FACTORS AFFECTING THE OCCURRENCE, ISOMER RATIO AND ENANTIOSELECTIVE DEGRADATION OF HEXACHLOROCYCLOHEXANE IN ARCTIC AND TEMPERATE AQUATIC SYSTEMS

by

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ABSTRACT

An investigation to determine factors affecting the biodegradative losses of HCHs in arctic, subarctic and temperate aquatic systems was performed. Concentrations of α- and γ-HCH were highest in large and cold lakes and lowest in warmer temperate lakes. The α-HCH / γ-HCH ratio was highest in arctic / subarctic lakes, indicating long range transport and the low ratios in small temperate systems are due to fresh γ-HCH. The enantiomer ratio (ER), was lowest in lakes with high concentrations of α-HCH, in cold and/or oligotrophic lakes with long residence times. ERs were highest in small temperate lakes with low α-HCH concentrations. These factors are significantly related to the contact between α-HCH and oligotrophic microbes. Enantioselective degradation was also greatest in wetlands and northern streams due to contact with the biofilm. Microcosms were unable to isolate factors enhancing enantioselective degradation of α-HCH due to the inability to mimic the conditions for the degrading microbes.

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CHAPTER 1: INTRODUCTION

The magnitude of xenobiotics chemicals in our environment has necessitated the ability to predict pollutant transport in the ecosystem, their transformation to other molecules, their impacts on biota, and most importantly, their effects on human health. Our concern with chemical fate is due mainly to our inability to control chemical movement within the environment and the chemical persistence. To describe chemical fate, a variety of mechanisms can be defined. Among these processes biodegradation is a significant, but often poorly quantified process, and the knowledge of which is necessary for the evaluation of chemical fate in the environment.

1.1 Alpha-Hexachlorocyclohexane Contamination

The arctic was once believed to be a pristine place on our polluted Earth. However, since the discovery of Arctic haze in 1956 by Mitchell, this belief is no longer true. In the last 20 years, organochlorine pesticides have been found throughout the arctic archipelago (Twitchell, 1991). The pesticides are not local to the arctic environment: their main sources are southern sites of application. Many studies in the arctic have found relatively high concentrations of pesticides in air, water, soil and animals (e.g., Bidleman *et al.*, 1995, Muir *et al.*, 1988, Falconer *et al.*, 1995, Gregor and Gummer, 1989). These pesticide residues have become a cause for concern due of their ability to bioaccumulate through the food chain, and possibly lead to toxicity in higher plants and animals, including humans (Jensen *et al.*, 1997, Bidleman and Falconer, 1999). In Canada, contamination of northern food chains is particularly important because freshwater fish, marine mammals and birds are subsistence foods for aboriginal people (Schindler *et al.*, 1995). A further concern is that

the reduced sunlight, extensive ice cover and the cold, could slow the degradation of chemicals, and increase contaminant persistence in the arctic environment (Twitchell, 1991).

The most abundant organochlorine in arctic air, freshwaters and the Arctic Ocean is hexachlorocyclohexane (HCH) (Iwata *et al.*, 1993, Jantunen and Bidleman, 1998). Alpha-hexachlorocyclohexane (α-HCH) is the most abundant HCH isomer in Northern Hemispheric air and water (Tanabe *et al.*, 1982) including the Great Lakes (Ridal *et al.*, 1996). Although HCH is not as toxic and bioaccumulative as other organochlorines, its high concentrations and environmental persistence in the arctic and, indeed, world wide is alarming, and can be used to provide a better understanding of the pathways and fate of other more toxic and less abundant compounds.

HCH and other persistent organochlorines travel to the arctic via atmospheric transport or oceanic circulation. It is now well established that long range transport of pollution to the arctic occurs (e.g., Barrie *et al.*, 1981, Oehme and Ottar, 1984). Researchers have found that ground level pollution moves to the arctic from areas with similar air temperatures. In winters, the polar front air mass will move into Eurasia and North America, increasing the input of pollutants into the air mass (Joranger and Ottar, 1984, Oehme and Ottar, 1984). The air mass in the Canadian Arctic originates from Siberia and North America, although some pulses from Europe also occur (Barrie *et al.*, 1981).

A second mechanism of pollutant travel to high latitudes is the global distillation model. "Global distillation" (Figure 1.1) introduced by Goldberg (1975) and recently popularized by Wania and Mackay (1993), accounts for the movement of chemicals such

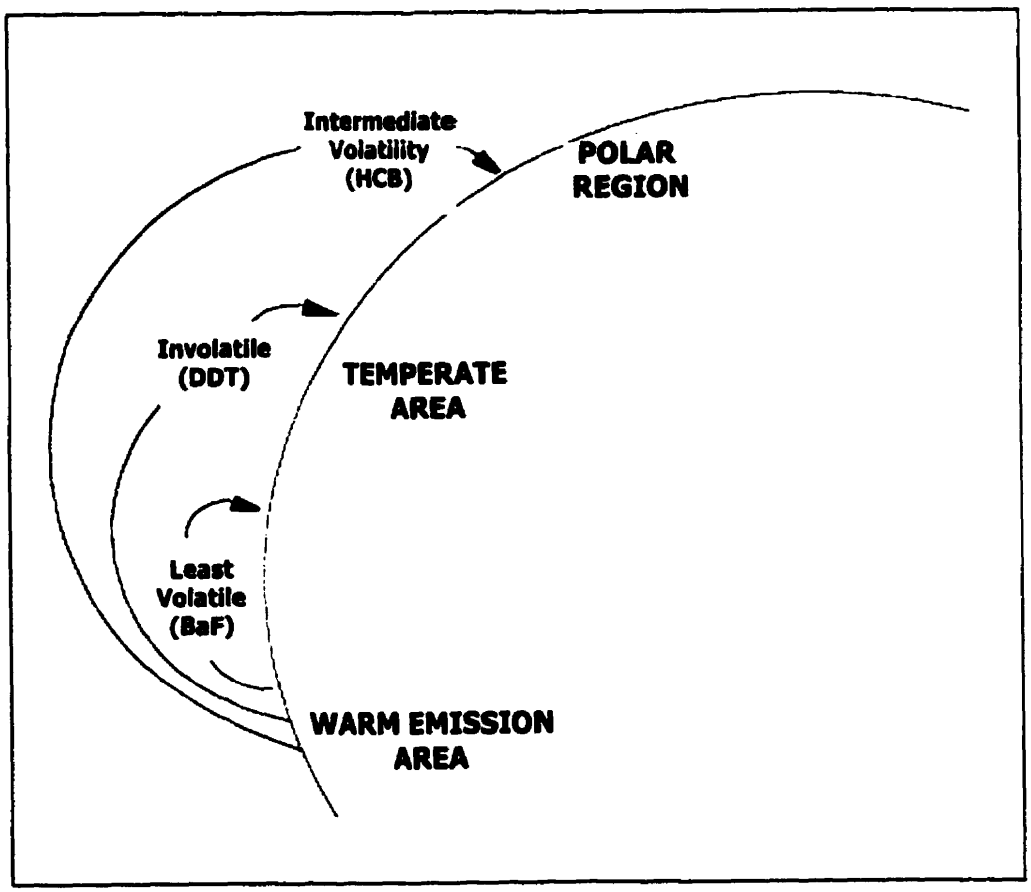


Figure 1.1: The global distillation model by Goldberg (1975). Volatile organic contaminant volatilize from warm areas of application and depending on the chemical's vapour pressure at ambient temperatures, will partition along a latitudinal gradient. Source: Wania and Mackay (1995).

as persistent organochlorines from warmer, mid-latitude regions where they are applied to cooler areas where they condense.

The transportation and persistence of HCH in the arctic is due to its physical-chemical properties. It has a relatively high vapour pressure among the semi-volatile organic compounds, which allows it to escape from the site of application, a low octanol-air partition coefficient, a long atmospheric residence time (low susceptibility to OH radical attack), and a low Henry's Law constant (allowing it to remain in the water column) (Harner *et al.*, 1999, Brubaker and Hites, 1998, Gregor and Gummer, 1989, Kucklick *et al.*, 1991).

The global production and usage of HCH is difficult to quantify (Willet *et al.*, 1998). Voldner and Li (1995) estimated that about 550 000 metric tons of HCH in total, were used worldwide. More recently, Li *et al.* (1998) estimated that as much as 6 million tons were consumed on a global basis.

Cotham and Bidleman (1991) calculated that annual loadings of α-HCH to the arctic region is approximately 98 000 kg/year. Since the banning of technical HCH in most industrialized countries, and recently in India and China (Voldner and Li, 1995) there has been a drastic decline in the concentrations of α-HCH in arctic air (Li *et al.*, 1998). This rapid decline has caused a disequilibrium between air and water, resulting in a net volatization of α-HCH from surface waters (Jantunen and Bidleman, 1996). Atmospheric concentrations of α-HCH above the Bering and Chukchi Seas have decreased from 91 to 23 pg/m^3 and have a summer reversal of flux from water to air (Jantunen and Bidleman, 1995). While atmospheric concentrations have declined due to reductions in global HCH usage, re-

emissions from oceans will continue to augment the atmospheric concentrations (Willett *et al.*, 1998, Walker *et al.*, 1999).

1.2 Rationale for the Project

The fate of a chemical in the environment is dependent on its physical-chemical properties and reactivity (Walker *et al.*, 1999). This research addresses gaps in our knowledge of the environmental fate of HCH, notably the abundance and factors affecting the abundance of HCH isomers. Here, we can exploit information such as the ratio of HCH isomers and the ratio of the enantiomers of α-HCH (ER) to assist us with estimating the relative age and source of HCH and factors affecting its enantioselective degradation. This research builds on that reported by Falconer *et al.* (1995) and Helm *et al.* (2000) who used enantioselective degradation to identify microbial degradation as a loss mechanism of α-HCH in arctic watersheds. This research aims to improve our knowledge of factors affecting the occurrence of HCHs and enantioselective biodegradation in the temperate zone to compare the importance of these factors in subarctic and arctic zones.

1.3 The Objectives

The overall aim of this research is to improve our understanding of the fate of HCHs and particularly degradation, using an isomer ratio and chirality as investigative tools. The main objectives of this study were to investigate likely source and age of HCH and factors affecting the enantioselective degradation of α-HCH in high arctic, subarctic, and temperate lakes and streams that vary in limnological characteristics.

The following hypotheses were examined:

1) Ratios of α-HCH / γ-HCH indicate the relative age and source of chemical with high ratios indicative of an aged mixture and vice versa.

2) Enantioselective degradation is related to the nutrient or trophic status of lakes at northern and southern latitudes. Enantioselective degradation is greatest in lakes of low trophic status, especially low in organic carbon, causing α-HCH to be used or inadvertently used as a source of carbon.

3) The enantioselective degradation of α-HCH is controlled by the delivery or availability of α-HCH to microbial populations that are most abundant at the sediment-water interface. Thus, in lakes, the enantioselective degradation is positively related to water residence time. In streams and wetlands, it is related to contact time with sediments, especially those supporting microbially-rich biofilms.

1.5 Thesis Outline

This thesis consists of five chapters. Chapter 2 reviews the relevant literature of HCH in the environment and summarizes information on the microbial degradation of xenobiotics.

Chapters 3 and 4 are written in the form of scientific articles. They include a short introduction, a summary of the methods used, and a concise presentation of results and discussion. Chapter 3 presents the results of the field survey of lakes, wetlands and streams in the arctic, subarctic, Great Lakes and southern Ontario study sites, as well as a discussion of the statistical analyses of the data set. This chapter includes the data of other researchers and will be submitted for publication in a refereed journal. It was written by the author of this thesis. The co-authors contributed data, analytical support and comments on data interpretation. Chapter 4 provides an overview of the laboratory microcosm experiments performed to investigate the parameters related to enantioselective degradation. Finally, Chapter 5 presents conclusions regarding HCH and the enantioselective degradation of α-

HCH in arctic and temperate aquatic systems, and the factors that control the magnitude of the degradation. The thesis ends with recommendations for further study.

CHAPTER 2: BACKGROUND

The following chapter reviews the history of HCH usage and summarizes the available information on α-HCH in arctic and temperate areas. This review will then focus on the microbial degradation of organochlorines and the factors that affect the degradation of xenobiotics.

2.1 Hexachlorocyclohexane: Past and Present

Faraday first synthesized HCH in 1825 by the chlorination of benzene under ultraviolet light (Deo *et al.*, 1994). Its insecticidal properties were not recognized until 1942 (Li *et al.*, 1998). The product, known as the technical mixture, is made up of 60-70% alpha- (α), 10-15% gamma- (γ) , 5-12% beta- (β) and 7% of 5 other theoretically possible isomers (IPCS, 1992). Only the γ- isomer exhibits insecticidal properties (IPCS, 1992). The technical mixture can be treated with methanol or acetic acid and recrystallized to produce a 99% pure γ-HCH mixture (also called lindane); (Willett *et al.*, 1998).

2.1.1 Deregistration of Technical HCH

When the infamous pesticide DDT was banned in the late 1970s, HCH (also known as the misnomer benzenehexachloride) became a popular substitute due to its low cost and high effectiveness (Twitchell, 1991, Li *et al.*, 1998). It was used as a broad-spectrum insecticide for a variety of purposes in agriculture, forestry and public health (Haugen *et al.*, 1998). Typical uses have been for growing fruits, vegetables, rice, Christmas trees and as a seed preservative. China used HCH to prevent locust infestations and the German military used it as a fog inducer (Voldner and Li, 1995). Although the γ-isomer has a relatively shorter lifespan than other

isomers, it is the persistence, toxicity and potential carcinogenicity of the other isomers that have caused technical HCH to be banned in most countries (Deo *et al.*, 1994).

Canada and the United States deregistered the use of technical HCH in 1971 and 1978, respectively (Barrie *et al.*, 1986, U.S. EPA., 1978). However, the technical mix was still produced until 1983 in the U.S. (U.S. Dept. of Health, 1998). In 1979, there were more than 70 countries that continued to use technical HCH (Walker *et al.*, 1999). In 1983, China banned the production and usage of technical HCH, representing the greatest drop in worldwide usage (Wu *et al.*, 1997). China produced 4.5 million tons of HCH between 1952 and 1983, three times the volume used by the rest of the world (Li *et al.*, 1998). India banned technical HCH in 1990 on some crops, but allowed its use in public health protection and on certain foodstuffs, until they could gradually phase out production by 30 000 tons per year and finish the use of old stockpiles (Li *et al.*, 1998). The last major user of HCH was the former Soviet Union, which banned HCH in 1990. However, they continued to use remaining stockpiled HCH until 1991 (Voldner and Li, 1995).

Currently, technical HCH and lindane are still used in Mexico and Central America. Lindane usage in Canada and the U.S. amounts to a few hundred tons per year (Barrie *et al.*, 1992). Africa and the Near East are reported to have about 2785 tons of technical HCH, 304 tons of lindane and 45 tons of unspecified HCH mixtures in dump sites (FAO, 1998). Some Eastern European countries may also have remaining stockpiles from previous HCH manufacturing (Pruszynski and Stobiecki, 1996).

Gamma-HCH is still used as a seed preservative, a topical medicine for head lice and scabies in humans (Voldner and Li, 1995) and for ectoparasite control in livestock (Lane *et al.*, 1991). However, usage of γ-HCH has declined in recent years due to restrictions based on its

carcinogenicity and the increased use of organophosphate insecticides (Lane *et al.*, 1992). In Ontario, lindane is deregistered and no longer used for agriculture, forestry or domestic use (lindane was used for structural protection and for grub and ant control). Presently, lindane is being phased out as seed treatment for canola and rapeseed and expected to be deregistered as a seed treatment by July 2001 (PRMA, 2000).

2.2 Alpha-Hexachlorocyclohexane: Physical-Chemical Properties

HCH is a halogenated cyclic chlorinated compound. Of the eight theoretically possible stereoisomers, α-HCH is the only one with chiral properties. The α-, β-, γ- and δ- isomers are persistent because they have strainless bonds (Faller *et al.*, 1991). All the isomers are stable to light, high temperature, hot water and acid but can be dechlorinated in alkali (Deo *et al.*, 1994). Alpha-HCH has a melting point of 159-160°C, a vapour pressure of $1.6 \pm 0.9 \times 10^{-2}$ Pa and a log K_{ow} of 3.9 ± 0.2 (Mackay, 1994). The Henry's Law constant is 0.68 Pa·mol/m^3(Mackay, 1982).

The abiotic processes that can transform α-HCH are hydrolysis in water and photooxidation in the atmosphere. Dehydrochlorination can occur in basic aqueous solutions to produce pentachlorocyclohexenes and trichlorobenzenes with a hydrolysis half-life of 26 years for α-HCH in 5°C and pH 8 conditions (Ngabe *et al.*, 1993). In the Eastern Arctic Ocean, Harner *et al.* (1999) calculated the hydrolysis half-life of α-HCH to be 63 years.

Schroeder and Lane (1988) concluded that atmospheric reactions are important for the removal of organic pollutants such as α-HCH. Atkinson (1987) estimated (from structure activity relationships), the half-life of α-HCH to be two to three days, but Brubaker and Hites (1998) found it to be 120 days from laboratory extrapolation.

2.3 Alpha-HCH Chirality

Technical mixtures of HCH are comprised of three main isomers, alpha- (α), beta- (β), and gamma (γ)- HCH. These isomers differ only in their placement of the chlorine atom, either in the axial or equatorial position (Figure 2.1). Alpha-HCH is the only isomer that exists as two chiral enantiomers (Figure 2.2). Enantiomers are stereoisomers in which the atoms are arranged such that the molecules are mirror images of each other. The two enantiomers can rotate polarized light in different directions. The ratio of the (+)-α-HCH to (-)-α-HCH enantiomer abundance is called the enantiomer ratio (ER). In HCH technical mixtures, the α-HCH isomer exists as a racemate (having an ER of 1). Abiotic processes such as transportation (e.g., volatilization, advection and leaching) and degradation (e.g., photolysis and hydrolysis) do not discriminate between the two enantiomers (Walker *et al.*, 1999) and are said to be non-enantioselective.

The chiral property of α-HCH has made it useful for determining microbial degradation. In the early 1990s, capillary gas chromatography, with the use of special chiral (heptakis-(3-O-butyrl-2, 6-di-O-pentyl)-β-cyclodextrin stationary phase) columns allowed the two enantiomers to be separated from seawater (Faller *et al.*, 1991) and a suite of environmental samples (Muller *et al.*, 1992). An ER that deviates from 1 is indicative of enantioselectivity. The loss of one enantiomer over the other is very likely due to microbial processes (Faller *et al.*, 1991). Thus, a detection of non-racemic ER can be due to α-HCH that has had contact with microbial degradative enzymes.

Bidleman and Falconer (1999) have discussed how we can exploit chirality of α-HCH to investigate the relative age, source and degradation rate of chiral compounds. For example, Falconer *et al.* (1995) found ERs <1 in arctic streams, a phenomenon they attributed to

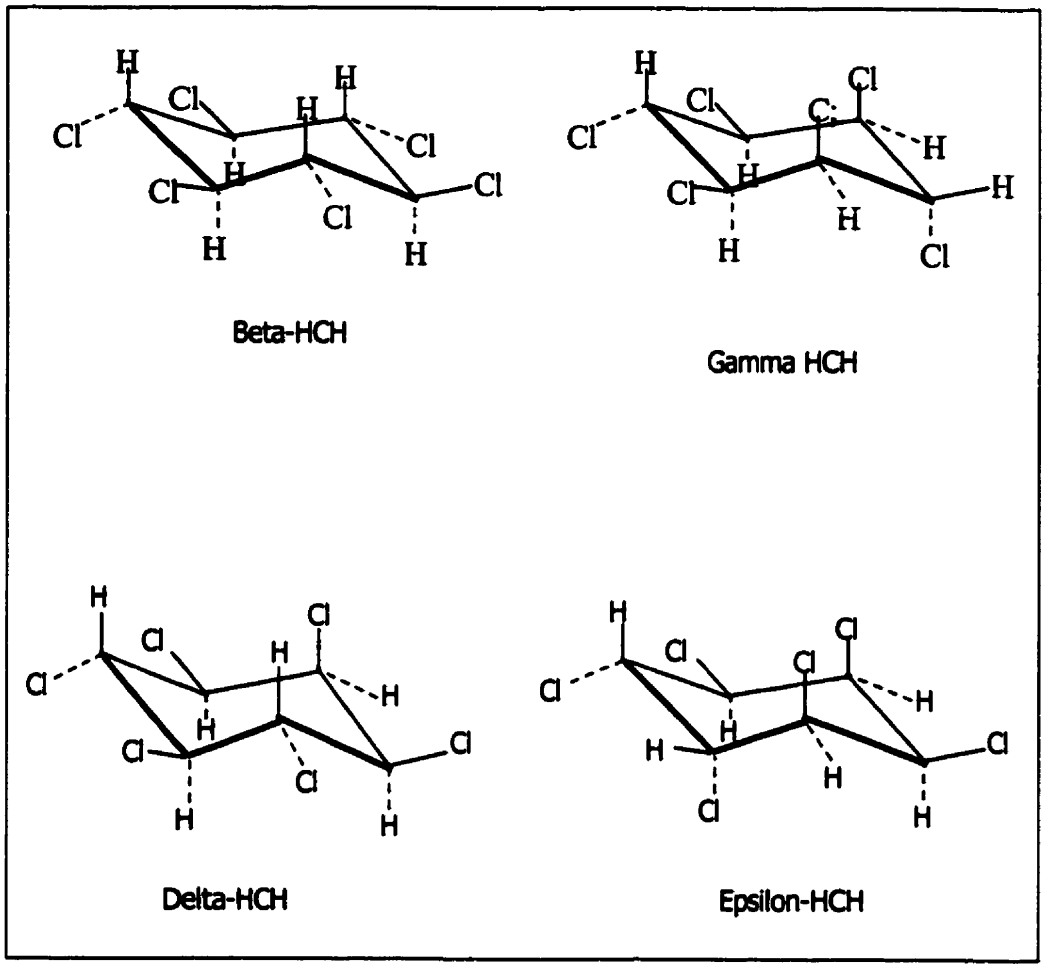


Figure 2.1: The structures of beta-, gamma-, delta-, and epsilon- HCH isomers. The isomers differ only in the placement of chlorine atoms, either in the axial or equatorial position.

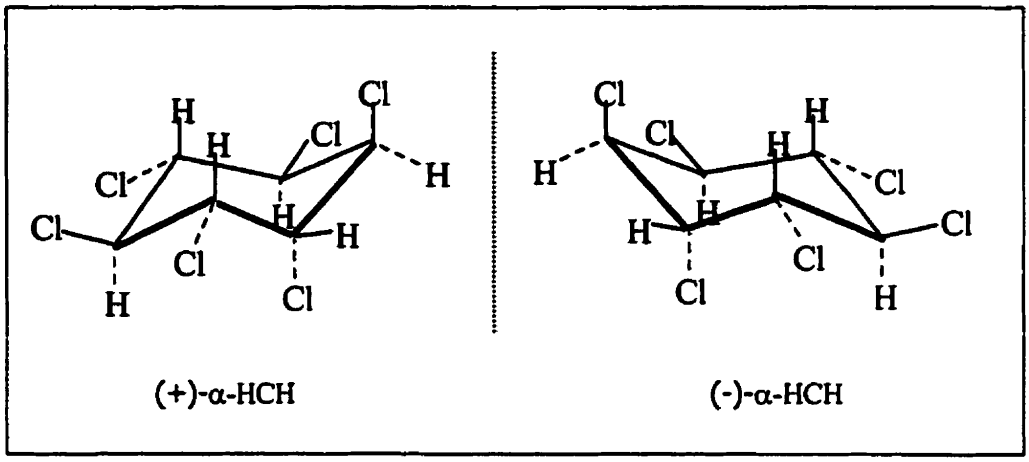


Figure 2.2: The two chiral enantiomers of α-HCH. They are non-superimposable mirror images of each other. The enantiomer ratio (ER) is the ratio calculated by the abundance of (+)-α-HCH and the abundance of (-)-α-HCH.

microbial degradation. Faller *et al.* (1991) and Helm *et al.* (2000) also used ER to investigate biodegradation losses of α-HCH. This research will also use ER as an indicator of microbial degradation of α-HCH.

2.4 Accumulation and Toxicity of Alpha-Hexachlorocyclohexane

Many researchers have studied HCH accumulation in humans and animals. Although β-HCH is the most persistent and metabolically inactive isomer, α-HCH is also important because of its abundance. In 1978, Indian males were found to have a concentration of 0.9 mg/kg of α-HCH in their bodies which was attributed to the heavy technical HCH usage (Siddiqui *et al.*, 1981). Tanabe *et al.* (1990) found that total HCH residues were higher than PCBs and DDT metabolites in the breast milk of Indian women. The transfer of HCH isomers from breast milk to babies has been studied in Germany, Denmark, Japan, Sweden, Finland,

Italy, India, Kenya, Hong Kong, the Czech Republic and Canada and found to be in the range of 3 to 27 μg/g of β-HCH, <0.01 to 0.21 ug/g of γ-HCH and 0.04 to 2.6 μg/g of α-HCH on a lipid basis (Fooken and Butte, 1987, Andersen and Orbaek, 1984, Noren, 1983, Mes *et al.*, 1993, Larsen *et al.*, 1994, Schoula *et al.*, 1996, Ip *et al.*, 1989). Since the health effects of HCH to infants are unknown, its presence in breast milk is being further investigated (Willett *et al.*, 1998).

Recently, concern over high concentrations in the arctic environment has prompted more research into α-HCH and other organochlorines. Despite the low bioconcentration factor and K_{ow} of α-HCH, bioaccumulation and biomagnification to top predators occur. Hargrave *et al.* (1997) found that HCH biomagnified in the Arctic Ocean food chains from epontic particulate matter to zooplankton, pelagic and benthic anthropods, and then to abyssal fish. To complete this chain, Norstrom *et al.* (1988) and Hummert *et al.* (1995) found high levels in seals and polar bears at the top of the food chain. A cause for concern, as identified by the Arctic Environmental Strategy's Northern Contaminants Program (1994), is the potential human health implications arising from the dependence of local people on natural foods that contain contaminants. There is a possibility that traditional diets can exceed the daily organochlorine intake standards (Muir *et al.*, 1989, Kinloch *et al.*, 1992).

A detailed toxicity review can be found in Willett *et al.* (1998). After γ-HCH, α-HCH isomer is the next most toxic isomer (U.S. Dept. of Health, 1998). Alpha-HCH is a hepatocellular carcinogen, and is listed as a probable human carcinogen with an estimated oral cancer potency factor of 6.3 $(mg/kg/day)^{-1}$ (U.S. Dept. of Health, 1998). Alpha-HCH, as well as the other isomers, can be excreted in urine, but can also rapidly break down in the body to produce pentachlorophenol, which is known to be harmful (U.S. Dept. of Health, 1998). The LC_{50}'s of α-HCH to guppy (*Poecilia reticulata*), zebra fish (*Brachydanio rerio*) and neon fish (*Paracheirodon axelrodi*) over 24 hours are 2.65, 1.41, and 1.64 mg/L, respectively (Oliveira-

Filho and Paumgartten, 1997).

2.5 Concentrations and Enantiomeric Ratios of α-HCH in the Environment

2.5.1 Concentrations and ERs of α-HCH in Arctic and Temperate Air

Alpha-HCH is the most abundant compound in arctic air (Stern *et al.*, 1997, Hargrave *et al.*, 1988). Studies by Hargrave *et al.* (1988), Fellin *et al.* (1996), Bidleman *et al.* (1995) and Halsall *et al.* (1999) have measured the concentrations of α-HCH in arctic air (Table 2.1). Concentrations ranged from 60 to 425 pg/m^3, depending on location and date of sampling.

Alpha-HCH in air is usually racemic. Jantunen and Bidleman (1996) found ERs close to 1.0 in air above ice covered regions, but in the range of 0.85 to 0.91 over the Greenland Sea. Falconer *et al.* (1995) also found ERs of 1.0 in air near Resolute Bay.

Table 2.1: Average α-HCH concentrations in Arctic air at various locations.

Location	Sampling Period	Alpha-HCH Conc. (pg/m^3)	Reference
Ice Island	May-June 1986	425	Hoff and Chan (1986)
	Aug.-Sept 1986	253	Hoff and Chan (1986)
	Aug.-Sept. 1986	546	Hargrave *et al.* (1988)
	June 1987	340	Patton *et al.* (1991)
Alert	May-Sept. 1992	57	Fellin *et al.* (1996)
	Oct.-April 1992	61.7	Fellin *et al.* (1996)
Resolute Bay	August 1992	114	Bidleman *et al.* (1995)

In temperate regions, Poissant and Koprivnjak (1996) reported a concentration of 56 pg/m^3 of α-HCH in the air around Villeroy, Quebec. Ridal *et al.* (1997) found that air above Lake Ontario average 106 ± 25 pg/m^3 of α-HCH. In the Great Lakes, atmospheric loadings of total HCHs to Lakes Superior, Michigan and Erie are reported to be 420, 270 and 600 kg/year,

respectively. Lake Huron has a net volatilization of 470 kg/year of total HCHs to the air, while Lake Ontario is in equilibrium with overlying air (Hillery *et al.*, 1998).

2.5.2. Concentrations and ERs of α-HCH in Temperate Waters

Atmospheric deposition is the main source of many organochlorines, including HCHs, to the Great Lakes (Lane *et al.*, 1992). Thus, the concentration of atmospherically-borne organochlorines to the Great Lakes are not greatly influenced by the drainage area (Eisenreich *et al.*, 1981, Schroeder and Lane, 1988). There are few studies of α-HCH in small temperate lakes and streams.

Ridal *et al.* (1997) found that α-HCH in Lake Ontario water averaged of 720 ±107 and 1020 ± 85 pg/L at the surface and hypolimnion, respectively. They attributed the difference in concentrations to volatilization, rather than to biodegradation. They also found that precipitation, due to atmospheric scavenging, had concentrations of 870 to 3030 pg/L. Ridal *et al.* (1997) found ERs of 0.86 ± 0.02 in the hypolimnion of Lake Ontario and similar ratios in surface waters which they attributed to *in situ* degradation by benthic biota.

2.5.3 Concentrations and ERs of α-HCH in Arctic Waters

Alpha-HCH is found below the thermocline at concentrations similar to surface water concentrations which is indicative of persistence caused by a lack of photolysis and volatilization (Ngabe *et al.*, 1993). The α-HCH tends to remain in the water column due to high water solubility, but also its inability to partition onto particles and sediments. Strand and Hov (1994) estimated that over 90% of the HCH present in the environment is now held in the world's oceans.

Table 2.2 lists the average concentrations of α-HCH in northern seawaters. Despite the heaviest usage of HCH being in tropical and temperate zones, the levels found in northern

oceans are about an order of magnitude higher then source regions (Jantunen and Bidleman, 1996). Concentrations vary between 1400 - 4700 pg/L (e.g., Kawano *et al.* (1988), Hinckley *et al.* (1991), Iwata *et al.* (1993), Jantunen and Bidleman (1995), Bidleman *et al.* (1995), Hargrave *et al.* (1997), Hargrave *et al.* (1988), Patton *et al.* (1989)). Falconer *et al.* (1995) and Helm *et al.* (2000) found concentrations of 2100 ±800 pg/L in Amituk Lake. Concentrations of α-HCH are declining due to reduced use of technical HCH and thus inputs are now from source regions and volatilization (Jantunen and Bidleman, 1996) and the degradation occurs for older α-HCH (Helm *et al.*, 2000).

Table 2.2: Average concentrations of α-HCH in arctic seawater.

Location	Sampling Period	α-HCH Conc. (pg/L)	Reference
Bering-Chuckchi Sea	1981	2750	Kawano *et al.* (1988)
	1988	2350	Hinckley *et al.* (1991)
	1990	1400	Iwata *et al.* (1983)
	1990	1500	Iwata *et al.* (1993)
	1993	2000	Jantunen and Bidleman, 1995
	1994	1820	Jantunen and Bidleman, 1997
Resolute Bay	1992	4700	Falconer *et al.* (1995)
	1993	3640	Hargrave *et al.* (1987)
Canadian Basin	1986	4470	Hargrave *et al.* (1988)
	1987	7100	Patton *et al.* (1989)
	1994	2310	Jantunen and Bidleman, 1996, 1998

There have been many studies of α-HCH ERs in arctic regions. Jantunen and Bidleman (1996, 1997) found that the ERs ranged from 1.05 to 1.14 in the Bering and Chukchi Seas to less than one in the Canada Basin and Greenland Sea surface waters (Jantunen and Bidleman, 1996).

ERs in all locations decreased with depth. ERs varied in different regions in the North Sea with values>1, <1 or ~1 (Faller *et al.*, 1991). The waters of Resolute Bay had an ER of 0.93 (Falconer *et al.*, 1995).

A study of arctic freshwaters by Falconer *et al.* (1995) and Helm *et al.* (2000) found that ERs ranged from 0.35 to 0.85 in inlet streams. They found that ERs decreased with increasing temperature and contact time with stream sediments.

2.5.4 Concentrations and ER of α-HCH in Biota

Despite its low bioconcentration factor, α-HCH does bioaccumulate and biomagnify. The high concentrations found in top predators are of concern because they are often an important source of food for aboriginal people in arctic areas. This diet likely exposes them to higher levels of contamination relative to the majority of Canadians (Twitchell, 1991). For example, Norstrom *et al.* (1988) found a trend of increasing α-HCH concentration along a south to north gradient in polar bears. Their adipose tissues were found to have 490 ng/g of total HCH isomers in the arctic regions. Levels as high as 100 ng/g of blubber have been found in arctic narwhales (Muir *et al.*, 1992). Cod muscles were found to have about 0.01 ng/g of α-HCH (Muir *et al.*, 1988). Although β-HCH constituted 91% of the HCH isomers in the blubber, liver and lungs of neonatal fur seals, α-HCH made up 91% of the HCH isomers in the brain (Mossner *et al.*, 1992). Similarly, Kawano *et al.* (1988) reported that α-HCH made up 75%, 73% and 83% of HCHs in the cerebrum, cerebellum and medulla oblongata in striped dolphins. All of these studies indicate that α-HCH has the ability to cross the blood/brain barrier and raises concerns about potential toxicological effects.

The ERs in biota have also been studied in the last few years. Hummert *et al.* (1995) found that harbour porpoises, white-beaked dolphins, harbour seals, grey seals and harp seals

showed a preferential accumulation of (+)-α-HCH in the blubber with ERs of 1.77, 1.26, 1.91 and 1.25, respectively. The exception was hooded seals that had a preferential accumulation of the (-)-α-HCH with an ER of 0.75. Tanabe *et al.* (1996) also found that small cetaceans had average ERs of 1.6 – 2.8, depending on the species, their feeding habits and species-specific metabolism. Blue mussels, flounder livers and eider ducks in the North Sea were found to have ERs of 0.84, 0.80 and 1.4, respectively (Pfaffenberger *et al.*, 1992). The high ER in eider ducks suggest that there may be a different enzymatic pathway of α-HCH degradation in higher trophic levels (Moller and Huhnerfuss, 1993), although this is not consistent with hooded seals. Northern fur seals were found to have ERs between 1.2 and 1.9 in the blubber, milk, lungs and liver (Mossner *et al.*, 1992). They also found ERs as high as 32 in brain tissues, supporting evidence that (+)-α-HCH can preferentially cross the blood-brain barrier. The ERs in roe-deer livers ranged from 0.03 to 0.40, values much lower then those found in the livers of marine animals (Pfaffenberger *et al.*, 1994).

2.6 Microbial Degradation of Alpha-Hexachlorocyclohexane

2.6.1 Microbial Degradation

Contaminants such as HCH are subject to various degradative mechanisms in the environment. Microbes contribute to the removal and mineralization of persistent organic pollutants (POPs) by transforming the parent compound to another metabolite. Unlike higher animals that can convert xenobiotics to a more polar form to promote excretion, and can do so in a few specialized organs (such as the liver), microbes can metabolize POPs for the production of energy. Since most organic chemicals can serve as a fuel source for microbes, only a few chemicals are foreign to microbes (Klein, 1989).

There are several conditions that must be met for microbial degradation to take place.

1) Microbes need the necessary enzymes for degradation.

2) The degrading microorganism must be present in the environment in which the chemical is present.

3) The degrading microbe must have access to the chemical, such as a preferred carbon source in the microenvironment.

4) If the degrading enzyme is extracellular, than it must be exposed to a catalyst to function properly.

5) If the degrading enzyme is intracellular, then the chemical must be able to penetrate the cell membrane.

6) The population of the degrading organisms must be large enough to destroy the chemical and have the optimal environmental conditions for the population to proliferate (Alexander, 1985, 1999).

In incidental metabolism the chemical is metabolized as a result of general microbial activities, often stimulated by the availability of nutrients, moisture, optimal temperature and pH (Klein, 1989). Trace concentrations (e.g., at μg/L levels) of xenobiotics cannot support microbes as sole-electron donors. In this situation, the xenobiotics may be transformed incidentally by microbes that obtain most of their energy and carbon from a more abundant carbon source. This co-metabolized chemical shares the same enzymatic pathway as the primary carbon source and the enzymes at the initial sequence of metabolic pathways are often non-specific. However, more specific enzymes are required for intermediates of the primary carbon source and will lead to partially oxidized intermediates of the xenobiotic (Bouwer, 1989).

Some studies have found that microbes can degrade α-HCH applied after the application of technical HCH (Deo, 1994). However, there is currently no information regarding whether α-HCH is degraded by common metabolic pathways, or if the degradation is by incidental metabolism or co-metabolism. In non-source areas such as arctic and temperate areas where technical HCH is no longer used, incidental metabolism is possible, due to low α-HCH

concentrations in the arctic that are insufficient for use as an energy source.

In general, the two limiting factors of degradation in the arctic and temperate region are: first, the low temperatures that can cause biological processes to proceed slowly; and second, the reduction of degradation because of the reduced microbial activity. According to the Q-10 rule, reaction rates change by a factor of two in response to temperature changes of 10°C. Thus, microbial degradation is twice as fast at 10°C than at 0°C.

2.6.2 Microbial Degradation Pathways

Microbial degradation can be an enantioselective process, while abiotic processes, with the possible exception of sorption on clays, is not enantioselective. The ER deviation from 1 provides an indication of microbial degradation. Microbes can transform HCH isomers by reductive dechlorination, dehydrochlorination, dehydrogenation and oxidation (Deo *et al.*, 1994, Johri *et al.*, 1996). Although reductive dechlorination has been considered a major pathway (Deo *et al.*, 1994), other studies have shown that α-HCH degradation under aerobic conditions is faster than under anaerobic conditions, with an intermediate step of dehydrochlorination to pentachlorocyclohexene (Bachmann *et al.*, 1988). Pathways such as oxidation and dehydrogenation also occur in higher organisms (Johri *et al.*, 1996, Deo *et al.*, 1994).

Many studies have concentrated on the degradation of α-HCH under controlled conditions. Buser and Muller (1995) found that the degradation of HCH isomers and enantiomers in sludge follows the order γ- >(+)- α-HCH > (-) α-HCH >δ- >β. The rates of (+)-α-HCH and (-)-α-HCH degradation differed by a factor of two or three (Buser and Muller, 1995). Deolman *et al.* (1990) concluded that α-HCH can be degraded in well aerated soils with 6% moisture or soil slurries (50% water content) under temperate conditions (e.g., Netherlands).

In their experiment, concentrations of α-HCH slowly decreased from 367 mg/kg of soil to 107 mg/kg in 23 weeks.

2.6.3 Factors Affecting Microbial Degradation

The degradation of α-HCH has been widely studied in recent years, mostly under *in situ* conditions. An important laboratory study by Bachmann *et al.* (1988) found that the degradation of α-HCH depends on temperature, substrate type, soil inhomogeneities, and the presence of any auxilliary carbon sources. The addition of other carbon sources as well as inhomogeneities in the soil, such as clumps were found to decrease the degradation. They reported that a temperature range of 20-30 °C is optimal for degradation and temperatures below 4 °C inhibited degradation. Wu *et al.* (1997) found that the addition of glucose and yeast enhances the degradation of α-HCH under aerobic and anaerobic conditions because the organic nutrients increased microbial biomass and activities in the sediment tested. In high arctic watersheds, it was found that enantioselective degradation of α-HCH was positively related to stream temperatures (Falconer *et al.*, 1995, Helm *et al.*, 2000). Helm *et al.* (2000) also found that degradation was greatest when contact time between chemical and productive sediments was maximized, as occurring during low flows through wetlands or biologically productive rills.

2.6.4 Microbes with α-HCH Degradation Abilities

Many researchers have been able to isolate the microbial species capable of degrading α-HCH at sites of repeated application of technical HCH. Sahu *et al.* (1990) observed that an aerobe, motile, gram-negative, non-spore-forming, straight or curved rod that is catalase and

oxidase positive can degrade α-HCH. Senoo and Wada (1989) found that *Pseudomonas paucimobilis* used HCH as its only source of carbon. This strain was also isolated by Sahu *et al.* (1990) in Japanese rhizosphere soils that had been extensively treated with technical HCH for many years. Johri *et al.* (1996) found that anaerobic *Clostridium spp.*, aerobic *Bacillus spp* and some Enterobacteriaceae were able to degrade α-HCH. No studies have identified α-HCH degrading microbes in non-source areas or when exposed to α-HCH only.

Oligotrophic microbes are defined as microbes that can live under conditions of low carbon flux of less than 1 mg/L/d (Alexander, 1999). These organisms do not comprise a special taxonomic grouping of organisms, but come from almost any group of bacteria. They are generally adapted to life under low nutrient conditions, but can readily be adapted to high nutrient conditions: there is no evidence of reverse adaptation from high nutrient conditions to a low condition, hence oligotrophs can only be cultured from low-nutrient environments. Since the minimum carbon concentration needed for measurable growth is lower than that required for high nutrient (eutrophic) organisms, their maximum growth is lower. Oligotrophic bacteria prefer an attachment rather than a free-living existence, and thus, are usually found living as biofilms. These organisms often appear to have multiple inducible enzymes, that can shift metabolic pathways, and can often take up and use mixed carbon sources (Alexander, 1985).

Oligotrophs are potentially useful in the removal of trace concentrations of organic contaminants from water such as effluent from wastewater treatment processes. Oligotrophs have high affinities for the organic molecules they use as a carbon source for growth (Alexander, 1985). When nutrient concentrations are high, diffusion provides molecules to the cell surface at a rate that is sufficiently rapid to meet the energy needs for maintenance and growth alike. At lower concentrations, diffusion to the cell satisfies carbon maintenance, but not for growth.

The threshold limit is the lowest concentration that will support growth and varies with populations. Thresholds are lower for oligotrophs than for eutrophics. Very low levels of xenobiotics present in the environment may be a result of the inability of microorganisms to destroy substrates readily at very low levels (Alexander, 1985).

2.7 Alpha-Gamma HCH Ratios

The ratio of α-HCH to γ-HCH can be useful in identifying sources and the approximate age of HCHs (Falconer *et al.*, 1995, Bidleman *et al.*, 1998, Jantunen and Bidleman, 1996). Ratios ranging from 1 to 4 are indicative of European sources, whereas North American sources have ratios typically between 7 and 10 (Pacyna and Oehme, 1988).

The ratios found in the atmosphere are composed of γ-HCH superimposed on the α-HCH / γ-HCH ratio from a technical HCH background (Voldner and Li, 1995, Bidleman and Falconer, 1999). The expected ratio, between 4 and 7 is based solely on the ratio of the isomers in the technical mixture. Generally, the higher the ratio is, the more indicative it is to long-range transport. High latitude areas are subject to high ratios. Although Oehme (1991) reported that ratios between 5 and 10 are indicative of recently transported air masses, ratios as high as 50 have been found in arctic air and more indicative of aged, residual HCH (Willett *et al.* 1998).

Two explanations can account for α-HCH / γ-HCH ratios higher than that in the technical mixture. First, γ-HCH is more likely to be scavenged by precipitation and is more likely to undergo air-to-water exchange with surface waters, due to its lower Henry's law constant than α-HCH (Kucklick *et al.*, 1991, Iwata *et al.*, 1993). The second explanation is the isomerization of γ-HCH to α-HCH (which is more photochemically stable) with photochemical energy during atmospheric transport (Steinwandter, 1976, Steinwandter, 1978a, 1978b, Benezet and

Matsumura, 1973, Malaiyandi and Shah, 1984) and presumably by microbial activity in sediments (Malaiyandi *et al.*, 1982). Strachan *et al.* (1980) used this explanantion of isomerization to account for the higher levels of α-HCH over γ-HCH in rain and snow samples in Ontario.

2.8 Trophic Status of Lakes

Lakes can be categorized by their nutrient status. Oligotrophic lakes are distinguished by their low concentration of nutrients, a poor development of phytoplankton and low primary production of organic matter (Kuzmetsov, 1970). Bacterial abundance is normally between 50 and 340 (x 10^3 mL^{-1}) (Rheinheimer, 1992). In contrast, eutrophic lakes have an abundance of nutrients. They are usually shallow lakes with an abundance of phytoplankton in the summer and transparency is usually limited (Kuzmetsov, 1970). Oxygen levels decrease drastically in the hypolimnion over the summer and winter due to its consumption for respiration. Bacterial numbers in eutrophic lakes range from 220 to 2300 (x 10^3 mL^{-1}); (Kuzmetsov, 1970, Rheinheimer, 1992). Mesotrophic lakes have intermediate levels of nutrients and have about 450 to 1400 (x 10^3 mL^{-1}) bacteria (Kuzmetsov, 1970, Rheinheimer, 1992). Trophic status is also determined by concentration of limiting nutrients such as N, P and C.

Arctic lakes are usually nutrient-deficient and have very low productivity rates (Hobbie, 1973). Levels of productivity can be as little as 100 $g.C/m^2$/year (Hobbie, 1973). Nitrogen and phosphorus are the limiting nutrients in arctic lakes, due to low inputs from precipitation (Schindler *et al.*, 1974, de March, 1975). Arctic lakes also have low concentrations of organic carbon in comparison to temperate lakes. The organic carbon fraction was found to be 3% or less in the arctic waters (Muir *et al.*, 1995). In Char lake, organic carbon ranged only from 0.3-

2.4% (de March, 1978).

CHAPTER 3: FACTORS AFFECTING THE OCCURRENCE, ISOMER RATIO AND ENANTIOMERIC DEGRADATION OF ALPHA-HEXACHLOROCYCLOHEXANE IN ARCTIC AND TEMPERATE AQUATIC SYSTEMS

Preamble

The following chapter outlines a field survey that was conducted to investigate the factors that may control the enantioselective degradation of α-HCH. The chapter is written in the form of a scientific article for submission to a refereed scientific journal. The co-authors of this paper supplied data, recommendations and analytical support for the study. M.L. Diamond supervised and funded the project. P.A. Helm collected samples from the arctic and provided data from his 1997 arctic research. L.M. Jantunen provided data from the Great Lakes, and T.F. Bidleman provided recommendations and use of laboratory facilities. M. Alaee provided data for the Yukon Lakes.

Abstract

Alpha-HCH concentrations, γ-HCH concentrations, α-HCH / γ-HCH ratios and ERs were measured in lakes, streams and wetlands in temperate, high arctic, subarctic, the Great Lakes and small temperate lakes. High concentrations of α-HCH were found in colder and larger lakes such as those in the arctic, subarctic and the Great Lakes. Smaller temperate lakes had considerably less α-HCH concentrations. High α-HCH / γ-HCH ratios indicate aged HCH being transported to high latitude areas, while the low ratios in small temperate systems indicate recent γ-HCH usage and residual α-HCH concentrations. The greatest enantioselective degradation was found in small high arctic lakes and streams, in large lakes in the subarctic and the Great Lakes, and wetlands. Wetlands in both arctic and temperate areas maximize contact between chemical and sediments and supported greater degradation. Minimal enantioselective degradation occurred in temperate small lakes, despite the warmer temperatures, greater microbial populations and the availability of nutrients, compared to arctic systems. Statistical analyses showed that enantioselective

degradation is positively related to the α-HCH concentration which is greatest in cold, oligotrophic lakes with long residence times which may be attributable because of contact between microbial populations and α-HCH. Watershed area is also positively related to enantioselective degradation since most degradation occurs in streams. The results suggest that enantioselective degradation is optimized by maximal contact between chemical and sediment substrate in nutrient-poor waters where it is hypothesized that oligotrophic bacteria may act as biofilms.

3.1 Introduction

Hexachlorocyclohexane (HCH) is a pesticide that is distributed worldwide due to its heavy usage, persistence and movement by long range transport (Li *et al.*, 1998, Wania and Mackay, 1995). For example, HCHs are the most abundant organochlorine in arctic air and surface waters (Willett *et al.*, 1998) where concentrations range from 60 to 425 pg/m^3 and 1400 to 4700 pg/L, respectively (Hargrave *et al.*, 1988, Fellin *et al.*, 1996, Bidleman *et al.*, 1995, Jantunen and Bidleman, 1995, Bidleman *et al.*, 1995, Hargrave *et al.*, 1997). Technical HCH was used as a broadbased insecticide in forestry, agriculture and public health (Twitchell, 1991, Li *et al.*, 1998, Haugen *et al.*, 1998). Among the isomers of HCH, α-HCH is the most abundant organochlorine in systems, ranging from 2310 to 4700 pg/L in the Arctic Ocean (Falconer et al., 1995, Jantunen and Bidleman, 1996, 1998) to 720 pg/L in the Great Lakes (Ridal *et al.*, 1997).

Technical HCH is comprised of eight theoretical isomers, of which only γ-HCH has insecticidal properties. Alpha-HCH is the most abundant isomer, constituting 60 to 70% of technical HCH. Recently, Li *et al.* (1998) estimated that as much as 6 million tons have been consumed on a global basis. Currently, technical HCH is still used in Mexico and Central America, while some eastern European, Near East and African countries have remaining stockpiles (Barrie *et al.*, 1992, FAO, 1998, Pruszynski and Stobiecki, 1996).

The deregistration of HCH in many countries has prompted the use of purified γ-HCH, also

known as lindane. It is currently used in Canada and the U.S. for grub control, for wood preservation, as seed preservative and a topical treatment for lice and scabies (Voldner and Li, 1995, PRMA, 2000). The deregistration of technical HCH has caused a dramatic decline in α-HCH concentrations in arctic and Great Lakes air and surface water concentrations (Li *et al.*, 1998, Hillery *et al.*, 1998). As a result of declining usage and emissions, surface waters and soils, with their historically elevated concentrations and fugacities, will continue to be a source of HCH to the environment as a new steady-state condition is approached (Jantunen and Bidleman, 1996, Bidleman *et al.*, 1995).

Alpha-HCH exists as a chiral compound, having two enantiomers that are non-superimposable images of each other. The two enantiomers can be separated by gas chromatography on chiral columns and an enantiomer ratio (ER) obtained by the calculation of the ratio of (+)-α-HCH to (-)-α-HCH (the signs refer to the direction of optical rotation) (Faller *et al.*, 1991, Bidleman and Falconer, 1999). The chirality of α-HCH can be exploited to differentiate between enantioselective processes such as microbial degradation from nonenantioselective processes such as transportation (e.g., volatilization and advection) or biotic degradation (e.g., photolysis and hydrolysis); (Bidleman and Falconer, 1999, Walker *et al.*, 1999).

Studies of enantioselective degradation have focused mainly in arctic regions. For example, ERs in the Bering and Chukchi Seas varied between 1.05 and 1.14 in surface waters and < 1 in the Canada Basin and Greenland Sea (Jantunen and Bidleman, 1997). ERs along this transect decreased with increasing depth in the Arctic Ocean (Jantunen and Bidleman, 1996, 1998, Harner *et al.*, 1999). Falconer *et al.* (1995) and Helm *et al.* (2000) reported ERs between 0.35 and 0.5 in freshwaters on Cornwallis Island, in the Canadian high arctic. ERs less than one have also been reported in Yukon basin lakes, located in the Canadian subarctic (Alaee, 1997).

Fewer studies have concentrated on temperate areas. Lake Ontario had ERs of 0.86 ± 0.02 in both surface and hypolimnetic waters that was attributed to *in situ* degradation by benthic biota (Ridal *et al.*, 1997). No other studies to date have explored enantioselective degradation in temperate aquatic systems, especially small lakes, wetlands and streams.

In addition to ERs, isomer ratios can be used as an indicator of contaminant source and age (Falconer *et al.*, 1995, Bidleman *et al.*, 1998, Jantunen and Bidleman, 1996). Ratios of α-HCH to γ-HCH reflect signatures of γ-HCH or lindane superimposed on the α-HCH / γ-HCH ratio derived from technical HCH (Bidleman and Falconer, 1999). The expected α-HCH / γ-HCH ratio is between 4 and 7, based solely on the ratio of isomer abundance in technical HCH mixtures. Lower ratios are indicative of a fresh lindane source, whereas higher ratios suggest long range transport as the source of HCHs.

Alpha-HCH to γ-HCH ratios range from 7 to 50 in northern regions, because the air masses are aged and the HCH originates from historical loadings (Oehme and Manø, 1984, Barrie, 1986). The ratio increases as γ-HCH is preferentially removed from air, either by wet precipitation or air-to-water partitioning due to its lower Henry's Law constant than that of α-HCH. Gamma-HCH, with a Henry's law constant of 0.257 Pa m^3/mol (at 20°C in freshwater), partitions from air to water more readily than α-HCH with a constant of 0.524 Pa m^3/mol (Kucklick *et al.*, 1991). This preferential removal of γ-HCH will leave proportionately more α-HCH in the atmosphere (Iwata et al., 1993). For example, Brubaker and Hites (1998) reported that α-HCH has a 120 day atmospheric lifetime compared to 96 days for γ-HCH and thus, α-HCH is subject to greater long range transport. To some extent, high ratios in high latitudinal areas are due to volatilization of already altered and/or degraded HCHs from treated surfaces (Kurtz and Atlas, 1987). High isomer ratios can also be due to the photochemically induced isomerization

of γ-HCH to α-HCH under photochemical energy (Benezet and Matsumura, 1973, Malaiyandi and Shah, 1984). However, there is no evidence that this isomerization occurs in the atmosphere.

The aim of this study was to investigate and compare the status of HCH in temperate and arctic aquatic systems by means of assessing α-HCH concentrations, the ratio of α-HCH to γ-HCH and the ER of α-HCH. To accomplish this, a selection of lakes and streams of varying trophic status and limnological characteristics in the arctic, subarctic and temperate regions were sampled and analyzed for α-HCH, γ-HCH and ER.

3.2 Methods

3.2.1 Study Sites

Descriptions of study sites, sampling procedures and analytical methods are described by Helm *et al.* (2000) for high arctic lake and streams sampled by Helm, during the summer of 1997 and 1998 and Alaee (1996) for the subarctic Yukon lakes sampled by Alaee in the summer of 1993. Lakes Superior, Huron and Erie were sampled by Jantunen in August, 1996 while additional samples of Lake Superior were taken in May of 1997 and samples for Lake Ontario were taken in July 1998 using the methods of Jantunen and Bidleman (1998). The methods below focus on the temperate water samples taken from lakes, wetlands and streams by the author from May to August, 1999. Table 3.1 summarizes the locations and morphometric data for these aquatic systems. The temperate lakes (Figure 3.1) are located in the Muskoka region in south central Ontario and the Kawarthas region in south eastern Ontario. Lakes with short and long residence times were chosen in the Muskoka region and were oligotrophic and the Kawartha region that were mesotrophic and eutrophic.

Samples collected from wetlands were located in southern Ontario. Cootes Paradise is a

Table 3.1: Table of locations and morphometic characteristics of sampled lakes, streams and wetlands

System	Name	Location (lat. / lon.)	Residence Time (y)	Watershed Area (ha)	Volume (m3)	Surface Area (ha)
Muskoka Lakes	Harp	45°23' N, 79°08'W	3.5	5.00E+05	8.26E+05	7.60E+01
	Dickie	45°12' N, 78°79'W	1.9	5.00E+06	4.64E+06	9.10E+01
	Plastic	45°11' N, 78°50'W	4.0	1.25E+06	2.58E+05	3.21E+01
	Blue Chalk	45°12' N, 78°56'W	6.0	1.58E+05	4.21E+05	5.24E+01
	Joseph	45°30' N, 79°06'W	180.1	1.27E+08	1.30E+09	8.27E+02
	Rosseau	45°28' N, 79°07'W	3.5	7.75E+08	1.50E+09	8.52E+02
	Muskoka	45°19' N, 79°02'W	1.1	4.61E+09	2.70E+09	1.05E+03
Kawartha Lakes	Moira	40°30' N, 77°27'W	.4	5.46E+08	3.10E+07	8.30E+02
	Sturgeon	44°78' N, 78°50'W	7.0	1.40E+11	1.60E+09	4.50E+03
	Scugog	44°10' N, 78°50'W	37.7	5.08E+08	9.60E+09	8.26E+03
	Stoco	47°15' N, 77°32'W	.0	2.23E+09	1.90E+07	7.50E+02
	Rice	44°12' N, 78°10'W	7.6	9.13E+09	2.80E+08	9.16E+03
	Swaugers	44°59' N, 78°25'W	.0	0.00E+00	5.52E+05	2.80E+01
Great Lakes	Huron	45°N, 83°W	22.0	1.34E+11	3.54E+09	5.96E+06
	Erie	42°N, 81°W	2.6	7.80E+10	4.84E+08	7.80E+06
	Superior	47°N, 87°W	191.0	1.28E+11	1.21E+10	8.21E+06
	Ontario	43°N, 78°W	6.0	6.40E+10	1.64E+09	1.90E+06
Yukon Lakes	Atlin	59°35'N, 133°48'W	18.0	6.81E+09	5.44E+07	5.89E+04
	Bennett	60°09'N, 134°42'W	5.4	1.68E+08	6.00E+06	9.60E+03
	Tagish	60°32'N,134°0'W	3.2	1.74E+10	2.18E+07	3.53E+04
	Marsh	60°25'N,134°58'W	.2	1.90E+10	1.20E+06	9.70E+03
	Lebarge	61°26'N, 135°11'W	4.0	3.08E+10	1.50E+06	4.70E+03
High Arctic Lakes	Char Lake	74°42'N, 94°56'W	9.0	4.35E+06	5.37E+06	5.30E+01
	Meretta Lake	74°41'N, 95°02'W	3.0	4.35E+06	8.45E+05	2.60E+01
	Amituk Lake	75°02'N, 93°45'W	1.4	2.65E+06	7.37E+06	3.80E+01
Wetlands	Meretta Wetland	74°41'N, 95°02'W				
	Harding Park	43°46' N, 79°20'W				
	Grenadier Pond	43°38' N, 79°28'W				
	Lynde Shores	43°29' N, 79°09'W				
	Cootes Paradise	43°14' N, 79°51'W				
Streams	Harp 5	45°25' N, 79°09'W				
	Harp 4	45°23' N, 79°06'W				
	Harp 6	45°22' N, 79°09'W				
	Harp 6A	45°22' N, 79°09'W				
	Don River	45°55' N, 79°20'W				
	Highland Creek	45°55' N, 79°25'W				

Muskoka Lake information from Dillon (MOE, pers. comm.), except Joseph, Rosseau and Muskoka (estimated).
Kawartha Lake information from Kitchen (Trent-Severn Waterways, pers.comm.)
Great Lakes information from Saunders (Water Survey of Cananda, pers. comm.)
Yukon Lake infromation from Kirkland and Gray (1986) and Jack et al. (1983)
High Arctic Lakes information from Helm (1999) and Semkin (1997).

drowned river mouth on the western coast of Lake Ontario, near the City of Hamilton, Ontario that receives sewage from the Dundas sewage treatment plant. Water enters the wetland via Spencer Creek, Borer's Creek and Chedoke Creek (Chow-Fraser, 1999). Grenadier Pond is a shallow, hypereutrophic wetland on the western boundaries of High Park in Toronto, Ontario, and Harding Park is an artificially constructed wetland in the suburban town of Richmond Hill, north of Toronto. Lynde Shores is a wetland on the eastern edge of Lake Ontario in the town of Whitby, Ontario.

Stream samples were collected from four inlet streams into Harp Lake in the Muskokas that varied in watershed characteristics. The Don River, in downtown Toronto, and Highland Creek in suburban Toronto are two urban rivers that were sampled for comparison to the Harp Lake streams.

Samples from the high arctic were taken from Char and Meretta Lakes. Char Lake is a small, oligotrophic arctic lake that supplies drinking water to the nearby village of Resolute (Hobie, 1984, Schindler *et al.*, 1973). Meretta Lake is anomalous since it received sewage effluent from 1949 until recently, causing it to become mesotrophic (Schindler *et al.*, 1974, Morgan and Kalff, 1972). Inlet streams that were sampled included both Char and Meretta Lake inlet streams.

The five subarctic lakes are part of the southern Yukon River basin. Atlin Lake receives most of its water from the Llewellyn Glacier and is the largest lake in the system with a residence time of almost 19 years. Atlin lake flows into Tagish, and Tagish and Bennett flow into Marsh Lake. Lake Laberge is about 70 km downstream of Marsh Lake. Laberge is the most productive of the lakes followed by shallow Marsh Lake (Kirkland and Gray, 1986).

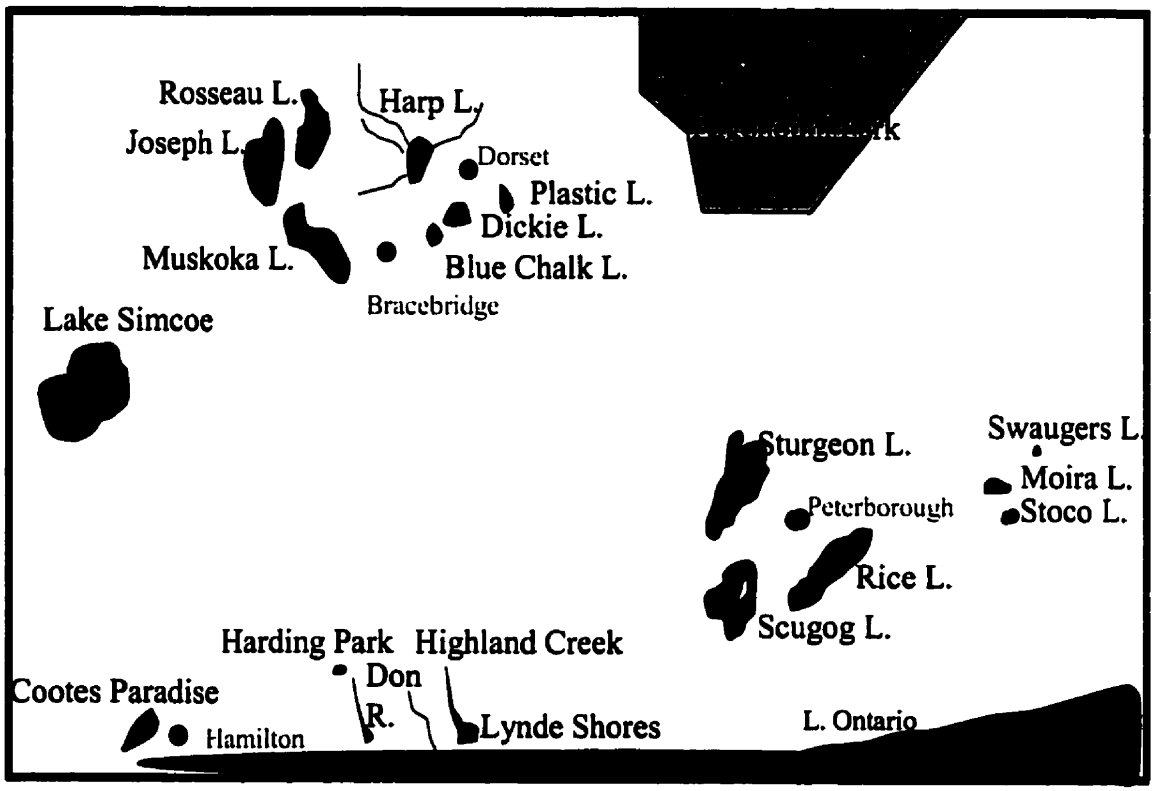


Figure 3.1: Map of temperate area study sites. The lakes in Muskoka are oligotrophic and the Kawartha lakes are mesotrophic to eutrophic. The streams that were sampled included Harp 4,5,6 and 6A, the Don River and Highland Creek. Wetlands included Cootes Paradise, Grenadier Pond, Harding Park and Lynde Shores.

3.2.2 Sample Collection and Extraction

About 60L of surface water were collected from each temperate site in stainless steel, 20L cans for HCH analysis. Stream samples were taken from V-notched weirs, except for the Don River and Highland Creek from which samples were obtained from stream side. Extraction and clean-up procedures are described by Falconer *et al.* (1995). The following modifications of the method were made. Each water sample was spiked with 54 ng of d_6-α-HCH in acetone as a surrogate standard. About 18 L of water from each 60L sample was passed through pre-cleaned glass fibre filters and through 1g Isolute ENV+ solid phase extraction cartridges (Jones Chromatography, Lakewood, Colorado) to extract the dissolved α-HCH. Three cartridges per sample were used and the elutes later combined before the clean-up procedure.

Once the three dichloromethane (DCM) extracts were obtained and combined they were exchanged into isooctane and reduced under N_2 gas (>97.5%) to about 0.75 mL in a Turbovap™. The samples were eluted with 10% DCM-petroleum ether through a column of 0.5 g neutral alumina (6% water) topped with 0.7 g of anhydrous sodium sulfate. Exactly 97 ng of Mirex was added as an internal standard before the extracts were vortexed with concentrated sulfuric acid as the final clean-up step.

3.2.3 Instrumental Analysis

Concentrations of α-HCH and γ-HCH in the 1998 temperate and 1997 high arctic samples were quantified using the GC-ECD method detailed by Falconer *et al.* (1995). The methods of separating the two enantiomers and calculation of ER were based on the GC-MS (NIMS) method also described by Falconer *et al.* (1995). However, the primary separation columns used were the Rt-βDEXcst (proprietary cyclodextrin material doped into 14% cyanopropyl/phenyl/ 86%

dimethyl polysiloxane, Restek, USA) and BGB-172 (20% tert-butyldimethysilated β-cyclodextrin in OV-1701, BGB Analytik AG, Switzerland) with the results confirmed on a Beta-DEX 325 column (2,3-di-O-methyl-6-O-TBDMS-β-cyclodextrin embedded in SPB-20 poly(20% phenyl/ 80% dimethylsiloxane, Supelco, USA). All columns were 30 m in length with 0.25 mm i.d. and 0.25 μm film thickness. The (+)-α-HCH enantiomer elutes first on the Rt-βDEXcst and Beta-DEX 325 column and second on the BGB-172 (Falconer *et al.*, 1995, Jantunen and Bidleman, 1996). Ion 255 was used in calculations and ion 257 was monitored for qualification.

Samples from the Great Lakes were also quantified following procedures in Falconer *et al.* (1995). The α-HCH and γ-HCH concentrations used for calculation of arctic isomer ratios were quantified according to Helm *et al.* (2000). Alaee (1997) describes methods used for quantifying α-HCH, γ-HCH, and ERs for the Yukon Lakes.

3.2.4 Nutrient Analysis

Surface water samples from temperate and high arctic sites were also taken for analysis of nitrate, nitrite, ammonia, total phosphorus, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC). Samples were collected in 500 mL, acid-washed glass bottles and stored at 4°C before analysis. Samples were filtered through 0.5 μm glass fibre filters and analyzed at the Soil and Nutrient Laboratory, University of Guelph following colourimetic methods with an autoanalyzer. The methods were based on Tabatabi and Bremner (1970) for particulate carbon analysis, Environment Canada (1994) for dissolved organic and inorganic carbon, Kenney *et al.* (1982) for nitrate, nitrite and ammonia, and Menzel and Corwin (1965) for total phosphorus.

Nutrient data for the Yukon Lakes are reported in Kirkland and Gray (1986) and Jack *et*

al. (1983). Nutrient data of the Great Lakes were provided by Environment Canada (unpubl.).

3.2.5 Data Analysis

Data were analyzed statistically by means of SPSS (version 9 for Windows)™. The objective of the principal components analysis (PCA) is to find a combination of the original variables, in this case, morphometric and limnological characteristics, that account for as much of the original variation as possible. Multiple linear stepwise regression, a general linear model (GLM), and a Pearson correlation analysis were also used to evaluate the factors related to α-HCH degradation.

3.3 Results and Discussion

3.3.1 Quality Control

The quality control for the 1997 arctic α-HCH and γ-HCH samples are detailed in Helm *et al.* (2000). Alaee (1997) and Jantunen (unpubl.) discuss quality control of the Yukon Lake and Great Lakes samples, respectively.

The following description pertains to the 1998 arctic and 1999 temperate samples. Four Milli-Q water samples (18L) were extracted as blanks and concentrations of α-HCH and γ-HCH were not detectable. System blanks (n=30), taken through the clean-up and analytical procedures, also had non-detectable concentrations with a limit of detection of 10 pg/L. Thus, blank corrections were not applied. Alpha-HCH and γ-HCH concentrations were corrected for individual recoveries of d_6-α-HCH surrogate spikes. Recoveries for the surrogate spike ranged from 50 % to 98% with an average of 88% ± 14% (n=70). Three samples were additionally spiked with 80 ng of α-HCH and had average recoveries of 91% ±7% which were corrected for native

amounts. Suspended sediments, analyzed from glass fiber filters, did not contain α-HCH.

The enantiomers in samples were resolved on three columns with an average difference of 1.08% ± 2.04 (n=39). Standard α-HCH ERs were 1.00 ± 0.01 (n=7) for the Rt-βDEXcst, 0.99 ± 0.01 (n=4) for the BGB-172, and 0.99 ±0.01(n=4) for the Beta-DEX 325. Reported sample ERs had 255 / 257 ion ratios within ±5% of the standard ion ratio.

To determine the precision of sampling and sample handling, triplicate samples were taken on four occasions. The average precision of α-HCH concentrations and ERs for those samples were 1.6 and 1.9 %, respectively.

3.3.2 HCH Concentrations

Table 3.2 summarizes the α-HCH and γ-HCH concentrations, α-HCH / γ-HCH ratios, ER and water chemistry for all the study sites. Concentrations of α-HCH were highest in high arctic and subarctic lakes, ranging from 0.64 in Char Lake to 1.7 ng/L in Atlin Lake, which are lower than those reported in the late 1980's to early 1990's for the Arctic marine waters (e.g., Hinckley *et al.*, 1990, Hargrave *et al.*, 1988). Lakes Ontario and Erie had low α-HCH concentrations of 0.34 and 0.55 ng/L, respectively, while larger Lakes Superior and Huron had higher concentrations of 2.12 and 1.7 ng/L, respectively. The lowest α-HCH concentrations were found in the small temperate lakes, ranging from 0.04 to 0.43 ng/L. Temperate wetlands also had low α-HCH concentrations of between 0.093 and 0.205 ng/L.

In contrast to α-HCH, small temperate lakes had the highest concentrations of γ-HCH, ranging from 0.14 to 0.94 ng/L, whereas the northern lakes had lower levels of 0.13 to 0.41ng/L. Temperate wetlands had concentrations between 0.16 and 0.45 ng/L of γ-HCH.

Table 5.2: Table of a-HCH / g-HCH ratios, ER and nutrients for arctic, Yukon, Great Lakes and temperate lakes, streams and wetlands.

System	Name	Location (lat. / lon.)	a-HCH Conc. ng/L	g-HCH Conc. ng/L	HCH / g-HCH Ratio	ER	NO2 mg/L	NO3 mg/L	NH4 mg/L	Total Dissolved Carbon mg/L	DIC mg/L	DOC mg/L	Total P mg/L	POC mg/kg sed.
Muskoka	Harp	45°23' N, 79°08'W	.43	n.d.	n.a	.98	.01	.24	.01	5.10	1.60	3.50	0.0	.85
Lakes	Dickie	45°12' N, 78°79'W	.12	n.d.	n.a	.95	.01	.17	.03	5.96	1.26	4.70	0.0	1.08
	Plastic	45°11' N, 78°50'W	.40	n.d.	n.a	.99	.01	.03	.03	2.66	0.66	2.00	0.0	1.10
	Blue Chalk	45°12' N, 78°56'W	.19	n.d.	n.a	1.06	.01	.05	.02	3.24	1.54	1.70	0.0	.95
	Joseph	45°30' N, 79°06'W	.25	.60	.42	.97	.11	.22	.45	5.94	1.70	4.24	0.1	1.12
	Rosseau	45°28' N, 79°07'W	.30	.91	.33	.99	.01	.20	.19	15.31	5.20	10.11	0.1	1.69
	Muskoka	45°19' N, 79°02'W	.19	.42	.45	.89	.11	.14	.20	8.07	2.60	5.47	0.1	1.54
Kawartha	Moira	40°30' N, 77°27'W	.18	n.d.	n.a	.99	.06	.11	.39	19.12	10.30	8.83	0.1	1.07
Lakes	Sturgeon	44°78' N, 78°50'W	.22	.25	.88	.96	.00	.02	.05	19.61	8.02	11.60	0.1	2.52
	Scugog	44°10' N, 78°50'W	.17	.42	.40	.91	.05	.02	.19	20.14	9.05	11.09	0.1	2.78
	Stoco	47°15' N, 77°32'W	.04	.42	.10	.94	.06	.02	.05	13.68	5.97	7.72	0.1	1.62
	Rice	44°12' N, 78°10'W	.09	.28	.32	.91	.06	.01	.02	14.19	7.32	6.87	0.1	1.28
	Swaugers	44°59' N, 78°25'W	.13	.14	.90	.98	.88	.06	.13	6.91	2.07	4.84	0.1	1.34
Great Lakes	Huron	45°N, 83°W	1.70	n.a.	n.a	.85	.01	.36	.01	2.44	0.00	2.44	0.0	.00
	Erie	42°N, 81°W	.55	n.a.	n.a	.88	.01	.66	.06	3.31	0.00	3.31	0.0	.38
	Superior	47°N, 87°W	2.12	n.a.	n.a	.85	.01	.66	.01	2.18	0.00	2.18	0.0	.17
	Ontario	43°N, 78°W	.34	n.a.	n.a	.89	.01	.43	.01	2.77	0.00	2.77	0.0	.25
Yukon	Atlin	59°35'N, 133°48'W	1.70	.41	4.10	.31	.01	.00	.00	13.53	11.00	2.53	0.0	.02
Lakes	Bennett	60°09'N, 134°42'W	.92	.13	7.30	.88	.01	.04	.01	8.97	6.00	2.97	0.0	.02
	Tagish	60°32'N,134°0'W	1.10	.21	5.20	.58	.01	.00	.00	10.27	7.40	2.87	0.0	.02
	Marsh	60°25'N,134°58'W	1.40	.38	3.70	.41	.01	.00	.00	12.43	9.40	3.03	0.0	.02
	Leberge	61°26'N, 135°11'W	1.60	.34	4.75	.46	.01	.00	.00	12.30	9.47	2.83	0.1	.02
Arctic Lakes	Char Lake	74°42'N, 94°56'W	.64	.13	4.80	.56	.01	.08	.01	20.30	19.17	1.15	0.0	.40
	Meretta Lake	74°41'N, 95°02'W	.87	.21	4.12	.70	.01	.70	3.33	33.43	26.30	7.13	0.1	1.30
	Char Lake*	74°42'N, 94°56'W	1.02	0.186	5.49									
	Meretta Lake*	74°41'N, 95°02'W	0.76	0.226	3.35									
	Resolute Lake*	74°29'N, 95°05'W	0.82	0.152	5.39									
	Amituk Lake	75°02'N, 93°45'W	0.64	1.313	0.49	0.76	.01	.02	.01		16.31	0.63	0.0	.03
Wetlands	Meretta Wetland	74°41'N, 95°02'W	0.08	.02	4	0.55	.02	.27	.06	35.5	25.00	10.50	0.0	.01
	Meretta Wetland*	43°46' N, 79°20'W	0.43	0.102	4.25									
	Harding Park	43°38' N, 79°28'W	0.15	.45	0.34	0.79	.09	.70	.20	14.66	8.45	6.22	80.0	2.46
	Grenadier Pond	43°29' N, 79°09'W	0.11	.23	0.47	0.875	.12	.03	.99	44.47	36.80	7.67	0.1	22.23
	Lynde Shores	43°14' N, 79°51'W	0.09	.16	0.6	0.79	.16	2.91	5.35	52.83	45.00	7.83	0.1	26.00
	Cootes Paradise	45°25' N, 79°09'W	0.21	.44	0.47	0.772	.30	2.84	1.92	18.4	13.92	4.48	0.1	6.19
Streams	Harp 5	45°23' N, 79°06'W	0.30	n.d.	n.a.	0.641	.01	.01	.02	21.26	4.56	16.70	0.0	.85
	Harp 4	45°22' N, 79°09'W	0.71	n.d.	n.a.	0.652	.01	.96	.02	6.24	1.54	4.70	0.0	.85
	Harp 6	45°22' N, 79°09'W	0.86	n.d.	n.a.	0.65	.01	.20	.02	11	3.20	7.80	0.0	.85
	Harp 6A	45°55' N, 79°20'W	0.19	n.d.	n.a.	0.67	.01	.01	.01	17.76	3.46	14.30	0.0	.85
	Don River	45°55' N, 79°25'W	0.4	n.d.	n.a.	0.73	.03	1.36	.12	33.05	27.63	5.42	0.1	9.73
	Highland Creek	75°02'N, 93°45'W	0.24	n.d.	n.a.	0.706	.02	7.58	7.69	33.25	27.70	5.55	0.1	16.60
	Mud Creek*	75°02'N, 93°45'W	0.62	0.161	3.85									
	Gorge Creek*		0.73	0.215	3.41									

N.B. Great Lakes nutrient data obtained from Environment Canada (unpubl.)
n.d. - concentrations below limit of detection
* Additional Sites used for a-HCH / g-HCH ratios from 1997 arctic survey
Yukon Lakes nutrient data obtained from Kirkland and Gray (1986) and Jack et al. (1983)
Amituk Lake nutrient data obtained from Semkin (1997)
Amituk Lake a-HCH, g-HCH and ratios obtained from Helm (1999)

The α-HCH concentrations in the arctic/subarctic lakes were significantly greater than those of the small temperate lakes ($t=2.3$, $p<0.05$). Although statistical analyses could not determine factors influencing γ-HCH concentrations, results of a stepwise regression indicated that DOC explained 42% of the variability in α-HCH concentrations ($\beta=-0.531$, $p<0.05$). This was further confirmed with a GLM in which 51% of the variance in α-HCH concentrations was explained by total carbon (DOC, DIC, POC) concentrations.

PCA separated small temperate lakes with their low α-HCH concentrations, from the Great Lakes, Yukon and high arctic lakes that had higher concentrations along Components 1 and 2. Principal Component 1, that accounted for 32% of the variance in α-HCH concentrations, had high loadings for lake volume, which is related to the potential for direct atmospheric, and lake volume that can be explained through the influence water temperature and hence potential for transformation via hydrolysis. Principal Component 2 (26% variance) had high loadings for factors related to trophic status, as found with the regression analyses (e.g., total phosphorus, DOC, DIC and POC).

The results indicate that high α-HCH concentrations are highest in colder and larger lakes, such as the high arctic, subarctic and Great Lakes while smaller temperate lakes have considerably less α-HCH. The high levels of γ-HCH in the small temperate lakes suggest local, rather than long range atmospheric source of γ-HCH. However, some caution must be used, however, in interpreting these results, particularly with respect to the α-HCH values in systems with short residence times. These systems respond rapidly to decreases in atmospheric concentrations, making the year of sampling an important variable. For example, the concentration of α-HCH in the water of Amituk Lake declined from ~1.6 to 0.56 ng/L from 1992 to 1994, whereas β and γ-HCH concentrations remained stable (Semkin, unpubl. data). Secondly, the small temperate lakes

were sampled during summer when α-HCH loss by hydrolysis was likely significant but the concentration may not accurately represent a year-long average.

3.3.3 Alpha-Gamma Ratios

Alpha-HCH / γ-HCH ratios in the high arctic lakes ranged from 5.2 to 9.4, from 3.5 to 8 in the Yukon lakes, and 0.25 to 0.37 in small temperate lakes. The observed mean ratios of the temperate and arctic/subarctic ratios are significantly different ($t = 3.12$, $p<0.05$), however the arctic and subarctic ratios are not significantly different.

A Pearson correlation matrix of all sites indicates that the ratio among all lakes is significantly, negatively correlated to lake DOC ($R^2 = 0.548$, $p<0.05$, Figure 3.2). The characteristically nutrient-poor high arctic and subarctic lakes, which have low DOC, have correspondingly higher ratios in contrast to aquatic systems in temperate areas that generally have higher DOC concentrations. A stepwise regression analysis also confirmed that nutrient status in this case particulate organic carbon, ($\beta = -0.912$, $p<0.05$), is inversely related to the α-HCH / γ-HCH ratios.

These results agree well with those reported in the literature. The ratios reported here for the high arctic lakes of 5.2 to 9.4 are greater than those reported for the Arctic Ocean of 4.5 (Jantunen and Bidleman, 1998), Bering and Chukchi Seas of 2.6 to 6.4 (Hinckley *et al.*, 1992) and Eastern Arctic Ocean of 1.5 to 5 (Harner *et al.*, 1999). This difference likely illustrates the more rapid response of lakes than oceans to changes in loadings (Freitas *et al.*, 1997, Helm, 1999) as α-HCH concentrations in air from long range atmospheric transport decline more rapidly than those of γ-HCH (Kucklick *et al.*, 1991, Iwata *et al.*, 1993). The ratios of the small temperate lakes

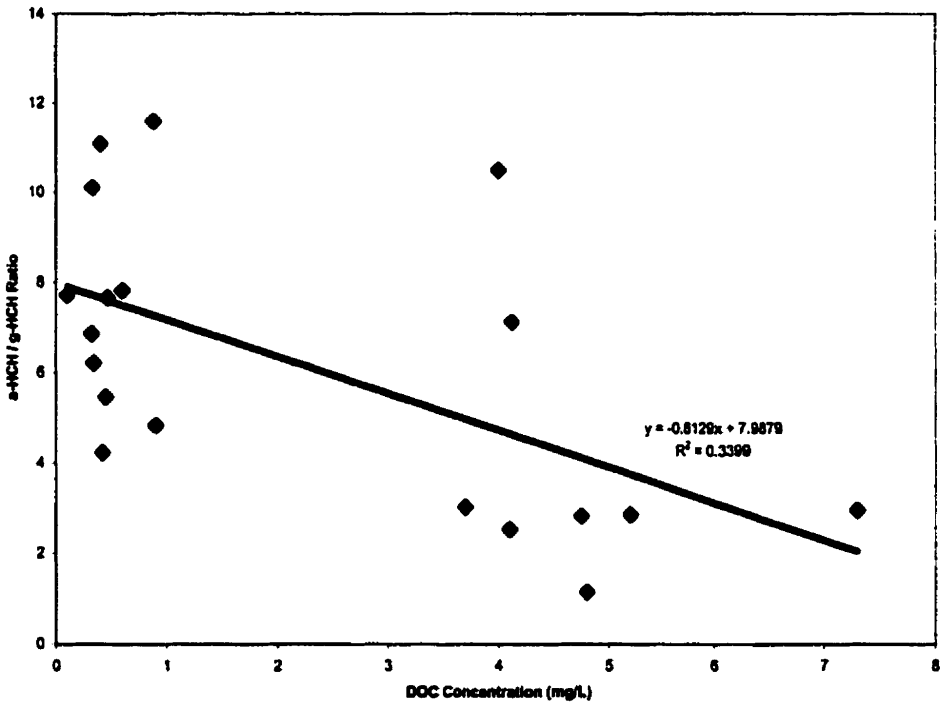


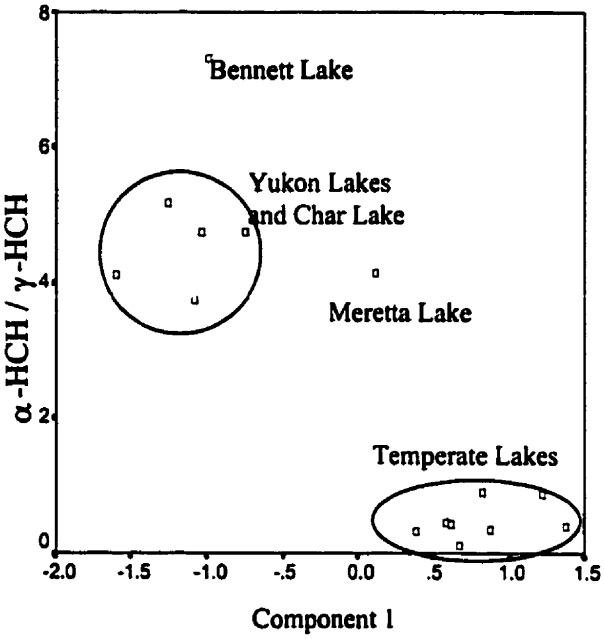
Figure 3.2: Bivariate plot of a-HCH / g-HCH ratio and DOC concentration in lakes.

are lower than those reported for the Great Lakes of 2.4 to 4.5 (McConnell *et al.*, 1993, Walker *et al.*, 1999) (α-HCH / γ-HCH could not be calculated for the Great Lakes because of the extremely low concentrations of γ-HCH). Again, this is probably due to the quicker response time of smaller lakes relative to large lakes. As well, the low ratios in these lakes ringed by cottages may indicate direct inputs of γ-HCH that is used for wood preservation (e.g., docks) and domestic insect control (PRMA, 2000).

Finally PCA shows that ratios are dependent on the α-HCH concentration and trophic status of the lake (Figure 3.3). Two components were extracted from the analysis, with Component 1 (explaining 35% of variance) having loadings comprised of α-HCH concentration, ER, carbon (POC and DOC), total phosphorus and surface area and loadings for Component 2 (25% of the variance) of ammonia, nitrate and DIC. Graphs of the α-HCH / γ-HCH ratios versus Component 1 and 2 separated the temperate sites from arctic and subarctic sites. In both plots, Meretta Lake was an outlier because it is a mesotrophic lake in the high arctic. Bennett Lake was also an outlier, the reason for which is unclear.

3.3.4 Enantiomeric Degradation of α-HCH in Streams and Wetlands

Alpha-HCH in wetlands and streams under went appreciable enantioselective degradation (Table 3.2), which supports the findings by Helm *et al.* (2000) who attributed the phenomenon to enhanced time between the chemical and substrates. Although significant controlling factors in stream data could not be found with a GLM, a stepwise regression or Pearson correlation matrix, PCA was able to separate groups of temperate polluted streams from arctic and temperate oligotrophic streams according to Component 1 (64%) that had high loadings for POC, DIC and nitrate concentrations (Figure 3.4). Generally, ERs were lower in cold, nutrient poor streams and



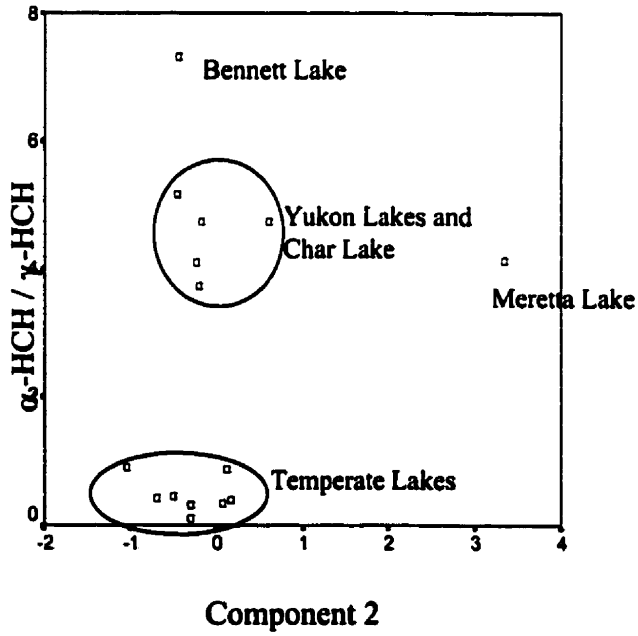


Figure 3.3 (a,b): Bivariate plot of α-HCH / γ-HCH ratio against Principal Components 1 and 2. Loadings for both components are related to nutrient status. Both plots show a separation of temperate and subarctic/arctic lakes. In both cases, Meretta and Bennett Lake are outliers.

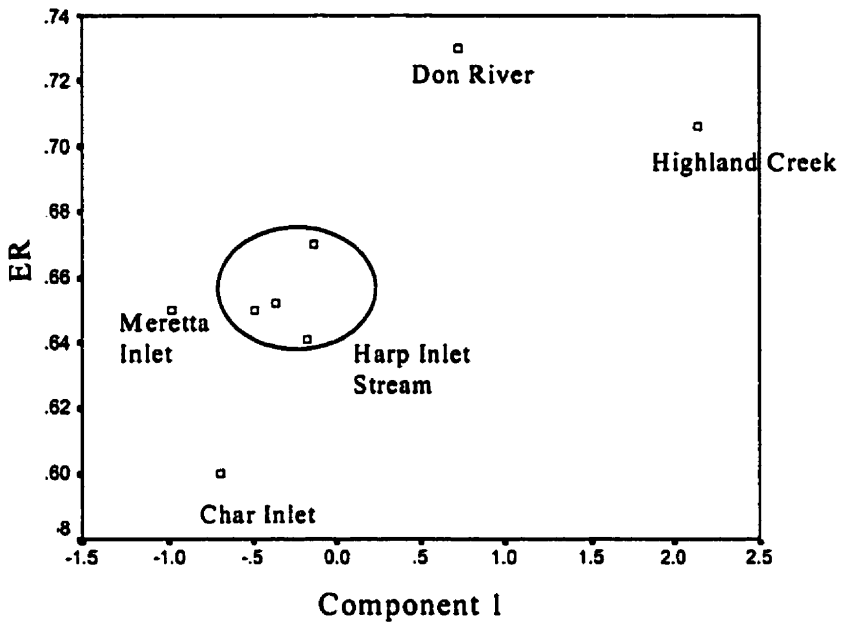


Figure 3.4 : Bivariate plot of ER versus Principal Component 1 which separates streams according to nutrient concentrations.

higher in warm, nutrient rich streams.

Both arctic and temperate wetlands had lower ERs than lakes. Biologically rich substrates such as those found in wetlands can support greater degradation than low productivity substrates (Helm *et al.*, 2000). No factors were found to be statistically related to wetland ERs. Similarly to streams, a graph of wetland ER versus Principal Component 1 separated the wetlands with greater nutrient concentrations, such as those in southern Ontario, that are subject to less enantioselective degradation, from the high arctic wetland (Figure 3.5). This dependency of degradation on nutrient availability is believed to be related to native microbial populations where

those adapted to eutrophic environments can preferentially use other sources of carbon than α-HCH in contrast to those in oligotrophic systems. Among the temperate wetlands, those with deeper water such as Grenadier Pond (mean depth = 2.9 m) and Lynde Shores (mean depth = 3 m) (Gartner Lee, 1979, 1995) had higher ERs than shallow systems such as Harding Park (mean depth = ~1 m) and Cootes Paradise (mean depth = 0.7 m) (Chow-Fraser, 1999) in which contact between chemical and sediment is maximized.

The greater enantioselective degradation of α-HCH in oligotrophic systems is likely due to the presence of oligotrophic bacteria that have the ability to survive under low nutrient conditions to induce multiple enzymes, shift metabolic pathways and can often take up and use mixed carbon sources (Atlas and Bartha, 1972). Thus, the shortage of other carbon sources in oligotrophic systems causes microbes to degrade xenobiotics such as α-HCH (Alexander, 1999).

Ultimately, microbial degradation is controlled by the rate and amount of enzyme activity and the supply of α-HCH to the microbes. The data indicate that enantioselective degradation is greatest in streams at periods of low flow when chemical is delivered to the microbial population and the supply of chemical is not a limiting factor, as may occur in stagnant systems. As well, degradation is greatest where contact time is maximized between chemical and substrate. Thus, degradation is maximized in wetlands that provide a suitable substrate for microbial communities (Woo and Young, 1998) and maximum contact between water and substrates. These results are consistent with Scrow and Alexander (1992) who found that the longer the water path, the greater the influence on biodegradation. This observation is also consistent with streams and wetlands acting as biotrickling filters that are designed to maximize contact time and degradation rates (Headley, 1995). Indeed, degrading microbes have the propensity to live attached to substrates and are usually found living as biofilms, rather than having a free-living existence (Alexander,

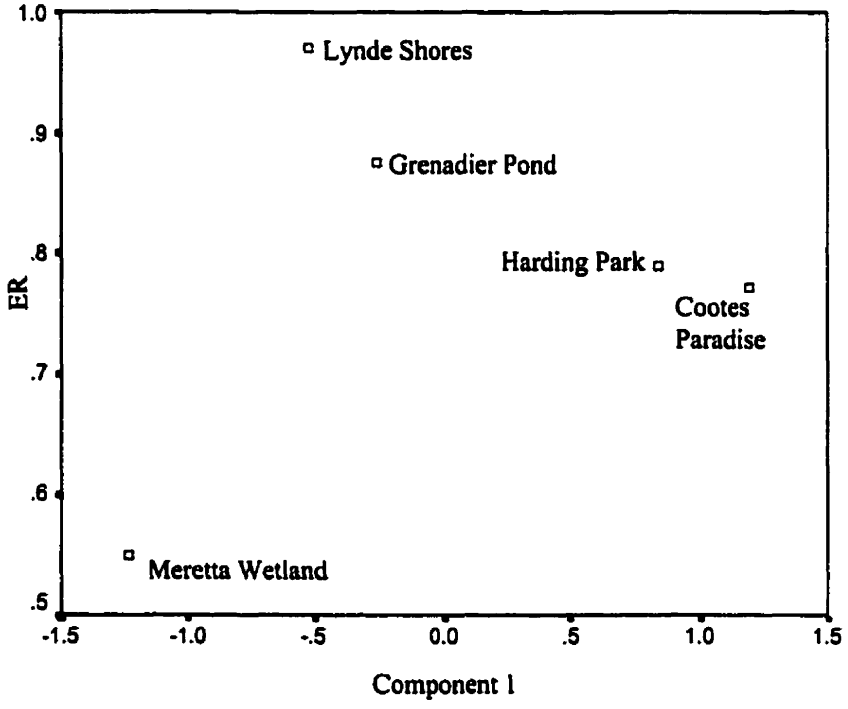


Figure 3.5: Bivariate plot of wetland ERs versus Component 1, which shows arctic Meretta wetland with the greatest enantioselective degradation. Temperate wetlands such as Lynde Shores and Grenadier Pond have deeper water and less enantioselective degradation than Harding Park and Cootes Paradise which are shallower.

1985).

Thus, the accumulation of microbes on surfaces as biofilm will increase the potential for biodegradation of organic pollutants as the attached microbes can remain near the source of fresh carbon and nutrients in the aqueous phase that flow past them.

3.3.5 Enantiomeric Degradation of α-HCH in Lakes

Table 3.2 summarizes the findings of ERs in temperate, subarctic and high arctic lakes. Since microbial degradation was expected to be greater with warmer temperatures and greater nutrient availability (Klein, 1989), lower ERs were expected in temperate lakes. On the contrary, small temperate lakes had minimal enantioselective degradation (ERs of 0.91 to 1.06) while low ERs between 0.31 to 0.7 were found in the subarctic and arctic lakes. ERs in the Great Lakes between 0.85 to 0.89 were intermediate between these extremes.

ERs were significantly and negatively related to α-HCH concentration (R^2=0.71, $p<0.05$) and DIC (R^2=0.56, $p<0.05$) and positively related to POC concentration (R^2=0.55, $p<0.05$) and total phosphorus levels (R^2=0.36, $p>0.05$). The latter variables are related to the trophic status of the lakes.

The importance of α-HCH concentration for enantioselective degradation was confirmed with a GLM that showed α-HCH concentration was significantly correlated with ER (F=6.8, R^2=0.73, $p<0.05$) and a stepwise regression that identified α-HCH as the factor that explains 86% of the variability in ER (slope= -0.44, $p<0.05$).

PCA was used to further explore factors influencing ER. Table 3.3 summarizes the component loadings and Figure 3.6 (a,b) illustrates graphs of ER versus Components 1 and 2. The loadings for Component 1 are α-HCH concentration, surface area, and variables related to

Table 3.3: Component Loadings in Principal Components Analysis for Lakes

	Component 1	Component 2
α-HCH Concentration	-0.564	0.572
Nitrite	0.330	-0.390
Nitrate	-0.246	0.156
Ammonia	0.444	0.622
Total Carbon	0.740	0.634
Dissolved Inorganic Carbon	0.470	0.830
Dissolved Organic Carbon	0.883	-0.154
Total Phosphorus	0.730	-0.304
Particulate Carbon	0.831	-0.353
Residence Time of Water	-0.29	0.502
Watershed Area	-0.585	0.498
Volume of Water	-0.381	0.414
Surface Area	-0.774	0.417

Extraction Method: Principal Component Analysis.

trophic status (total carbon, DOC, total phosphorus and POC). Loadings on Component 2 are α-HCH concentration, water residence time and watershed area. The small temperate lakes separated from the Great Lakes and the subarctic and arctic lakes (Figure 3.6 (b)). Bennett Lake is anomalous due to its high ER, the reason for which is not known. Meretta Lake is also anomalous because it is an artificially mesotrophic system in the high arctic.

This analysis indicates that several factors are related to the enantioselective degradation of α-HCH. First, it is well known that microbial degradation is a second order process in which the rate is a function of chemical concentration. Concentrations of α-HCH are highest in lakes with a large surface area available for atmospheric deposition and cold, deep lakes that act as cold traps and in which loss by hydrolysis and volatilization is minimized. Secondly, lake trophic status is inversely related to enantioselective degradation, as was found with the streams and wetlands. As mentioned above, this inverse relationship may be due to the ability of oligotrophic microbes to metabolize or cometabolize α-HCH whereas eutrophic microbes may have a low

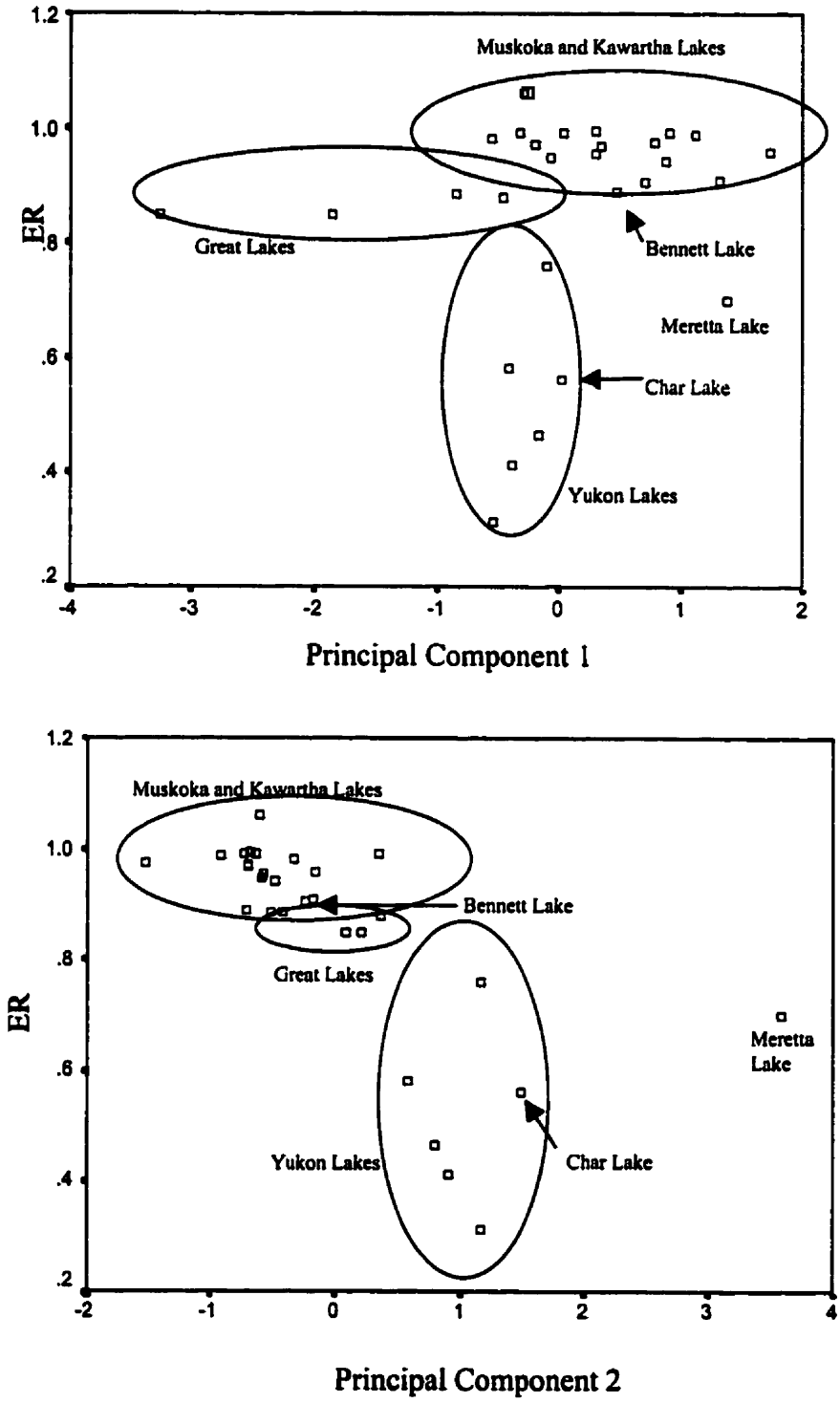


Figure 3.6 (a, b): Bivariate plots of ER versus Components 1 and 2. There is a separation of the small temperate, Great Lakes and arctic/subarctic lakes.

affinity for α-HCH. Third, enantioselective degradation is greatest in lakes with long water residence times in which the α-HCH concentration may be elevated due to past loadings and the long residence time maximizes contact between chemical and sediments, as suggested by Alaee (1997). Finally, enantioselective degradation is positively related to watershed area, which can be explained by the observations here, that degradation is greatest in streams in which contact time between chemical and sediments is maximized and supports a similar finding by Helm *et al.* (2000) for a high arctic watershed and lake.

3.4 Conclusions

Alpha- and γ-HCH concentrations, α-HCH / γ-HCH ratios and ERs were measured in streams, wetlands and lakes from the high arctic, subarctic, Great Lakes and small temperate systems. Alpha-HCH concentrations increased with latitude and lake size, whereas γ-HCH concentration decreased with latitude. High α-HCH / γ-HCH ratios suggested an aged, long range source of HCH in high latitude systems whereas temperate systems had low ratios indicative of recent γ-HCH usage and residual α-HCH concentrations. Enantioselective microbial degradation of α-HCH was greatest in small, high arctic lakes and streams, large lakes in the Yukon and Great Lakes, and wetlands, and was least in nutrient-rich temperate lakes and urban streams and wetlands. These results were unexpected, condsidering the thermodynamics of degradation and higher microbial populations in eutrophic systems. Statistical analysis showed that enantioselective degradation is positively related to α-HCH concentration, which is greatest in cold, oligotrophic systems with long residence times, due to the ability of oligotrophic bacteria, existing as biofilms, to metabolize or cometabolize HCH and in which it is suggested, contact between microbial populations and chemicals is maximized. As well, degradation is related to

watershed area since most degradation likely occurs in streams.

CHAPTER 4: INVESTIGATION OF FACTORS AFFECTING THE ENANTIOSELECTIVE DEGRADATION OF ALPHA-HEXACHLOROCYCLOHEXANE BY MEANS OF MICROCOSM EXPERIMENTS

Preamble

The following chapter outlines the laboratory experiments that were performed to determine controlling factors that may enhance the enantioselective degradation of α-HCH. This chapter is written in the form of a scientific article, although it will not be submitted for publication.

Abstract

An investigation into the factors that control the magnitude of enantioselective degradation of α-HCH by use of controlled, laboratory experiments was performed with the use of microcosms. The calculation of the enantiomer ratio (ER) was used to assess enantioselective degradation. Substrates from the littoral and profundal sediments of temperate Harp, Dickie and Moira Lakes, high arctic Char and Meretta Lakes, and wetlands from both regions were used in a modified factorial designed experiment with different treatments. Treatments included the addition of nutrients such as nitrate, phosphate and yeast extract as a carbon source, exposure to light and darkness, and at warm (ambient room temperature) and cool (4°C) temperatures. All microcosm experiments with both temperate and arctic substrates under selective treatments and additions of oxygen did not elicit enantioselective degradation. The results indicate that the microcosms could not mimic the natural conditions found *in situ* for microbes to enantioselectively degrade α-HCH. This study concludes that laboratory microcosm studies were not able to isolate the factors that enhance enantioselective degradation of α-HCH.

4.1 Introduction

The presence of organic pollutants in the environment is a cause for concern due to their toxicity and persistence. Fortunately, numerous studies have found that biological mediation can reduce concentrations of many organic pollutants by biotransforming them into innocuous forms. Microbes accomplish this by degradation - mineralization to carbon dioxide and water under aerobic conditions, or by anaerobically decomposing them to carbon dioxide and methane (Kobayashi and Rittmann, 1982). Researchers have found that some organic compounds can be mineralized at levels below 1 ng/L, however many persistent compounds are resistant to microbial attack under any condition or they are metabolized extremely slowly (Alexander, 1999).

Microbial metabolism of an organic contaminant is influenced by a variety of parameters such as pH, redox potential, nutrients, contaminant concentration. Nutrient limitation is a critical factor that controls microbial activity, often on a species specific basis. Nutrients can be limiting if they are not bioavailable, for example, phosphate is unavailable at high pH and high bivalent cation concentrations. Organic matter content also frequently limits microbial growth.

Nutrient-poor substrates from areas such as the arctic contain oligotrophic bacteria (Alexander, 1999) that are defined as microbes that can live under conditions of low carbon flux of less than 1 mg/L/d. Taxonomically, they come from almost any group of bacteria and are generally acclimated to life under low nutrient conditions and grow at a slow rate. They can readily be adapted to live under high nutrient conditions but there is no evidence of reverse adaptation from high to low nutrient conditions (Alexander, 1999). They are often used in the removal of trace concentrations of organic contaminants from water and effluent from

wastewater treatment processes because of their efficiency in degrading xenobiotics (Alexander, 1985). Oligotrophic bacteria prefer to be attached, rather than free-living and thus, are usually found living as biofilms. These organisms often appear to have multiple inducible enzymes, which can shift metabolic pathways, and can often take up and use mixed carbon sources (Headley, 1995).

Normally, microbes cannot grow and live under low-nutrient conditions because of the minimum maintenance conditions that may not be met by the availability of nutrients. Oligotrophs have high affinities for the organic molecules they use as a carbon source for growth (Alexander, 1985). When nutrient concentrations are high, diffusion will provide molecules to the cell surface at a rate that is sufficiently rapid to meet the energy needs for maintenance and growth. At lower concentrations, diffusion to the cell satisfies requirements for maintenance, but not for growth. This implies that there is a threshold or a lower concentration limit that will support growth. Thresholds vary with different organisms. For oligotrophs, the values will be lower than for eutrophic microbes. Low concentrations of xenobiotics can be a result of the inability of microbes to destroy these compounds readily at these threshold levels (Alexander, 1985).

Studies of biodegradation require a method of differentiating between degradation by microbial action, versus those of abiotic processes. In the case of α-HCH, enantioselective microbial degradation can be identified with the calculation of enantiomer ratios (ER). Alpha-HCH exists as two, non-superimposable chiral enantiomers from which microbes can preferentially degrade the (+)-α-HCH. A physical separation of the two enantiomers is possible with the use of specialized chiral columns and the ratio of the abundance of the (+)-α-HCH to the (-)-α-HCH can be expressed as an enantiomer ratio (ER). Alpha-HCH from technical

mixtures is racemic with an ER of 1 and a deviation from 1 is indicative that the α-HCH has had contact with microbial enzymes.

Microbes need a variety of nutrients for their survival. Carbon sources are required as a source of energy and food to microbes. Nitrogen is a requirement for the formation of proteins with ammonium, nitrate and nitrite forms as the most available forms of N for microbial usage. Phosphorus, in the form of phosphate, is needed for ATP generation and the synthesis of nucleic acids and the phospholipid membrane. Although essential, a high concentration of phosphate can limit the growth of microorganisms.

The aim of this research was to investigate the environmental factors that can influence enantioselective degradation of α-HCH by means of controlled laboratory experiments. More specifically, the experiments sought to determine the effect of nitrogen, phosphorus, carbon and darkness on the enantioselective degradation of α-HCH. The measurement of ER was used to assess the extent of enantioselective degradation of α-HCH.

4.2 Method

4.2.1 Microcosm Experiments

The laboratory degradation experiments were performed in microcosms consisting of 4 L glass jars with substrates and water. The substrates were lake sediments from a deep hole and littoral zones of Harp, Dickie, and Moira Lakes, littoral zone sediment from Meretta and Char Lakes and sediment from the inlet streams and substrate from both temperate and arctic wetlands. Table 4.1 summarizes the locations and characteristics of lakes, streams and wetlands from which sediments were taken and sediment types. For each substrate, a series of microcosms was set up according to a modified factorial design that is summarized in Table 4.2.

Table 4.1 Locations and characteristics of lakes and streams from which sediments were taken and sediment characteristics.

System	Region	Location	System Characteristics	Substrate Type	Substrate Characteristics	% Organic Carbon
Harp Lake	Temperate	45°23' N, 79°08'W	Oligotrophic, headwater lake	Deep Hole	Rocky, coarse grained sediment	<3%
				Littoral	sand	<3%
Dickie Lake	Temperate	45°12' N, 78°79'W	Oligotrophic, headwater lake	Deep Hole	Rocks, very little sediments	<3%
Moira Lake	Temperate	40°30' N, 77°27'W	Eutrophic	Deep Hole	Fluffy sediment, fine grained loam	~7%
Harp Wetland	Temperate	74°41'N, 95°02'W	Shallow (<0.5 m), developed by beaver dams	mid-wetland	Rocky, coarse grained sand	<3%
Char Lake	High Arctic	74°42'N, 94°56'W	Oligotrophic	Littoral	Small rocks, medium-grained san	<3%
Meretta Lake	High Arctic	74°41'N, 95°02'W	Eutrophic	Littoral	Medium to fine grained loam	~5%
Meretta Wetland	High Arctic	74°41'N, 95°02'W	Biologically productive, supports mosses and plants	mid-wetland	Medium to fine grained loam	~5%

N.B. The %organic carbon was determined by the difference in weight after combustion of dry sediment in a muffle furnace.

Table 4.2: Modified factorial design of microcosm experiments

Part 1 (b) using Meretta wetland sediment

	20 C	4 C	Light	Darkness	Nitrate	Phosphate	Yeast Extract
20 C	▲						
4 C		▲					
Light			▲				
Darkness	▲	▲		▲			
Nitrate	▲	▲	▲	▲	▲		
Phosphate	▲	▲	▲	▲	▲	▲	
Yeast Extract	▲	▲	▲	▲	▲	▲	▲

Part 2 experiments using Moira Lake sediments

	20 C	4 C	Light	Darkness	Nitrate	Phosphate	Yeast Extract
20 C	▲						
4 C		▲					
Light	▲		▲				
Darkness	▲	▲		▲			
Nitrate	▲	▲	▲	▲	▲		
Phosphate	▲	▲	▲	▲	▲	▲	
Yeast Extract	▲	▲	▲	▲	▲	▲	▲

Part 3 experiments uing Harp Inlet 4, Dickie Lake, Moira Lake, Meretta Lake and Char Lake substrate

	20 C	4 C	Light	Darkness	Nitrate	Phosphate	Yeast Extract
20 C	▲						
4 C		▲					
Light	▲		▲				
Darkness	▲	▲		▲			
Nitrate	▲	▲	▲	▲	▲		
Phosphate	▲	▲	▲	▲	▲	▲	
Yeast Extract	▲	▲	▲	▲	▲	▲	▲

Triplicates were run on each treatment. Exactly 40 ng of α-HCH in 1 mL of acetone was added to the Milli-Q water in each microcosm. The jars were monitored for pH and oxygen concentration and adjusted with hydrogen peroxide when necessary. Hydrogen peroxides was added to increase dissolved oxygen in the water rather than air bubbling that could potentially strip α-HCH from solution. Sodium hydroxide was used to adjust if pH levels.

For each treatment of experiments, blanks were prepared consisting of a jar with water only and water plus sediment. System blanks were performed during clean-up procedures to ensure samples were contaminant-free. All blanks were tested to ensure α-HCH contamination did not occur. Three samples were also spiked with 80 ng of α-HCH to measure recoveries. A surrogate spike of d_6-α-HCH (exactly 18 ng) was added to each jar before the water was removed and α-HCH extracted.

At the end of the experiments, the overlying water was removed using a vacuum pump. The water was then passed through a pre-cleaned glass fibre filter, and into an ISOLUTE ENV+ solid phase extraction cartridge (SPEC). The SPEC is a specialized sorbent that can sorb dissolved polar organic compounds like α-HCH from the water. The volume of water was measured and recorded.

4.2.2 Clean-up Procedures

Samples were eluted from the SPEC and exchanged into isooctane and reduced under N_2 gas (>97.5%) to about 0.75 mL in a Turbovap™. The samples were eluted with 10% DCM-petroleum ether through a column of 0.5 g neutral alumina (6% water) topped with 0.7 g of anhydrous sodium sulfate. Exactly 97 ng of Mirex was added as an internal standard before the extracts were vortexed with concentrated sulfuric acid as the final clean-up step.

4.2.3 Instrumental Analyses

The concentrations of α-HCH were quantified using the GC-ECD method detailed Falconer et al. (1995). The method of separating of the two enantiomers and calculating the ER were based on the GC-MS (NIMS) method by Falconer et al. (1995). The primary separation columns used were the Rt-βDEXcst (proprietary cyclodextrin material doped into 14% cyanopropyl/phenyl/ 86% dimethyl polysiloxane, Restek, USA) and BGB-172 (20% tert-butyldimethysilated β-cyclodextrin in OV-1701, BGB Analytik AG, Switzerland) with the results confirmed on a Beta-DEX 325 column (2,3-di-O-methyl-6-o-TBDMS-β-cyclodextrin embedded in SPB-20 poly(20% phenyl/ 80% dimethylsiloxane, Supelco, USA). All columns were 30 m in length with 0.25 mm i.d. and 0.25 μm film thickness. The (+)-α-HCH enantiomer elutes first on the Rt-βDEXcst and Beta-DEX 325 column and second on the BGB-172 (Falconer *et al.*, 1995, Jantunen and Bidleman, 1996). Ion 255 was used in calculations and ion 257 was monitored for qualification.

4.2.4 Part I (a): Degradation Experiments in the High Arctic

The aim of this set of experiments was to determine which substrate achieved the highest amount of enantioselective degradation. Substrates from high arctic lakes were obtained by using an Eckman grab sampler and shovels for substrates from streams and wetlands. The substrates were placed in plastic 'Ziploc' bags or plastic buckets, and stored at 4°C for a few days until usage.

Microcosm experiments were set up outdoors at Resolute Bay, Cornwallis Island, in July 1998. Substrates from Char Lake, soil near Char Lake, wetland sediment and soil/moss substrate near Char Lake were weighed and placed in jars. About 300 g of substrates and 3.5 L of water from Char Lake water were used for each jar. The jars were then filled with about

250 mL of tap water, covered with an aluminum foil, and set outside. These substrates were not spiked with α-HCH. Average atomospheric temperatures were about 12°C and dissolved oxygen levels were monitored throughout the 13 day duration of the experiment.

4.2.5 Part 1(b) with Meretta Wetland Substrates in the High Arctic

A second set of experiments was performed using only Meretta wetland substrates because Helm (1999) found that enantioselective degradation was greatest in wetlands. In these tests that were preformed at Resolute Bay, Cornwallis Island, the following factors were varied: light/dark, temperature and nutrient concentration. The Milli-Q water in the jars were spiked with 40 ng of α-HCH, 50 ug/L of phosphate, 500 ug/L of nitrate and 50 mg/L of yeast extract for the nutrient addition experiments (Table 4.2).

4.2.6 Part 2: Preliminary Degradation Experiments in the Temperate Zone

A set of preliminary degradation experiments were set up at the University of Toronto using temperate profundal sediments from Moira Lake, a small eutrophic lake in the Kawarthas region of southern Ontario. This set of experiments was used to determine whether enantioselective degradation was possible with temperate substrates. The preliminary results were used to design Part 3 of the experiments. Triplicate jars were set up with conditions varying in temperature, nutrients and light/darkness. For the nutrient manipulations, 50 ug/L of phosphate, 500 ug/L of nitrate or 50 mg/L of yeast extract were added. All the jars contained Milli-Q water and were spiked with 40 ng of α-HCH in 1 mL of acetone. These jars were monitored for dissolved oxygen and pH over 7 days at ambient room temperature with dissolved oxygen and pH monitoring.

4.2.7 Part 3: Degradation Experiments of Arctic and Temperate Substrates with Dissolved Oxygen and pH Monitoring

Substrates from Harp Inlet 4, Dickie Lake, Moira Lake, Meretta Lake and Char Lake were used for the final set of degradation experiments conducted at the University of Toronto. The same factors as in Part 2 were manipulated. Again, Milli-Q water in jars were spiked with 40ng/mL of α-HCH in acetone. The oxygen levels were monitored, and corrected to 8 mg/L of dissolved oxygen by the addition of hydrogen peroxide when necessary (the maximum concentration at 20°C) (Kuzmetsov, 1970). The pH was constant throughout the 14 day experiment.

4.3 Results and Discussion

4.3.1 Quality Control and Assurance

Milli-Q water samples (4L) were extracted as blanks and concentrations of α-HCH were not detectable. System blanks (n=15) were also found to have non-detectable concentrations. Substrates used in the experiments were analyzed for α-HCH and were found to have negligible concentrations of α-HCH so that blank corrections were not applied. However, α-HCH concentrations were corrected for individual recoveries of d_6-α-HCH surrogate spikes. Recoveries for the surrogate spike ranged from 50 % to 98% with an average 53% ±20% (n=131). Three samples were additionally spiked with 80 ng of α-HCH and had average recoveries of 83% ±4% and were corrected for native amounts in the sample. The ERs of 39 experimental samples were confirmed on a Beta-DEX 325 (Supelco) and BetaDEXcst (Restek). A comparison of the average percent difference was1.08% ± 2.04.

4.3.2 Microcosm Experiments

Figure 4.1 shows the average ERs of the Part 1 (a) degradation experiments performed at Resolute Bay. All samples had ERs very close to the blank ER of 0.90. Similarly, experiments to determine degradation enhancement by differing light and nutrient conditions on wetland substrate (Part 1 (b)) produced results close to the blank (Figure 4.2).

A general linear model (GLM) of the data reveal the ERs from treatment jars were not significantly different from the blank ($F=3.8$, $p<0.18$). The observed ERs in blank jar, and consequently in the treatment jars, are due to the ERs already present in the lake water that was used.

The results from Part 2 of the jar experiments also indicate that no enantioselective degradation occurred ($F=6.5$, $p<0.14$). Table 4.3 summarizes the experimental results. The dissolved oxygen levels declined from 8 mg/L at the start of the experiment to less than 1 mg/L within 1 day. The anoxic conditions may have been unfavourable for the degrading microbes, which are presumed to live in aerobic conditions. Thus, hydrogen peroxide was added to jars in the next set of experiments.

Figure 4.3 shows that all experiments in Part 3, regardless of substrate type, nutrient concentration or in light or darkness, all resulted in ERs not significantly different from the control. It would appear, that although oxygen and pH conditions were monitored closely, the conditions were not favorouable for enantioselective degradation of α-HCH. Thus, these degradation experiments were unable to identify the factors controlling enantioselective degradation of α-HCH or to mimic natural conditions.

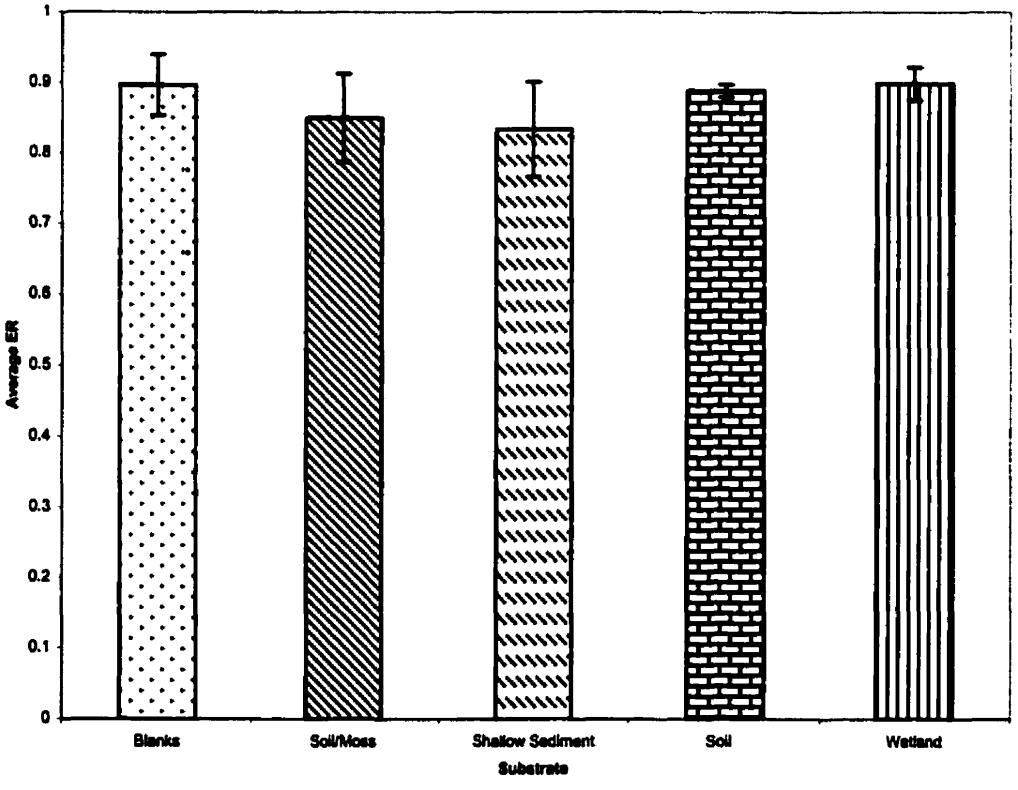


Figure 4.1: Average ERs of microcosm experiments using substrates from high arctic aquatic systems.

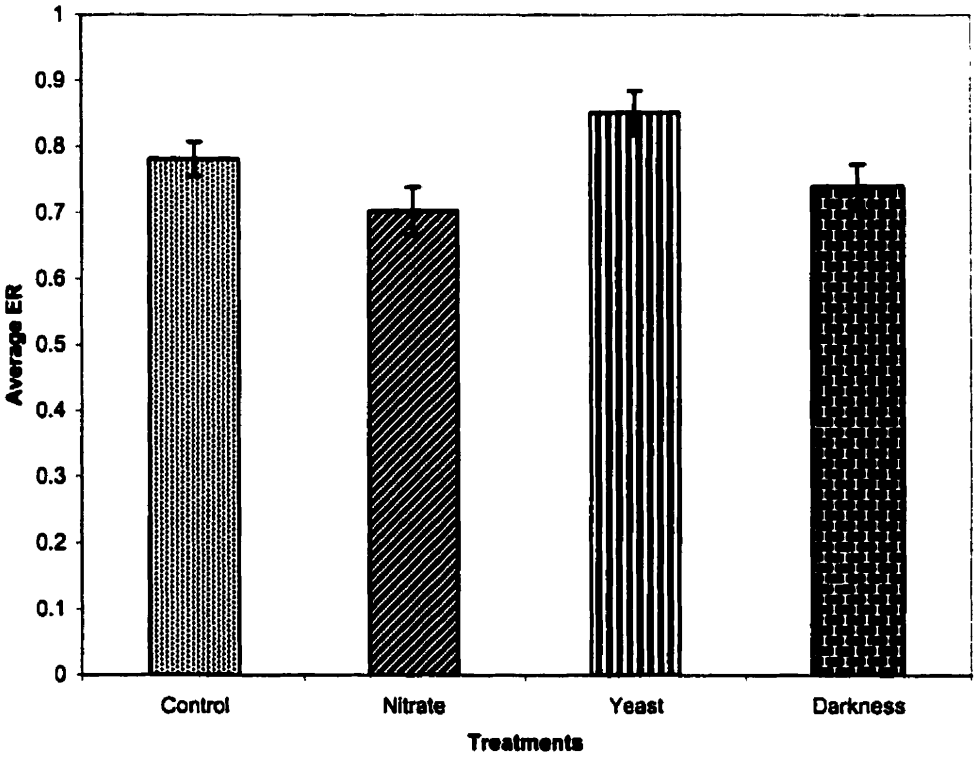


Figure 4.2: Part 1 (b) average ERs in degradation experiments using arctic wetland substrate under nitrate, yeast and darkness treatments.

Table 4.3: Preliminary degradation experiments using temperate substrates with treatments.

Substrates	Treatment	Average ER
MilliQ Water and a-HCH Spike	Standard a-HCH spike	1.02
Moira Lake	1 week duration	1
Moira Lake	Blank	1.01
Moira Lake	Air Bubbling	0.99
Moira Lake	Added Spike/ Control	1
Moria Lake	Blank	n.d.
Moiral Lake	Phosphate Addition	0.97
Moira Lake	Nitrate Addition	0.99
Plastic Lake	Phosphate Addition	1.02
Plastic Lake	Nitrate Addition	0.99
Plastic Lake	Added Spike/Control	1
Plastic Lake	Blank	n.d.

N.B. The duration of the experiments were 2 weeks, unless otherwise noted.

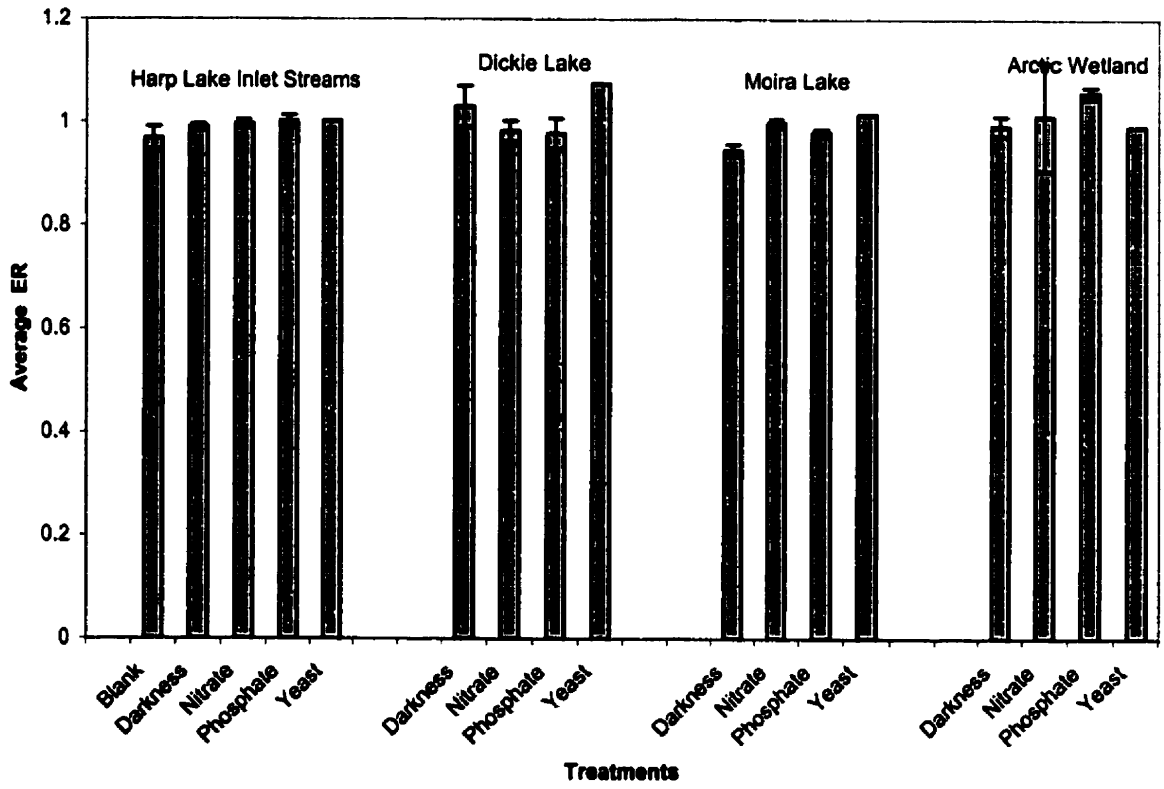


Figure 4.3: Part 3 of the experiments show the ERs of a-HCH of four substrates under different treatments

Other researchers have also found that laboratory biodegradation studies cannot mimic the natural processes found in the field (Madsen, 1991, Kobayashi and Rittmann, 1982). There are numerous reasons why microbes and substrates brought in from their natural habitats cannot degrade xenobiotics similarly in laboratory experiments. As many researchers have discussed, microbes are very sensitive to their environment, and any change in the parameters that exceed their tolerance level, will cause them to become either metabolically inactive or dead. Kobayashi and Rittmann (1982) have discussed the inability of scientists to accurately mimic the natural conditions for microbes under laboratory conditions. Thus, it may have been our inability to reconstruct the environmental conditions necessary for the microbes in our degradation experiments.

Biodegradation in the field reflects a delicate and intricate balance of nutritional and biological relationships, as well as environmental factors such as oxygen, pH, redox potential. The environmental conditions for microbes is extremely important for their survival. For example, microbial populations are very sensitive to pH. Under acidic conditions, the microbial enzymes can become deactivated, and under alkaline conditions, cell components can be hydrolyzed. Changes in pH can alter enzyme activity as well as water chemistry that controls the availability of the nutrients to the microbes (Atlas and Bartha, 1972). The complex and interactive nature of environmental factors for microbial populations complicates the task of defining the controlling factors in a microbial ecosystem. Atlas and Bartha (1972) mention that changes in the environment can allow a different microbial population to dominate. Complicating this scenario even more, is the evidence that some microbes can change the environmental conditions to become more favourable to themselves, and consequently, making it unfavourable to other populations.

When a sample of substrate is removed from the field, it cannot be assumed that the physiological status of the accompanying microorganisms is unaltered. It has been shown that microbial activity, when disturbed in the environmental sample and incubated in the laboratory, is likely to be quantitatively and qualitatively different from the microbial activity *in situ*. However, biodegradation of organic contaminant compounds in the field is also is very difficult to measure because environmental parameters can not be easily manipulated and the spatial heterogeneity. The problems of extrapolating from laboratory data to the field have never been solved (Madsen, 1991).

Although a simple study designed to isolate factors and study mechanisms can be valuable, they cannot always be used in predicting biodegradability or transformation in natural systems. The difficulty of studying degradation is yet more difficult if the compound is subject to cometabolism in which it is incidentally transformed by organisms using other, similar compounds. Moreover, the products of the initial transformation by one organism may be subsequently broken down sequentially by a series of different organisms until compounds can be metabolized by normal metabolic pathways are formed (Kobayashi and Rittmann, 1982). Researchers have assumed that if a compound is cometabolized or resistant to microbial conversions at the levels normally used for biodegradation tests, it would be similarly cometabolized or resistant in nature. Unfortunately, many studies, including our study, has found that controlled biodegradation studies is not feasible for explaining the degradation occurring in nature.

4.4 Conclusions

Our results indicate that enantioselective microbial degradation of α-HCH did not occur in laboratory degradation experiments in which α-HCH was added to microcosms containing water (natural and deionized) and natural substrate. The addition of nitrate, phosphate and yeast extract, in light and darkness and at two temperatures from arctic or temperate lakes, streams and wetlands, did not enhance the degradation of a-HCH. The inconclusive results can stem from many factors that have been reported by other researches. The main reason for the lack of results is likely our inability to mimic the natural conditions required for the microbes and enzymes responsible for the degradation.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The concentrations and isomer and enantiomeric ratios of HCHs, and factors affecting these parameters were investigated in arctic, subarctic and temperate lakes, streams and wetlands. The following conclusions have been drawn from the survey of aquatic systems and laboratory degradation experiments.

- The highest α-HCH concentrations were found in cold, large lakes such as those in the high arctic, subarctic and the Great Lakes, while lower concentrations were found in the small temperate lakes. These high concentrations are due to the minimal losses by hydrolysis and minimal volatilization. The low concentrations of α-HCH in small temperate lakes is due to losses by hydrolysis, volatilization and non-enantioselective degradation.

- Alpha-HCH / γ-HCH ratios are significantly higher in arctic and subarctic lakes, than in temperate lakes. The ratios are consistent with high loadings of α-HCH in high latitude areas due to long-range transport and fresh γ-HCH usage in temperate areas.

- The enantioselective degradation of α-HCH in wetlands and streams is a function of α-HCH delivery to the microbes and maximizing the contact time between the chemical and microbes in the form of biofilm. The degradation is greatest in wetlands that are more biologically productive and have smaller volumes of water than faster moving streams.

- Enantioselective degradation was appreciable in temperate wetlands and streams, but limited in temperate lakes. These were unexpected results considering the warmer

temperatures and greater availability of nutrients and microbial populations in temperate systems.

- The greater magnitude of enantioselective degradation that occurs in the arctic in comparison to the temperate zone is hypothesized to be due to the presence of oligotrophic bacteria. These microbes are adapted to systems with limited carbon flux and are able metabolize a variety of carbon compounds. Microbes in warmer aquatic systems may be unable to degrade α-HCH or will use carbon sources other than α-HCH.

- Enantioselective degradation in lakes can be explained by α-HCH concentration, which in turn, are related to factors that affect α-HCH loadings such as lake surface area, volume and temperature.

- Enantioselective degradation is also related to lake residence times, where long residence times increase α-HCH contact with microbes in the substrate, as well as higher historical loadings of α-HCH.

- The degradation experiments were unsuccessful due to the inability to mimic the natural conditions needed to successfully maintain viable microbes that have α-HCH degradative abilities.

5.2 Recommendations

This study identifies several factors related to the concentration, isomer ratios and enantiomeric degradation of HCH. Whereas statistically significant relationships were found with several variables, the study was unable to make direct cause-effect linkages. The following recommendations are made for future research.

- Further efforts are needed to isolate the factors that may enhance microbial degradation of α-HCH. This includes using microcosms under controlled conditions, as well as investigating the parameters *in situ*, especially streams and wetlands where most of the degradation is occurring.

- Research should aim to isolate and identify the microbes and characterize the biofilm responsible for enantioselective degradation in non-source areas of α-HCH.

- Future monitoring of enantioselective degradation of α-HCH in temperate systems should be conducted. ERs should also be measured in the overlying air of the study sites to determine fluxes of α-HCH between water and air in temperate sites.

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Table A1: Enantiomer Ratios of Part 1 and 2 Degradation Experiments.

Substrate Type	Rt-BDEXcst	Beta-DEX 325
Blank	0.93	
Blank	0.85	
Blank	0.91	0.95
Blank	0.84	
Blank	0.84	
Soil and Moss	0.87	
Soil and Moss	0.78	0.8
Soil and Moss	0.90	
Soil and Moss		
Char Sediment	0.78	
Char Sediment	0.82	
Char Sediment	0.91	
Soil near Char	0.88	0.89
Soil near Char	0.88	
Soil near Char	0.90	
Wetland	0.92	0.95
Wetland	0.91	
Wetland	0.87	
Part 2		
Wetland	0.77	0.8
Wetland	0.76	
Wetland	0.81	
Wetland with nitrate	0.73	
Wetland with nitrate	1.28	
Wetland with nitrate	0.66	
Wetland yeast	0.82	
Wetland yeast	0.88	
Wetland yeast	1.03	0.9
Wetland darkness	0.78	
Wetland darkness	0.72	
Wetland darkness	1.13	0.78
Blank	0.69	
Blank	0.70	
Blank	0.67	0.7
Blank	0.67	
Blank	0.67	

Table A2: Enatiomeric Ratios of Part 3 Degradation Experiments. All samples were found to have near racemic values.

SAMPLE	Rt-bDEXcst	Beta-DEX 325	BGB-172
Harp 4 Sediment	0.99	0.92	
Harp 4 Sediment	1.01	1.00	
Harp 4 Sediment	1.01	1.00	
Harp 4 Blank	1.00	0.99	
Harp 4 darkness	0.97	0.99	0.99
Harp 4 darkness	0.99	0.99	
Harp 4 darkness	1.01	1.00	
Harp 4 Nitrate	0.99	0.98	
Harp 4 Nitrate	1.00	1.01	
Harp 4 Nitrate	0.99	0.99	
Harp 4 Phosphorus	1.01	0.99	
Harp 4 Phosphorus	1.00	1.00	
Harp 4 Phosphorus	1.00	1.01	
Harp 4 Yeast	1.00	1.00	
Harp 4 Yeast	1.01	1.00	
Dickie(spike)	0.99	1.00	
Dickie(spike)	1.00	1.00	
Dicke Darkness	0.98	0.99	0.98
Dicke Darkness	0.99	0.99	
Dicke Darkness	1.12	1.10	
Dickie Nitrate	1.02	0.99	
Dickie Nitrate	0.99	0.99	
Dickie Nitrate	0.94	0.99	
Dickie Phosphorus	0.99	1.00	1.00
Dickie Phosphorus	0.98	0.98	
Dickie Sediment	0.94	0.96	
Dickie Sediment	1.00	0.99	
Dickie Sediment	0.99	1.00	
Dickie Yeast	1.10	1.00	
Dickie Yeast	1.08	1.30	
Dickie Yeast	1.04	1.02	1.01
Dickie Blank	n.d.		
Moira Darkness	1.00	1.00	
Moira Darkness	0.83	0.99	
Moira Darkness	1.01	1.00	
Moira Nitrate	1.01	1.00	
Moira Nitrate	1.00	1.30	
Moira Nitrate	0.99	0.99	0.99
Moira Phosphorus	0.97	0.98	
Moira Phosphorus	0.99	0.96	
Moira Phosphorus	0.82	1.00	
Moira Sediment	1.01	1.00	
Moira Sediment	0.99	0.99	
Moira Yeast	1.01	0.99	
Moira Yeast	1.01	0.99	1.00
Moira Yeast	1.02	1.00	
Meretta Darkness	1.01	0.99	
Meretta Darkness	0.98	0.99	1.00
Meretta Darkness	1.01	1.00	
Meretta Nitrate	1.00	1.00	
Meretta Nitrate	1.00	1.00	
Meretta Nitrate	1.03	1.00	
Meretta Phosphorus	1.00	0.99	
Meretta Phosphorus	1.00	1.00	0.99
Meretta Phosphorus	1.18	0.99	
Meretta Sediment	1.03	1.00	
Meretta Sediment	0.99	0.99	
Meretta Sediment	0.99	0.99	
Meretta Spike	0.98	0.98	
Meretta Spike	1.02	1.00	
Meretta Yeast	1.00	1.00	0.99
Meretta Yeast	0.98	0.99	
Meretta Yeast	1.00	0.99	
Char Sediment	1.00	0.98	1.00
Char Sediment	0.83	0.95	
Char Sediment	1.01	1.04	
Char Nitrate	1.01	1.00	
Char Nitrate	1.00	0.98	
Char Nitrate	0.99	0.99	
Char Darkness	1.00	0.99	
Char Darkness	1.00	0.99	0.99
Char Darkness	1.01	1.00	
Char Yeast	0.99	1.00	
Char Yeast	1.00	1.00	
Char Yeast	0.83	0.95	0.99
Char Phosphorus	1.01	1.00	
Char Phosphorus	1.01	1.03	
Char Phosphorus	1.00	0.99	
Char Blank	n.d.		
JAR SPIKE	1.01	1.00	1.00

Table A 3: Enantiomeric and Ion Ratios of a-HCH Standard (0.06 ng/L) on Three Columns

Column	ER (+)/(-) 255	ER (+)/(-) 257	AVERAGE ER	IR (-)255/(-)257	IR (+)255/(+)257	AVERAGE IR
Rt-BDEXcst	0.97	1.06	1.02	1.37	1.26	1.31
Rt-BDEXcst	1.01	1.00	1.01	1.57	1.58	1.57
Rt-BDEXcst	1.01	0.98	1.00	1.58	1.65	1.61
Rt-BDEXcst	1.02	0.98	1.00	1.52	1.58	1.55
Rt-BDEXcst	1.02	0.99	1.00	1.49	1.54	1.52
Rt-BDEXcst	1.01	1.01	1.01	1.46	1.47	1.46
Rt-BDEXcst	1.00	0.99	1.00	1.50	1.53	1.51
BGB-172	1.00	1.01	0.99	1.57	1.57	1.57
BGB-172	0.99	1.01	1.00	1.58	1.56	1.57
BGB-172	0.99	1.01	0.99	1.50	1.50	1.50
BGB-172	1.00	0.99	0.99	1.49	1.50	1.49
Beta-DEX 325	0.99	0.99	0.99	1.51	1.50	1.50
Beta-DEX 325	0.99	1.01	1.00	1.51	1.51	1.51
Beta-DEX 325	0.99	0.99	0.99	1.51	1.51	1.51
Beta-DEX 325	0.99	0.99	1.03	1.51	1.49	1.50

Table A4: Average and Ranges of ER in High Arctic Samples. Averages are from three chiral columns and replicate samples.

Char Inlet	0.60 (0.53 - 0.65)	n=3
Char Lake	0.64 (0.62 - 0.66)	n=2
Meretta Inlet	0.64 (0.55 - 0.69)	n=25
Meretta Lake	0.70 (0.65-0.76)	n=2
Slope Rill	0.70 (0.61 - 0.89)	n=4
Wetland Inlet	0.76 (0.73 - 0.78)	n=3
Wetland Mid	0.6 (0.45 - 0.75)	n=3
Wetland Outflow South	0.45 (0.43 - 0.46)	n=3
Wetland Outflow North	0.56 (0.52 - 0.6)	n=3

Table A5: Pearson Coefficients (R^2) for alpha-gamma ratios.

	Ratio	α-HCH	ER	NO2	NO3	NH4	Total C	DIC	DOC	Total P	POC	Residence Time	Watershed Area	Volume	Surface Area
RATIO	1.000														
α-HCH	-.750	1.000													
ER	-.645	-.791	1.000												
NO2	-.327	-.329	.333	1.000											
NO3	-.261	-.263	.046	.200	1.000										
NH4	-.158	-.174	.058	.090	.805	1.000									
Total C	-.111	-.278	-.053	-.124	.486	**.724**	1.000								
DIC	.026	-.132	-.196	-.113	.514	**.745**	**.971**	1.000							
DOC	**-.548**	-.637	**.519**	-.084	.054	.156	.435	.206	1.000						
Total P	-.193	-.155	.034	-.013	.078	-.078	-.081	-.091	.013	1.000					
POC	-.369	-.356	.201	.123	.571	.703	**.794**	**.797**	.248	-.038	1.000				
Residence Time	-.183	-.119	.212	-.042	-.072	-.033	-.256	-.254	-.058	-.092	-.100	1.000			
Watershed Area	-.098	-.029	.066	-.171	-.215	-.149	.153	-.037	.392	.167	.296	-.128	1.000		
Volume	-.420	-.372	.326	-.076	-.085	-.067	.155	-.129	.574	.240	.662	.188	-.014	1.000	
Surface Area	.371	**.607**	**-.609**	-.208	-.314	-.214	-.134	.029	-.338	-.597	-.456	-.083	-.018	-.115	1.000

Bold type is significant at the 0.05 level.

Table A6: Pearson Coefficients (R^2) for streams.

	ER	CONC.	NO3	HN4	TOTAL C	DIC	DOC	TOTAL P	Total C for SS
ER	1.000								
α-HCH	-.561	1.000							
Nitrate	-.117	-.336	1.000						
Ammonia	-.129	-.383	**.984**	1.000					
Total C	.336	-.680	.603	.573	1.000				
DIC	.227	-.412	.724	.643	.915	1.000			
DOC	.168	-.459	-.478	-.341	-.087	-.482	1.000		
Total P	.271	-.285	.400	.309	.868	.913	-.368	1.000	
POC	.095	-.413	.910	.856	.838	**.944**	-.510	.737	1.000

Bold type is significant at the 0.05 level.

Table A7: Pearson Correlation Coefficients (R^2) for Wetland Sites

	ER	α-HCH	Nitrite	Nitrate	Ammonia	Total C	DIC	DOC	Total P	POC	Residence Time
ER	1.000										
α-HCH Conc.	.429	1.000									
Nitrite	.234	.785	1.000								
Nitrate	.004	.434	.779	1.000							
Ammonia	.044	-.102	.437	.792	1.000						
Total Carbon	-.260	-.768	-.227	.035	.592	1.000					
DIC	-.187	-.689	-.122	.114	.657	**.993**	1.000				
DOC	-.634	-.942	-.841	-.527	-.134	.573	.471	1.000			
Total P	.640	.282	-.257	-.257	-.387	-.630	-.637	-.282	1.000		
POC	.288	-.304	.213	.280	.741	.799	.860	-.003	-.419	1.000	
Residence Time	.165	-.915	-.509	-.781	-.189	.967	**.947**	.908	-.372	.949	1.000

Bolded type is significant at the 0.05 level.

Table A8: Pearson Coefficients (R^2) for streams.

	ER	CONC.	NO3	HN4	TOTAL C	DIC	DOC	TOTAL P	Total C for SS
ER	1.000								
α-HCH	-.561	1.000							
Nitrate	-.117	-.336	1.000						
Ammonia	-.129	-.383	**.984**	1.000					
Total C	.336	-.680	.603	.573	1.000				
DIC	.227	-.412	.724	.643	.915	1.000			
DOC	.168	-.459	-.478	-.341	-.087	-.482	1.000		
Total P	.271	-.285	.400	.309	.868	.913	-.368	1.000	
POC	.095	-.413	.910	.856	.838	**.944**	-.510	.737	1.000

Bold type is significant at the 0.05 level.

Table A9: Pearson Coefficients (R2) for All Study Lakes.

Correlations	ER	a-HCH Concentration	Nitrite	Nitrate	Ammonia	Total Carbon	DIC	DOC	Total Phosphorus	Particulate Carbon	Residence Time	Watershed Area	Volume of Water	Surface Area
ER	1													
a-HCH Conc.	**-0.71**	1.00												
Nitrite	0.22	-0.23	1.00											
Nitrate	0.21	0.11	-0.12	1.00										
Ammonia	-0.04	-0.02	-0.03	0.30	1.00									
Total Carbon	-0.33	-0.03	0.04	-0.17	0.61	1.00								
DIC	**-0.56**	0.18	-0.09	-0.13	**0.62**	0.92	1.00							
DOC	0.34	**-0.47**	0.30	-0.15	0.23	0.58	0.22	1.00						
Total Phosphorus	0.36	**-0.51**	0.38	-0.15	0.27	0.29	0.06	**0.62**	1.00					
Particulate C	**0.55**	**-0.70**	0.28	-0.14	0.18	0.38	0.06	0.83	**0.61**	1.00				
Residence Time	0.06	0.32	-0.06	0.19	-0.02	-0.21	-0.19	-0.13	-0.09	-0.07	1.00			
Watershed Area	-0.03	0.47	-0.18	0.21	-0.18	-0.18	-0.21	-0.02	-0.22	-0.15	0.31	1.00		
Volume	0.08	0.33	-0.03	0.16	-0.09	-0.06	-0.14	0.16	-0.09	0.18	0.63	0.48	1.00	
Surface Area	0.02	**0.49**	-0.13	0.44	-0.12	-0.39	-0.33	-0.28	-0.35	-0.37	0.42	0.74	0.54	1

Bold type is significant to p>0.05 level.