are proteins with chiral surfaces that are slightly different in different enzymes. Enzymes work by allowing a molecule (called a substrate) to temporarily fit into the contours of the enzyme's surface where it can undergo a chemical reaction. Referring to the example of the human hand, we can illustrate this point by adding gloves to our example. Gloves, like hands, are chiral. A glove fits well only on its corresponding hand, so that the left glove only fits the left hand. In the same manner, an enzyme can only accept a substrate molecule that has the right fit, usually only one of the enantiomers. If the enzyme molecule were not chiral it would not be able to discriminate between enantiomers. Chirality does not affect reactions with achiral compounds. Therefore, enantiomers have identical chemical properties toward all reactants whose molecules are achiral. Referring to our hand example, a ball is achiral and can fit into either the left or right hand.

CHIRAL-PHASE ANALYSIS

Chiral-phase analysis can be used to solve stereochemical problems as well as to resolve the enantiomers of a wide variety of compounds including lactones, alkyl halides, olefins, and amino acids.[33] It was first used to differentiate the enantiomers of flavors and drugs. Aromas of many fruits are composed of alkyl-substituted γ- and δ-lactones and are widely used in flavor formulation. Enantiomeric composition of chiral aroma compounds in natural products is related to biogenic pathways. The ability to identify enantiomers provides a way to differentiate between flavor compounds of natural origin

and synthetic racemates. This knowledge is useful to enforce food laws that regulate the use of natural, nature identical, and artificial aroma compounds.[34]

Because enzymes are often enantioselective, one enantiomer of a drug may be responsible for biochemical activity while the other may be the cause of toxic side effects. An example of this is the (+) enantiomer of chiral barbiturates which may have convulsive properties, while the (-) enantiomer shows narcotic activity.[35] For the antidepressant drug tranylcypromine, the (-) enantiomer is an inhibitor of catecholamine uptake and responsible for the drug's antidepressant effect, whereas the (+) enantiomer is the more potent monoamine oxidase inhibitor and contributes unnecessary toxicity.[35] This type of information is important for preparing drug formulations and may lead to regulations requiring enantiomerically pure drugs.[36]

Another area where specific enantiomer information could be useful is in agrochemistry. As with chiral drugs, chiral pesticides may also show enantiomer differences in non-target toxicity and pesticidal properties. Pesticide industries could use enantiomeric analysis in the synthesis of single enantiomer products as well as to study toxicity and metabolism of racemic products. Residue analysis of chiral pollutants in environmental samples could also help determine new means of decontamination by biological degradation[35] or to track releases to the atmosphere by giving an 'old' source signature.

An important challenge for analytical chemists has been to determine individual enantiomers in mixtures. Many advances have been made in this area in the last 10 years. Even though enantiomers possess the same physical and chemical properties, separations

of enantiomeric mixtures can now be accomplished by using chiral substances within the separatory medium. Some of the present chiral analytical techniques are: chiral-phase gas chromatography, chiral-phase liquid chromatography, and capillary electrophoresis with chiral modifiers. These techniques have led to the separation of a wide variety of structural, positional, and optical isomers.

Chiral-Phase GC

In the past, researchers have used cyclodextrins (CDs), and their substituted derivatives, as mobile phases in thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gel permeation chromatography (GPC), or as stationary phases in gas chromatography (GC), HPLC, and GPC. CDs are cyclic oligosaccharides with 6, 7, or 8 *D*-glucose units covalently attached by (1→4)-glycosidic bonds (Figure 8). The binding of different isomers with the particular CDs present in the stationary or mobile phase is the basis of the separations. Enantioselective CD-based GC separations are distinguished from HPLC separations on CD bonded phases by two factors: lower analyte size selectivity and easy resolution of enantiomers with little functionality.[37] HPLC separations generally require aromatic moieties and good hydrogen bonding groups close to the stereogenic center. Unlike HPLC, GC does not need to account for solvent effects, which can be pronounced. Capillary GC is highly efficient and advantageous for enantiomer separation as it often allows baseline separation of the two enantiomers with a relatively low selectivity factor.[38-40] Advantages of GC include simplicity, speed, sensitivity, reproducibility, and ease of detection.[37] König et al.,[41]

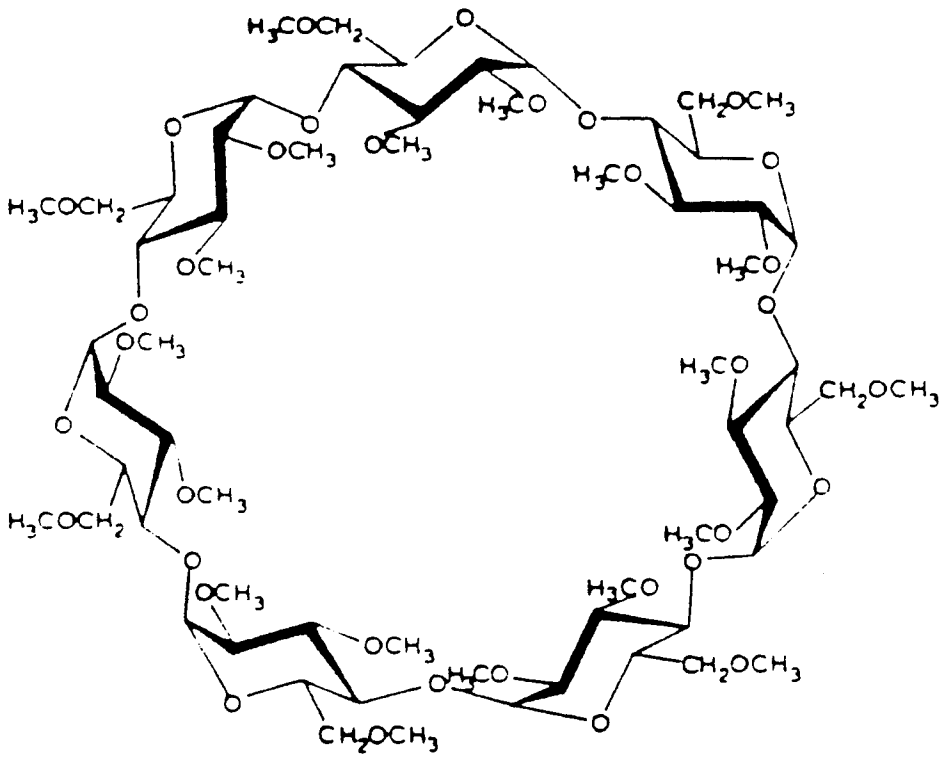


Figure 8. An Example of a Cyclodextrin Molecule

listed the following fields of application for enantioselective GC:

- Determination of absolute configuration of chiral natural products
- Determination of enantiomeric excess of enantioselective synthesis
- Determination of enantiospecificity of enzymatic and microbial transformations
- Determination of enantiomeric purity of chiral building blocks for organic synthesis
- Determination of enantiomeric purity of chiral pharmaceuticals
- Investigation of mechanistic aspects in chemical conversions of chiral compounds

The three main classes of chiral GC stationary phases are: amino acid derivatives, metal complexes, and derivatized CDs.[37] Of these, the derivatized CD phases are the most common for environmental analysis. Much of the early work using native CDs as GC stationary phases was done by Smolkova-Keulemansova and co-workers[42,43] and Koscielski et al.[44] Due to the highly crystalline nature of native CDs, researchers found them at first difficult to use as well as inefficient. However, the above authors work demonstrated that CDs are highly selective and form inclusion complexes with vaporized solutes. CDs were used in GC mainly for separation of geometrical isomers and only occasionally enantiomers until 1987.[44-46]

The first CD GC capillary columns had the CD (or its derivatives) coated directly onto the column. However, low thermal stability and poor inertness led to excessive retention times and high background.[47] Researchers then tried diluting the high-melting CD derivatives in apolar polysiloxane stationary phases.[47] Most columns presently

available for environmental analysis contain different derivatives of α-, β-, and γ-CDs diluted into relatively non-polar stationary phases similar to those in DB-5 and SE-54 columns. Moderate column temperatures must still be used, usually less than 250°C, for all CD GC columns.

Although the use of CDs and their derivatives for GC separations has been receiving increasing attention, very little work has been done to determine the main factors governing the separations. Armstrong et al.[48] demonstrated that when used in HPLC, CDs form inclusion complexes (host-guest complexes) between the CD cavity and the individual enantiomers. For CD GC columns, multiple retention mechanisms have been postulated including: hydrogen bonding, dipole-dipole interactions, inclusion complexes and surface interactions.[37,41,49] Hydrogen bonding plays a major role in the chiral recognition in HPLC and with native underivatized CDs.[37] In GC, however, many of the compounds separated contain no hydrogen bonding groups and, after derivatization, many CD GC stationary phases were no longer good hydrogen bond donors as were the native underivatized CDs.

Common derivatizations of the α-, β-, and γ-CDs include: permethylation, acylation, alkylation, tert-butyldimethylsilation and perethylation. It has been found that, in comparing the properties of the α-, β-, and γ-CD derivatives, the enantioselectivity is strongly influenced by the size of the cavity.[41] However, it has been questioned whether a substrate must actually intrude into the cavity or if the overall chirality of the CD matrix is sufficient. Berthod et al.[37] postulated two retention mechanisms: a classic inclusion complex formation, and a loose, external, multiple association with the outer part of the

CD. NMR studies had revealed the inclusion complexes to be rather weak[50] and chiral separation studies with open-chain polysaccharides have added strength to the argument for the importance of the outside wall in separating enantiomers.[51] A large temperature dependence indicates that the interaction between host and guest molecules is very delicate and relatively weak.[49] As soon as more detailed information about the retention mechanisms is available, CDs could be selectively derivatized (tailor-made) for special applications.

Application to Pesticide Analysis

Commercial pesticides are produced as racemic mixtures in which the ratio of the enantiomers is 1:1. If degradation in the environment is primarily caused by non-enzymatic processes (such as photolysis and hydrolysis), this ratio would remain the same since the enantiomers have identical chemical and physical properties. Biotic processes, however, are enzymatically controlled. Since enzymes are chiral molecules which may be enantioselective, chiral pesticides that are degraded by biological systems may show enantiomeric differences from those of the technical product.

Enantiomers of pesticides representing several chemical classes (OCs, coumarin rotenticides, organophosphates, carbamates, herbicides) as well as chiral low molecular weight halocarbons, have been resolved on a suite of chiral-phase columns.[17,28,35,38,46,47,52-55] The first GC separation of a chiral OC pesticide was reported by König et al.[41] in which the enantiomers of α-HCH were separated using γ-CD with 3-O-butyryl and 2,6-di-O-pentyl residues. The same group later published methods for the separation of the enantiomers of chlordane, HEP, HEPX, trichlorfon, fonofos and

methamidophos using derivatized β- and γ-CDs.[35] Since then, several groups have developed chiral GC columns for agrochemicals using derivatized α-, β- and γ-CDs. Specific chiral OC pesticides and the columns on which they have been separated are listed in Table 1.

Meanwhile, several research groups began investigating the distribution and fate of toxic pollutants in the environment using chiral-phase GC. However, because no optically active reference materials were commercially available at the time, the order of elution of the enantiomers could not be determined. The order of elution is strongly dependent on the type of column.[17,39,56] Falconer et al.,[56] found a reversal of elution order for the enantiomers of α-HCH using a γ-CD vs. a β-CD column. They determined this by preparing a standard enriched in the (-) enantiomer by dehydrochlorination of racemic α-HCH with brucine[57] and injecting on the two columns.

In 1994, Hardt et al.[58] used preparative enantioselective GC to obtain enantiomerically enriched or pure samples for eight pesticides, including CC, TC, HEP, HEPX, OXY and α-HCH, which were then analyzed on several CD columns. Müller and Buser[55,59] used chiral HPLC with chiroptical detection to separate, isolate and identify the (+) and (-) enantiomers of CC, TC, HEP, HEPX, OXY, α-HCH, chlordene. o,p'-DDT and o,p'-DDD. These small isolated fractions were then used to determine the elution order for two β- and one α-CD column. The commercial availability of enantiomerically enriched and/or pure enantiomer standards will help relieve the confusion caused by differing elution orders on different CD columns.

Table 1. Chiral Phase Columns Used to Separate OC Compounds

Column	Compounds Separated	Reference
dimethyl-t-butylsilylated heptakis(2,3,6-tri-O-methyl)-β in polysiloxane	toxaphene	a
heptakis(2-O-methyl-3,6-di-O-pentyl)-β-CD	OXY	b
heptakis(2,3-di-O-methyl-6-O-tert-hexyldimethylsilyl)-β-CD	OXY	c
heptakis(2,3,6-tri-O-n-pentyl)-β–CD in OV1701	α-HCH	d, e
heptakis(2,6-di-O-methyl-3-O-pentyl)-β-CD	TC, CC	c
heptakis(3-O-acetyl-2,6-di-O-pentyl)-β	α-HCH	c
heptakis(3-O-butyryl-2,6-di-O-pentyl)-β–CD	α-HCH	c, d, f, g, h
octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-CD in OV1701	α-HCH	b, i
perethylated α-CD in OV1701	TC, CC	j, k
permethylated β-CD in OV1701	α-HCH	l
permethylated β-CD in phenyl polysiloxane or DB1701	α-HCH	h,m, n, o
permethylated heptakis(2,3,6-tri-O-methyl)-β-CD in OH-terminated methylpolysiloxane	TC, CC	p
permethylated heptakis(2,3,6-tri-O-methyl)-β-CD in OH terminated polysiloxane	α-HCH	j, k, q
permethylated γ-CD in phenyl polysiloxane	α-HCH	m
tert-butyldimethylsilyl-2,3-dimethyl-β-O in OV1701	DDT	a
tert-butyldimethylsilyl-β-CD in OV1701	α-HCH, OXY, toxaphene,	n, o, r
	DDT, HEPX, TC, CC	
a: Buser & Müller, 1995	l: Tanabe et al., 1996	
b: Pfaffenberger et al., 1994	m: Falconer et al., 1995a	
c: Hardt et al., 1994	n: Falconer et al., 1996	
d: Ludwig et al., 1992	o: Jantunen et al., 1996	
e: Moller et al., 1993	p: Müller et al., 1992	
f: Huhnerfuss et al., 1992	q: Buser et al., 1992	
g: Faller et al., 1991	r: Buser & Müller, 1994	
h: Mössner et al., 1992		
i: Pfaffenberger et al., 1992		
j: Müller & Buser, 1994		
k: Buser & Müller, 1993		

Many of the chiral GC columns used for environmental studies have been custom made by the researchers and are just now becoming commercially available. Since the analytical techniques for these separations are still in the development stage, columns must be screened for their ability to resolve the compounds of interest. At present, there is no way to select chiral columns in advance. For example, the PS086/PMCD (OH-terminated dimethylpolysiloxane/permethylated β-CD) column used by Buser and Müller[17] resolves enantiomers of CC and TC, but not the structurally similar OXY.

CHIRAL PESTICIDES IN THE ENVIRONMENT

Since the early 1990s many articles have appeared which document the occurrence of non-racemic pesticide residues in the environment. Selective accumulation or degradation of one enantiomer over the other is often described by the enantiomer ratio (ER), the ratio of peak areas of the (+)/(-) enantiomer. A racemic mixture would therefore have an ER = 1.0, an excess of the (+) enantiomer an ER >1 and an excess of the (-) enantiomer an ER <1. The occurrence of chiral OCs and their ERs in various environmental compartments are reviewed in the following subsections.

Water

Investigations of enantioselective breakdown in water have largely been with the pesticide α-HCH, since it is more water soluble than most other OCs and is thus found in lake and ocean water at higher concentrations. Early papers by Faller et al.,[60,61] Hühnerfuss et al.[62] and Pfaffenberger et al.[63] describe the occurrence of non-racemic α-HCH in the North Sea and nearby waters. Faller et al.[61] obtained ERs ranging from 0.80

to 1.15, showing degradation of the (+) enantiomer in some regions and the (-) enantiomer in others. They postulated that in regions with high γ-HCH concentrations, transformation of achiral γ-HCH to α-HCH would give rise to an excess of the (-) enantiomer of α-HCH (ER < 1), while in regions of high α-HCH concentration, the (-) α-HCH is preferentially degraded (ER > 1). Later studies by Hühnerfuss et al.[62] and Ludwig et al.,[64] however, determined that achiral γ-HCH is isomerized to α-HCH non-enantioselectively. Another study (in the same region where Faller found an ER = 0.83) reported a mean ER of 0.84 ± 0.03.[63]

Enantioselective degradation of α-HCH in freshwater was investigated by Falconer et al.[56] who measured ERs in a small Arctic lake and its watershed on Cornwallis Island, N.W.T., Canada. Whereas a 5-15% deficiency of the (+) enantiomer had been seen in seawater off the coast,[65] the enantioselectivity was greater in the freshwater system with ERs for the lake itself averaging 0.77 ± 0.004. The ERs in streams feeding into the lake changed with time showing degradation occurring most rapidly in the creek with the highest sediment load and temperature. ERs of α-HCH in the snowpacks were essentially racemic (0.98 ± 0.03). The differing ER values in the streams vs. the lake allowed the authors to follow the flow of fresh meltwater under the ice and over the bulk lake water.[56]

ERs can also be utilized to investigate air-water gas exchange. Studies done in the Canadian Arctic revealed an ER of 1.00 ± 0.04 in air[65] which agreed well with Müller et al.[66] who found an ER of 1.02 in ambient air in Norway. Jantunen et al.,[67] measured seawater and air from the North Pacific across the North Pole to the North Atlantic. They

found the ratio of (+)-α-HCH/(-)-α-HCH in surface water was 1.10-1.15 in the Bering-Chukchi seas and 0.75-0.88 in the Arctic Ocean, indicating a difference in enantiomeric breakdown processes in the different regions of water. Air sampled 40 m above the sea surface resulted in deficits of (-)-α-HCH over the Bering-Chuckchi Seas, (+)-α-HCH over the Greenland Sea and near racemic over the Canadian Basin at high latitudes.[67] The appearance of non-racemic α-HCH in air confirms sea-to-air volatilization which is predicted from fugacity calculations for these regions.[67]

Biota

Enantioselective accumulation may result from either selective metabolism or selective permeation through membranes.[68] Selective accumulation has been reported in several types of biota for α-HCH, chlordane, OXY, HEP, HEPX, and o,p'-DDT. These studies are reviewed below.

α-Hexachlorocyclohexane Enrichment of the (+) enantiomer of α-HCH has been found in marine mammal blubber, neonatal northern fur seals, sheep liver and eider duck liver.[66,68-71] Other studies found enrichment of the (-) enantiomer in roe-deer liver, sheep brain, flounder liver and blue mussels.[68,70] Tanabe et al.[71] suggested that the ERs are dependent on factors such as feeding habits, transport processes, and specific enzymes as well as differences in trophic levels. They postulated that lower trophic level organisms have a lower metabolic capability and ERs therefore reflect their environment. For instance, the ERs of blue mussels (0.88) and flounder (0.89) were close to the values

of the water in which they live (0.84). On the other hand, ERs for fur seal brain (higher trophic level) were greater than 30, suggesting a higher metabolic capacity.[71]

Chlordane. Past research has shown selective degradation of both CC and TC as well as OXY. The chiral components of chlordane were analyzed by Müller and Buser[55] in which they found opposing trends for CC and TC enantiomers in herring oil and salmon muscle tissue. Herring showed higher levels of (-)-TC and (+)-CC while in salmon there was an excess of (+)-TC and (-)-CC. It was also reported that TC was metabolized in pigs to give an excess of (+)-OXY, whereas CC gave optically inactive (racemic) OXY. In an earlier study, Buser and Müller[17] determined the ERs of OXY in herring oil, salmon muscle, grey seal liver and human adipose tissue and found a higher abundance of (+)-OXY (1.3-1.6) in all samples.

Heptachlor Epoxide. Müller and Buser[55] examined the enantiomeric composition of HEPX in herring oil, salmon muscle, grey seal liver and human adipose tissue. HEP was absent from the samples due to its rapid conversion to HEPX. All aquatic species showed enrichment of the (-)-HEPX while the adipose tissue had more (+)-HEPX. Photolysis of HEP has been shown to lead to racemic HEPX[28] while incubation of racemic HEP with rat liver homogenate yielded an excess of (+)-HEPX[55] Analysis of the unreacted HEP, however, showed (-)-HEP as reacting faster. No explanation was given for this controversial finding, however, in a later paper, the authors found that although the absolute configuration (R, S) of an enatiomer is conserved during degradation, optical activity (+, -) is not always conserved.

DDT. DDT reportedly binds to steroid hormone receptors and mimics the action of natural estrogens.[72] McBlain et al.[73] found that the two enantiomers of o,p'-DDT differed in estrogenic activity, the (-) enantiomer being far more active. Buser and Müller[54] reported an ER of ≈0.8 for o,p'-DDT in human adipose tissue and ≈0.7 for cod liver oil, indicating that the estrogenically more potent (-) enantiomer is less abundant.

Soil

Very little work has been published to date on chiral OCs in soils. Müller et al.[66] examined α-HCH in soil near a former HCH factory and found an ER close to racemic with a slight depletion of the (-) enantiomer. In a study where sewage sludge was incubated with α-HCH, degradation was found to be highly enantioselective with the (+) enantiomer degrading much faster.[54,59] The ER values changed from the initial ≈1.0 (0 h) to 0.17-0.21 after 48 h, whereas no enantioselectivity was observed in sterilized sludge.[59] Falconer et al.[74] determined ERs of several OCs in agricultural soils from British Columbia and found enantiomeric differences for α-HCH, OXY, o,p'-DDT and HEPX, but none for TC or CC.

Ludwig et al.[64] used bacteria from marine sediments to degrade α-HCH and lindane in laboratory experiments to verify that enantiomeric degradation does occur. They found that achiral γ-HCH degrades to γ-pentachlorocyclohexene (γ-PCCH) non-enantioselectively (ER = 1.0), whereas the chiral α-HCH is converted to β-PCCH enantioselectively, suggesting that a chiral substrate is required to obtain enantioselective

decomposition. They also found that the (+)-α-HCH and its corresponding β-PCCH enantiomer were degraded faster than the corresponding (-) enantiomers.

CHAPTER TWO

STATEMENT OF PURPOSE

Past research has illustrated that selective enzymatic degradation of enantiomers by microorganisms does occur (Chap. 1, Chiral Pesticides in the Environment). By measuring the ERs of OC pesticides in soils, we may be able to distinguish between 'old' and 'new' atmospheric sources. Freshly applied pesticides which volatilize into the atmosphere should be subject to only non-biological degradation processes such as photolysis and attack by hydroxyl radicals. Therefore, pesticides and their breakdown products from new sources are expected to be racemic. Pesticides are lost from soils by physical processes (volatilization, leaching), chemical breakdown and microbial attack. Residues in soils that have been subjected to microbial degradation might be non-racemic due to the enantioselectivity of enzymatic processes. Enantioselective breakdown in soils may thus result in pesticide residues that give an 'old' source signature as they volatilize, and such signatures could be used to track releases to the atmosphere.

This work was done to determine the enantiomeric ratios and concentrations of several chiral OC pesticides in soils from across Ohio. This type of information could be used for a variety of purposes such as tracking releases to the atmosphere, determining new means of decontamination through biological degradation[35] or as tracers of geochemical processes.[56]

CHAPTER THREE

MATERIALS

Solvents were pesticide grade (Fisher Scientific, Pittsburgh, PA), or Omnisolv (EM Science, Cherry Hill, NJ). Other reagents were 100-mesh silicic acid (Mallinckrodt, Chesterfield, MO), 70-230 mesh neutral alumina (EM Science), anhydrous granular sodium sulfate and sulfuric acid (BDH Inc., Toronto, Ontario). Pesticides and other analytical standards were purchased from Accustandard (New Haven, CT) or Supelco (Bellefonte, PA). Helium, nitrogen, methane and hydrogen for the chromatographic instruments were ultra pure carrier grade. Nitrogen for concentrating samples was ultra-high purity grade. Pure cellulose thimbles (Whatman, Fairfield, NJ) were used for Soxhlet extraction of soils (Fisher Scientific).

The silicic acid was precleaned by baking at 120°C for 24 h; the alumina, and sodium sulfate at 400°C for 24 h. The alumina and silicic acid were deactivated with 6% and 3% water, respectively. Cellulose thimbles were precleaned by soxhlet extraction for 16 h in dichloromethane. Sulfuric acid was precleaned by shaking with petroleum ether. Nitrogen for concentration of samples was further cleaned with a Tenax-GC resin trap.

CHAPTER FOUR

EXPERIMENTAL

SAMPLE COLLECTION

Soil samples were taken during winter 1995 and spring 1996 across Ohio and have been designated as eastern (E1-E4), western (W1-W5) or central (C1-C7) (Figure 9). Samples were collected with a soil probe (when available) or clean garden tools to a depth of ≈25 cm at eight sites from the field then pooled to yield a representative sample, except for the C2 sample which was only from one site. The GS soil was not an agricultural soil, but a representative sample from an 8' x 8' garden located in Youngstown, Ohio. Each pooled sample was placed in clean aluminum foil, sealed in a plastic bag and frozen at 4°C until analyzed.

EXTRACTION AND CLEAN-UP

The soil was allowed to thaw, then was thoroughly mixed. Approximately 15 g of wet soil was mixed with anhydrous granular Na_2SO_4 to remove water. The samples were placed into pre-cleaned cellulose thimbles and Soxhlet extracted >20 h with dichloromethane (DCM). The extracts were reduced and transferred into isooctane by rotary evaporation, concentrated to 1-2 mL with nitrogen, and placed onto a silicic acid/alumina column (SA/Al_2O_3) similar to that of Keller & Bidleman[75] for cleanup and to fractionate compounds of interest before analysis. The SA/Al_2O_3 column was

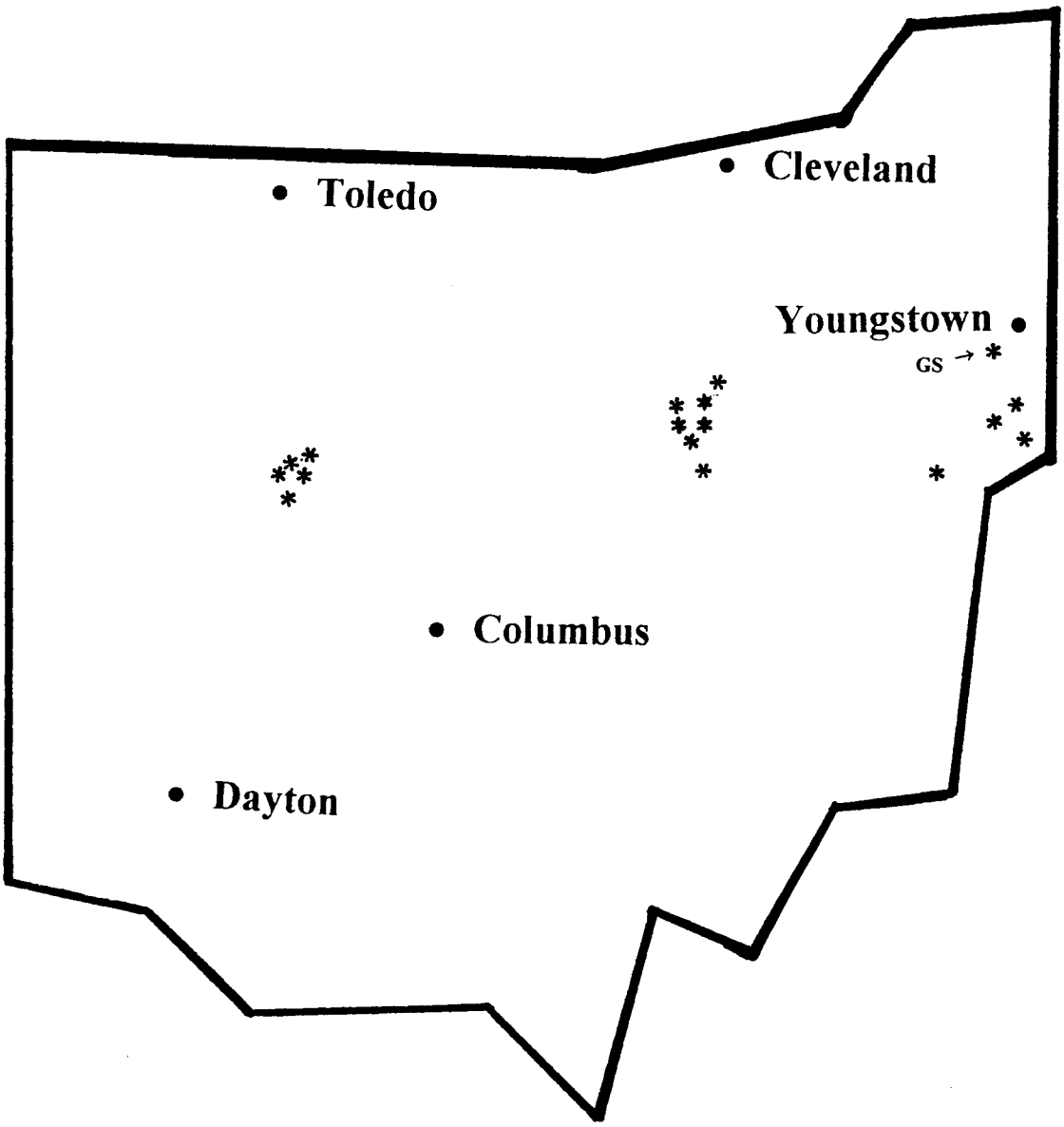


Figure 9. Sampling Sites in Ohio

composed of three layers (bottom to top):

1. 3 g silicic acid (3% added water)
2. 2 g alumina (6% added water)
3. 1 cm sodium sulfate

The layers were dry-packed into a 1 cm i.d. column with glass frit bottom and the column was prewashed with 30 mL DCM followed by 30 mL petroleum ether (PE). Solvents were pushed through the column with nitrogen. The sample was placed on the column and eluted in two fractions. Fraction 1 was eluted with 20 mL PE and contained HEP, p,p'-DDE and a portion of the o,p'-DDT. Fraction 2, 35 mL DCM, contained CC, TC, TN, dieldrin, HEPX, HCH, p,p'-DDD, p,p'-DDT, and the remainder of the o,p'-DDT. Both fractions were reduced and the solvent was exchanged to isooctane by rotary evaporation and concentration with nitrogen and brought to a known volume. A portion (10-20%) of Fraction 2 was adjusted to a suitable volume for analysis, spiked with mirex as an internal standard, and used for determination of dieldrin and HEP. Mirex was chosen as the internal standard because it is an OC pesticide with properties similar to the target compounds, but is not expected to be found in soil. The remaining portion of Fraction 2 and all of Fraction 1 were spiked with mirex and cleaned by shaking with 18 M H_2SO_4 on a vortex mixer for one minute. After centrifuging for five minutes the isooctane layer was removed and the volume was adjusted for analysis.

Wet soil from each location was weighed, air dried and reweighed to determine the percent water, which was used to adjust the sample weight to a dry weight.

ANALYSIS

Quantitative Analysis

Quantitative analysis of samples was done with a Hewlett Packard 5890 gas chromatograph, equipped with dual ^{63}Ni electron capture detectors (GC-ECD), using a DB5 column (60 m, 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific). Samples were injected splitless (split opened after one 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 4-5 standards which spanned a 50-100 fold concentration range (e.g. 0.001-0.2 ng/μL) using mirex at 0.1 ng/μL as an internal standard. Chromatographic data were collected and processed using a Hewlett Packard Chemstation. Examples of calibration plots are shown in Figures 10-18.

Enantiomeric Analysis

Determination of enantiomers was done with a Hewlett Packard 5890 GC-5989B MS Engine mass spectrometer (GC-MS) operated in the negative ion (NIMS) or electron impact (EIMS) mode. Separations were carried out using either a Betadex-120 or BSCD column. The Betadex-120 column (30 m, 0.25 mm i.d., 0.25 μm film thickness; Supelco) was 20% permethylated β-cyclodextrin in SPB-35 (35% diphenyl, 65% dimethylpolysiloxane). The BSCD column (30 m, 0.25 mm i.d., 0.20 μm film thickness; BGB Analytik, Switzerland) was 20% tert-butyldimethylsilylated β-cyclodextrin in OV-1701 (14% cyanopropylphenylmethylpolysiloxane). Samples (2 μL) were injected

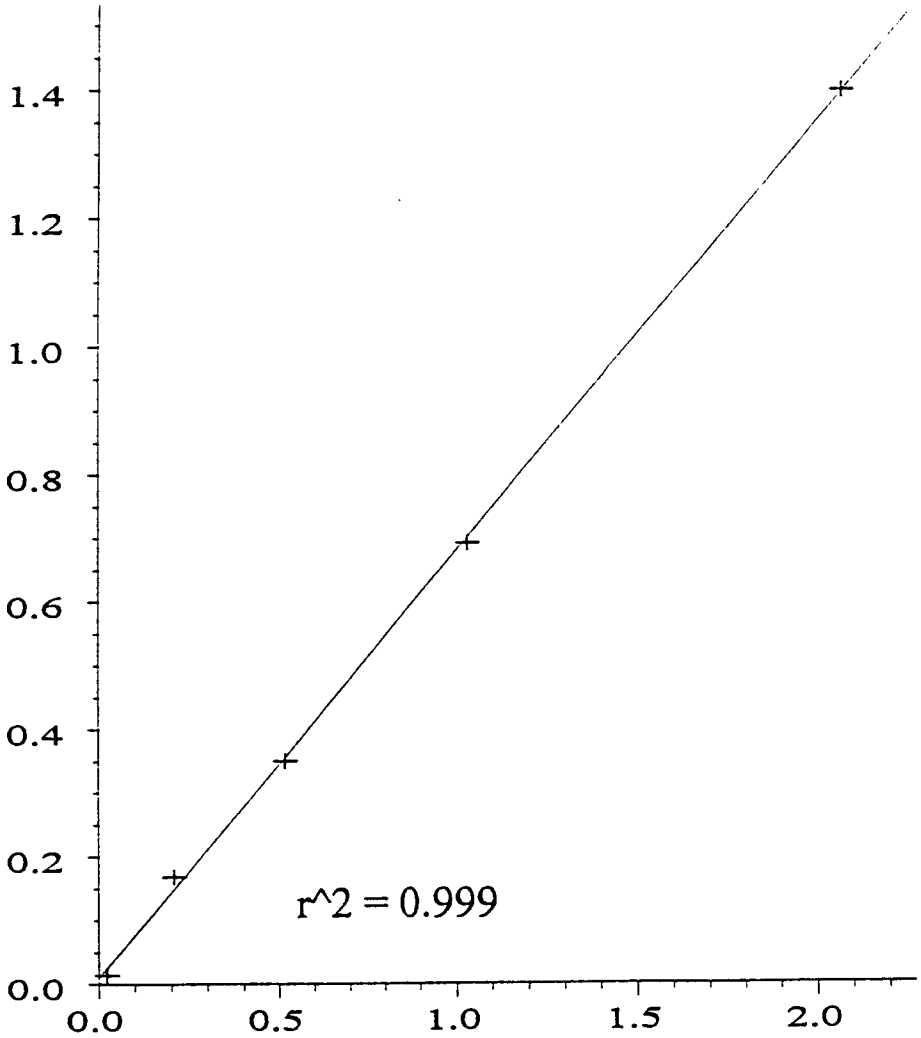


Figure 10. Calibration Plot of p,p'-DDT Standards

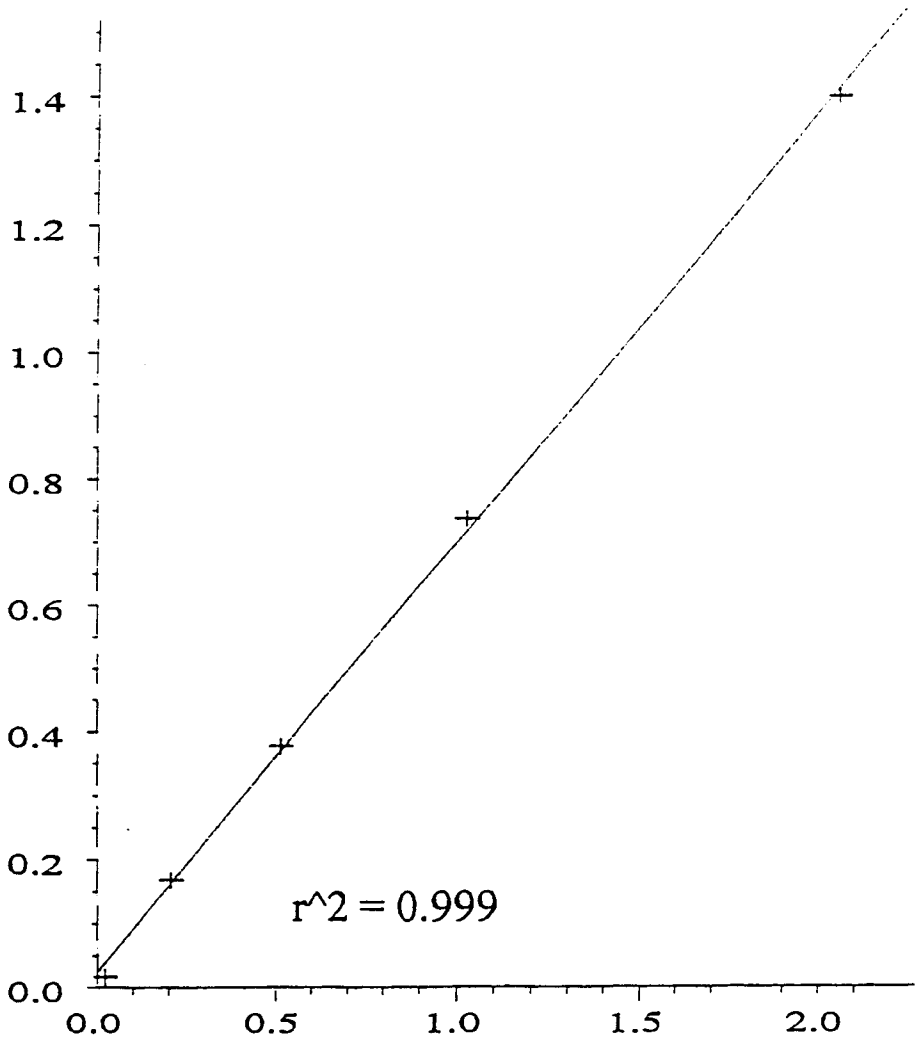


Figure 11. Calibration Plot of p,p'-DDD Standards

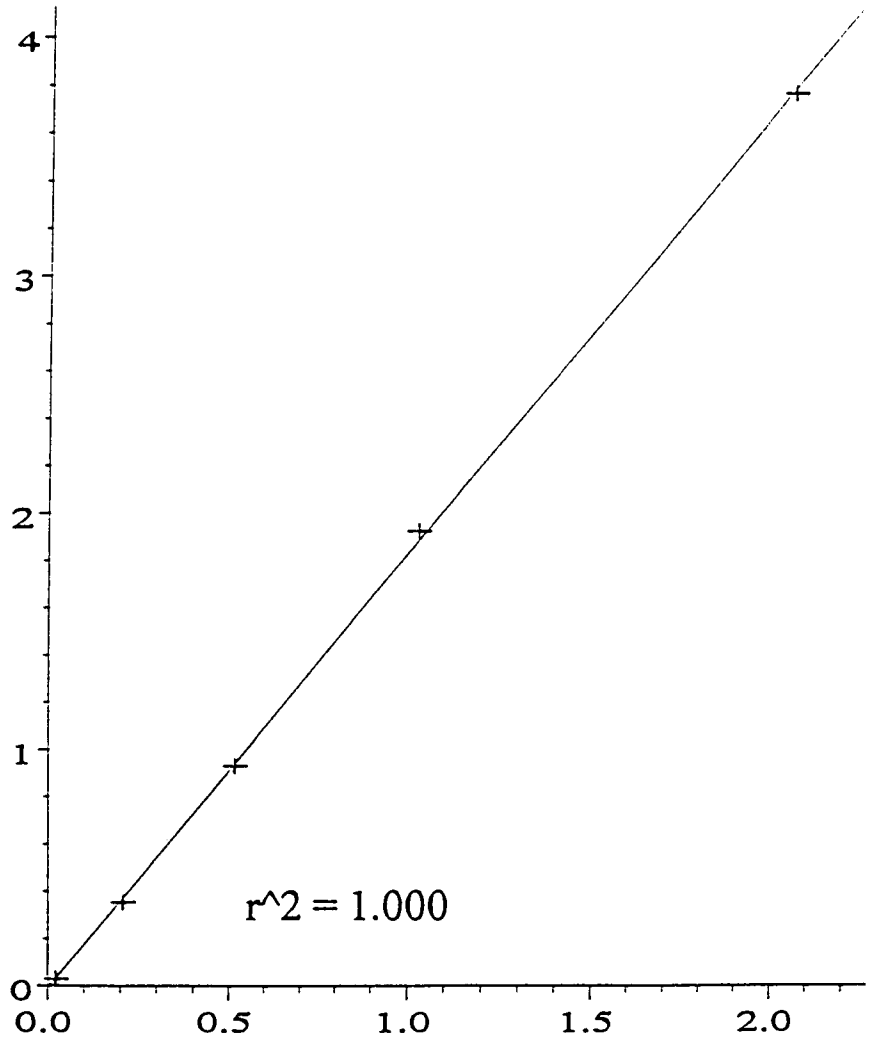


Figure 12. Calibration Plot of p,p'-DDE Standards

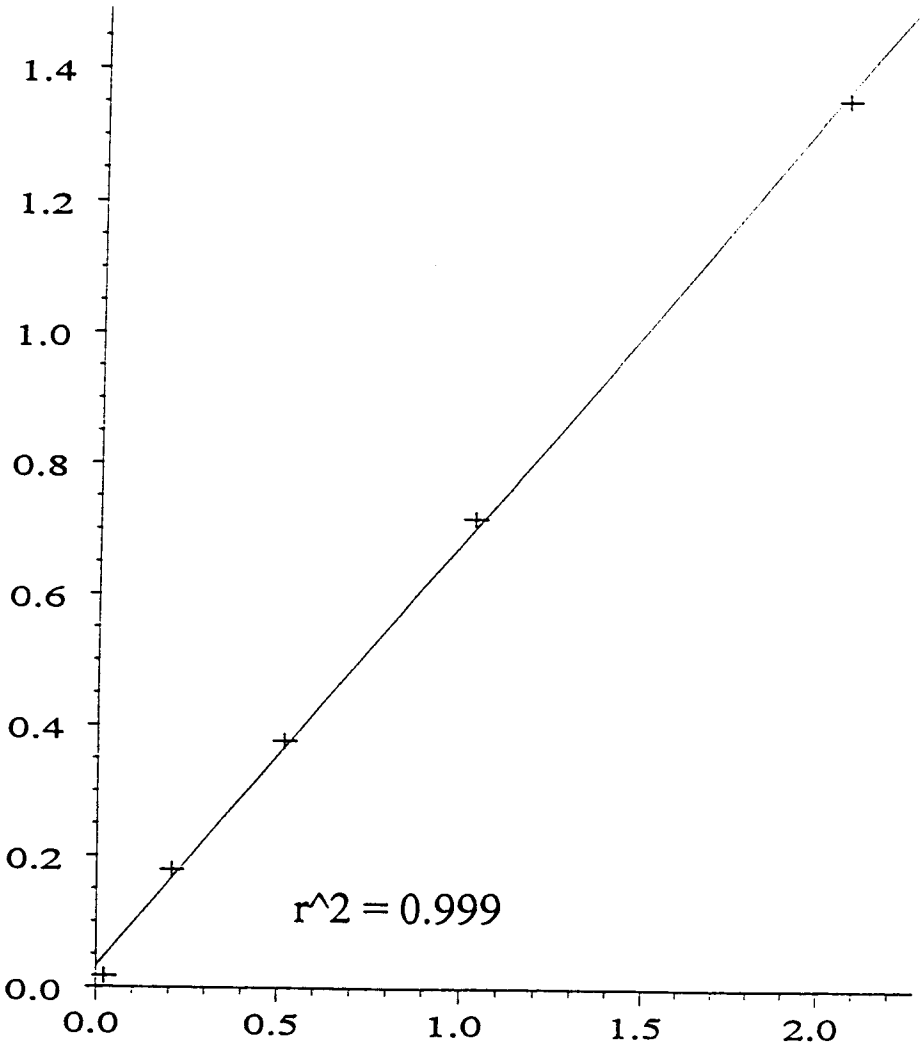


Figure 13. Calibration Plot of o,p'-DDT Standards

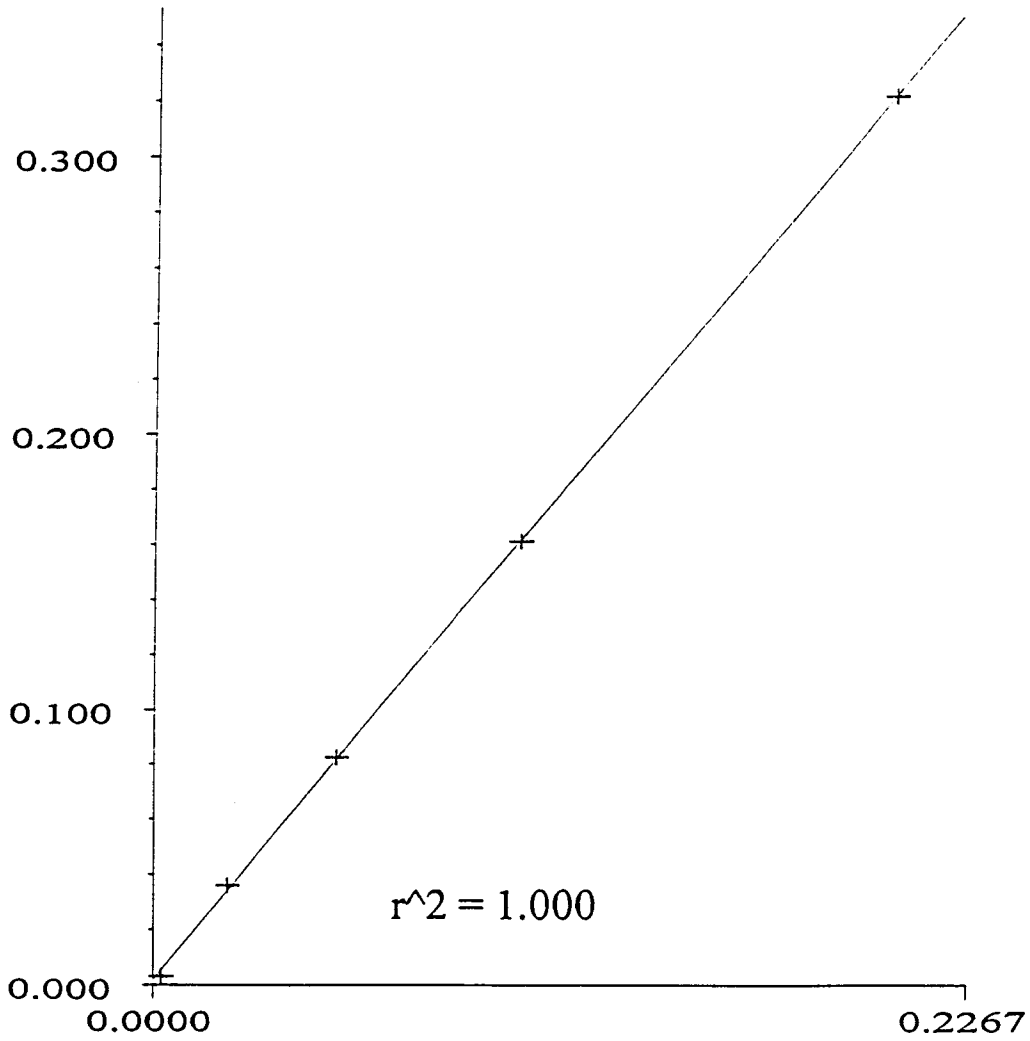


Figure 14. Calibration Plot of TC Standards

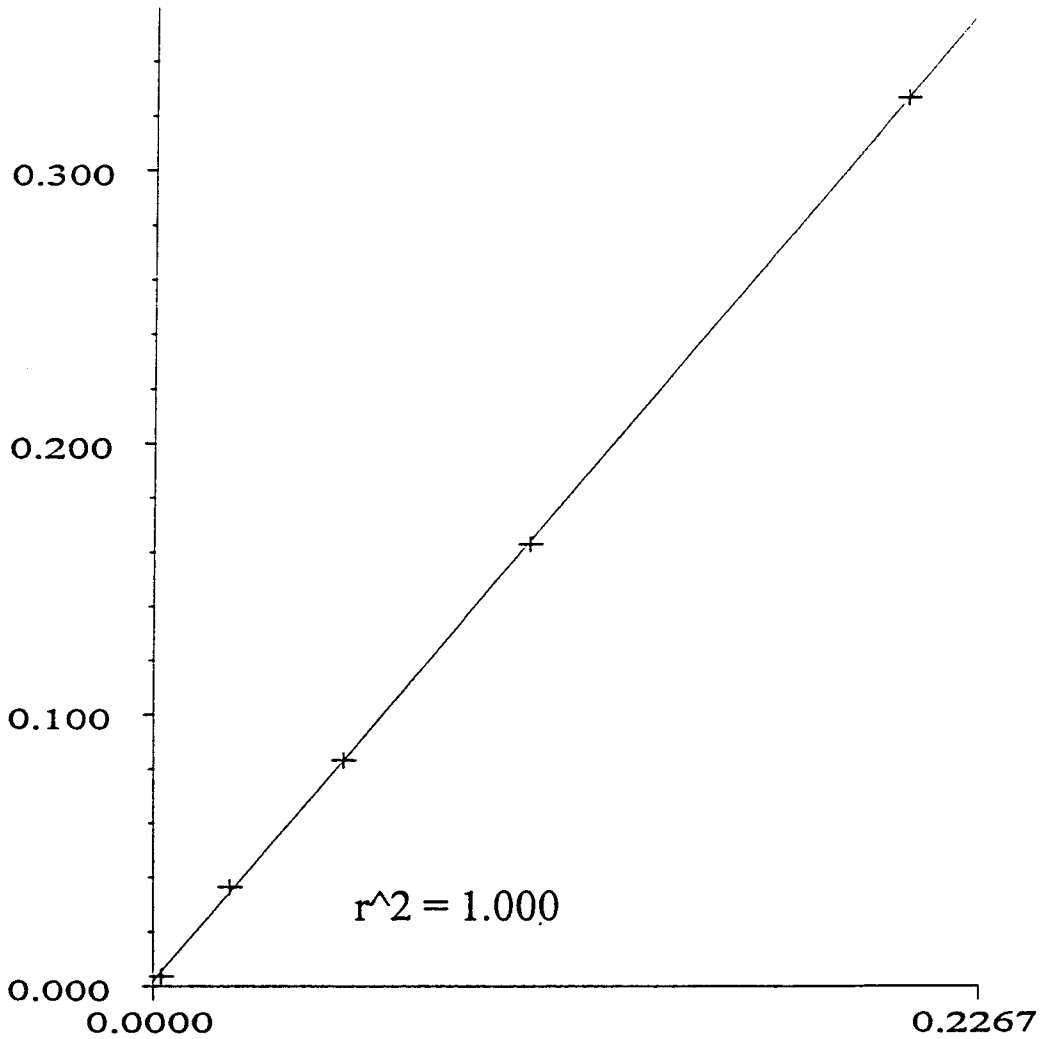


Figure 15. Calibration Plot of CC Standards

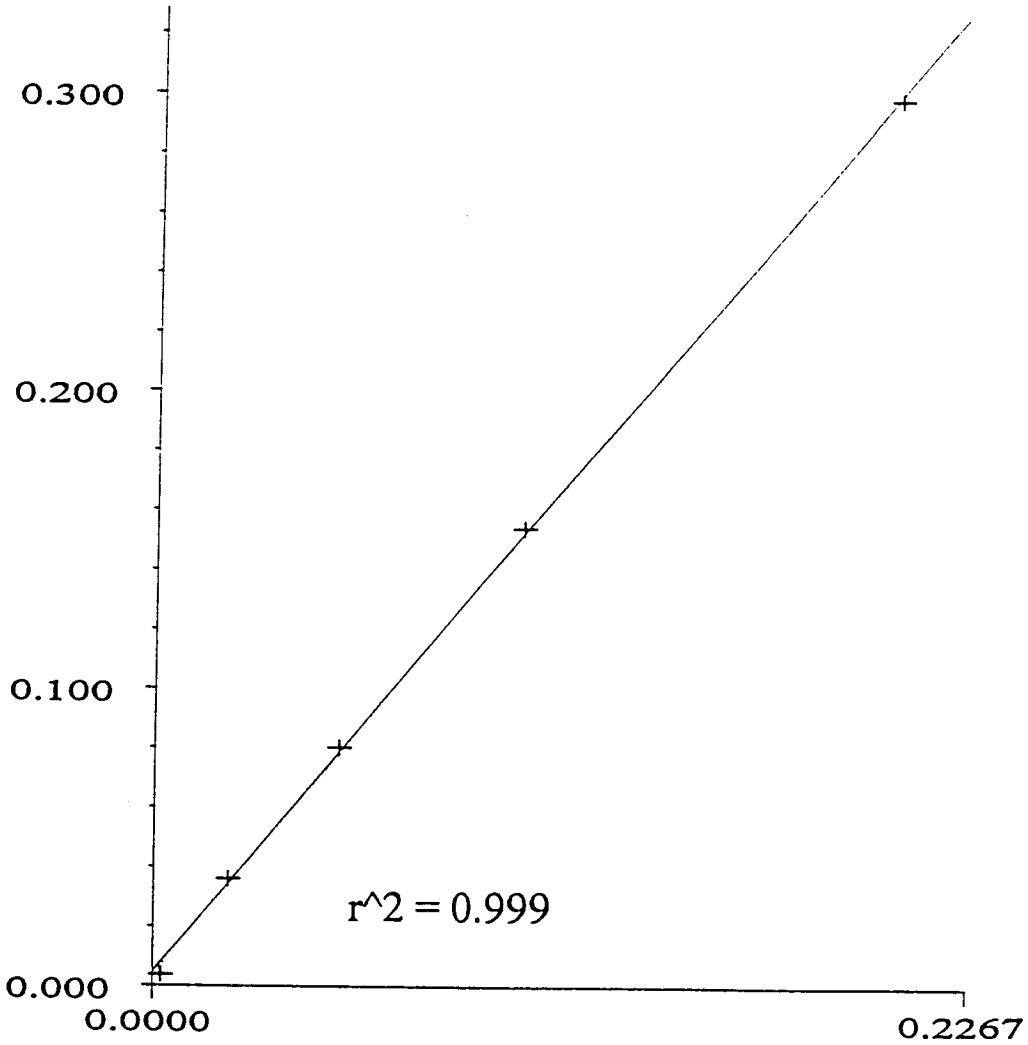


Figure 16. Calibration Plot of TN Standards

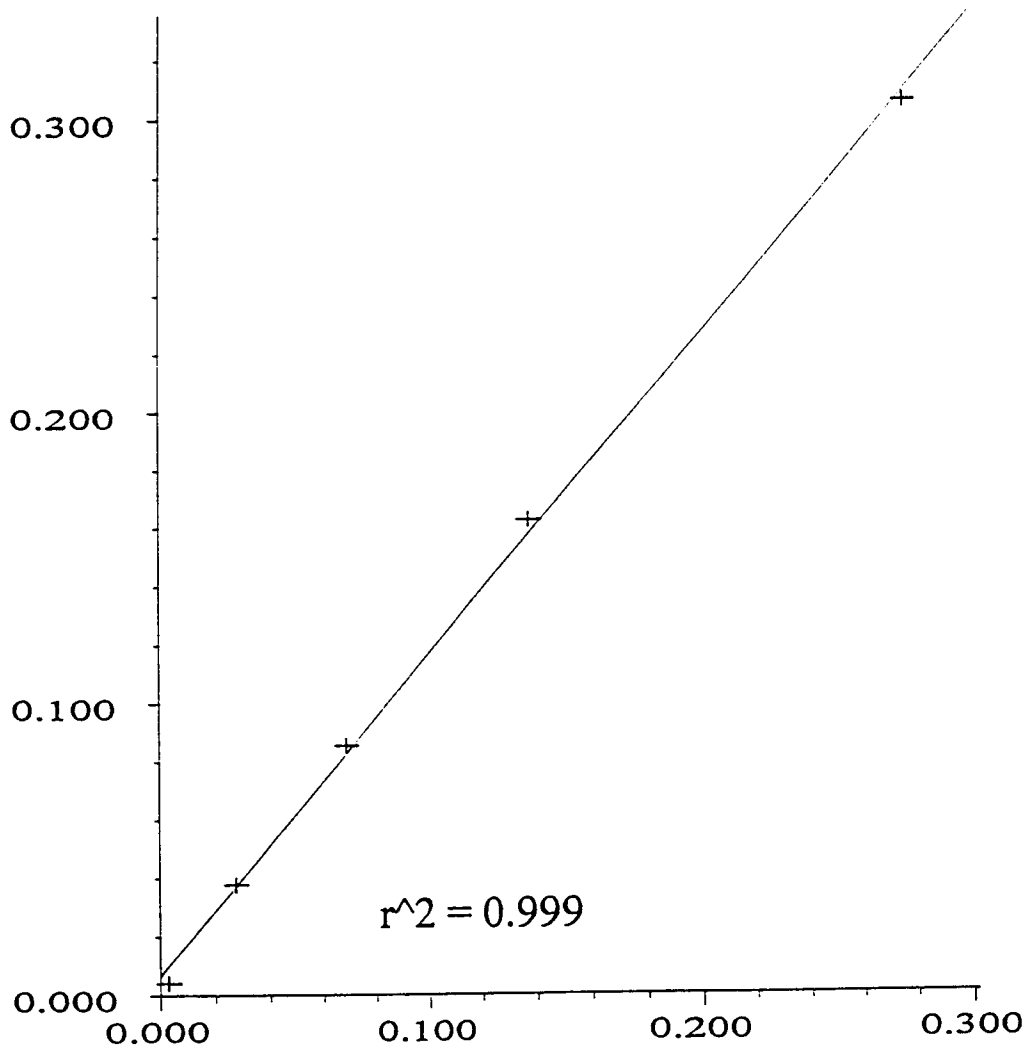


Figure 17. Calibration Plot of HEPX Standards

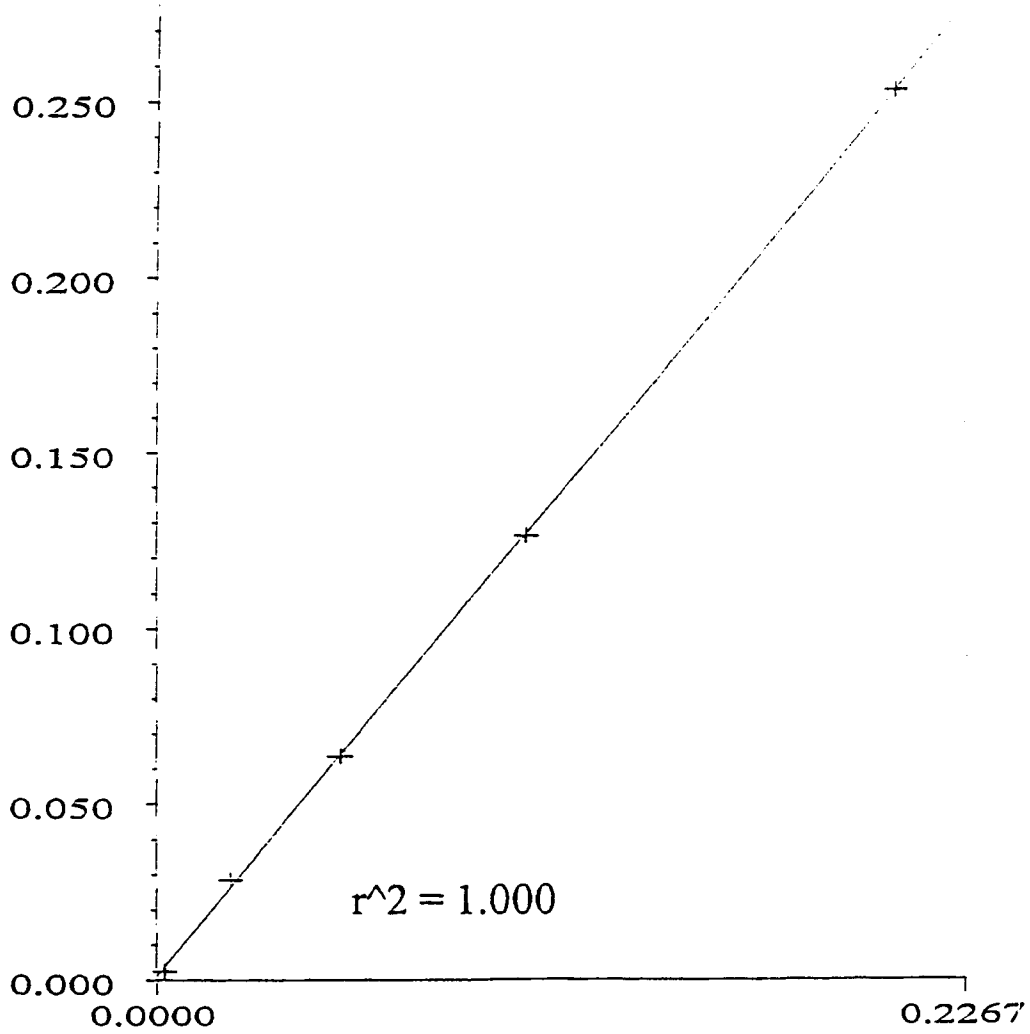


Figure 18. Calibration Plot of Dieldrin Standards

splitless (split opened after 1.0 min) at an oven temperature of 90°C. After a 1-min hold, the oven was ramped at 15°C min^{-1} to 150°C, 2°C min^{-1} to 235°C, held 2 min, ramped at 20°C min^{-1} to 250°C, and held 5 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. Methane pressure was 1.0 Torr. The BSCD column was used for separating enantiomers o,p'-DDT, HEPX, and OXY, while the Betadex column was used for TC and CC. The elution order of the enantiomers on each column is listed in Table 2. Table 3 lists the column (BSCD or Betadex), mode (EIMS or NIMS), and the ions monitored for each compound.

Table 2. Elution Orders of Chiral OCs on BSCD and Betadex Columns

Compound	BSCD	Betadex	Reference
(+)-TC	1	1	a,d
(-)-TC	2	2	a,d
(+)-CC	1	1	a,d
(-)-CC	2	2	a,d
(+)-α-HCH	2	1	c,d
(-)-α-HCH	1	2	c,d
(+)-o,p'-DDT	2		b
(-)-o,p'-DDT	1		b
(+)-HEPX	1		a
(-)-HEPX	2		a
(+)-OXY	1		a
(-)-OXY	2		a

a: Müller and Buser, 1994
b: Buser and Müller, 1995
c: Müller et al., 1992
d: Falconer et al., 1996

Table 3. GC Methods for Enantiomeric Analysis

Negative Ion	Ions
TC, CC	410, 412
o,p'-DDT	246, 248
OXY	420, 422; 316, 318
HEPX	386, 388
Electron Impact	
TC, CC	373, 375

CHAPTER FIVE

RESULTS AND DISCUSSION

QUANTITATIVE RESULTS

Quality Control

Blanks were processed by placing ≈15 g Na_2SO_4 into a Soxhlet thimble, then extracting and analyzing using the same procedure described in Chapter 4 for samples. The concentrations of OCs in blank extracts at 1.0 ml volume were all lower than the lowest calibration standard (0.002 ng/μL). Therefore the limits of detection (LOD) for OCs in the soils were estimated by multiplying the extraction volume (1.0 ml) by 0.002 ng/μL and dividing by the dry weight of an average soil sample (10 g). The LODs were thus 0.2 ng/g. Blank values were all less than the detection limit and therefore no blank corrections were made.

A summary of spike recoveries is presented in Table 4. Three spike recovery experiments were done with soil where residues were close to or below the detection limit. Approximately 15 g of soil was spiked with 200 ng TC, CC, p,p'-DDE, p,p'-DDD and p,p'-DDT, 250 ng TN and 214 ng o,p'-DDT, extracted and analyzed as for samples. After correcting for the native amounts in the soil, recoveries were all within acceptable levels (± 86-105%) so no recovery corrections were made.

OC Pesticide Concentrations in Soil

Of the sixteen agricultural soils sampled, only eleven were used for determination of pesticide concentrations; five of the seven central soils were not analyzed due to

Table 4. Spike Recoveries (ng/g); n=3

Compound	Mean (%)	(±) s.d.
HEPX	86	± 11
TC	88	± 9.1
CC	89	± 12
TN	90	± 13
p,p'-DDE	105	± 20
p,p'-DDD	95	± 13
o,p'-DDT	105	± 20
p,p'-DDT	100	± 4.0

contamination which yielded a complex and poorly resolved mixture upon GC analysis. The concentrations (mean ± s.d) of all compounds (ng/g dry weight of soil) are listed in Table 5. The mean and standard deviation were calculated from replicate extractions of the same soil. The residue information is discussed for each compound in the following subsections.

DDT. Levels for ∑DDT (p,p'-DDT + p,p'-DDD + p,p'-DDE + o,p'-DDT) ranged from 1.6-1072 ng/g. All soils analyzed contained all four DDT components with the exception of W4 which had o,p'-DDT levels below the LOD. The four eastern soils had the highest levels (205-1072 ng/g) and the two central soils the lowest (2.2-3.0 ng/g), while the western soils showed the most variability (1.6-345 ng/g). The GS soil had a ∑DDT = 30 ng/g. Forest soils in Maine which had been exposed to aerial application of DDT from 1958-67 were sampled in 1993 for DDT levels.[12] ∑DDT in these soils ranged from 270-1898 ng/g in sprayed areas and from 0-11 ng/g in unsprayed locations. After comparison to earlier studies in the same location, the authors suggested a 'half time' for disappearance of DDT residues of 20-30 years. Szeto and Price[22] found ∑DDT levels in agricultural soils from British Columbia, Canada ranging from 194-763 ng/g in silt loam soils and 2984-7162 ng/g in muck soils. Some of these soils were later analyzed for enantiomeric composition by Falconer et al.[74] and are discussed in Chapter 5, Enantiomeric Results.

Percentage of the parent compound (%PC) is a measure of the proportion of the initial compound (parent compound) to the sum of the remaining parent compound and its metabolites. For DDT, %PC was determined by dividing the concentration of p,p'-

Table 5. Average Concentrations (ng/g) of OC Pesticides in Ohio Soils

Sample	p,p'-DDE	p,p'-DDT	p,p'-DDD	o,p'-DDT	ΣDDT	TC	CC	TN	HEPX	Dieldrin
	Mean ± s.d.(N)	Mean ± s.d.(N)	Mean ± s.d.(N)	Mean ± s.d.(N)	ΣMean	Mean ± s.d.(N)	Mean ± s.d.(N)	Mean ± s.d.(N)	Mean ± s.d.(N)	Mean ± s.d.(N)
E1	77 ± 9.5 (4)	90 ± 23 (4)	23 ± 2.8 (2)	15 ± 6.3 (4)	205	13 ± 2.9 (4)	23 ± 4.2 (4)	33 ± 5.7 (4)	11 ± 1.2 (4)	20 ± 0.71 (2)
E2	502 ± 54 (2)	239 ± 7.1 (2)	2.4 (1)	31 ± 22 (2)	774	ND*	ND	ND	ND	ND
E3	570 ± 125 (2)	408 ± 76 (2)	16 (1)	78 ± 4.9 (2)	1072	ND	ND	ND	ND	ND
E4	319 ± 45 (2)	171 ± 0.71 (2)	2.9 ± 1.5 (2)	37 ± 4.9 (2)	530	ND	ND	ND	ND	ND
GS	5.0 ± 2.5 (3)	9.2 ± 3.7 (3)	14 ± 6.9 (3)	1.4 ± 0.55 (3)	30	7.1 ± 2.9 (3)	17 ± 6.6 (3)	21 ± 6.0 (3)	3.1 ± 1.6 (3)	4.3 ± 0.71 (2)
C1	0.47 (1)	1.3 (1)	0.79 (1)	0.40 (1)	3	0.25 (1)	0.39 (1)	0.58 (1)	0.63 (1)	0.20 (1)
C2	0.51 (1)	0.76 (1)	0.53 (1)	0.38 (1)	2.2	ND	ND	0.22 (1)	ND	0.09 (1)
W1	0.79 ± 0.30 (2)	5.2 ± 4.4 (2)	0.89 ± 0.08 (2)	0.94 ± 0.65 (2)	7.8	0.44 ± 0.06 (2)	0.68 ± 0.04 (2)	0.63 ± 0.16 (2)	0.16 ± 0.21 (2)	35 ± 0.00 (2)
W2	132 ± 16 (2)	161 ± 16 (2)	9.3 ± 1.1 (2)	43 ± 14 (2)	345	0.16 ± 0.11 (2)	0.13 ± 0.13 (2)	0.15 ± 0.06 (2)	0.26 (1)	0.41 ± 0.29 (2)
W3	84 ± 9.9 (2)	126 ± 45 (2)	8.6 ± 1.2 (2)	49 ± 9.9 (2)	268	0.33 ± 0.11 (2)	0.31 ± 0.25 (2)	0.61 ± 0.18 (2)	0.46 ± 0.28 (2)	2.5 ± 1.2 (2)
W4	0.29 (1)	0.48 (1)	0.76 (1)	ND	1.6	0.90 (1)	0.35 (1)	0.78 (1)	2.6 (1)	0.22 (1)
W5	1.9 (1)	3.5 (1)	0.33 (1)	0.41 (1)	6.1	ND	0.02 (1)	ND	ND	0.12 (1)

DDT by the sum of p,p'-DDT + p,p'-DDD + p,p'-DDE. In general the average %PC in the western soils was highest (55 ± 16), the eastern was lowest (38 ± 6) and central was in the middle (46 ± 6). The GS soil value was similar to the eastern soils at 33%. Using the average concentrations reported by Szeto and Price,[22] average %PC values were calculated for the British Columbia silt loam (84%) and muck soils (88%). No correlation between concentration and average %PC was found for either of these studies. Dimond and Owen[12] determined a average %PC for the Maine forest soils as 39%.

Chlordane. Levels for chlordanes in samples above the LOD ranged from 0.16-13 ng/g for TC and from 0.02-23 ng/g for CC. Of the four eastern soils only one (E1) had concentrations above the LOD, however, this soil had the highest concentration of both TC and CC for all soils (13 ± 2.9 ng/g TC, 23 ± 4.2 ng/g CC). All the western soils had relatively low levels of chlordane (0.16-0.90 ng/g TC, 0.02-0.68 ng/g CC) with the W5 soil being below detection limit for TC. One of the central soils (C1) was above the LOD with levels similar to that of western soils (TC = 0.25 ng/g, CC = 0.39 ng/g). The GS had concentrations almost as high as E1 (7.1 ± 2.9 ng/g TC, 17 ± 6.6 ng/g CC). Szeto and Price[22] reported the mean concentration for CC as 48 ng/g dry weight in silt loam and 174 ng/g in muck soils and for TC as 63 ng/g in silt loam and 508 ng/g in the muck soil from British Columbia.

TN concentrations for samples above the LOD ranged from 0.15-33 ng/g. The trends in concentrations for eastern, western and central soils for TN followed that of TC and CC. Of the eastern soils, only E1 was above the detection limit for TN (33 ng/g). Four of the five western soils were above the LOD, but all were relatively low (0.15-0.78

ng/g). One central soil had a concentration of 0.58 ng/g for TN, falling in the range for the western soils, while the other was not detected. The GS soil showed one of the highest concentrations of TN (21 ± 6 ng/g), similar to the trend for TC and CC. In British Columbia, Szeto and Price[22] found an average concentration of 59 ng/g in silt loam soils and 148 ng/g in muck soils.

Heptachlor epoxide. Seven samples were above the LOD for HEPX ranging in concentration from 0.16-11 ng/g. Concentration trends based on location were similar to that of TC, CC, and TN. One eastern soil (E1) was above LOD for HEPX (11 ± 1.2 ng/g), whereas, all western soils, except for one (W5), were above the LOD ranging from 0.16-2.6 ng/g. One central soil, (C1), had 0.63 ng/g HEPX, while the other was below LOD. The GS soil had a higher concentration (3.1 ± 1.6 ng/g) than the western and central soils but only about 1/3 that of the E1 soil. In British Columbia soils, HEPX averaged 16 ng/g in silt loam soils and 174 ng/g in muck soils.[22]

Dieldrin. Levels of dieldrin in samples above the LOD ranged from 0.09-35 ng/g. Dieldrin followed the same trends as TC, CC, TN and HEPX, except for one sample. This sample (W1) had the highest concentration of all samples at 35 ± 0.00 ng/g. This value falls quite far from the other western soils which range from 0.12-2.5 ng/g for dieldrin. As with the chlordanes and HEPX, only one eastern soil (E1) was above the LOD for dieldrin at 20 ± 0.71 ng/g. Dieldrin was, however, found in both central soils (0.09 & 0.20 ng/g). Szeto and Price[22] reported that dieldrin in muck soils averaged 692 ng/g but was not detected in silt loam soils.

ENANTIOMERIC RESULTS

Quality Control

Full scan mass spectra for each compound are shown in Figures 19-22. Two ions from the major cluster were monitored for each compound and ERs were calculated from the responses (each ER value is an average of the enantiomeric ratios from the two ions). Sample ion ratios were compared to standard ion ratios and had to be within ± 5% to be accepted. Repeated injections of individual standards resulted in the ERs ± standard deviations (s.d.) listed in Table 6. The accuracy of this analysis technique can be seen by the low standard deviations for the standards (± 0.01). Figures 23-26 are chromatograms of standards for TC, CC, HEPX, OXY and o,p'-DDT.

Buser et al.[47] stated that losses encountered during extraction and cleanup cannot change ERs, making enantiomeric composition much less subject to analytical variations than isomer composition. They also stated that ERs can be determined without pure enantiomeric standards due to the identical enantiomeric response of SIM detection. To determine the effect of analytical technique on ER for this study, the following checks were done:

1. comparison of ERs using both EIMS and NIMS modes
2. comparison of ERs using two different ion pairs
3. determination of ER for spike recovery sample

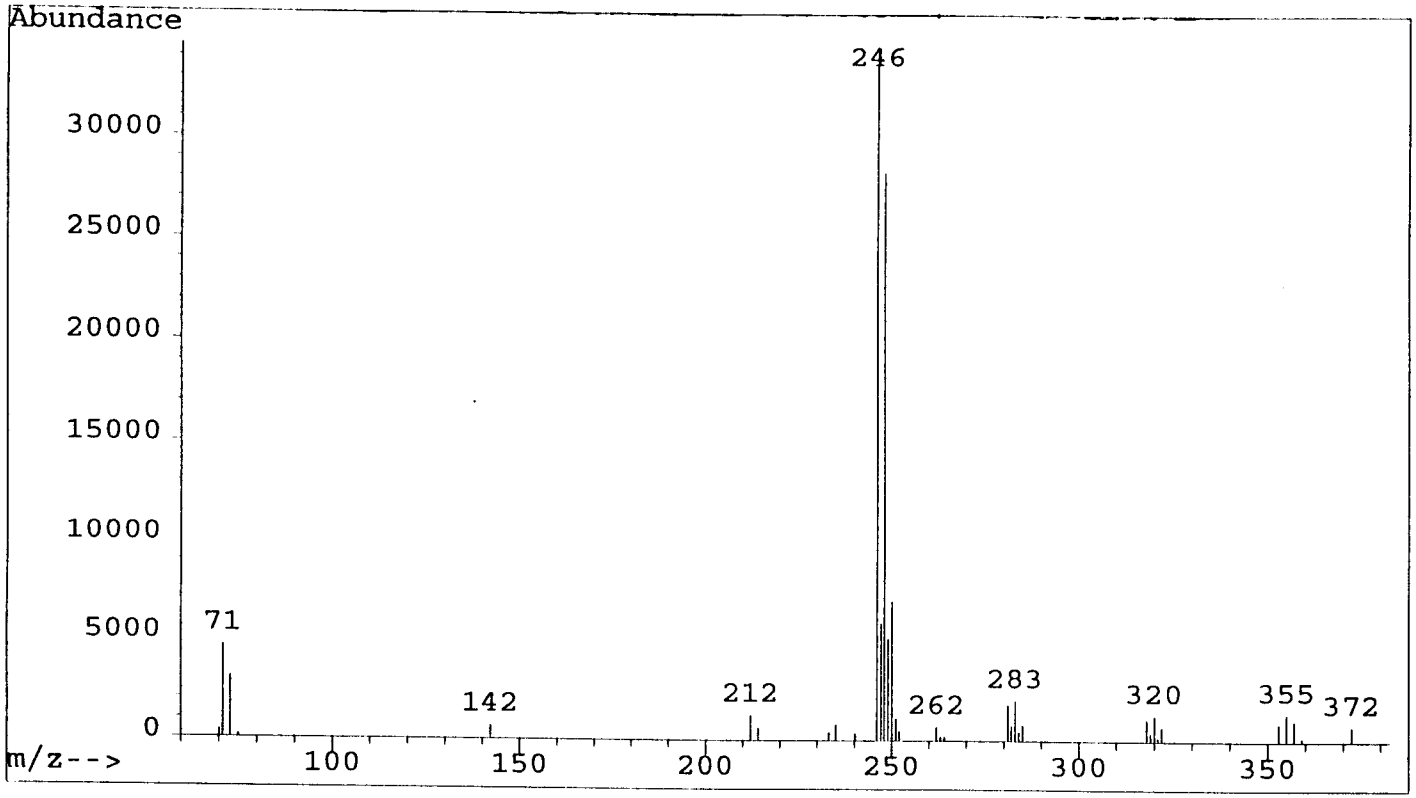


Figure 19. Full Scan Mass Spectrum of o,p'-DDT

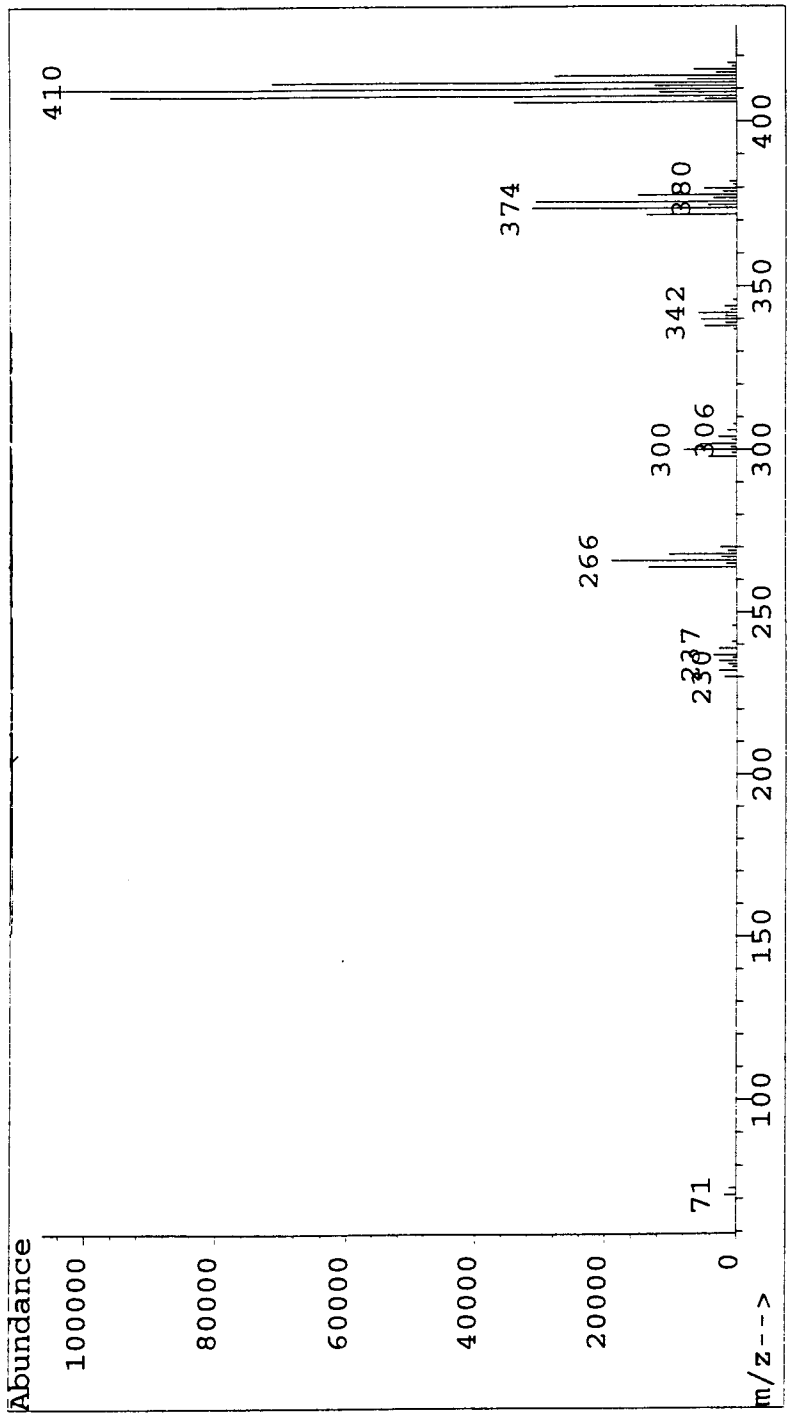


Figure 20. Full Scan Mass Spectrum of TC and CC

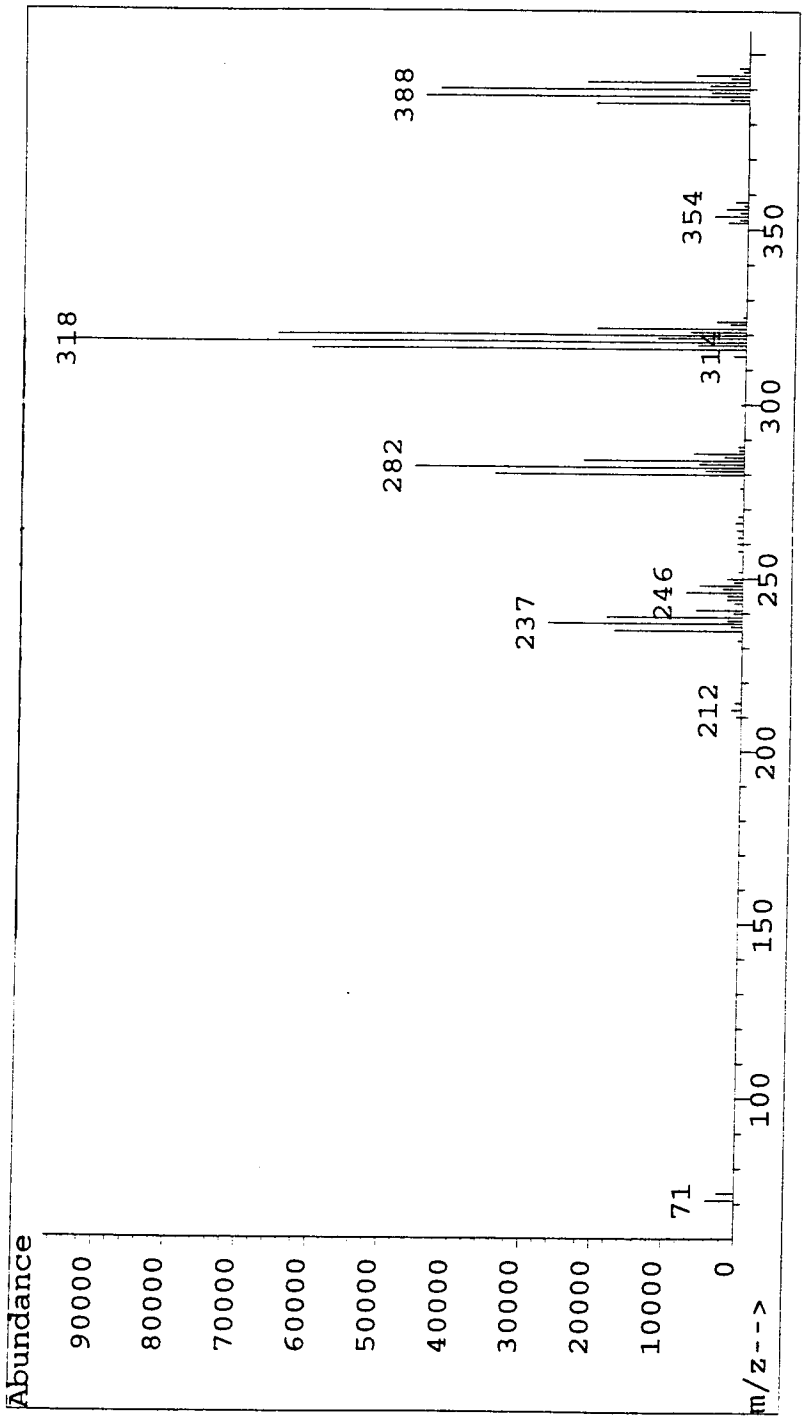


Figure 21. Full Scan Mass Spectrum of HEPX

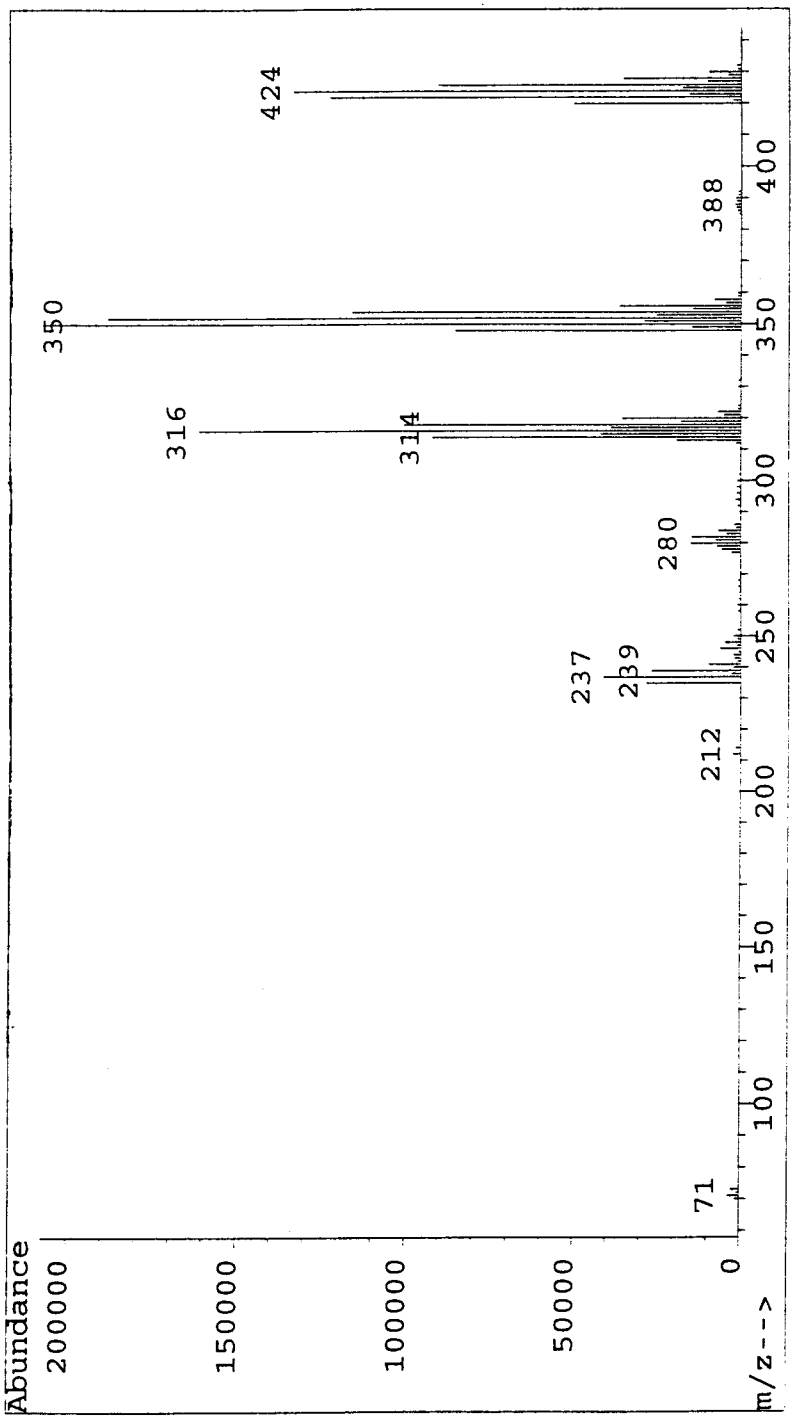


Figure 22. Full Scan Mass Spectrum of OXY

Table 6. Enantiomeric Ratios of Pesticide Standards

Compound	ER ± s.d. (n)
TC	0.99 ± .01 (5)
CC	1.00 ± .01 (5)
o,p'-DDT	1.01 ± .01 (5)
OXY	1.00 ± .01 (4)
HEPX	1.00 ± .01 (5)

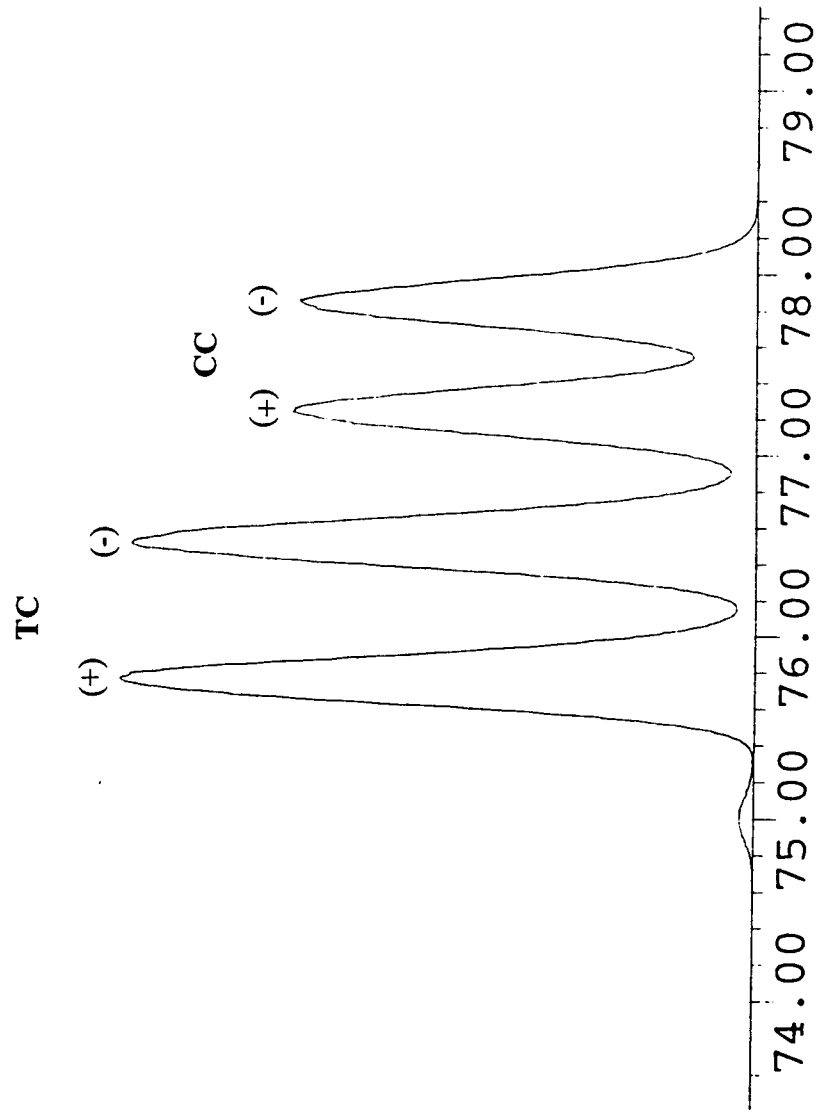


Figure 23. Chromatogram of Standard TC and CC Enantiomers

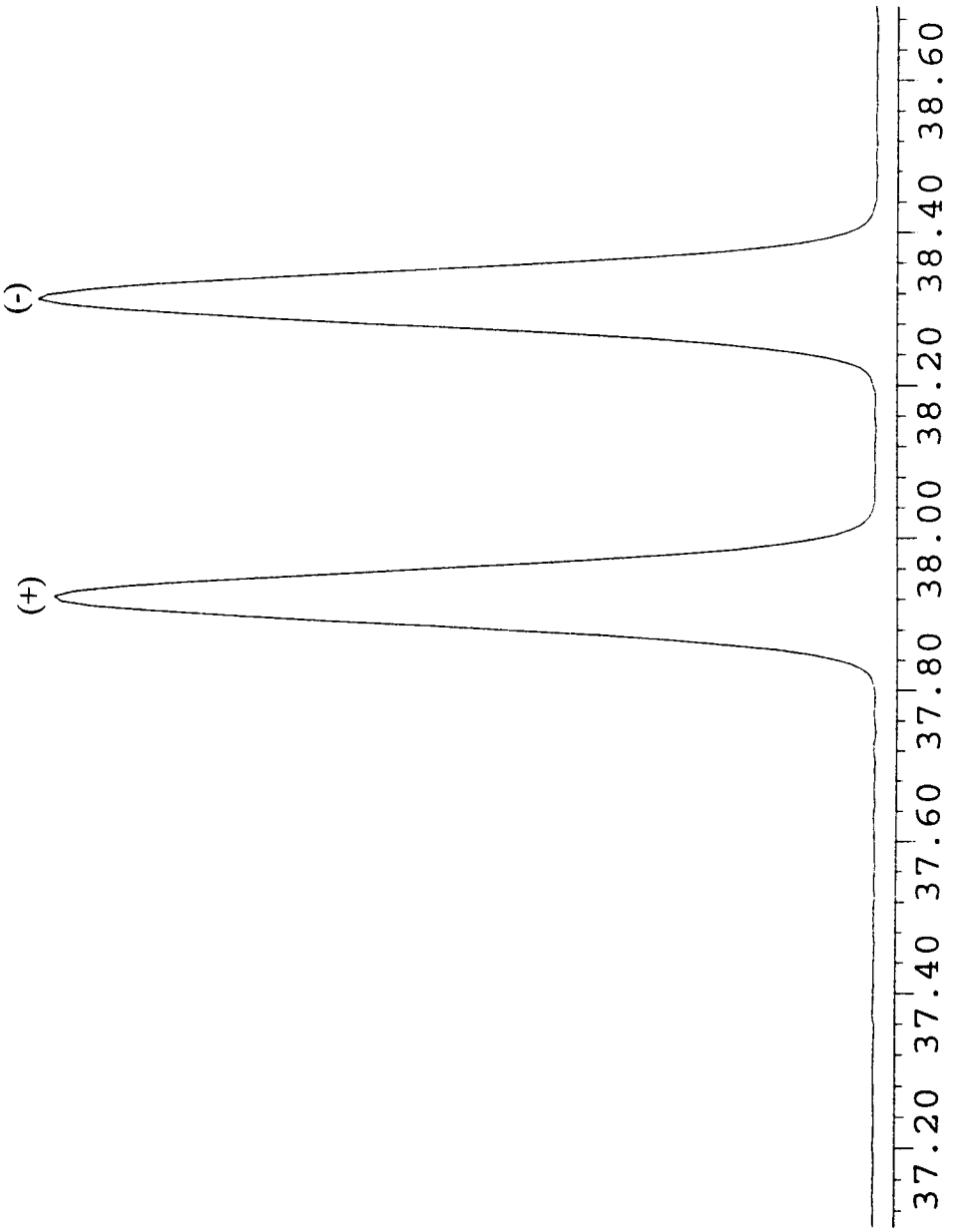


Figure 24. Chromatogram of Standard HEPX Enantiomers

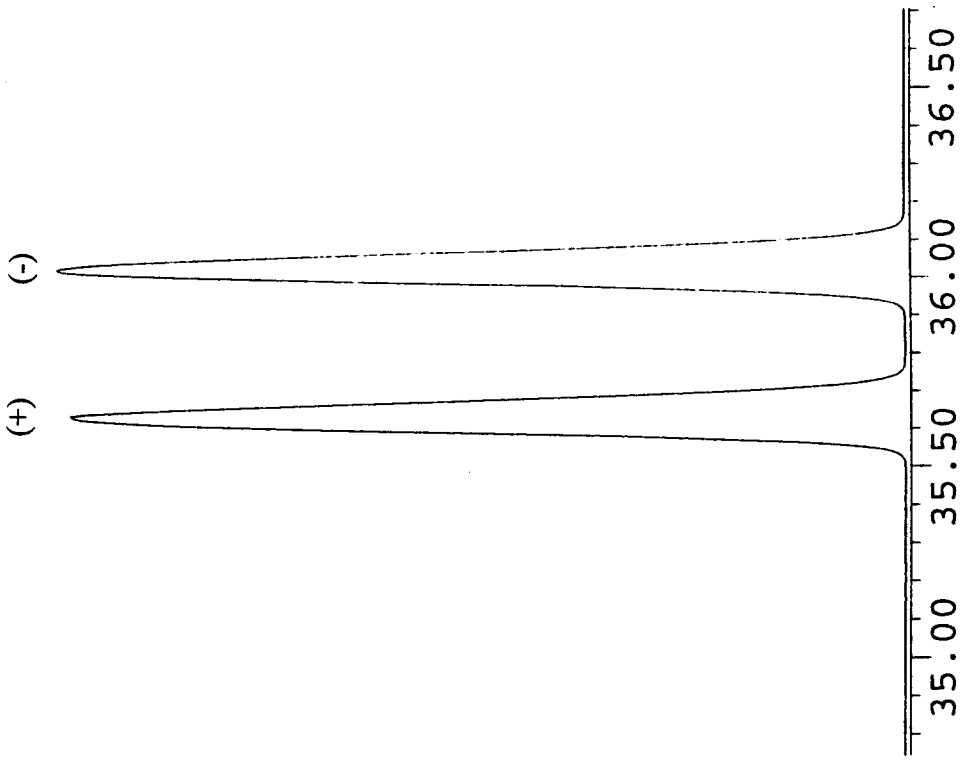


Figure 25. Chromatogram of Standard OXY Enantiomers

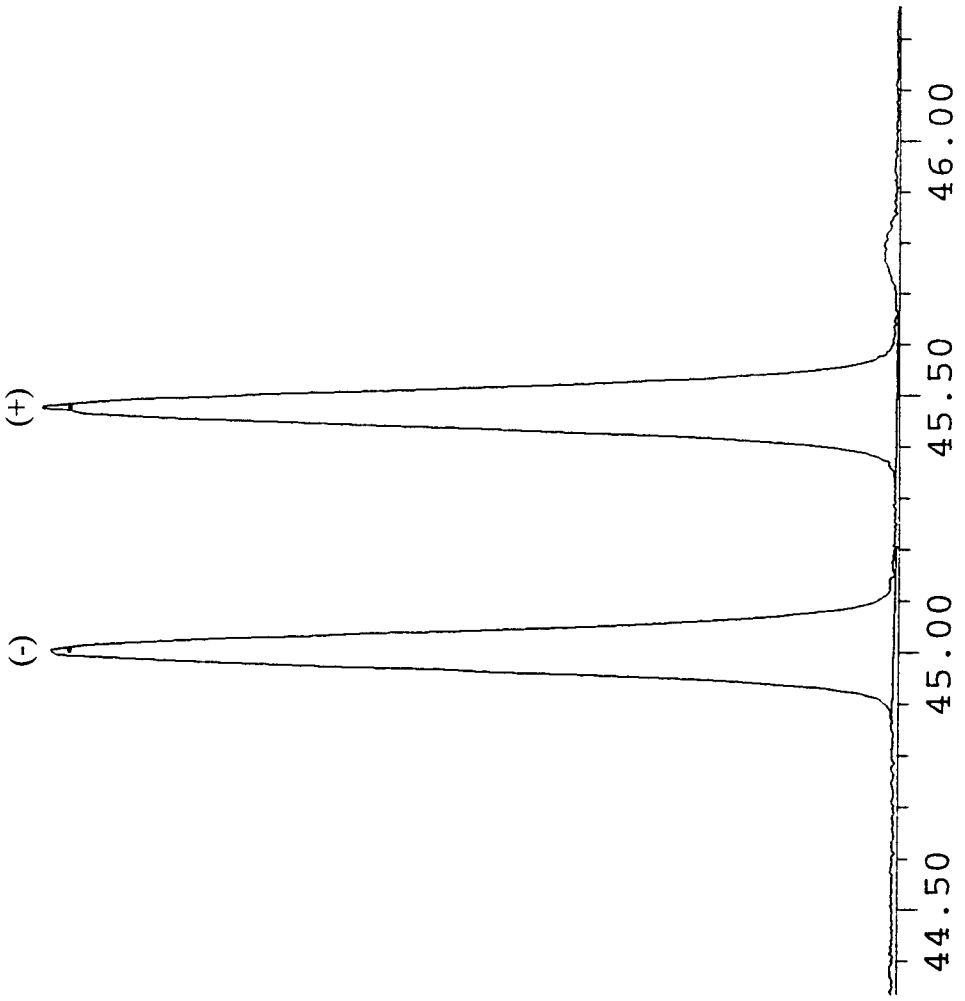


Figure 26. Chromatogram of Standard o,p'-DDT Enantiomers

Comparing CC and TC in two soils using both EIMS and NIMS modes gave the following ERs:

	NIMS	EIMS
CC-GS	1.25, 1.26	1.31
TC-GS	0.56, 0.57	0.59
CC-E1	1.11, 1.11	1.09, 1.11
TC-E1	0.79, 0.80	0.78, 0.78

For OXY (in NIMS mode), the ERs for the 316/318 ion pair were compared to the ERs for the 420/422 ions in two soils with the following results:

	316/318	420/422
GS	1.49	1.45
E1	0.89, 0.89	0.90, 0.90

The ER of o,p'-DDT was determined for one spike recovery soil to be 1.00 ± 0.03. These results show that the extraction and analysis procedures did not affect the ER value.

Enantiomeric Ratios

The enantiomeric ratios (mean ± s.d) for all compounds are listed in Table 7. The means and standard deviations were calculated from replicate extractions of the same soil.

Table 7. Average Enantiomeric Ratios (+/-) of OC Pesticides in Ohio Soils

Sample	o,p'-DDT	TC	CC	HEPX	OXY
	Mean ± s.d. (N)	Mean ± s.d. (N)	Mean ± s.d. (N)	Mean ± s.d. (N)	Mean ± s.d. (N)
E1	0.82 ± .01 (4)	0.79 ± .01 (4)	1.10 ± .01 (4)	1.60 ± .10 (4)	0.89 ± .01 (4)
E2	0.79 ± .01 (2)	ND*	ND	ND	ND
E3	0.76 ± .01 (2)	ND	ND	ND	ND
E4	0.86 ± .01 (2)	ND	ND	ND	ND
GS	0.99 (1)	0.57 ± .02 (3)	1.27 ± .03 (3)	3.05 (1)	1.47 ± .03 (3)
C1	ND	0.48 (1)	1.30 (1)	2.42 (1)	1.41 ± .05 (1)
C2	ND	ND	ND	ND	ND
W1	ND	0.60 (1)	1.56 (1)	7.27 (1)	1.65 ± .04 (2)
W2	1.05 (1)	0.76 ± .01 (2)	1.06 ± .01 (2)	ND	1.13 (1)
W3	1.19 (1)	0.79 (1)	1.07 (1)	2.69 (1)	1.09 ± .02 (2)
W4	ND	0.65 (1)	1.29 (1)	2.80 (1)	0.98 (1)
W5	1.03 (1)	ND	ND	ND	ND

The ERs are discussed for each compound in the following subsections. Figures 27-31 are a summary of the ERs for all soils.

o,p'-DDT. Enantioselective degradation of o,p'-DDT occurred in all eastern Ohio samples with the exception of the GS soil which was racemic (ER = 0.98). Selective degradation was observed for the (+) enantiomer in these soils with ERs ranging from 0.76-0.86 (Figure 32). Two soils in western Ohio were close to racemic (1.03, 1.05) while a third showed selective degradation of the negative enantiomer (ER = 1.19) (Figure 33). The final western soils as well as the central soils had residues below the detection limit for o,p'-DDT. A study conducted in British Columbia, Canada by Falconer et al.[74] found depletion of (+)-o,p'-DDT (ER = 0.8) in one silt loam soil out of the six agricultural soils analyzed. Two other silt loam soils and three muck soils contained racemic o,p'-DDT. Buser and Müller[54] found preferential degradation of (+)-o,p'-DDT in both human adipose tissue and cod liver oil with ER values of ≈0.8 and ≈0.7, respectively.

Chlordane. For eastern soils, levels of chlordane were only high enough to see enantiomeric degradation in one soil (E1). Both E1 and the GS soil showed preferential degradation of (+)-TC and (-)-CC enantiomers (Figure 34). In the E1 soil, the ER for (+)-TC was 0.79 and (-)-CC was 1.10. The GS soil ER was 0.57 for TC and 1.27 for CC. Four out of the five western soils had chlordane levels above the detection limit and displayed the same trend as the eastern soils for TC; degradation of the (+) enantiomer (0.60 - 0.79). CC, however, varied greatly in selective degradation for the western soils. Two of the four soils followed the trend of the eastern soils with ERs for CC being 1.29

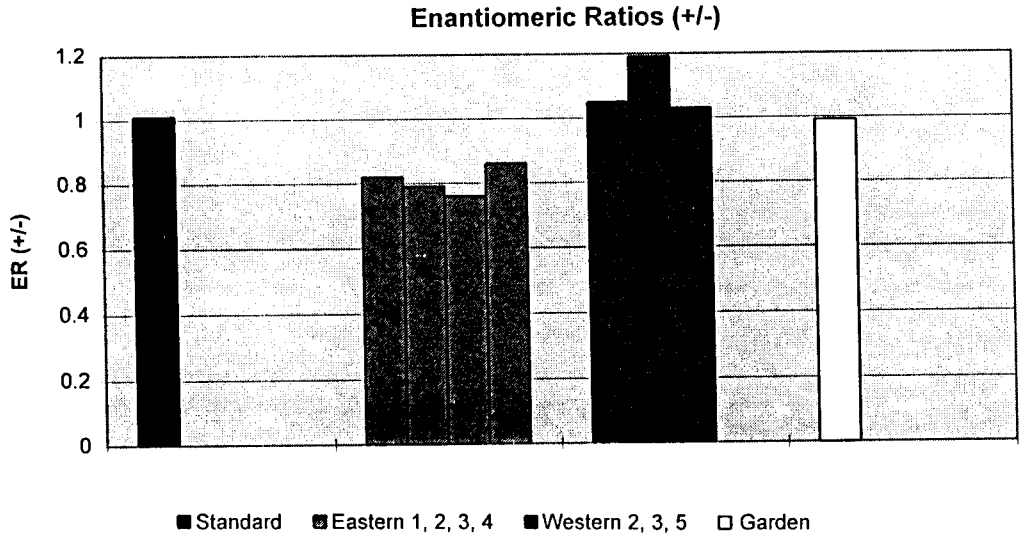


Figure 27. Enantiomeric Ratio of o,p'-DDT in all Soils

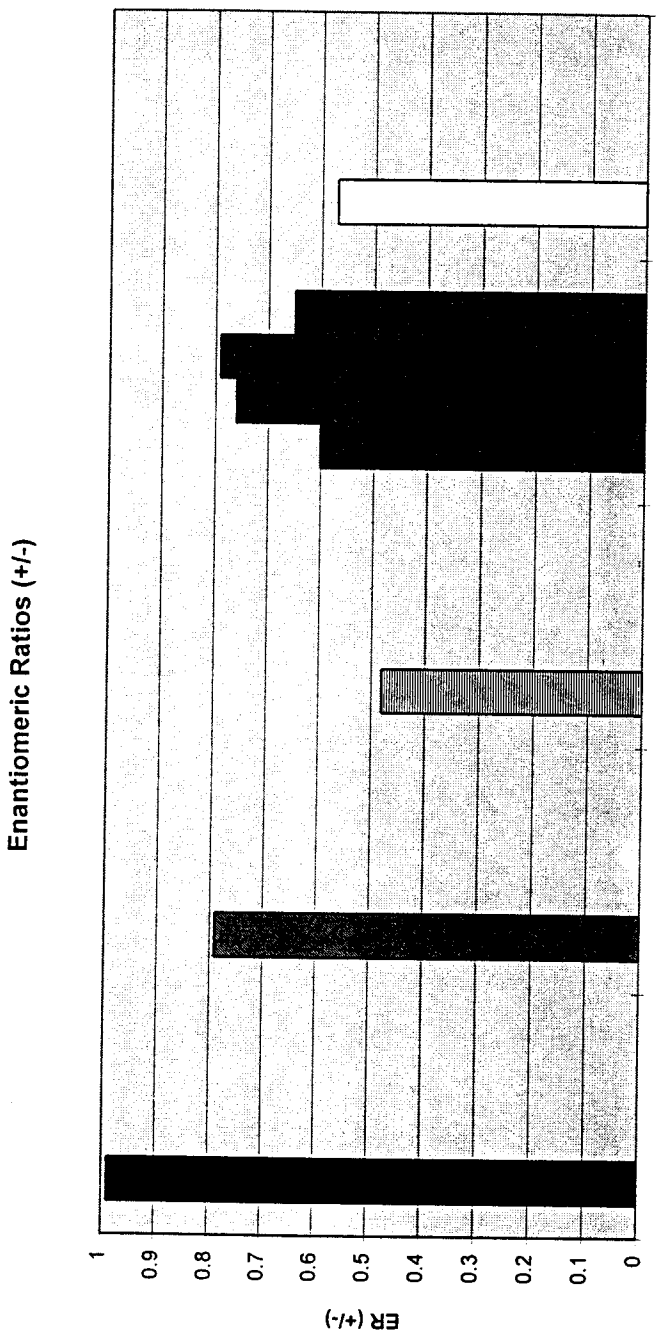


Figure 28. Enantiomeric Ratio of TC in all Soils

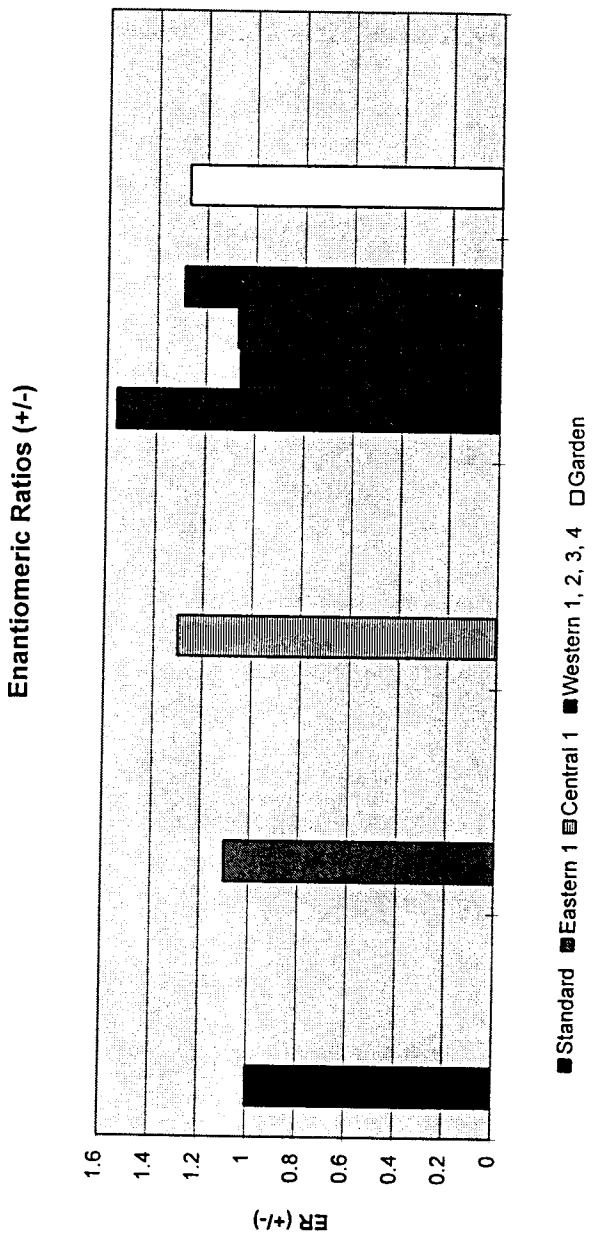


Figure 29. Enantiomeric Ratio of CC in all Soils

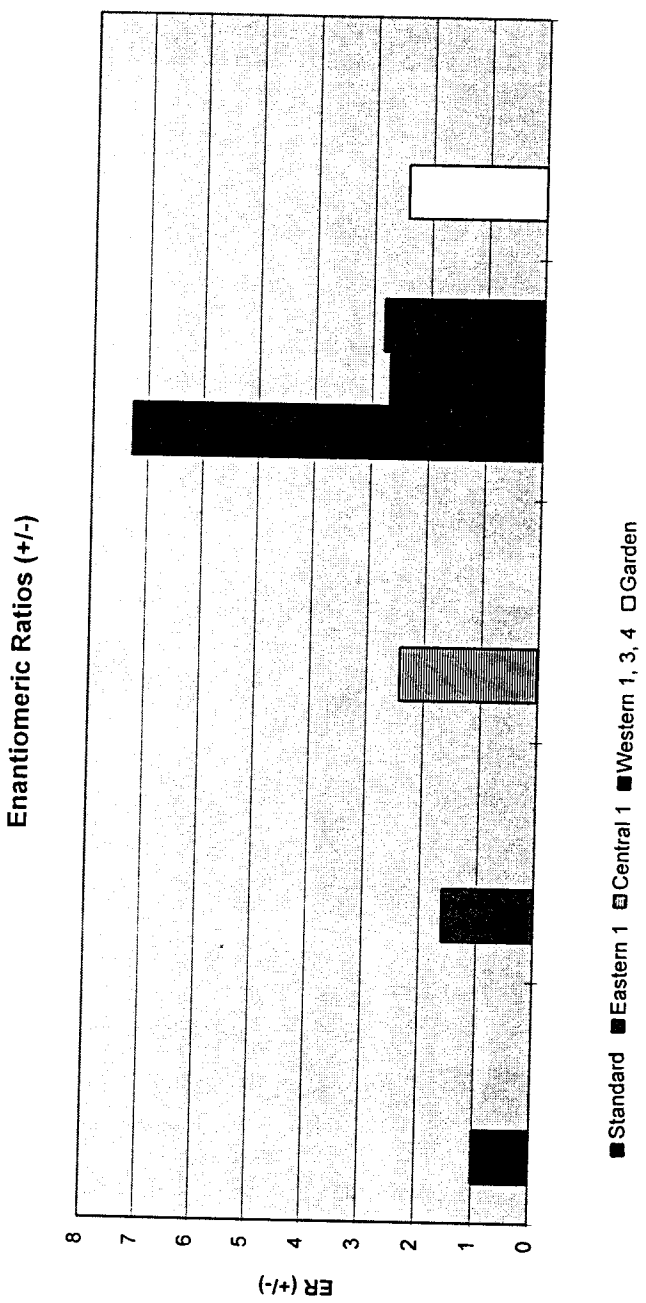


Figure 30. Enantiomeric Ratio of HEPX in all Soils

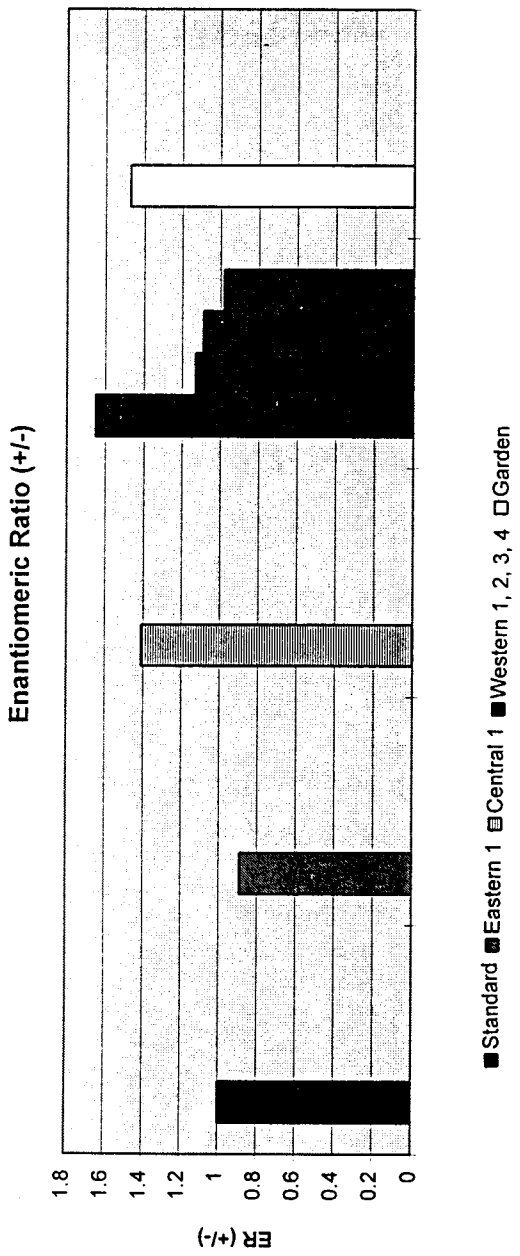


Figure 31. Enantiomeric Ratio of OXY in all Soils

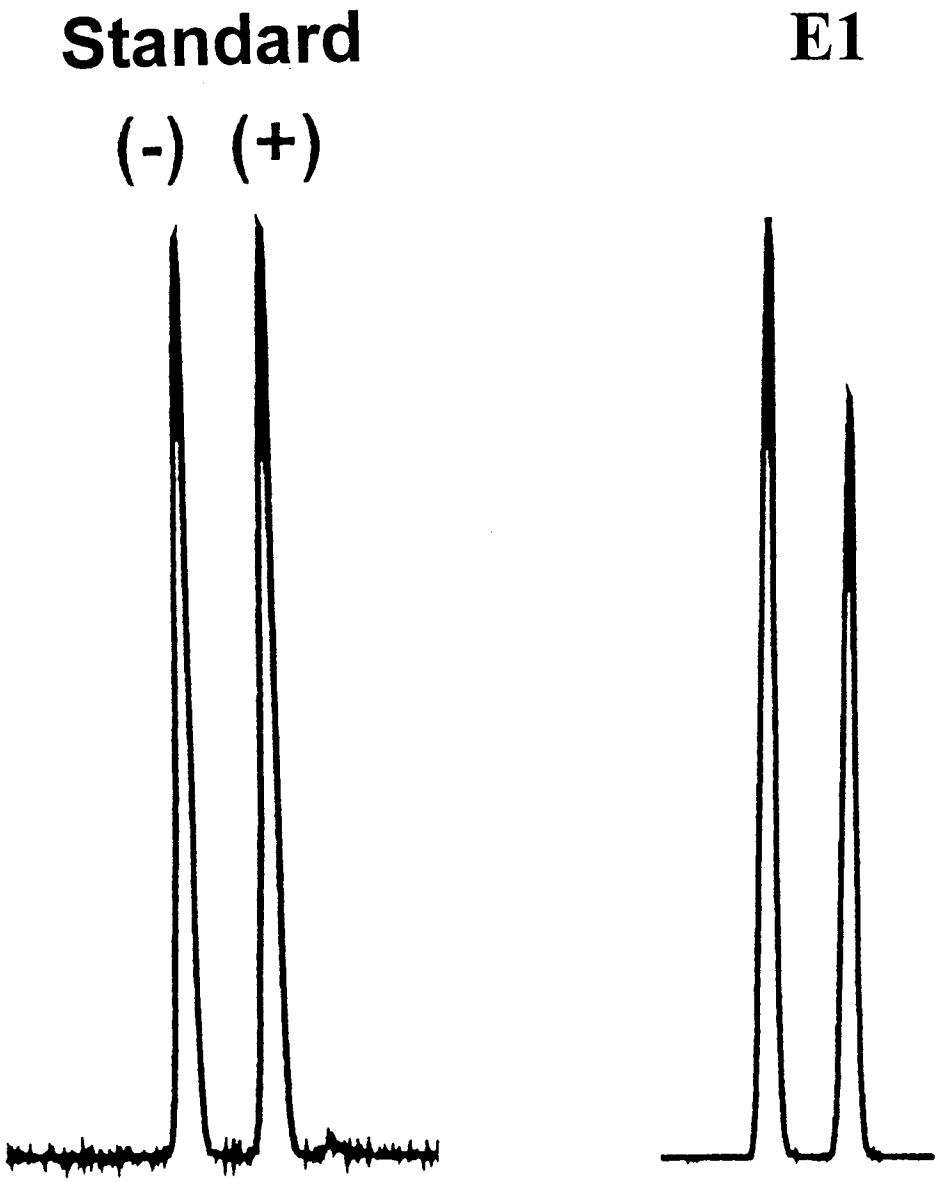


Figure 32. Comparison of o,p'-DDT in a Standard and Eastern Agricultural Soil

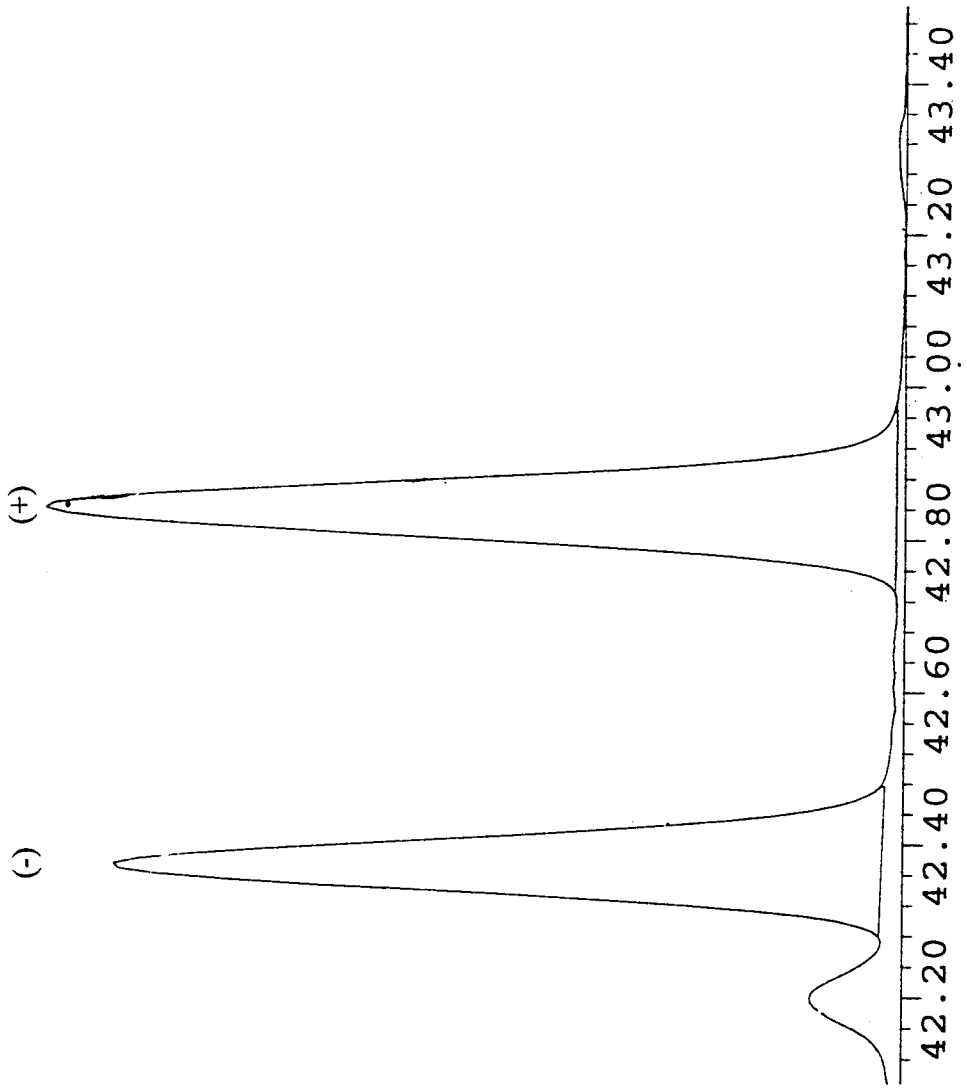


Figure 33. Chromatogram of o,p'-DDT Enantiomers in W3 Soil

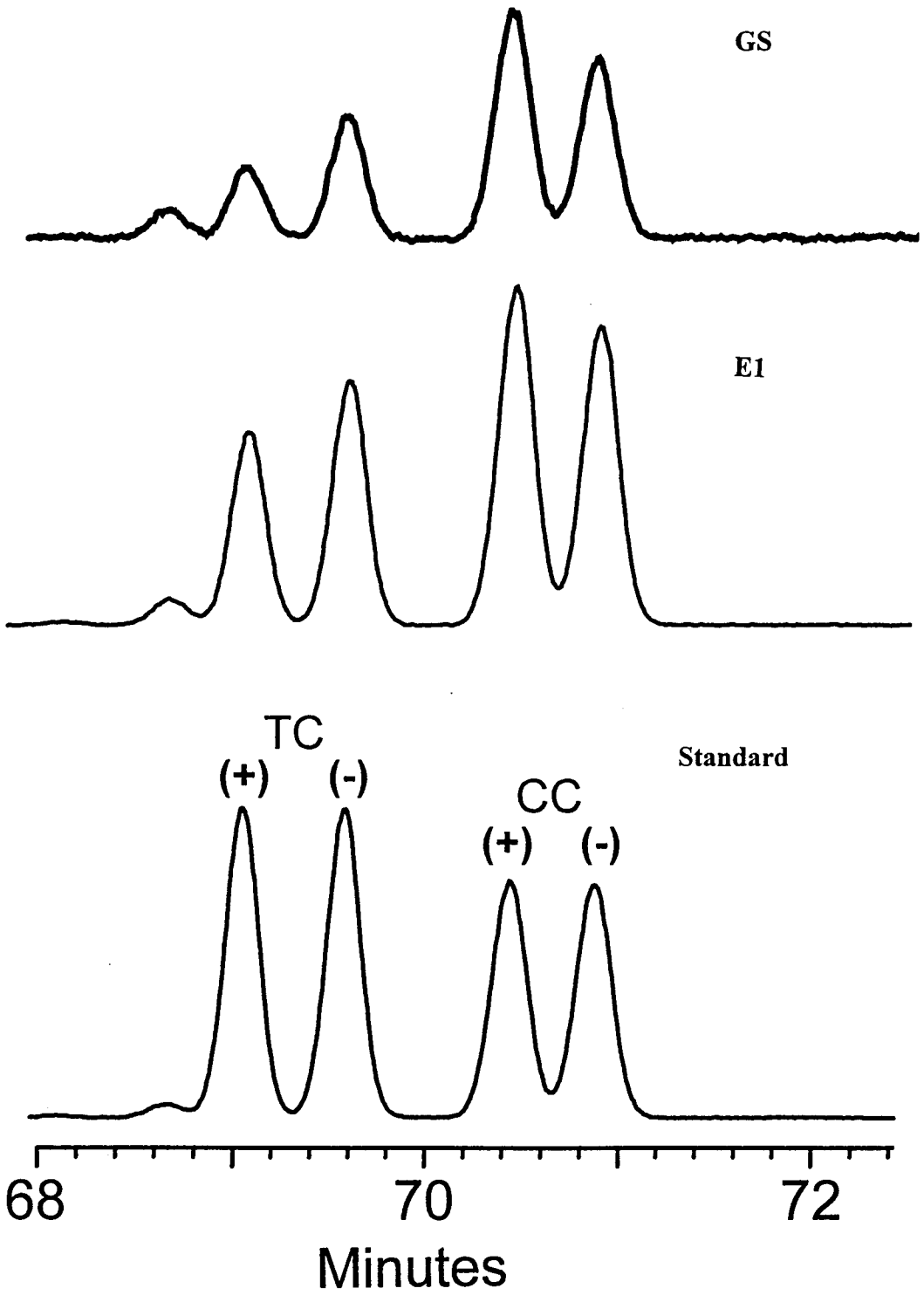


Figure 34. Comparison of TC and CC Enantiomers in a Standard, Eastern Agricultural Soil, and a Garden Soil

and 1.56, while in the other two soils CC was close to racemic with ERs of 1.06 and 1.07 (see Figures 35 & 36). The C1 soil had an ER of 0.48 for TC and 1.30 for CC. Falconer et al.,[74] found that both TC and CC were racemic in all six samples (both silt loam and muck soils) from British Columbia. Müller and Buser[17,55] found the same trend for TC and CC in Baltic herring oil as for eastern soils; an excess of (-)-TC (ER = 0.42 ± 0.02) and (+)-CC (ER = 1.35 ± 0.1). However, this could be either preferential degradation of (+)-TC and (-)-CC or preferential uptake of (-)-TC and (+)-CC. Salmon on the other hand showed an excess of (-)-TC (ER = 1.19 ± 0.02) and (+)-CC (ER = 0.38 ± 0.03). In this study, Buser and Müller[17] stated that one or the other enantiomer of a particular chiral compound may predominate in different species, although the original source was presumably racemic.

Heptachlor Epoxide. HEP was undetectable in all samples. However, this is understandable since HEP is rapidly converted into HEPX or other metabolites by most organisms.[55] HEPX was found in seven of the soils and showed a difference between its enantiomers in all. It is unclear whether the non-racemic HEPX arises from selective degradation of HEPX or selective formation from the degradation of HEP, or a combination of both. Only one eastern agricultural soil (E1) had sufficiently high concentrations of HEPX to obtain ER values, resulting in an ER = 1.60 (Figure 37). The GS soil ER for HEPX = 3.05 and one central soil (C1) had an ER = 2.42. Four out of five western soils had appropriate concentrations for determining enantiomeric composition. In these soils, the ER range of HEPX was 2.42-7.27 (Figure 38). Falconer et al.[74] found HEPX in four British Columbia soils (3 muck and 1 silt loam), all showing an excess of

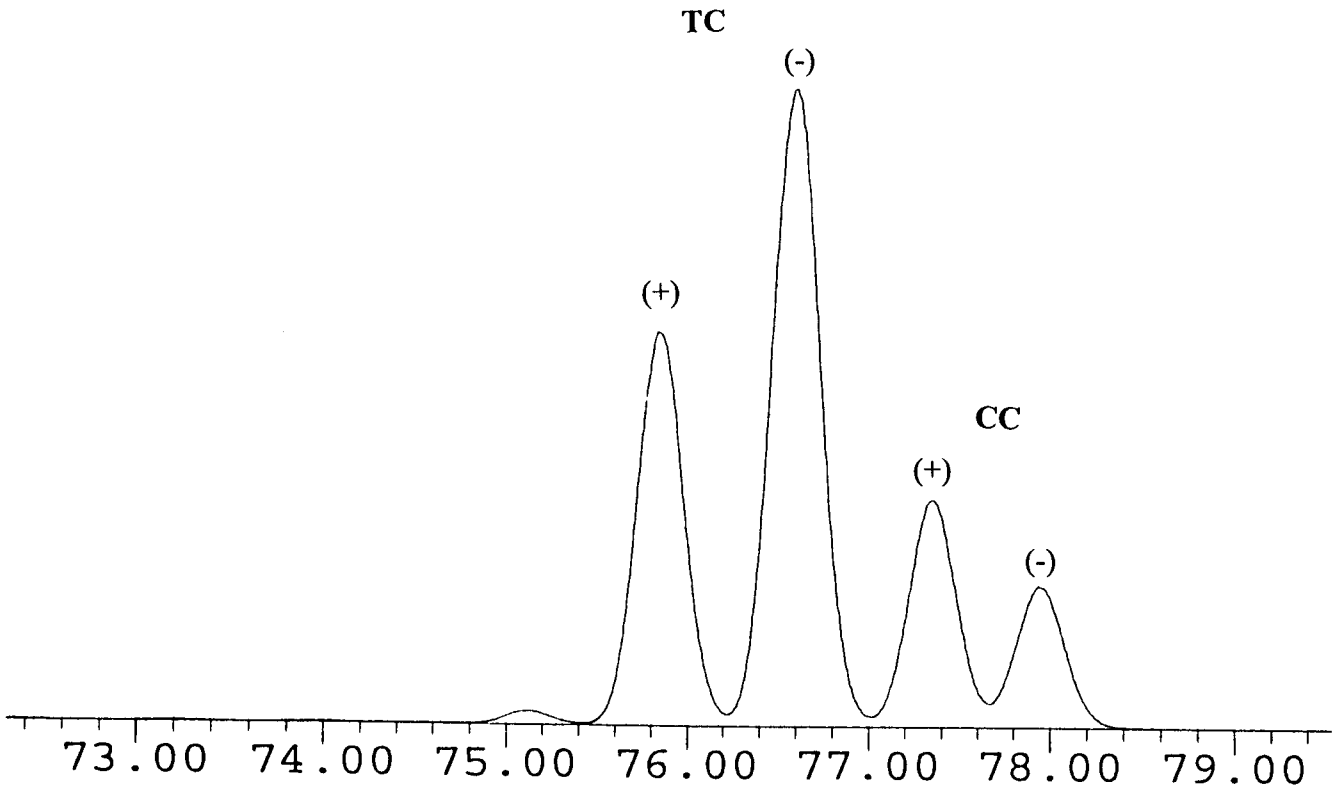


Figure 35. Chromatogram of Chlordane Enantiomers in W1 Soil

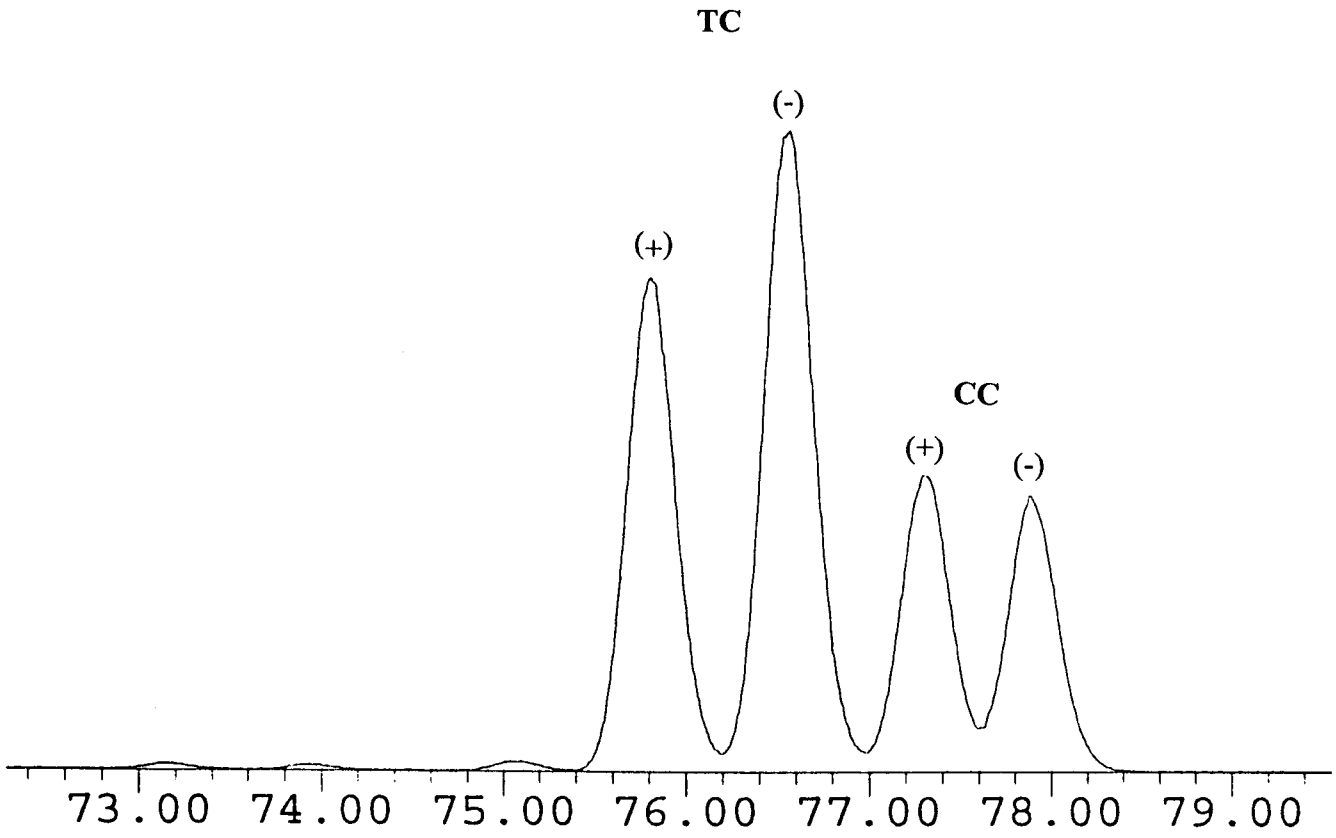


Figure 36. Chromatogram of Chlordane Enantiomers in W2 Soil

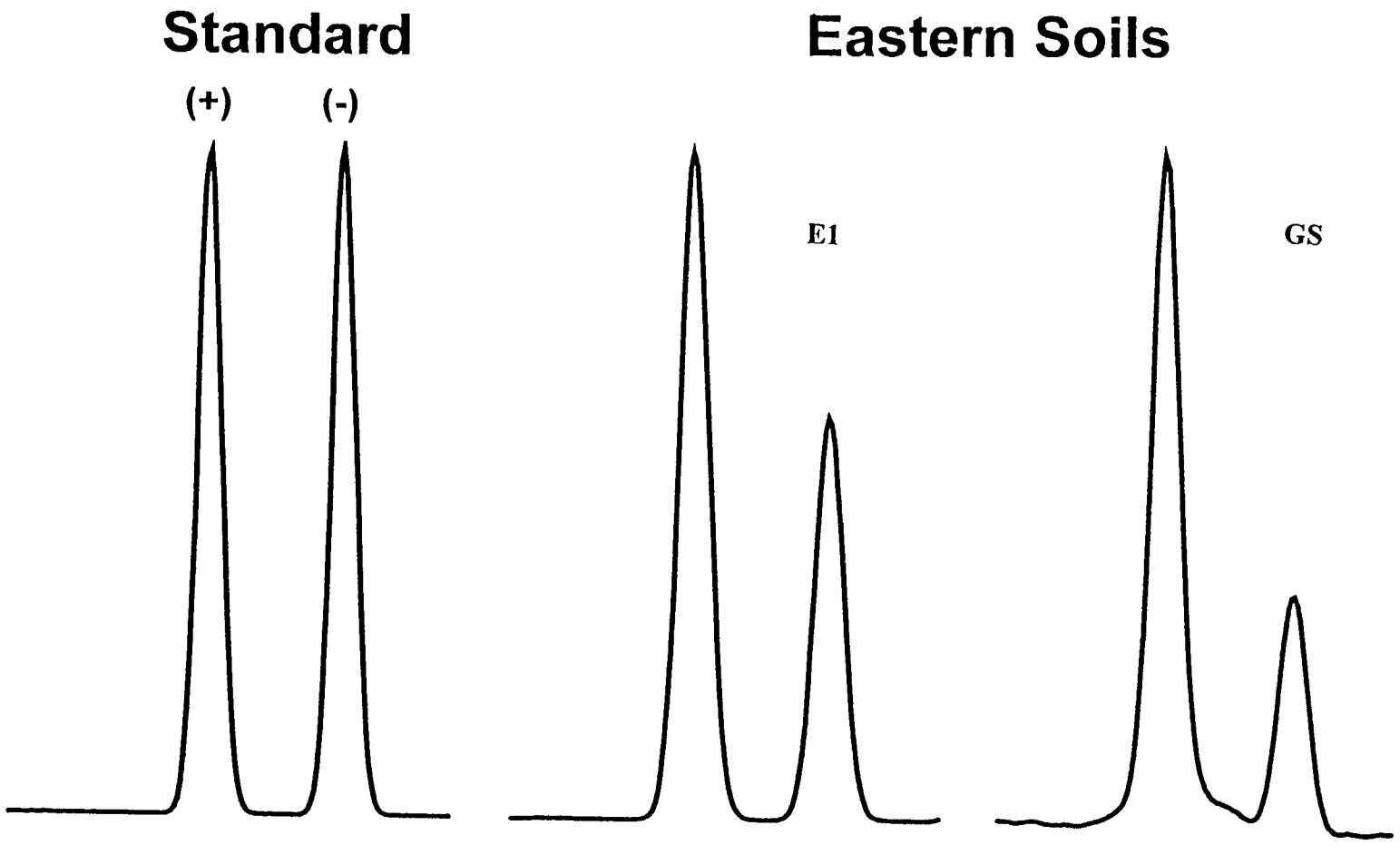


Figure 37. Comparison of HEPX Enantiomers in a Standard, Eastern Agricultural Soil, and Garden Soil

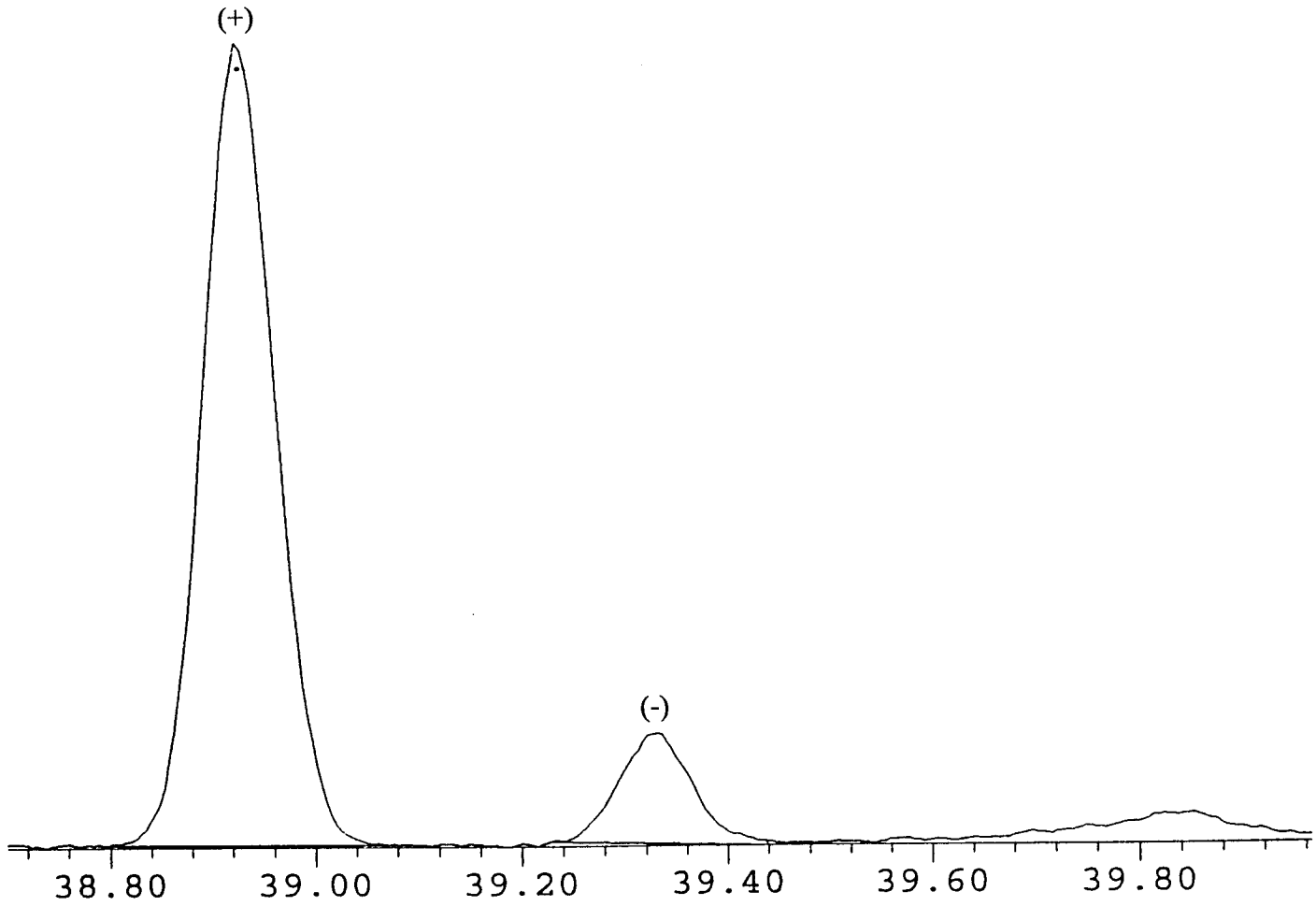


Figure 38. Chromatogram of HEPX Enantiomers in W1 Soil

the (+) enantiomer. Müller and Buser[17,54] found that rat liver homogenate incubated with racemic HEP as well as human adipose tissue yielded an excess of (+)-HEPX. Examination of herring oil, salmon muscle, and gray seal liver, however, found enrichment of the (-) enantiomer.[52]

Oxychlordane. OXY is the principal metabolite of CC, TC, and the nonachlors.[17] Seven soils in this study had concentrations above the LOD for OXY. As with HEPX, it is not known if enantiomeric excesses were due to preferential degradation, selective formation or both. One eastern soil (E1) showed an excess of the (-) enantiomer of OXY with an ER of 0.89 (Figure 39). Of the four western soils with levels of OXY above the LOD, one was almost racemic (ER = 0.98), two showed a slight excess of the (+) enantiomer (ERs = 1.09 and 1.13), and one showed a larger excess of the (+) enantiomer (ER = 1.65). Both of the central soils and the garden soil (Figure 40) showed an excess of the (+) enantiomer with ERs of 1.41, 1.54 and 1.47, respectively. Falconer et al.[74] found an excess of the (-) enantiomer of OXY in the two soils (one silt loam, one muck) where OXY was detectable. Buser and Müller[17] found a higher abundance of (+)-OXY in herring oil, salmon muscle, grey seal liver and human adipose tissue.

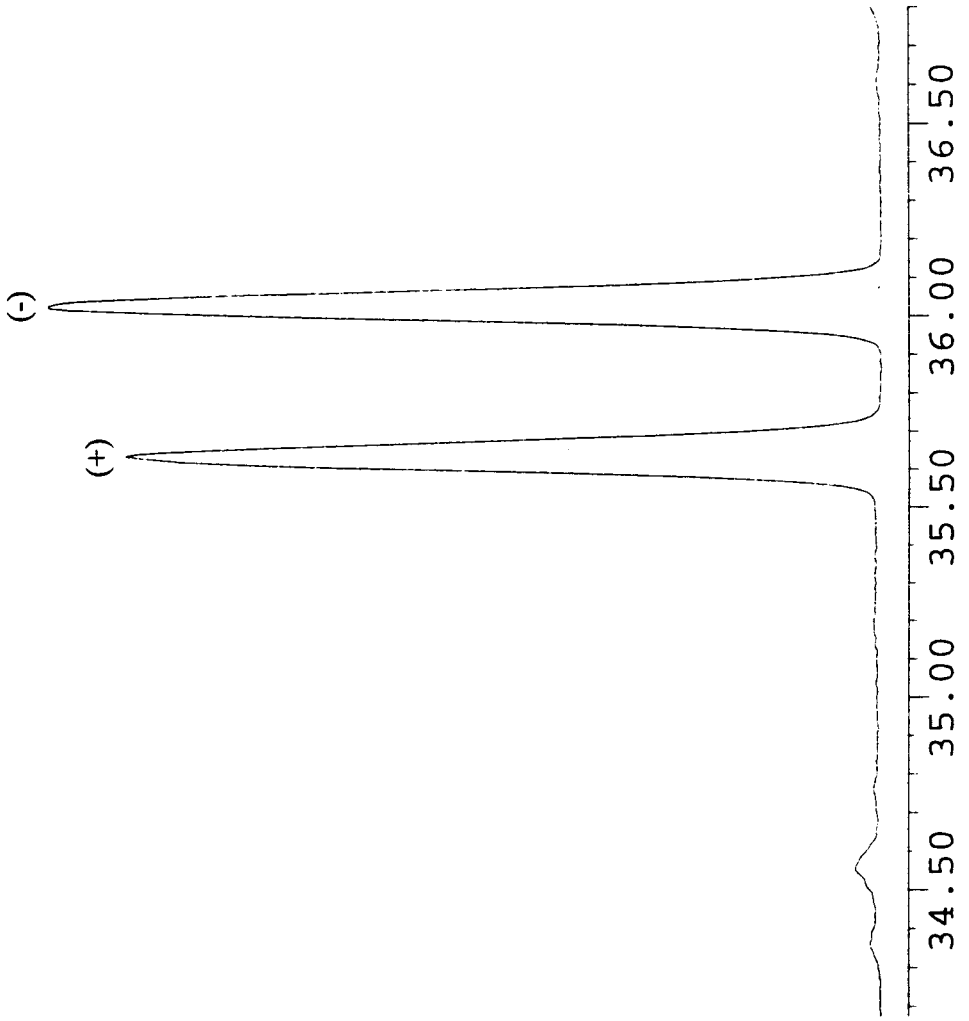


Figure 39. Chromatogram of OXY Enantiomers in W1 Soil

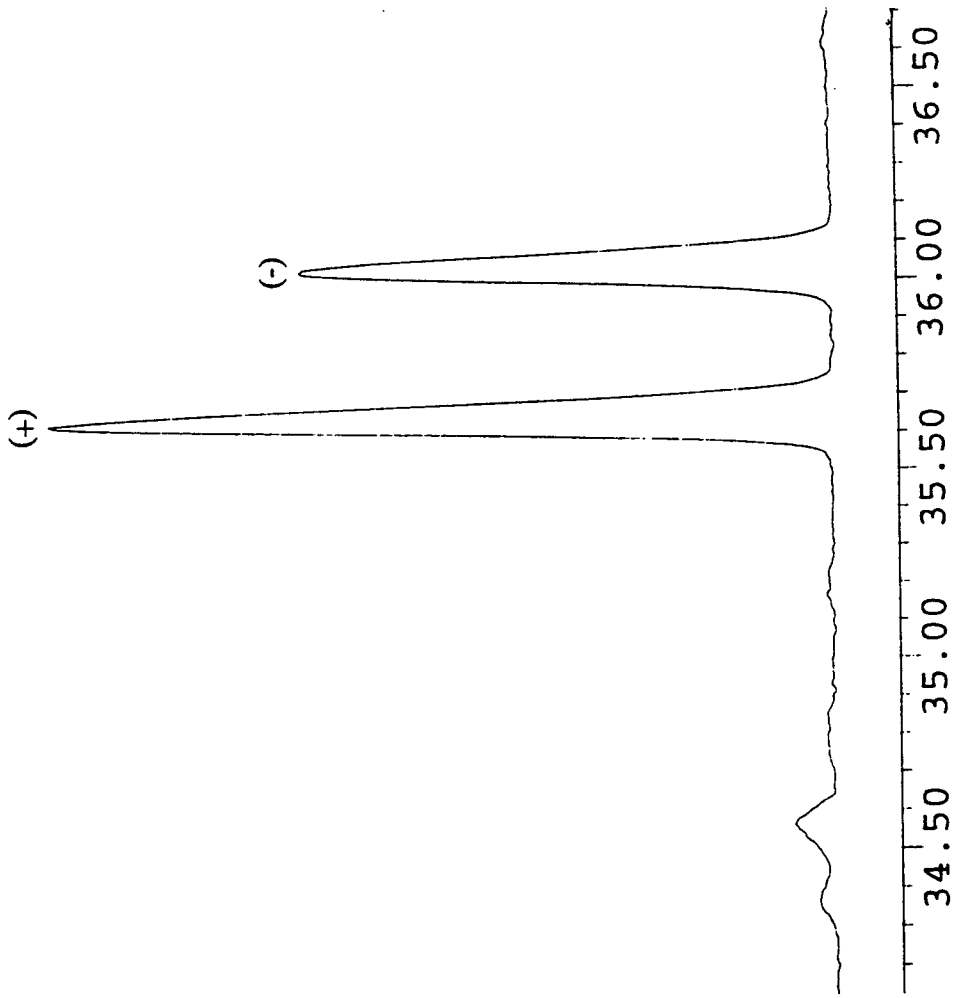


Figure 40. Chromatogram of OXY Enantiomers in Garden Soil

CHAPTER SEVEN

CONCLUSIONS

The purpose of this work was to determine the ERs and concentrations of several chiral OC pesticides in soils from across Ohio. Concentration values were determined for nine compounds (o,p'-DDT, p,p'-DDT, p,p'-DDD, p,p'-DDE, CC, TC, HEPX, dieldrin and TN) in eleven agricultural soils and one garden soil. The components of DDT were the only compounds present in all soils analyzed. Three eastern soils were below the LOD for all compounds except the DDT components. One eastern soil was above the LOD for all compounds and had the highest levels of TC, CC, TN, and HEPX in all soils. The western and central soils were typically a factor of two or more lower for these compounds. The garden soil had concentrations lower than the eastern soil and similar to or higher than the western and central soils.

Enantioselective degradation of OCs did occur in Ohio soils. The eastern soils showed enantioselective breakdown of (+)-o,p'-DDT, while the western soils varied from racemic to enantioselective breakdown of the (-) enantiomer. The garden soil was racemic for o,p'-DDT. All soils showed selective degradation of (+)-TC. The ERs of CC, however, varied with some being close to racemic and others showing selective degradation of the (-) enantiomer. Although it was unclear which enantioselective process was working on the enantiomers of HEPX and OXY (selective degradation or selective formation) both showed enantiomeric excesses. HEPX had the largest ER (7.27) of all compounds analyzed and all soils showed an excess of the (+) enantiomer.

No correlations were found between pesticide concentration and ER. More information is needed about soil type, pH, and past chemical use to determine if correlations between ER and soil properties exist.

Atmospheric transport and deposition is a major contributor of OCs to the Great Lakes. By measuring the ERs of OC pesticides in soils, it may be possible to distinguish between 'old' and 'new' atmospheric sources. Since freshly applied pesticides that volatilize into the atmosphere are subject to only non-biological degradation processes, their ERs should be racemic. Pesticide residues volatilizing from soils that have been subjected to microbial degradation, however, might be non-racemic. Enantioselective breakdown in soils may result in pesticide residues that give an 'old' source signature that could be used to track releases to the atmosphere. This information is important for designing control strategies to curb entry of pesticides into natural waters in the Great Lakes region. Combining a more extensive study of OCs in soils from states around the region with an examination of the air above the soil may allow us to infer possible source contributions to the Great Lakes.

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