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Persistent organic pollutant transport and fate: Assessment by molecular tracers

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PERSISTENT ORGANIC POLLUTANT TRANSPORT AND FATE: ASSESSMENT BY MOLECULAR TRACERS

A Dissertation

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In Partial Fulfillment

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APPROVAL SHEET

This Dissertation is submitted in partial fulfillment of

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DEDICATION

This dissertation is dedicated to
Aaron Bartholomew,
husband and best friend

TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Persistent organic pollutants (POPs) such as the organochlorine pesticide hexachlorocyclohexane (HCH) may undergo global distillation (via the atmosphere) and accumulate in regions remote from the source. However, HCHs have relatively long residence times in aquatic ecosystems in mid-latitudes during their transport to the poles, and thus may cause a variety of deleterious effects on biological organisms in these areas, as well as near the source and accumulation regions. Hence, it is important to develop reliable techniques that can help to apportion source and identify the transport or transformation processes to which HCHs and other mobile POPs may be subjected. Since chemical molecules carry intrinsic information in terms of the mass and arrangement of their atoms, molecular tracers such as compound specific stable isotope and enantiomer ratios may prove valuable in studying POP fate and transport. Thus, the main objective of this study was to further develop the use of two novel geochemical tools: compound-specific stable isotope ratios (CSIRs) and enantiomer ratios (ERs) to evaluate the sources, transport and environmental fate of POPs. This was done in the context of studying the fate and transport of α-HCH, which is a globally distributed POP. Two processes that are important in the fate of α-HCH and other volatile POPs are air-water transfer (the major pathway for transport of relatively volatile POPs), and microbial degradation, (a major process controlling long-term fate to which HCHs are susceptible, when they are retained by terrestrial and aquatic reservoirs). Hence, I used a biogeochemical tracer approach to study air-water flux and biodegradation of α-HCH.

In the first part of the study, I evaluated the potential for using stable isotope ratios to track POP source and estimate transport distance, using α-HCH and the hydrocarbon, phenanthrene, in laboratory simulations of global distillation. I compared the relative fractionation of carbon versus deuterium isotopes during air-water gas exchange of these POPs along a strong temperature gradient. The hypothesis, that perdeuterated compounds, but not necessarily carbon-labeled compounds would show measurable and significant fractionation during air-water transfer, was validated within the confines of the experimental system. The results suggest that it may be possible to use a dual tracer approach on a larger scale, in which carbon isotopes could be used to track POP source, while fractionation of hydrogen isotopes may be used to track POP transport distance.

In the second part of the study, I evaluated the potential for use of ERs to evaluate α-HCH biodegradation. The rationale for this study was that since most enzymatic processes are stereoselective, enantiomers (non-superimposable mirror image isomers) of pesticides are expected to microbially degrade at significantly different rates, leading to increased environmental persistence of the non-degradable isomer. It is often assumed that ERs reflect the extent of chiral pesticide biodegradation during retention in terrestrial or aquatic reservoirs, despite the lack of data on relationships between microbial parameters and ERs. In order to bridge the gap between microbial and chemical information on enantioselective processes, and to quantify fluxes of hexachlorocyclohexanes across the air water interface, I measured microbial activity and abundance, as well as the concentrations of lindane (the γ-isomer of HCH) and both enantiomers of α-HCH (the only chiral stereoisomer of HCH) in surface waters of the

York River estuary. Air and surface water samples were collected at six sites along the salinity gradient at various times over the course of a year. Chemical analyses were conducted using gas chromatography with a β-dex 120 chiral column. Seasonal and spatial variations in select parameters (microbial biomass and activity, temperature, salinity) that could change the partitioning, transport and transformation processes to which HCHs are subjected in this mid-latitude estuary, were also monitored. Microbial activity was measured using tritiated thymidine and leucine. Bacterial numbers were estimated by acridine orange direct counts using epifluoresence microscopy.

Use of mixing lines to predict α-HCH concentrations and ERs in surface waters along the estuary, in conjunction with data on air-water flux and precipitation, indicated that microbial degradation led to depleted HCH concentrations, as well as non-conservative mixing of α-HCH ERs, during later summer and early fall months when river flow and precipitation were low. Surprisingly, at fresh water sites with high bacterial activity, ERs were close to 1:1 despite lower overall α-HCH concentrations, whereas on average α-HCH concentrations were higher but ERs were non-racemic at the estuary mouth. Although α-HCH concentrations and ERs were correlated with microbial activity, some seasonal variations in enantioselectivity were observed, suggesting that seasonal as well as spatial differences in microbial community structure may affect α-HCH ERs.

These results suggest that the relationship between microbial parameters and enantio-selective degradation is complex and warrants further study before ERs can be used as effective tracers of chiral POP transport. Overall, this study indicates that both CSIRs as well as ERs have the potential to become powerful tools that can enhance our understanding of the physicochemical and biological processes that are important in the transport and fate of α-HCH and other POPs.

BIOGEOCHEMICAL TRACERS OF POLLUTANT FATE AND TRANSPORT

Chapter 1

GENERAL INTRODUCTION

Many classes of chlorinated compounds are persistent organic pollutants (POPs) that undergo bioaccumulation and bioconcentration due to their lipophilic nature (Schroeder and Lane, 1988, Schwarzenbach *et al.*, 1993) and exert a variety of toxic effects (Gardner *et al.*, 1992, Ceron *et al.*, 1995, Dodson and Hanazato, 1995; Facemire *et al.*, 1995, Lahvis *et al.*, 1995, Sleidernik *et al.*, 1995). Although the United States has banned the use of many chlorinated pesticides, some are still manufactured for export (US International Trade Commission, 1994), and certain developing nations continue to manufacture and use these chemicals. For example, although the US banned the use of commercial mixtures of the agrochemical pesticide hexachlorocylcohexane (HCH) as early as 1978 (Barrie *et al.*, 1992), the mixture was still used in Asia, Eastern Europe, Central and South America in the 1990s (Fischer *et al.*, 1991, Willet *et al.*, 1998).

In 1974, researchers hypothesized that some chemicals, including chlorinated pesticides, may volatilize at equatorial latitudes, undergo atmospheric transport and condense in high-latitude locations (Rappe, 1974). POPs may migrate in a series of short steps (repeated cycles of volatilization followed by deposition) termed the "grasshopper effect" (Ottar, 1981) or "cold condensation" (Wania and Mackay, 1996). Since a POP's physico-chemical characteristics such as subcooled liquid vapor pressure, octanol-air

partition coefficient, and condensation temperature affect the rate and extent of atmospheric transport (Wania and Mackay, 1996), this leads to segregation of individual POPs during air-earth exchange in different geographical regions (Goldberg, 1975; Risebrough, 1990). That is, the global distillation theory predicts that when a contaminant mixture is atmospherically transported from lower to higher latitudes, its components will fractionate according to their volatility. Non-volatile components will not partition readily into the gas phase, resulting in preferential accumulation and enrichment of non-volatile POPs in lower latitude source regions. In contrast, higher latitudes will tend to receive greater concentrations of the highly volatile POPs, which readily succumb to gas phase transport from warmer source locations. Thus, global atmospheric transport of semivolatile POPs is analogous to fractional distillation and gas chromatography, whereby components of a mixture separate due to different migrational velocities.

Evidence supporting the predictions of the POP global transport hypothesis has been supplied by numerous studies (e.g. Bidleman *et al.*, 1989; Muir *et al.*, 1988, Gregor and Gummer, 1989; Hargrave *et al.*, 1988 & 1992). For example, inverted latitudinal profiles (i.e. declining concentrations with increasing proximity to the source) have been observed for relatively mobile POPs, in accordance with the theory of global transport (Calamari *et al.*, 1991; Simonich and Hites, 1995; Wania and Mackay, 1996; Agrell *et al.*, 1999). Moreover, many chlorinated pollutants are susceptible to atmospheric transport on global scales, and there are numerous reports of chlorinated pesticide accumulation in air, water and soils distant from primary sources (e.g. Larsson *et al.*, 1990, Bidleman *et al.*,

1995).

POPs that are highly volatile and preferentially accumulate in polar environments, are likely to have long residence times in water and soil in these areas, due to slower microbial degradation at low temperatures. In addition, many animals in these regions accumulate and store high amounts of lipid. Since polar food webs are short, POPs may be quickly transferred to higher trophic levels including humans (Hargrave *et al.*, 1992). Furthermore, POPs may reside within animals as well as humans for extended periods due to the slower rates of metabolism at cold temperatures. POPs may also have detrimental sub-lethal effects, such as suppressed immune response and endocrine disruption on humans as well as wildlife inhabiting these remote locations (Skarre *et al.*, 2000). Consequently, it is of vital importance to develop tools that can help to identify key sources, predict persistence, and track the transport of POPs, in order to protect fragile ecosystems and resolve issues related to trans-boundary pollutant migration.

Information about the source of a POP and environmental transport and fate pathways may be sequestered within its molecules, in the isotopic composition of its constituent atoms (e.g. stable isotopic signatures), as well as in its three dimensional structure (e.g. stereoisomer ratios). Thus, the development of molecular level biogeochemical tracers for anthropogenic compounds could aid in apportioning source and evaluating the significance of transport pathways and degradation mechanisms. Although biogeochemists have successfully employed various molecular marker approaches, including the use of stable isotopic and stereo-isomeric data, these potentially valuable tools have not been widely used in environmental studies of pollutants.

Therefore, the primary aim of this study was to examine the viability of two biogeochemical tracing techniques, namely compound specific stable isotope ratios (CSIRs) and enantiomer ratios (ERs), to elucidate POP source, fate and transport in the environment.

CSIRs have the potential to become a powerful tool for environmental chemists, since a POP's stable isotopic composition may be modified by physicochemical processes such as molecular diffusion and volatilization, in addition to biological processes, such as uptake across cellular membranes. Many previous studies have used stable isotope ratios to glean information on biogeochemical cycling of compounds in various environments. For example, stable isotope signatures have been used by geochemists to provide information about the origin and transformation of organic matter (e.g. Fry and Sherr, 1984), and to reconstruct paleoclimate (e.g. Pluta and Zuber, 1995). Bulk measurements of natural isotopes of C, N, and S have been used to examine trophic structure and flow of these elements through an ecosystem (e.g. Peterson and Fry, 1987). Researchers have also used stable isotope techniques to provide insights on sources and environmental pathways of contaminants (Farran *et al.*, 1987; Gearing *et al.*, 1994; Macko *et al.*, 1998; Macko, 1994). For instance, stable isotope techniques have been successfully used to study the fate of pollutants after an oil spill (Macko *et al.*,1981). O'Malley *et al.* (1994; 1996) determined $\delta^{13}C$ signatures of PAH sources and used this information to quantitatively apportion different source contributions to isotopically characterized PAHs isolated from estuarine sediments. Ballentine *et al.* (1996) used CSIRs to provide clues about the sources of atmospheric PAHs and fatty acids. Stable isotopes have also been

used to study the trophic transfer of pollutants (Cabana and Rassmussen, 1994). However, prior to this study, no attempts were made to evaluate the potential for the use of CSIRs in studies involving atmospheric transport of POPs.

The primary hypothesis for the first portion of my research was that for a given POP, fractionation of isotopically heavy and light compounds would occur during atmospheric transport and due to faster movement of the isotopically light POP (Figure 1-1). However, in order to use CSIRs as tracers of long-range atmospheric transport, it is necessary that CSIR variations in source materials are lower than those induced by global transport, or that the source variations in CSIRs for a chemical are well characterized. Additionally, alteration of CSIRs by biogeochemical processes during global migration must be understood. Thus, it is necessary to first establish the potential for using stable isotope ratios as indicators of the extent of atmospheric transport of POPs. Hence, the first objective of this research was to evaluate the potential for using stable isotope ratios to gain insights into physico-chemical transport process of POPs, in a laboratory setting.

The second biogeochemical tracing technique that I investigated in my research, was the use of enantiomer ratios (ERs). Enantiomers are stereoisomers that are non-superimposable mirror images of one another. ERs have long been used by geochemists to study the origin and fate of organic compounds (e.g. Silfer *et al.*, 1994, Engel and Macko, 2001). However, application of ER measurements to investigate environmental problems involving semi-volatile organic pollutants, became possible only after the more recent development of chiral capillary gas chromatography techniques that enabled the separation and accurate determination of environmental concentrations of enantiomeric

pollutants (Faller *et al.*, 1991). Since development of chiral gas chromatography, ERs have been used to study biodegradation (Ludwig *et al.*, 1992, Lewis *et al.*, 1999) and to study the air-water flux of pollutants (Ridal *et al.*, 1997). Furthermore, knowledge of the enantiomer signatures of pesticides from different sources may help distinguish pesticide emissions from secondary sources. For example, Leone *et al.* (2000), found that chlordane emissions from house foundations were racemic, but those in other environmental samples were not. If different sources or sites have unique enantiomer ratios, source apportionment through ER measurements might be possible (Buser *et al.*, 2000).

In terms of pesticide biogeochemistry, ERs are important because enzymatic degradation of chiral pesticides is often enantio-specific, and enantiomers of chiral POPs may be biodegraded at significantly different rates, leading to increased accumulation of the form that is less susceptible to biodegradation (Figure 1-2). In support of this, numerous recent studies have reported enantiomeric excesses (ER not equal to 1) of chiral pesticides in the environment, attributable to microbial modification (Huehnerfuss *et al.*, 1993, Falconer *et al.*, 1995, Ridal *et al.*, 1997). Since about a quarter of anthropogenic pesticides are chiral, and since they are generally marketed and released into the environment as racemic mixtures (containing equal concentrations of both enantiomers), it is important to evaluate the relative persistence of pesticide enantiomers in the environment (Airens *et al.*, 1988; Eilel *et al.*, 1994). Research on the environmental dynamics of chiral pesticides using ERs can distinguish the fate of the more persistent enantiomers from those that are more readily degraded (Ludwig *et al.*,

1992).

Since physical processes are not enantioselective and only biologically mediated reactions can cause changes in ERs, the assumption has been made that changes in ERs measured in the environment should reflect the rate of biological modification of POPs (Harner *et al.*, 1999). However, this assumption ignores the possibility of non-enantioselective degradation, as well as the variety of factors that may cause changes in enantioselective degradation, such as nutrient enrichment, bacterial community structure and species differences (Lewis *et al.*, 1999). In addition, environmental factors may have an indirect effect on ERs to the degree that they can influence the speed and efficiency of microbial processes. Thus the first step towards using the ER of a particular chiral POP to quantify source, fluxes, rate of transport, age in an environment, or the overall significance of microbial modification in the POP's dynamics, is to study the relationship between the ER of this POP and the various factors that may modify it in the environment. Therefore, the second objective of this research was to conduct an inter-disciplinary field study to establish links between environmental (i.e. microbial) factors and the biodegradation and ER values of a globally relevant POP.

In order to accomplish my two major dissertation objectives, I chose to focus on α-hexachlorocyclohexane (α-HCH) as the model POP. α-HCH, a prevalent HCH isomer in environmental samples, is susceptible to global atmospheric transport as well as biodegradation, and it can be studied in terms of ERs since it is chiral. HCH has been one of the most abundant POPs present in the atmosphere and hydrosphere of the northern hemisphere over the last several years (Tatsukawa *et al.*, 1990, Willet *et al.*, 1998).

According to the classification scheme of pollutants susceptible to global migration, developed by Wania and Mackay (1996), α-HCH is a considered a highly mobile pollutant that will accumulate preferentially in polar latitudes, but will undergo seasonal deposition in temperate climates. During these periods of deposition and retention by terrestrial and aquatic reservoirs, α-HCH biodegradation has been observed (Bidleman *et al.* 1989, Falconer *et al.* 1995, Ridal *et al.*, 1997). However, the relative importance of microbial degradation during residence of this pesticide in temperate latitudes is unknown. Figure 1-3 presents some of the transport and transformation processes that are considered to be significant for volatile POPs such as α-HCH. In particular, fluxes to and from the atmosphere are important for such compounds, and microbial degradation during their residence in aquatic and terrestrial reservoirs is a major mechanism for removal from the environment. Hence, I evaluated CSIRs as a tool to track α-HCH transport during air-water exchange in the laboratory, and also attempted to further develop the use of ERs as a tool to evaluate the importance of α-HCH biodegradation in a field study conducted in the York River VA, a temperate estuary.

This dissertation is organized in manuscript format, where each chapter constitutes a complete individual paper. Chapter 3 addresses my first objective, and describes a laboratory experiment that compared deuterium versus carbon CSIR changes during the air-water exchange and atmospheric transport of POPs. Chapter 4 discusses a field study that was conducted to evaluate the relative influence of microbial degradation seasonally and spatially, in the fate of HCHs in the York River. Chapter 5 builds on the information obtained in the previous chapter, and details the relationship between α-HCH

ERs and microbial activity parameters, during seasons in which microbial degradation was found to be significant in the field study. Prior to these chapters, Chapter 2 outlines briefly my hypotheses, which in some cases were rejected. Finally, Chapter 6 integrates the findings and briefly summarizes the main conclusions, along with suggestions for future research. Together, these form a comprehensive study in which the use of two novel biogeochemical tracers was developed in the context of evaluating the major physicochemical and biological pathways by which the ubiquitous chlorinated pesticide α-HCH may be transported to and from, and degraded within, aquatic ecosystems.

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Figure **1-1.** Compound specific stable isotope fractionation during POP transport.

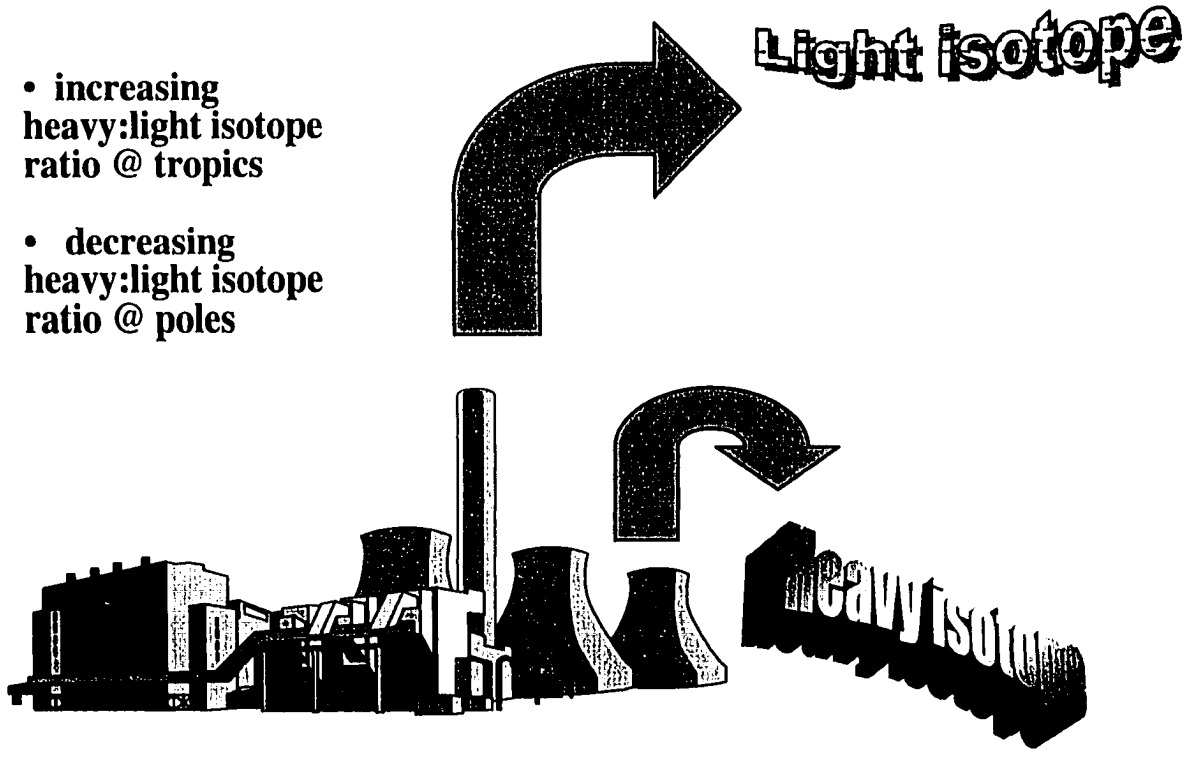

Figure **1-2.** Enantiomer ratio changes after enzymatic degradation in the environment.

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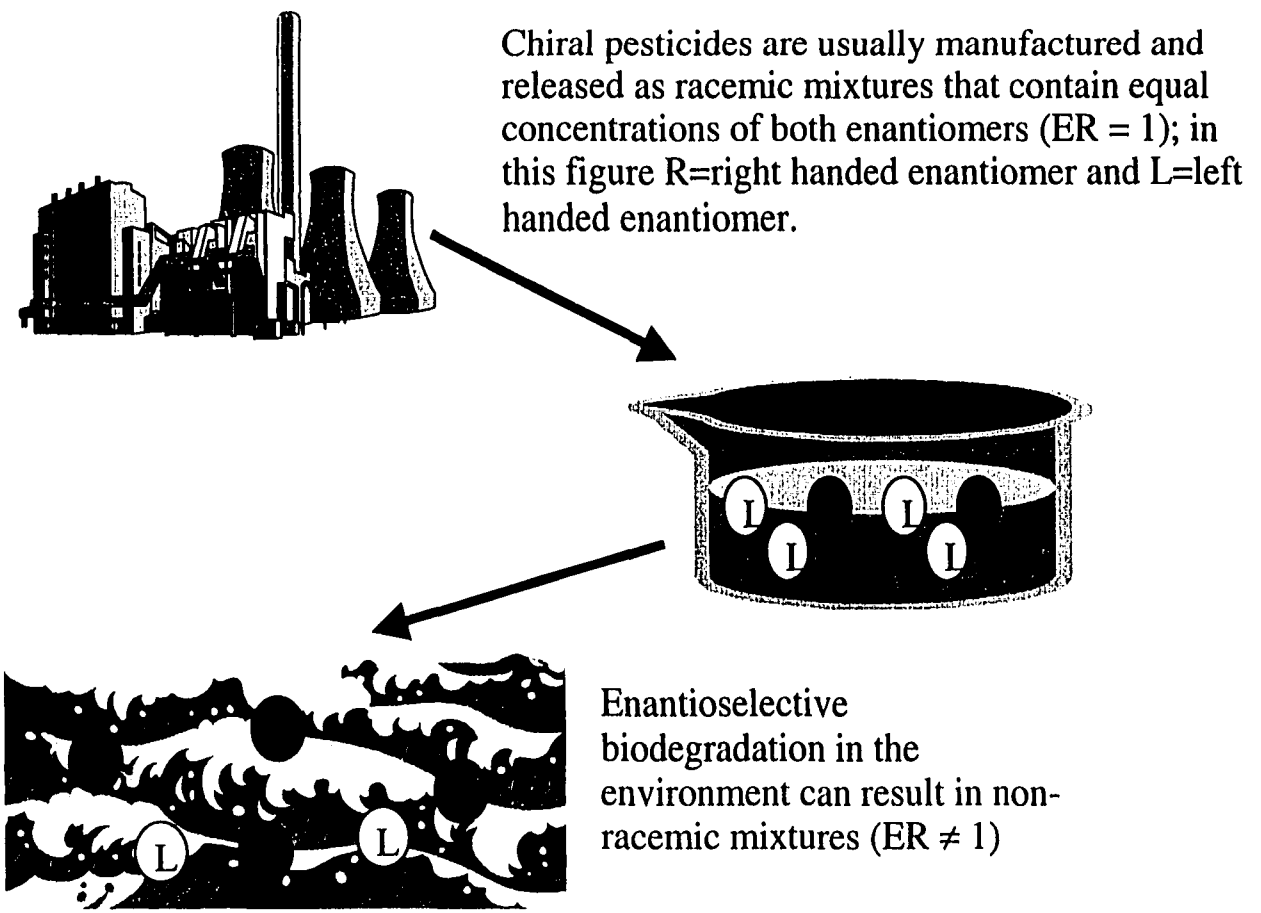
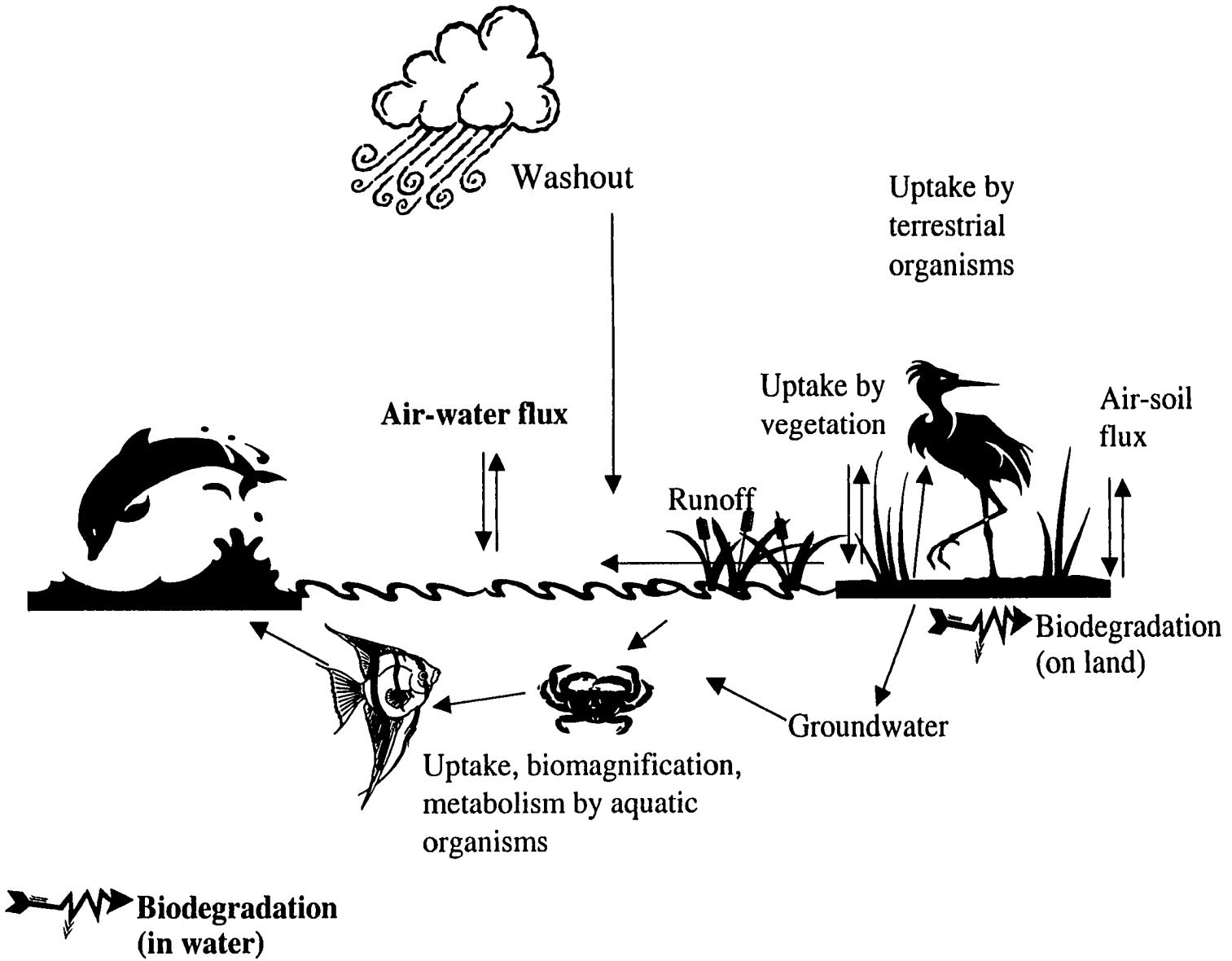


Chiral pesticides are usually manufactured and released as racemic mixtures that contain equal concentrations of both enantiomers (ER = 1); in this figure R=right handed enantiomer and L=left handed enantiomer.

Enantioselective biodegradation in the environment can result in non-racemic mixtures (ER ≠ 1)

Figure **1-3.** Some important processes in HCH transport and fate.



Chapter 2

HYPOTHESES

The underlying hypothesis of my dissertation was as follows:

During air-water transport and biodegradation of persistent organic pollutants (POPs), measurable changes in the isotopic and isomeric composition of the POPs will occur, such that POP isotope and isomer signatures can be used as tools to track the sources and physicochemical/biological processes affecting POPs in the environment.

This general hypothesis was broken down into more specific hypotheses that were tested in each chapter. In Chapter 3, a laboratory study was conducted in which stable isotopically labeled POPs were subjected to air-water transport along a strong temperature gradient in order to test the following working hypothesis:

During air-water exchange and vapor-phase and atmospheric transport of stable isotope labeled and unlabeled POPs of relatively high mobility, fractionation of the isotopically heavy and light compounds will occur, such that warmer source regions will be enriched in the heavy isotope, and the ratio of the heavy:light isotope will be higher than in the colder accumulation regions. Conversely, in the colder regions where the light isotope accumulates, there will be a lower heavy/light isotope ratio (equivalent to a more negative $\delta^{13}C$ or δD value for the compound, compared with the source region).

However, during the course of the experiment, it was found that perdeuterated ^{13}C labeled compounds underwent significant fractionation. Therefore, the working hypothesis was refined, such that the specific hypothesis tested was:

Perdeuterated isotopes will fractionate more than ^{13}C labeled isotopes due to intrinsic differences between the vibrational energy and rotational partition function within the hydrogen vs. carbon atoms.

In Chapter 4 and 5, the chiral POP α-HCH was chosen to be the focus of my research. Coincident with field testing the potential for using enantiomer ratios (mirror-image stereoisomers) as tracers of α-HCH biodegradation, information on the fate and transport of this POP in the York River estuary (field site) was required. Hence the objective of the work described in Chapter 4 was to constrain the factors affecting fate and transport of α-HCH in the York River system. Since α-HCH was thought to be delivered to the estuary from remote atmospheric sources via air-water gas exchange, the working hypothesis was the following:

α-HCH concentrations will not vary in surface waters along the length of the York River estuary.

Finally, Chapter 5 details the links between the spatial and seasonal variation in the microbial and chemical data collected (α-HCH concentrations and ER values) in surface waters. The following hypothesis was tested in this chapter.

Since enantiomer ratios of compounds are altered by biological processes alone due to the stereo-specificity of enzyme function, and physicochemical processes are not enantio-selective, greater deviations in ERs from 1:1 will be observed in those areas of the York River estuary at which the bacterial community is actively growing and metabolizing carbon sources including the substrate of interest relative to sites where the bacterial community is less active.

As will be explained in Chapters 4 and 5, these two final hypotheses were rejected, and the link between microbial processes and enantioselective biodegradation was found to be more complex than previously thought.

Chapter 3.

DIFFERENTIAL FRACTIONATION OF DEUTERIUM AND CARBON STABLE ISOTOPE LABELED PERSISTENT ORGANIC POLLUTANTS DURING AIR-WATER EXCHANGE

ABSTRACT

To test the potential for using compound specific isotope ratios as indicators of the global atmospheric transport of persistent organic pollutants (POPs), simplified distillation experiments were conducted in the laboratory using mixtures of stable isotope labeled (D and ^{13}C) and unlabeled POPs. Perdeuterated phenanthrene and α-hexachlorocyclohexane were transported much more slowly than their unlabeled analogs from warm to cold regions via air-water gas exchange in my experiments, resulting in significant isotopic fractionation of perdeuterated/unlabeled compound mixtures of both POPs. In contrast, fractionation of ^{13}C-labeled POPs relative to unlabeled compounds was not significant. The data indicate that stable carbon isotopes may be useful for identifying POP source material after release into the environment, whereas stable hydrogen isotopes may be useful for determining POP source region and transport distance. Such geochemical tracer techniques help answer questions related to the global atmospheric transport of POPs.

INTRODUCTION

Many classes of toxic organic compounds including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and organochlorine pesticides are considered persistent organic pollutants (POPs) (Schroeder and Lane, 1988). POPs degrade slowly and also undergo bioaccumulation and bioconcentration due to their lipophilic nature. These chemicals exert various sublethal effects such as endocrine disruption, immunological and neurological dysfunctions, reduced reproductive success, genotoxicity and teratogenicity (Gardner *et al.*, 1992; Dodson and Hanazato, 1995; Facemire *et al.*, 1995; Lahvis *et al.*, 1995, Sleidernik *et al.*, 1995).

Researchers have hypothesized that POPs may undergo a form of "global distillation" whereby they volatilize at equatorial and temperate latitudes, undergo atmospheric transport and condense in colder climates (Rappe, 1974; Goldberg, 1975; Wania and Mackay, 1993; Wania and Mackay, 1996). Evidence in support of this includes inverted latitudinal profiles (i.e. low concentrations near source regions with increased concentrations in polar regions) for hexachlorocyclohexanes (HCHs) and hexachlorobenzene in seawater (Tanabe *et al.*, 1982; Iwata *et al.*, 1993) and terrestrial vegetation (Calamari *et al.*, 1991; Simonich and Hites, 1995). Likewise, the accumulation of pesticides in air, water, soil and biota in polar regions remote from any primary source (Larsson *et al.*, 1990; Bidleman *et al.*, 1995; Weber and Goerke, 1996) supports the idea of global atmospheric transport of these xenobiotic compounds.

The physico-chemical characteristics of a POP such as its subcooled liquid vapor pressure, octanol-air partition coefficient and condensation temperature affect the rate

and extent of its atmospheric transport (Wania and Mackay, 1993; 1996). Consequently, it is proposed that specific compounds from various classes of POPs are deposited in different geographical regions during their global atmospheric transport poleward (Wania and Mackay, 1993; 1996). This geographic separation of moderately volatile (subcooled liquid vapor pressure $P_L = 10^{-4}$ - 1 Pa) organic contaminants during global atmospheric transport is similar to the process of fractional distillation, with highly volatile components readily undergo gas phase transport, while less volatile components undergo little if any atmospheric transport. Higher latitudes will therefore experience preferential accumulation of the more volatile fraction of a contaminant mixture, whereas relatively greater concentrations of the non-volatile components will remain in lower latitude source regions. Such fractionation has been observed for PCBs in the Baltic Sea region (Agrell *et al., 1999*).

Similarly, I hypothesize that POPs containing heavy stable isotopes are transported atmospherically to a lesser extent than those containing light isotopes, since heavier isotopes are less volatile. This phenomenon has been established for meteoric water (Craig, 1961; Ingraham and Taylor, 1986; Libes, 1992). In this case, water vapor generated by evaporation of ocean water in low latitude equatorial regions, becomes increasingly depleted in heavy isotopes (^{18}O, D) during atmospheric transport poleward. This well-documented example of latitudinal isotopic fractionation demonstrates the potential for using compound specific stable isotopes to track the long-range atmospheric transport of a substance.

It is proposed that during the long-range atmospheric transport of a POP via cyclic evaporation-condensation or distillation, isotopic fractionation occurs in an

analogous manner to that of meteoric water such that pollutants released in equatorial and tropical regions can be tracked and distinguished from those released at higher latitudes. As a POP is transported, its isotopically heavy components should be preferentially deposited near the source and its isotopically lighter components should be transported to higher latitudes, resulting in isotopic fractionations indicative of the region of origin of the compound. The original compound specific stable isotope ratio or CSIR (heavy/light isotope) should decrease (i.e. become isotopically lighter) with repeated distillation and condensation cycles, thus providing information on age and source region of POPs that have undergone long-range atmospheric transport. However, such interpretation requires that CSIR variations in source materials are lower than those induced by global transport, and that the source variations in CSIRs for a chemical are well characterized. In addition, alteration of CSIRs by biogeochemical processes during global migration must be understood.

Here, I report the results of laboratory experiments conducted to investigate the feasibility of using stable isotope ratios as tracers for the long-range atmospheric transport of POPs. Phenanthrene and α-HCH were chosen as model POPs, since both are classified as possessing "relatively high mobility" according to Wania and Mackay (1996) with the potential for long-range atmospheric transport and accumulation in polar regions. The experimental system for simulating evaporation-condensation cycles focused on air-seawater exchange since seawater covers more than 70% of earth's surface area. The specific hypothesis was that within the confines of a laboratory set up, the heavy/light isotope ratio of a POP undergoing repeated distillation would decrease from the area of release to the area of deposition, because isotopically light components

undergo faster gas-phase transport due to their increased preference for the gas phase, lower tendency to partition on surfaces and lower condensation temperatures relative to the corresponding isotopically heavy POPs. It was predicted that measurable fractionation would occur if stable isotopically labeled POPs (e.g. perdeuterated compounds) were used and that isotopic effects might cause differential fractionation of ^{13}C-labeled vs. D-labeled POPs.

MATERIALS AND METHODS

The system developed for simulating the global distillation of POPs consisted of temperature controlled (40, 20, 0 °C) gas washing bottles containing 500-1000 ml of artificial seawater (salinity = 35 ppt), which were connected to each other via an air stream (Figure 3-1). A Tenax trap was attached at the end of the system in order to collect any gas-phase POPs that were present in the overflow from the last (0 °C) bottle. At the start of each simulation, a labeled and unlabeled POP pair (e.g. D_{10}-phenanthrene and phenanthrene) was added (at a concentration lower than half solubility, in acetone) to the water in the first (40 °C) bottle, the bottles were sealed except for the air inlet and exit paths, and the air stream was turned on. The migration of the POPs from the first to the second (20 °C) and last (0 °C) bottles was monitored by sub-sampling the water in each of the gas washing bottles daily. Air flow was turned off during sub-sampling which was conducted through a sample port on the bottle in order to maintain a closed system during the experimental period for all except the first two experiments with phenanthrene (Table 1). In the first two experiments with D_{10}-phenanthrene and phenanthrene, the bottles were opened for sampling resulting in low mass balances for the experiments.

Consequently, a third experiment with D_{10}-phenanthrene/phenanthrene in which a closed system was maintained, was also performed.

Water samples (5-50 ml) collected during the distillation experiments were extracted by vortexing samples three times successively with hexane, after addition of perdeuterated anthracene (for quantitation of phenanthrene) and chlordane (for quantitation of hexachlorocyclohexane). Excess water was removed from the extracts by eluting extracts thorough a column of sodium sulfate. The concentrations of both the isotopically light and heavy compounds were then measured using gas chromatography/electron impact mass spectrometry (GC-MS; Hewlett Packard 5890 Series II gas chromatograph/ Hewlett Packard 5971A mass spectrometer). Compounds were separated using a 30m x 0.25 mm DBL-XLB column and peaks were detected using selective ion monitoring. Recoveries of standards from known solutions for triplicates for this procedure were greater than 90%. From these data, the concentration ratio of the isotopically heavy (labeled) and light (unlabeled) POPs was calculated for each reservoir in order to determine if there was fractionation during air/water exchange. At least two experiments each (between 8 and 21 days long) were conducted using D_{10}-phenanthrene, $^{13}C_6$-phenanthrene, D_6-α-HCH and $^{13}C_6$-α-HCH, (Cambridge Isotopes, MA, USA) along with the appropriate unlabeled form of each POP (Ultra Scientific, RI, USA). In the case of $^{13}C_6$-α-HCH alone, the replicate experiment was conducted using a system designed to increase the number of theoretical plates by including diatomaceous earth columns (50 cm x 2.5 mm inner diameter) after each seawater bottle through which the POPs had to travel after initial release in the 40 °C bottle. Atom enrichment was 99% in the case of both ^{13}C-labeled POP standards and 98% for both perdeuterated POP standards.

RESULTS

For all experiments (D_{10}-phenanthrene/phenanthrene; D_6-α-HCH/α-HCH; $^{13}C_6$-phenanthrene/phenanthrene and $^{13}C_6$-α-HCH/α-HCH), the change in concentration with time in each bottle during the air-water distillation simulations showed a similar pattern. An exponential decrease in concentration for both labeled and unlabeled POPs was always observed in the 40 °C bottle (Figure 3-2a). This decline was associated with a concurrent increase in concentrations of both the unlabeled and labeled compounds followed by a slow release in the 20 °C bottle (Figure 3-2b), and steadily increasing concentrations of the POPs in the 0 °C bottle (Figure 3-2c).

The recovery of spiked compounds calculated using a mass balance approach was low (20% to 60%) for the first two experiments with D_{10}-phenanthrene/phenanthrene in which a closed system was not maintained. However, mass balances improved in all later experiments including the third experiment using D_{10}-phenanthrene/phenanthrene. For all of the experiments in which sampling was conducted while maintaining a closed system, recoveries based on mass balance calculations ranged from 66% to over 90%.

In each experiment, the stable isotope (labeled/unlabeled compound) ratio reached a relatively constant value in all of the bottles after a period of time, usually 48 to 72 hours (Figure 3-3). Consequently, for each experiment, a mean heavy/light isotope ratio for each bottle was calculated after the ratio had attained a constant value. In some cases, lower isotope ratios were observed with increased gas exchange and decreasing temperature in the distillation simulations (Figure 3-4). Moreover, this result was reproducible; notice the trend in isotope ratio among bottles was similar for replicates of the D_{10}-phenanthrene/phenanthrene experiments regardless of whether or not a closed

system was maintained (Figure 3-4).

For all three replicates of the D_{10}-phenanthrene/phenanthrene experiments, differences in the mean heavy/light isotope ratio between bottles was significant at the >99% confidence level (ANOVA, $P < 0.01$). In contrast, the distillation simulations of α-HCH with ^{13}C-labeled α-HCH did not demonstrate fractionation (Figure 3-5). The relative concentrations of $^{13}C_6$-α-HCH and unlabeled α-HCH were not significantly different among the bottles at the 95% confidence interval either in the air/water (Figure 3-5a) or in the air/water/earth distillation simulations (Figure 3-5b). Subsequent experiments were conducted to examine whether this result was due to isotope effects (i.e. D vs ^{13}C label) rather than differences in POP physical-chemical properties. These latter experiments resulted in significantly different (99% confidence level) isotope ratios for D_6-α-HCH relative to α-HCH between the 0 °C and 40 °C bottles (Figure 3-6a). In addition, no significant fractionation of the ^{13}C-labeled phenanthrene relative to unlabeled phenanthrene was observed until both of these compounds reached the 0 °C bottle (Figure 3-6b).

DISCUSSION

The concentration profiles of all of the POPs in each experiment demonstrate that transport via the gas phase always occurred to each of the subsequent bottles in the system. The observed POP concentration profiles in the distillation simulations agree with those predicted by Wania and Mackay (1996) for chromatographic-like transfer of "relatively high mobility" POPs from warm to cold regions. Thus, the experimental system appears to simulate the global distillation process although presumably with fewer

and more abrupt cyclical air/earth exchanges than would likely occur in the environment.

The decreasing heavy/light isotope ratio observed during transport of perdeuterated isotopes of both phenanthrene and α-HCH from the 40 °C to the 0 °C bottles (Figure 3-4 and 3-6a) is the direct result of Rayleigh distillation, a process whereby the isotopic composition of a material undergoing reaction varies as a function of the extent of the reaction (Libes, 1992). In the global distillation simulations, the isotopically heavy perdeuterated POPs (i.e. D_{10}-phenanthrene and D_6-α-HCH) move much more slowly from the 40 °C to the 0 °C bottles, and are thus depleted relative to the unlabeled, isotopically light POPs as temperature decreases and the extent of reaction increases. In contrast, although POP transport was similar, no significant fractionation of ^{13}C-labeled α-HCH relative to unlabeled α-HCH was observed, even in the air/water/earth system (Figure 3-5), which was designed to increase the number of "theoretical plates" for fractionation to occur. In the case of $^{13}C_6$-phenanthrene/phenanthrene experiments, fractionation was observed only in the coldest bottle (Figure 3-6b). In this case, the isotopically heavy $^{13}C_6$-phenanthrene lagged behind phenanthrene, which was more readily exported from the system. The difference in the extent and nature of observed isotope fractionation for ^{13}C-labeled POPs in the distillation simulations may in part be related to kinetic isotope effects imparted by mass differences, but was most likely attributable to factors related to differences between ^{13}C and D isotopes.

A kinetic isotope effect may impart fractionation during air/water exchange due to the overall mass difference conveyed to the POP with the addition of heavy isotopic components. For example, air/water exchange of chemicals is largely controlled by their

rate of diffusion through stagnant air and water films (Liss, 1973). Consequently, if air/water exchange is occurring under non-equilibrium conditions, more rapid mass transfer of isotopically light compounds will favor fractionation with these POPs being more readily transported than isotopically heavy POPs. This can be calculated for ideal gases by equating the kinetic energies of each species:

$$1/2\, m_H v_H^2 = 1/2\, m_L v_L^2$$

which are the same if the molecules are at the same temperature and pressure (Castellan, 1971). The result is that the ratio of the velocities (v) of the molecules is inversely related to the square-root of the ratio of the masses (m) of the compounds:

$$\frac{v_L}{v_H} = \left(\frac{m_H}{m_L}\right)^{1/2}$$

where the subscripts L and H refer to the isotopically light and heavy compounds, respectively. Using this equation the relative mass transfer velocity of phenanthrene is predicted to be 2.8% greater than that for D_{10}-phenanthrene, but only 1.7% greater than $^{13}C_6$-phenanthrene due to a lower overall increase in mass (6 vs 10 amu) between the isotopically light and heavy POPs. Thus, a smaller difference in mass transfer velocity may contribute to the observed lack of fractionation of $^{13}C_6$-phenanthrene/phenanthrene compared with D_{10}-phenanthrene/phenanthrene. However, if isotope fractionation was simply the result of differences in mass transfer velocities of the isotopically heavy/light

components, similar fractionations should have been observed for $^{13}C_6$-α-HCH and D_6-α-HCH compared to unlabeled α-HCH.

A second and perhaps more important isotopic effect in the distillation of POPs may be vibrational energy effects on the equilibrium constant for a reaction. Molecules with heavy isotopes tend to be more stable, possessing a lower vibrational energy than molecules with corresponding light isotopes, due to a lower vibrational frequency imparted by the heavy atoms in a molecule (Castellan, 1971; Hoefs, 1997). Thus, isotopically heavy compounds are less likely to undergo reactions including phase transitions. However, the addition of excess neutrons to hydrogen as opposed to heavier atoms on a molecule appears to have a profoundly greater influence on the vibrational energy, and thus the fractionation of D enriched molecules as opposed to ^{13}C or ^{18}O enriched compounds. For example, as noted above, both the hydrogen and oxygen isotopes of water are fractionated during the Rayleigh distillation of meteoric water (Craig, 1961; Ingraham and Taylor, 1986) due to vapor pressure differences between H_2O, HDO, and $H_2{}^{18}O$ (Hoefs, 1997). However, the isotopic depletion of HDO is almost an order of magnitude greater than that of $H_2{}^{18}O$ during global distillation (Craig, 1961). This difference is related to the addition of an excess neutron to a hydrogen atom on the water molecule, as opposed to the addition of two excess neutrons to the oxygen atom of the molecule. A doubling of the atomic mass of a hydrogen atom significantly lowers its vibrational frequency rendering the heavy water more stable and more likely to condense. In comparison, two additional neutrons on the oxygen atom of a water molecule have a lower overall effect on the atomic mass of the oxygen, and hence, on the molecular vibrational frequency, energy, and stability of water.

It was hypothesized that the significant isotopic fractionation observed in the initial experiments with D_{10}-phenanthrene/phenanthrene (Figure 3-4), but not in the second set of experiments with $^{13}C_6$-α-HCH/α-HCH (Figure 3-5), was due to the use of a ^{13}C-labeled compound as the isotopically heavy component in the latter experiments, which may not undergo the same degree of fractionation during the distillation process as a perdeuterated compound. This hypothesis was supported by subsequent experiments that evaluated the fractionation of D_6-α-HCH/α-HCH and $^{13}C_6$-phenanthrene/phenanthrene. In this case, significant fractionation of the D-labeled α-HCH relative to α-HCH was observed (Figure 3-6a). In addition, no significant fractionation of ^{13}C-labeled phenanthrene relative to unlabeled phenanthrene was observed until both of these compounds reached the 0 °C bottle. This late fractionation for $^{13}C_6$-phenanthrene/phenanthrene (Figure 3-6b) as opposed to no fractionation for $^{13}C_6$-α-HCH/α-HCH (Figure 3-5) may be related to a differential effect of temperature on the vapor pressures, and hence air/water partitioning of the isotopically heavy and light compounds (Balabane and Letolle, 1985). Furthermore, $^{13}C_6$-phenanthrene/phenanthrene showed an increase in heavy:light isotope ratio with decreasing temperature (Figure 3-6b), in contrast to the decreasing trend in ratio compared with the warmest bottle as was observed for D_6-α-HCH/α-HCH (Figure 3-6a) and D_{10}-phenanthrene/phenanthrene (Figure 3-4). Such atypical behavior, resulting in isotope effects in the inverse direction to that of water, have been observed previously for certain organic compounds, and may be due to interactions within the complex structure of the molecule or to higher vapor pressures present in the heavy isotope (Harrington *et al.*, 1999; Poulson and Drever, 1999; Slater *et al.*, 2000).

To summarize, these experiments support the primary hypothesis that compound specific stable isotope fractionation is likely to occur during the global distillation of POPs. Isotopically heavy POPs labeled with deuterium were more slowly transported through a laboratory distillation system compared to the corresponding isotopically light unlabeled POPs. Isotope fractionation was observed with higher fractions of D-labeled contaminants remaining in warmer regions (e.g. low latitude simulations), whereas the isotopically light compounds were preferentially transported via the air to colder areas typical of high latitudes. In contrast, ^{13}C-labeled POPs did not demonstrate significant fractionation relative to unlabeled compounds, with the exception of phenanthrene at very cold temperatures. This phenomenon has interesting implications for using compound specific stable isotope measurements to track the global sources and transport of POPs.

If the results hold true for larger scales, it seems likely that D-labeled POPs will fractionate during volatilization and atmospheric transport in the environment, but that ^{13}C-labeled POPs will not. If so, by releasing and tracking both stable hydrogen and carbon isotopically labeled POPs simultaneously, fractionation of the deuterium labeled components could be used to determine the distance a POP has been transported from a source, whereas measurement of the ^{13}C composition of the POP may be useful in determining its source material (provided sources have distinct ^{13}C composition) and in distinguishing from mixing of a tracer with background air. This would allow for development of a quantitative isotopic description of the long-range atmospheric transport of POPs similar to that for the global meteoric water line. Development of such a geochemical tracer technique for evaluating the sources, transport and fate of POPs could eventually help resolve trans-boundary pollutant issues.

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Table 1. Summary of experiments performed

Pollutant pair used	Type of Isotope label	Conditions	# of replicate experiments
Unlabled and artificial stable isotope labeled Phenanthrene	D	Open air/water system	2
		Closed air/water system	1
	^{13}C	Closed air/water system	2
Unlabeled artifical stable isotope labeled HCH	D	Closed air/water	2
	^{13}C	Closed air/water system	1
		Closed air-water-diatomaceous earth system	1

Figure 3-1. Laboratory distillation system.

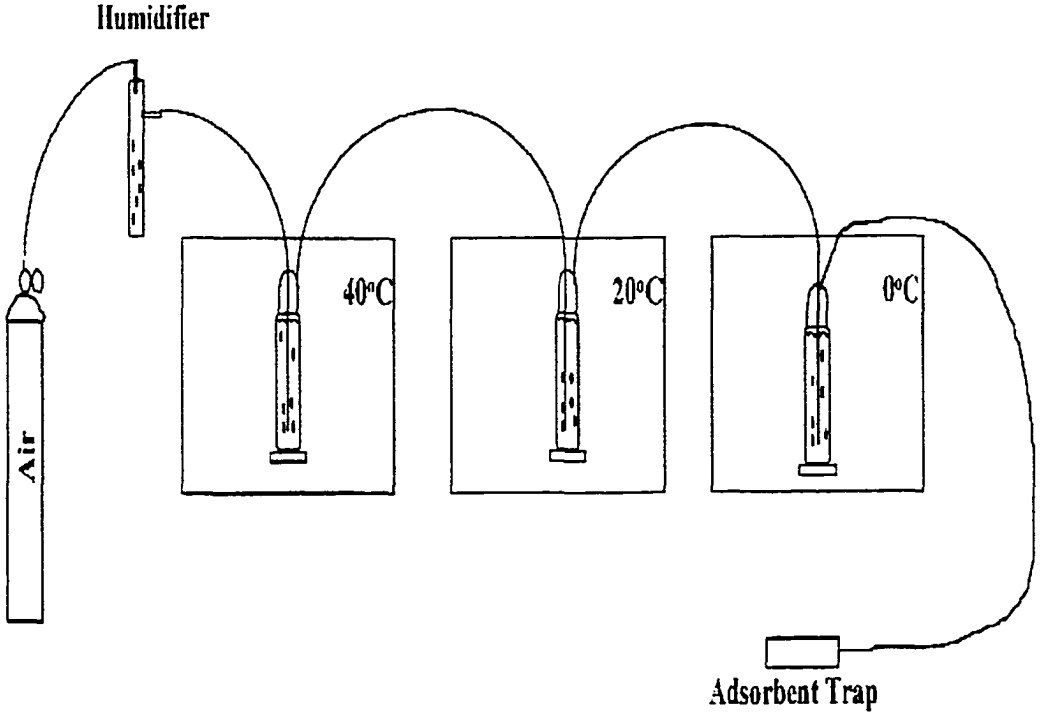


Figure 3-2. Results of an air-seawater distillation using phenanthrene and D_{10}-phenanthrene, showing the typical profile of concentration vs. time of each compound in **a:** 40°C bottle; **b**: 20°C bottle; **c**: 0°C bottle. Initial concentrations were calculated (based on the spiked standards) and not measured. Error bars around each observation represent the standard deviation of duplicate samples (always less than 0.01).

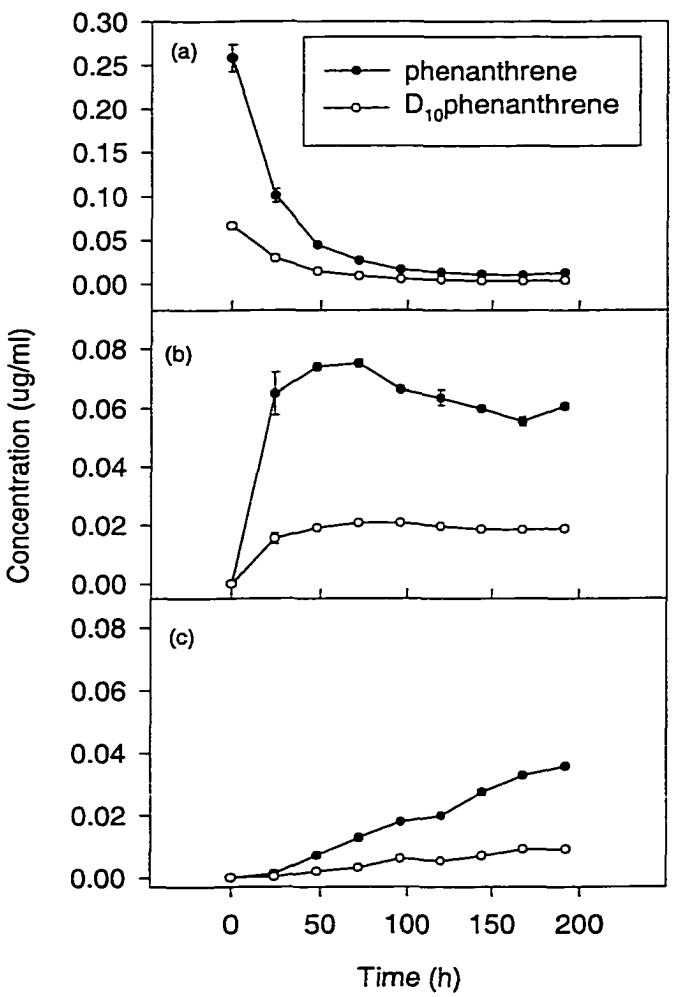


Figure 3-3. Change in heavy/light (labeled/unlabeled) $^{13}C_6\alpha$-HCH/HCH ratio for each bottle over time during a distillation experiment. Initial ratios were based on calculated concentrations of spiked standards.

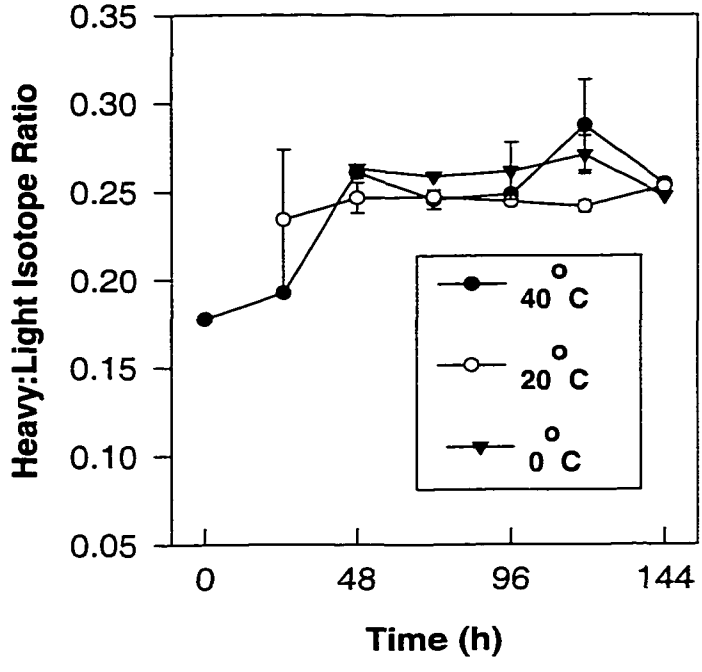


Figure 3-4. D_{10}-phenanthrene/Phenanthrene ratios after attainment of steady values in each bottle for **a**: replicate without maintaining a closed system and **b**: replicate in which a closed system was maintained. Ratios of the heavy to light isotope were significantly different among the three bottles ($p < 0.01$).

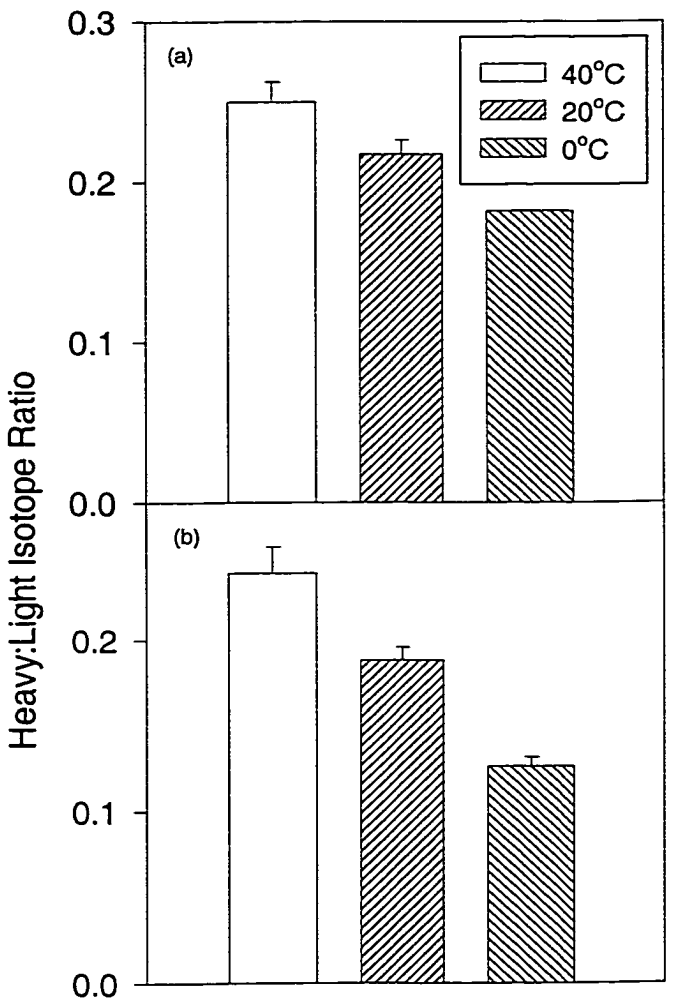


Figure 3-5. $^{13}C_6\alpha$-HCH/α-HCH ratio for **a**: air/water and **b**: air/water/earth distillation simulations. The ratios for each experiment are not significantly different at the 95% confidence level.

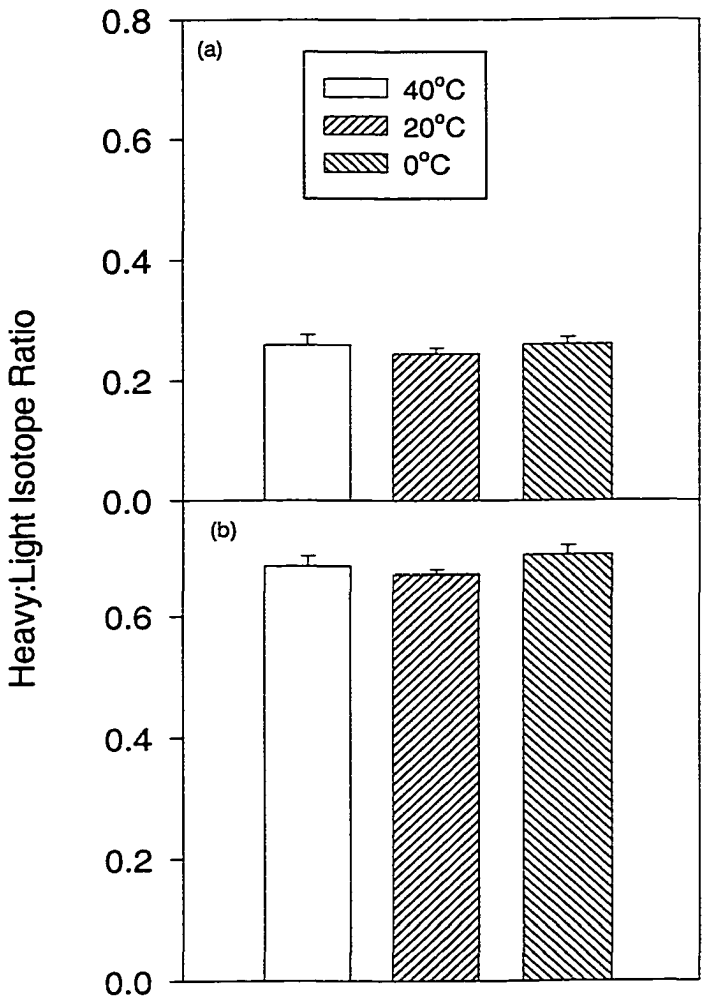
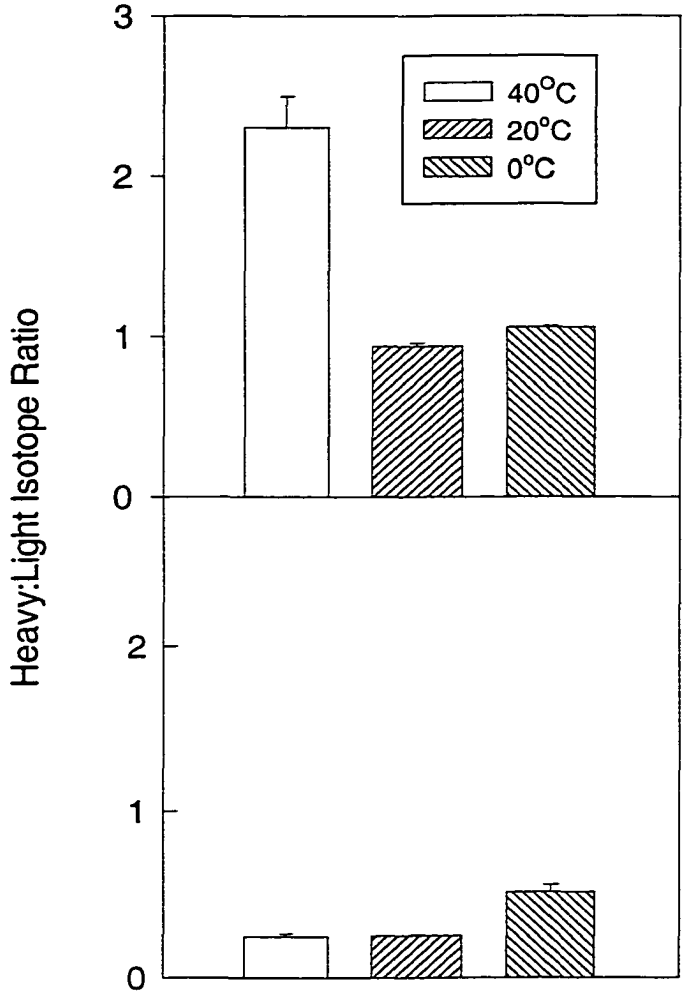


Figure 3-6. Heavy/light isotope ratios for air/seawater distillation simulations of **a**: D_6α-HCH relative to unlabeled α-HCH and **b**: $^{13}C_6$-labeled phenanthrene relative to unlabeled phenanthrene. The heavy: light isotope ratio in the 40 oC bottle is significantly different from the 20 oC and 0 oC bottles for **a**: the experiment with perdeuterated α-HCH. In contrast, the 0 oC bottle is significantly different from the 40 oC and 20 oC bottle for the experiment with $^{13}C_6$-labeled phenanthrene (**b**).



Chapter 4.

SPATIAL AND TEMPORAL VARIATION IN HEXACHLOROCYCLOHEXANE ISOMERS IN A TEMPERATE ESTUARY

ABSTRACT

Hexachlorocylcohexanes (HCHs) are pesticides that persist in air and water of the Northern hemisphere. To understand the spatial and temporal variability in HCH levels in estuarine surface waters I measured concentrations of two HCH isomers (α-HCH and γ-HCH) at six sites in the York River estuary at bimonthly intervals for a year. Bacterial abundance and activity were also monitored using acridine orange direct counts and uptake of tritiated substrates, respectively. α-HCH was consistently observed to be significantly higher in marine water compared to river water entering the estuary suggesting that the Chesapeake Bay is a larger source of this compound to the York River estuary compared to riverine input. Moreover, following periods of high freshwater flow into the estuary during spring and early summer, both α- and γ-HCH mixing curves indicated an additional source of these pollutants to the estuary such as land-derived runoff or groundwater discharge. In contrast, during low freshwater flow (late summer and fall) the estuary was a sink for HCHs, with γ-HCH more rapidly removed from the estuary than α-HCH. During the period of low freshwater flow, concentrations of both α- and γ-HCH were inversely correlated with bacterial activity. Bacterial activity as opposed to abundance appears to control HCH degradation in estuarine surface waters.

INTRODUCTION

Hexachlorocyclohexanes (HCHs) are neurotoxic pesticides (Rosa *et al.*, 1996) that may also affect the functioning of the blood, liver and kidneys (van Velsen *et al.*, 1986; Srinivasan *et al.*, 1991; Ceron *et al.*, 1995). The technical mixture of HCH, which contains about 60-70% α-HCH, 10-12% γ-HCH, and smaller amounts of β-HCH and δ-HCH, has been banned in the United States and Canada since the 1970's. However, pure γ-HCH (also called lindane) is still used in the United States.

Over the past decade, HCH isomers have been the most abundant organochlorine pesticides observed in air and water in northern latitudes (Hinckley *et al.*, 1991; Iwata *et al.*, 1993, Simonich and Hites, 1995; Willett *et al.*, 1998). The high concentrations seen in North American waters are thought to be due to continued input via atmospheric transport and deposition from remote sources such as Asia (Johnson *et al.*, 1990). Gas scavenging and rainout are likely to be the primary atmospheric removal processes for HCHs due to their high volatility and solubility (Johnson *et al.*, 1990, Cotham and Bidleman, 1991).

Although HCHs are considered persistent organic pollutants, microbial degradation is a primary mechanism for removal of these xenobiotic compounds from the environment. Aerobic degradation of α-HCH and γ-HCH by bacteria has been documented in a variety of environments (e.g. Bachmann *et al.*, 1988). Ludwig *et al.*, 1992 have also demonstrated enantioselective degradation of α-HCH, the only chiral HCH isomer, by marine bacteria in the laboratory.

I examined seasonal and spatial trends in α- and γ-HCH concentrations in the surface

waters of the York River, a temperate sub-estuary of the Chesapeake Bay (VA, USA) bimonthly for a year. Air concentrations of α- and γ-HCH were also measured to help determine the rates of atmospheric deposition or efflux of HCH isomers from the estuary. Microbial (bacterioplankton abundance and activity) and physical (temperature and salinity) parameters that could alter the partitioning, transport and transformation processes to which HCHs are subjected in this mid-latitude estuary were also examined. The objective of my study was to determine the major processes driving HCH input to and removal from the York River estuary.

METHODS AND MATERIALS

Sample collection and analysis

Surface water samples (36 L) were collected approximately bimonthly from June 1998 through April 1999 into clean stainless steel tanks from a depth of 1m using a high volume peristaltic pump at each of six sites (except in August, when replicate samples were collected at stations 2, 4, and 6) along the York River (Figure 4-1). In June 1998, bottom waters (1 m above the bottom) were also sampled at the same six stations, twenty days after the June surface samples were collected. Samples were processed immediately after return to the laboratory (within 10 hours of collection). The tanks were pressurized with ultra-high purity nitrogen and the water was filtered through a glass fiber filter (Gelman Type A/E) onto pre-cleaned Amberlite XAD-2 resin (Gustafson and Dickhut, 1997). Particulate samples consisted of those materials that were retained by the filter and these were processed according to the

methods described by Countway (1999). The dissolved phase (collected on pre-cleaned XAD-2) consisted of materials that passed through the 1 μm pore size filter and were processed as described below.

Air was sampled at stations 2 and 6 using a high volume air sampler (General Metal Works model GPTN 1123) according to the methods described by Gustafson and Dickhut (1997) for approximately ten hours concurrent with the surface water sampling dates. Briefly, particulate atmospheric samples were collected onto a pre-weighed, pre-combusted glass fiber filter (8 in. x 10 in., Gelman type A/E), while gas phase atmospheric samples were collected on two pre-cleaned polyurethane foam (PUF) plugs (Dickhut and Gustafson, 1995). Due to oversampling and breakthrough, only air samples collected at station 6 were quantified.

The XAD-2 was Soxhlet extracted with acetone and hexane, and PUF plugs with acetone and petroleum ether, after addition of PCB 65 as a surrogate standard. The acetone fraction from the surface water samples was then back-extracted with equal amounts of hexane and hexane-extracted water. The hexane fractions were then combined and reduced in volume using rotary evaporation. After sample clean-up using silica column chromatography (Dickhut and Gustafson, 1995) and further solvent reduction, the samples were analyzed using a gas chromatograph equipped with an electron capture detector (GC-ECD). All samples were analyzed using a β-dex 120 chiral column (Supelco) following the temperature program described by Falconer *et al.* (1995). GC-ECD performance was checked daily by monitoring the relative retention times and areas of standards at concentrations bracketing those in the samples. Each α-HCH enantiomer ((+) α-HCH and (-) α-HCH) and γ-HCH was quantified

relative to the surrogate standard. Results for the total α-HCH (∑(+) α-HCH,(-) α-HCH) are reported here; α-HCH enantiomer ratios are reported elsewhere.

Subsequent to GC-ECD analysis, perdeuterated α-HCH was added as an internal standard to a third of the samples and compound identifications were verified using negative chemical ionization mass spectrometry. Surrogate standard recoveries were greater than 70% in all cases.

Microbial parameters

Concurrent measurements of bacterioplankton abundance and activity were made between September 1998 and April 1999. Samples were processed in the manner described by Shiah and Ducklow (1997). Briefly, samples were preserved with a 25% glutaraldehyde solution (Sigma) prefiltered through a 0.2 μm filter such that the final concentration of the preserved sample was 2% glutaraldehyde. Samples were stored at 4 °C and slides were prepared within seven days of collection. Two to six ml of well-mixed sample were filtered onto 0.2 μm irgalan black stained polycarbonate filters (Poretics), and stained with a solution (final concentration 0.005%) of acridine orange (Sigma), while filtering. Filters were mounted on a glass slide in Resolve immersion oil and frozen until they were counted. Bacterioplankton numbers were estimated by acridine orange direct counts using epifluoresence microscopy (Hobbie *et al.*, 1977). A Zeiss Axiophot microscope at 1613 x magnification with a blue BP 450-490 excitation filter and an LP-520 barrier filter was used.

Bacterioplankton activity was estimated based on bacterioplankton incorporation of

tritiated methyl thymidine (^{3}H-TdR) and tritiated (4,5-^{3}H)-leucine (^{3}H-Leu) substrates. ^{3}H-TdR is incorporated into bacterial DNA and is an indicator of cell division and growth. ^{3}H-Leu incorporation indicates protein synthesis. Samples were processed according to Schultz (1999). ^{3}H-TdR (Dupont-NEN, specific activity approximately 80 Ci/mmol) and ^{3}H-Leu (Dupont-NEN, specific activity approximately 180 Ci/mmol) were added to centrifuge tubes prior to each cruise and stored on ice during the cruise. At each station, three replicate ^{3}H-TdR and three replicate ^{3}H-Leu tubes along with one killed control were incubated with 1.7 ml of well-mixed water sample. Killed controls consisted of a blank to which 100 μl of cold 100% trichloroacetic acid (TCA) had been added. Samples were incubated in an insulated water bath at near in situ temperatures for about 1 hour and then stopped by addition of 100 μl of cold 100% TCA. Samples were placed on ice and processed immediately upon return to the lab which was always within 7 hours.

RESULTS AND DISCUSSION

HCH concentrations

Total dissolved concentrations of the HCHs in York River surface and bottom waters ranged from tens to hundreds of pg/L (Figures 4-2, 4-3 and 4-4), which is within the range seen in previous studies in temperate and arctic latitudes (Falconer *et al.*, 1995; Jantunen and Bidleman, 1996; Ridal *et al.*, 1997). In addition, previous studies in lakes and seawater have found that HCHs were undetectable in particle samples (Jantunen and Bidleman, 1996); however, since the York River has a high concentration of suspended particulate matter, the

surface water particle samples were monitored for HCHs. As in previous studies, HCHs associated with suspended particulate matter in the York River were not quantifiable.

In all months sampled, α-HCH concentrations were significantly lower ($p<0.05$, paired t-test) in the freshwater regions of the York River compared with the marine endmember (Figure 4-2, 4-4). Since seawater enters the York River estuary at the mouth, forming a bottom layer of high salinity water below the freshwater runoff from land (estuarine circulation), greater concentrations of α-HCH in bottom waters at the mouth versus the head of the estuary would be expected if seawater is a source of this pesticide to the York River. Consequently, it appears that Chesapeake Bay or the coastal ocean is a larger source of α-HCH to the York River estuary compared to freshwater. However, it remains to be determined whether the elevated levels of this pesticide that currently enter the York River with saline water are derived from the Susquehanna River and other tributaries of the Chesapeake Bay or from levels built up over time in ocean water.

In contrast, γ-HCH concentrations in the freshwater region of the York River estuary were significantly higher ($p= 0.025$, paired t-test) compared to those in marine water following periods of higher river discharge respective to the overall discharge during the sampling period (June 1998, February and April 1999; Figures 4-3, 4-4, and 4-5), whereas the opposite trend was observed during periods of little to no river discharge (August - November 1998; Figures 4-3 and 4-5). This implicates freshwater input as a major source of γ-HCH to the York River estuary.

Since γ-HCH is still used in the United States, it is not surprising to observe input of this

pesticide through freshwater runoff. Moreover, the ratio of α-HCH/γ-HCH ranges generally from 4-7 in the technical HCH mixture, which is no longer used in the U.S., but was always lower than this in every sample taken in my study. These lower α-HCH/γ-HCH ratios have been observed in previous studies and are considered to be indicative of local γ-HCH use and input (Chernyak *et al.*, 1995, Lane *et al.*, 1992).

Concentrations of both α- and γ-HCH in surface waters of the York River estuary deviated from those expected based on conservative mixing, indicating both additional sources, as well as losses of these pesticides within the York River estuary, depending upon the time of year (Figures 4-2, 4-3, and 4-4). In June 1998, mixing curves for both α- and γ-HCH in surface waters of the York River estuary were convex (Figures 4-2 and 4-3), indicating an additional source of HCHs to the estuary at this time. This was also observed for γ-HCH in April, and to a much lesser extent in February 1999 (Figure 4-3). Likewise, in April, as well as February 1999 an additional source of α-HCH is apparent in the upper estuary, although the lower estuary appears to be a sink for α-HCH at these times (Figure 4-2). In contrast to surface waters, in later June 1998, the mixing curve for α-HCH in bottom waters indicated nearly conservative mixing , whereas the mixing curve for γ-HCH in bottom waters showed increases relative to expected concentrations at station 5 (Figure 4-4). Station 5 was located just downstream of where the waters of the Mattaponi, a second tributary of the York River estuary, enters into the system (Figure 4-1), adding approximately 37% to the total water flow during this period (Figure 4-5). Since land-use in both the Mattaponi and Pamunkey river watersheds, is similar (Bilkovic, 2000; Raymond and Bauer, 2001; US EPA Multi-resolution

land cover data set, 1996), assuming that the source contributions to both rivers are also similar, this added influx of water from the Mattaponi, could potentially account for the observed increase (by approximately a third of the concentration at station 5) in γ-HCH concentrations.

Influx of HCHs via air-water gas deposition was investigated as a possible source of these compounds to the York River estuary during spring and early summer. The direction of air-water gas exchange flux was determined using the fugacity ratio (FR):

$$FR = F_w/F_a = C_wH/C_aRT$$

where F_w and F_a are the compound fugacities in water and air, C_w and C_a are the dissolved concentration in water and gas concentration in air, respectively, R is the universal gas constant, T (K) is the air temperature, and H is the temperature specific Henry's law constant of the compound (Jantunen and Bidleman, 1995). Instantaneous gas exchange fluxes (J_{vol}) were also calculated using the two-film model for gas exchange (Liss and Slater, 1974). Since exchange of HCHs is under gas phase control, the overall mass transfer coefficient K_{ol} may be considered to be equal to the air side mass transfer coefficient K_a (Jantunen and Bidleman, 1995). Hence,

$$J_{vol} = (F_w\text{-}F_a)D_{aw}$$

where $D_{aw}=K_a/RT$ and K_a is calculated as a function of windspeed (McConnell *et al.*, 1993).

Fugacity ratios less than 1 indicate gas deposition fluxes into the York River estuary of γ-HCH, and to a lesser extent α-HCH, during June 1998, as well as February and April 1999 (Figures 4-6 and 4-7), when mixing curves (Figures 4-2 and 4-3) indicate additional sources of

these compounds to the system. However, gas deposition fluxes are insufficient to support the increased levels of γ- and α-HCH observed in the estuary at these times. For example, using an average volume and surface area of the York River estuary of $1055(10)^6$ m^3 and $211(10)^6$ m^2, respectively (Cronin, 1971), a gas deposition flux of 3.4 ng/m^2d as measured for γ-HCH during June 1998 would increase the dissolved load of this pesticide by only 0.7 pg L^{-1} d^{-1}. Thus, a sustained gas deposition loading of this magnitude would be required for more than two months to increase the γ-HCH concentration from 100 to 175 pg L^{-1}, the increase in γ-HCH observed between 0 and 6 psu during June 1998. As the residence time for water in the York River ranges from about a week to a maximum of two months (Schultz 1999, Sin *et al.*, 1999), sources other than gas deposition loading must be responsible for the observed increases of both α- and γ-HCH concentrations in the York River estuary during June 1998, as well as February and April 1999 (Figures 4-2 and 4-3).

Analysis of river discharge and precipitation data (Figures 4-5 and 4-8) demonstrate that the June 1998, as well as February and April 1999 samples were collected immediately following periods of higher rainfall and freshwater discharge into the estuary relative to late summer and fall samples. Thus, it is possible that atmospheric washout and subsequent runoff or groundwater discharge into the York River are additional sources of HCHs to the estuary following periods of prolonged precipitation. Wet deposition fluxes were calculated using air concentrations (C_a), compound specific Henry's law constants (Kucklick *et al.*, 1991), and rainfall intensity data. First, the gas scavenging ratios (W_gs) were calculated using the relationship: $W_g = RT / H$ (Cotham and Bidleman, 1991). The gas washout flux (J_{gwo}) was then

calculated for each compound using the equation: $J_{gwo} = W_g I C_a$, where I is the monthly average of rainfall intensity (Virginia institute of Marine Science archives). Based on these calculations, higher gas washout fluxes of α- and γ-HCH were observed in May 1998, and January and March 1999, immediately preceding the June 1998, and February and August 1999 sampling periods (Figure 4-9). However, the wet deposition flux to of γ-HCH during May 1998 was only 20% of the observed gas deposition flux to the York River estuary at this time (June 1998-Figure 4-7). Nonetheless, since the watershed area of the York River is approximately 7×10^9 m^2, which is about 35 times greater than the surface area of the estuary, gas washout to the watershed exceeded direct gas deposition to the estuary during May-June 1998.

Assuming efficient transfer of HCHs in wet deposition to the estuary via runoff and groundwater discharge, washout during May 1998 could have raised levels of γ-HCH in surface waters by up to 4 pg L^{-1} d^{-1}. In this case, increases in the pesticide levels in the York River could be observed on time scales similar to the hydraulic residence time for the estuary.

During August, September and November 1998, loss of HCHs within the estuary is indicated by the concave shape of the HCH mixing curves (Figures 4-2 and 4-3). Removal of α-HCH is apparent throughout this time, whereas loss of γ-HCH from the York River is most pronounced in August 1998, with only slight removal observed during September and November 1998. Moreover, this period of late summer to fall was also characterized by little freshwater flow into the estuary (Figures 4-5 and 4-8). Low river discharge rates, precipitation-derived runoff and groundwater discharge will increase the residence time of HCHs in theYork River estuary, and may promote their removal from the system.

Possible reasons for the observed mid-estuary depletion in HCH surface water concentrations during late summer and fall include particle scavenging, volatilization and biodegradation. However, since HCHs were not detectable in suspended particle samples, the observed losses are not likely to be due to particle scavenging. Likewise, previous studies have also shown that α- and γ-HCH are not very particle reactive (Jantunen and Bidleman, 1996). Furthermore, the lowest dissolved concentrations of both of the HCH isomers never coincided with the estuarine turbidity maximum (station 4) as would be expected if particle scavenging of HCHs were an efficient process.

Volatilization of HCHs was examined as a potential mechanism for removal of these compounds from the York River estuary during late summer and fall. Fugacity ratios greater than 1 indicate that volatilization exceeds deposition and the direction of flux is from the water into the air for both α- and γ-HCH during August, and to a lesser extent, September 1998 (Figures 4-6 and 4-7). In this case, a flux of 6 ng m^{-2} d^{-1} as observed for α-HCH during August 1998, would decrease the dissolved level of this pesticide in the York River by 1.2 pg L^{-1} d^{-1}. Thus, a sustained gas evasion at this rate for one month could account for losses of α-HCH from the estuary; however, this loss may be offset in part by the wet deposition flux, which could raise the dissolved level of the pesticide by up to 1 pg L^{-1} for the July-August period. In contrast, since deposition fluxes exceeded volatilization at all stations for both α- and γ-HCH during November 1998, and gas evasion of both of these compounds had slowed considerably by September 1998, it is unlikely that gas efflux was an important sink process for HCHs during the fall. Consequently, I propose that microbial degradation accounts for the

majority of the removal of HCHs from the York River estuary during late summer and fall.

Microbial degradation of HCHs

Bacterioplankton abundance increased non-linearly with increasing salinity throughout the sampling period (Figure 4-10). In comparing bacterioplankton abundances relative to the 1:1 conservative mixing line the influence of processes such as growth, grazing and other internal sources and sinks within the estuary may be evaluated. Bacterioplankton were depleted in the estuary relative to 1:1 mixing in most months, suggesting that there may be an internal sink for cells within the York River estuary.

Microbial activity as measured by incorporation of ^{3}H-TdR decreased as salinity increased in the York River estuary, whereas ^{3}H-Leu activity often reached maximum values at mid-estuary (Figure 4-11). Both indicators are highly sensitive and have previously been used to estimate the growth of coastal bacterioplankton in the presence of pollutants (Bauer and Capone, 1985; Hudak and Fuhrman 1988).

The spatial pattern of bacterioplankton activity along the estuary was the reverse of the abundance pattern (Figures 4-10 and 4-11). Activity of both TdR and Leu decreased non linearly along the estuary and were inversely correlated with abundance in all months sampled. This opposing trend of bacterial activity and abundance was also observed in a previous study conducted by Schultz (1999) in both surface and bottom waters of the York River in the two years prior to the current study.

Concentrations of both total α- and γ-HCH were correlated with microbial parameters

in the York River estuary during late summer and fall. HCH concentrations varied directly with bacterioplankton abundance (Figure 4-12) and inversely with microbial activity (Figure 4-13) during this period characterized by low freshwater flow. However, the correlation between bacterial abundance and HCH concentrations is unlikely to be causal, since HCH concentrations increase rather than decrease with microbial abundance. This trend is the opposite of what would be expected if microbial degradation of HCHs is controlled by bacterial abundance. Consequently, the relationship between HCH concentration and bacterial abundance is probably just due to the inverse relationship between abundance and activity within the estuary (Figures 4-10 and 4-11).

In contrast, HCH concentrations decrease with increasing microbial activity (as measured by thymidine incorporation) in the York River estuary during late summer and fall (Figure 4-13). Based on these trends, it appears that the removal of HCHs (likely due to microbial degradation) is greatest in the freshwater regions of the York River where microbial activity is high, and lowest in the marine regions of the estuary where microbial activity is low. Thus, during the late summer and early fall months when there are low river and runoff or groundwater derived inputs of freshwater, and hence, HCHs to the York River, the effect of bacterial activity on HCH concentrations in the estuary gains enhanced importance. In addition, Schultz (1999) showed that the bacterioplankton communities along the York River may be differentiated by temperature, which may also account, at least in part, for the observed seasonal variation in the relative importance of microbial degradation of HCHs in the estuary.

Finally, overall γ-HCH appears to be removed from the York River estuary more

rapidly than α-HCH. Total concentrations of γ-HCH in August 1998 were greater than α-HCH concentrations in freshwater, but became lower than α-HCH concentrations during the September 1998 sample which was taken about three weeks later (Figure 4-14). The α-HCH/γ-HCH ratio shows greater inputs of γ-HCH to the estuary during spring and summer, similar to previous studies in which seasonal inputs of γ-HCH have been observed during these periods (Brorström-Lünden *et al.*, 1994; Duinker and Hillebrand, 1979; van Zoest and van Eck, 1993). Since γ-HCH is likely to be more susceptible to bacterial breakdown than α-HCH (Buser and Muller, 1995), it may be degraded more quickly within the estuary. Thus, although γ-HCH input to the York River estuary exceeds that of α-HCH with freshwater influx during spring and early summer, γ-HCH is present at lower concentrations when biodegradation becomes the dominant sink process controlling the fate of HCHs in this system during late summer and fall. Moreover, as noted above, saline waters from Chesapeake Bay or the coastal ocean appear to be a consistently greater source of α-HCH to the York River estuary, as opposed to river water. Thus, the increased importance of salt water intrusion into the York River during periods of low fresh water flow may dampen the observed decreases in α-HCH due to microbial degradation. As temperatures and microbial activity decrease in winter, levels of both HCH isomers in the York River estuary are likely sustained by a balance between atmospheric input and microbial degradation.

CONCLUSIONS

HCHs are important chlorinated pollutants based on their toxicity and ubiquitous

distribution in the environment. During months of high river flow, overall concentrations and mixing curves for both α- and γ-HCH were indicative of additional sources to the York River estuary such as precipitation-derived runoff, groundwater discharge and influx from the Mattaponi during early spring and summer. In contrast, later summer and fall concentrations and mixing curves showed depletion of HCHs within the estuary, which appears to be due to microbial degradation. Inverse correlations between HCH concentrations and bacterial growth during late summer and fall indicate that bacterial growth and degradation are likely to be a major removal process for HCHs in estuarine surface waters. Overall, seasonal changes in the relative importance of precipitation/runoff, volatilization, and microbial degradation control the fate and transport of HCHs in this temperate estuary.

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Figure 4-1. Location of sampling sites along the York River estuary. Station 6 is located at the tidal freshwater region of the Pamunkey, a tributary of the York River estuary, and station 5 is located at the confluence of the Pamunkey and Mattaponi tributaries. The area shown lies is bounded by approximately 37.2°N to 37.5°N latitudes and 77°W to 76.3°W longitudes.

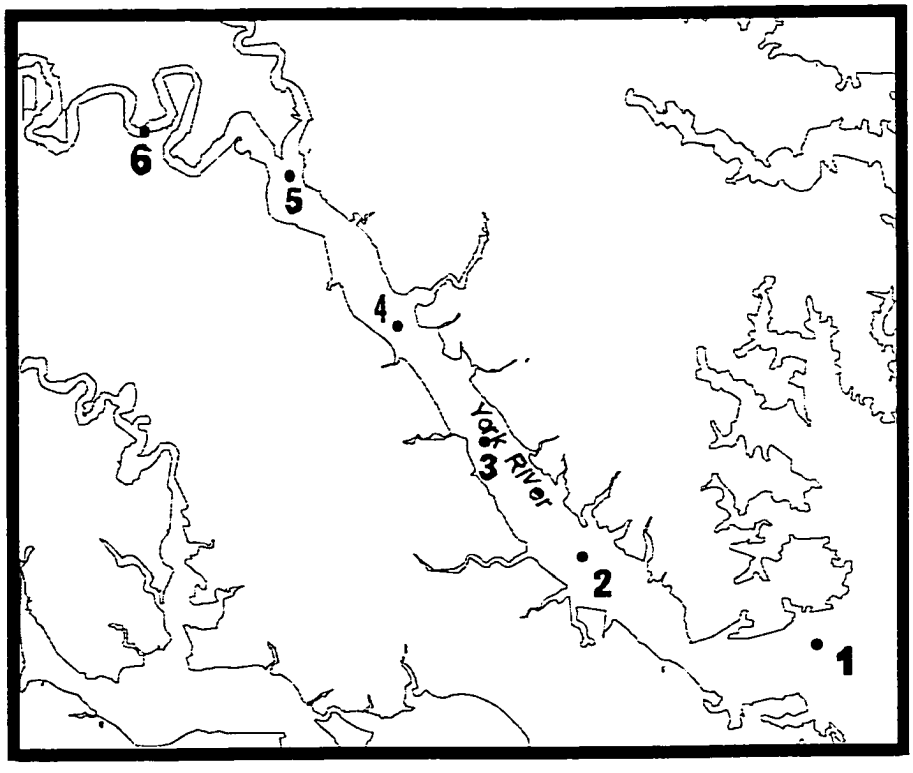


Figure 4-2. Mixing curves for α-HCH in the surface waters of the York River estuary. Station 1 coincides with the highest salinity sample and station 6 coincides with the lowest sample at all times except in Aug. 1998 when only stations 2, 4, and 6 were sampled. Dotted lines delineate the conservative (linear) mixing lines. August samples are the average values for two replicate samples; error bars illustrate the standard error for the replicate samples. In all other months, error bars represent the pooled estimate of variance based on the replicates taken in August.

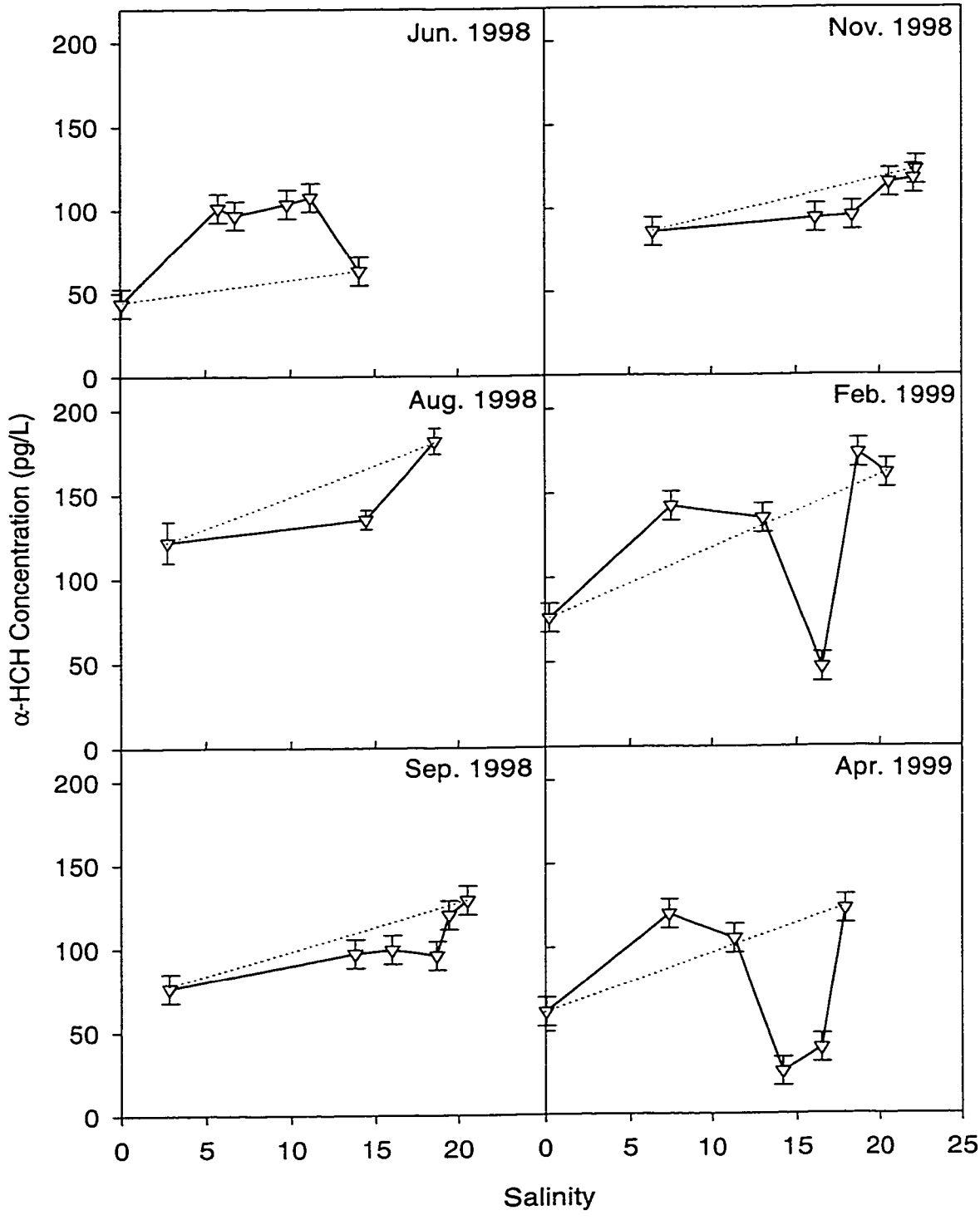


Figure 4-3. Mixing curves for γ-HCH in the surface waters of the York River estuary. Station 1 coincides with the highest salinity sample and station 6 coincides with the lowest sample at all times except in Aug. 1998 when only stations 2, 4, and 6 were sampled. Dotted lines delineate the conservative (linear) mixing lines. August samples are the average values for two replicate samples; error bars illustrate the standard error for the replicate samples. In all other months, error bars represent the pooled estimate of variance based on the replicates taken in August.

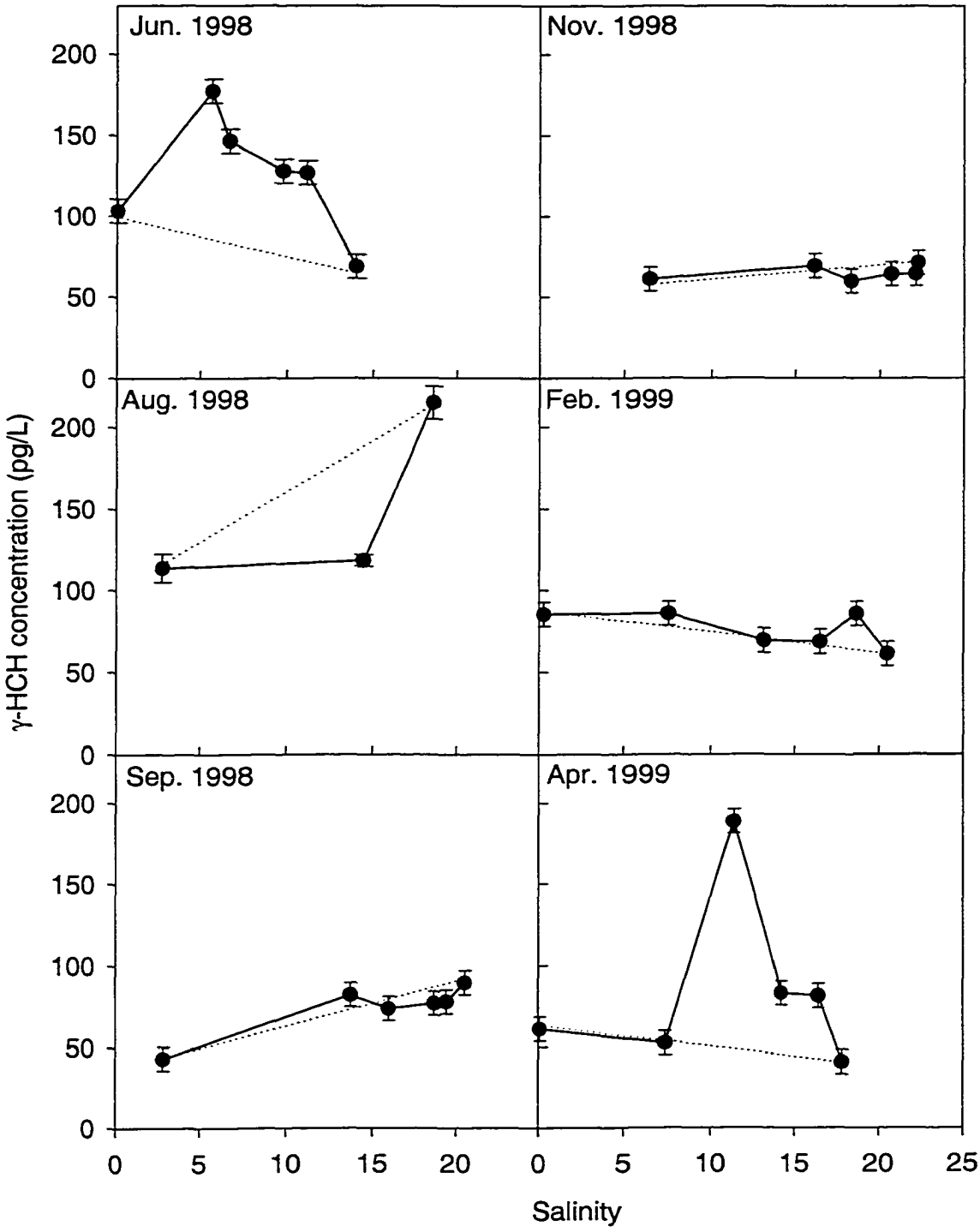


Figure 4-4. Mixing curves for α-HCH and γ-HCH in the bottom waters of the York River estuary in June 1998. Dotted lines delineate the conservative (linear) mixing lines. Data for station 1 was lost, hence data for the remaining five stations is reported. Station 2 coincides with the highest salinity and station 6 coincides with the lowest salinity. Error bars illustrate the estimated variance, based on the replicate surface water samples taken in August.

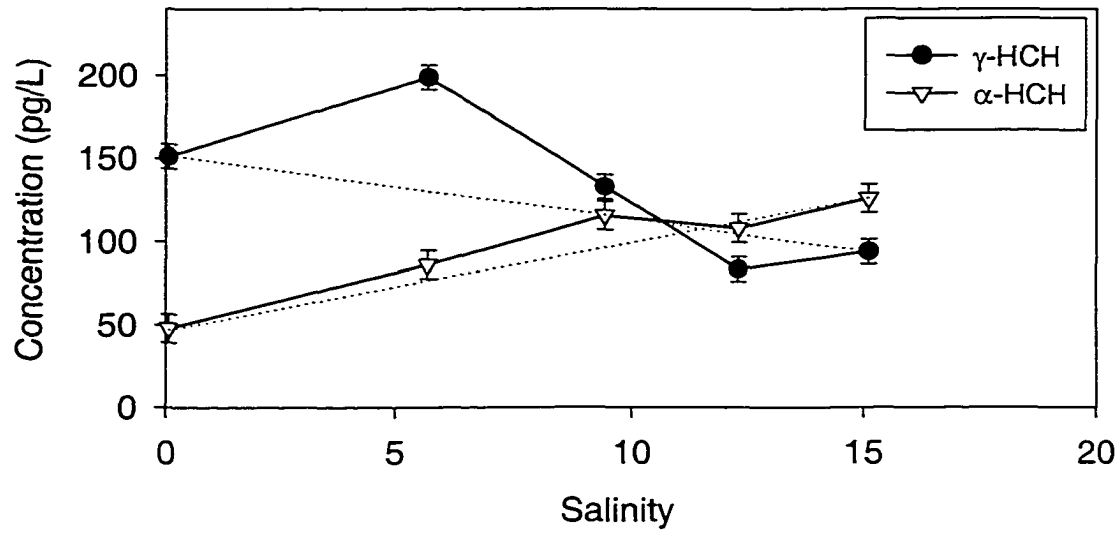


Figure 4-5. River discharge during the sampling period.

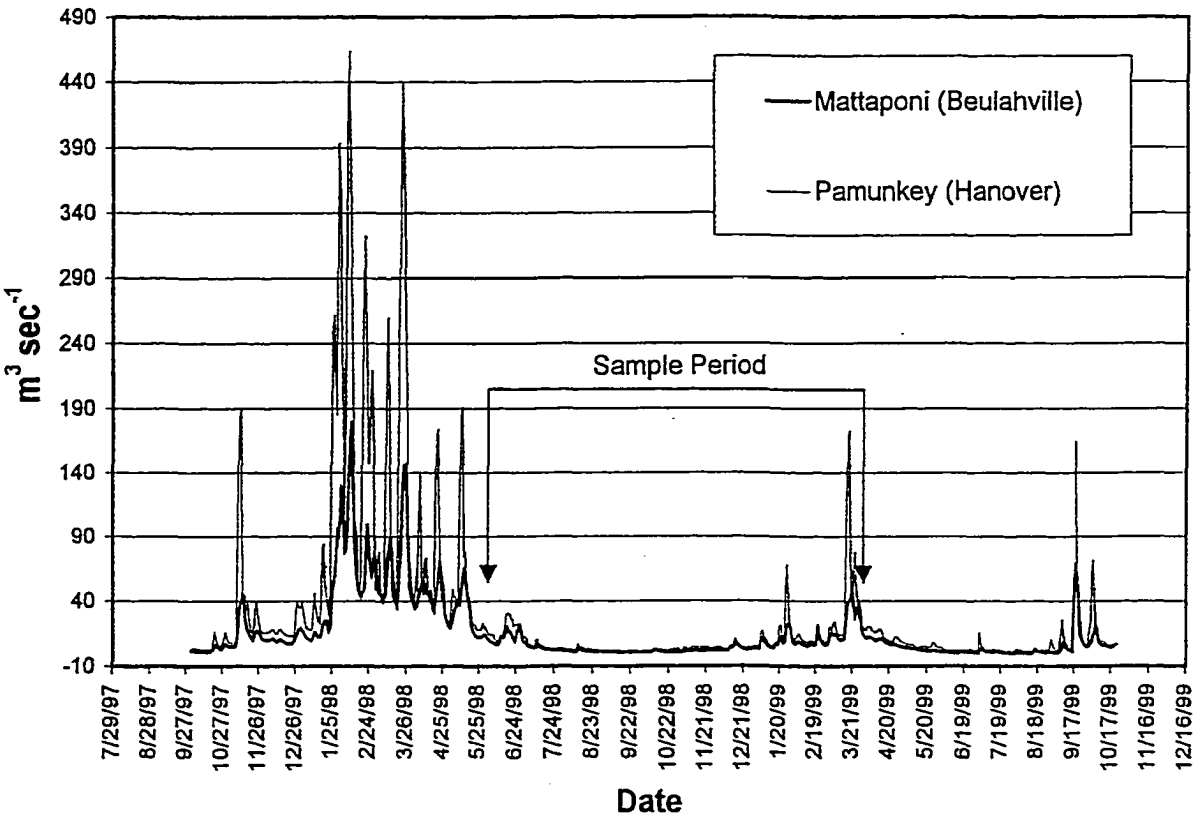


Figure 4-6. α-HCH fugacity ratios and air-water fluxes for the York River estuary between June 1998 and April 1999.

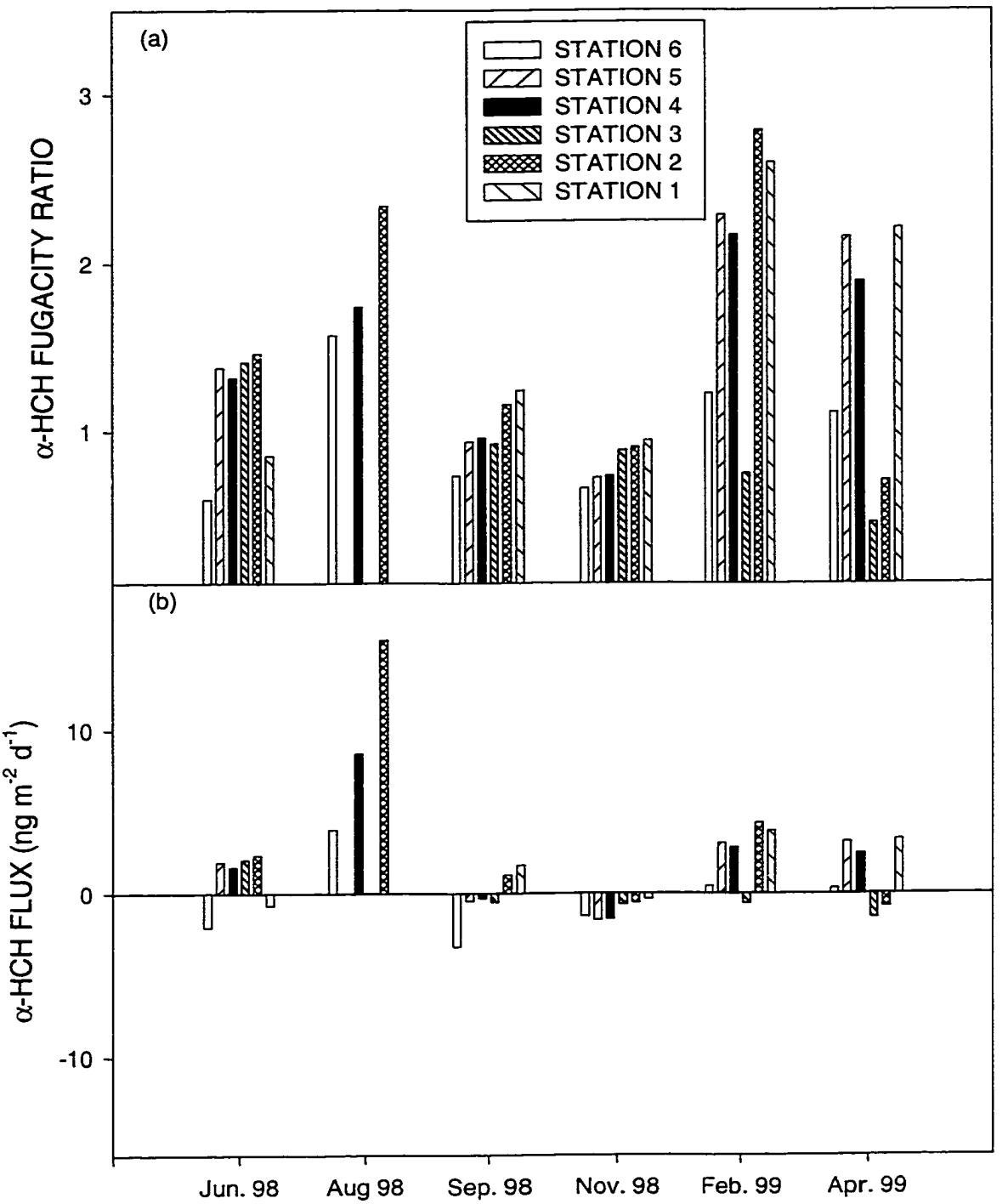


Figure 4-7. γ-HCH fugacity ratios and air-water fluxes for the York River estuary between June 1998 and April 1999.

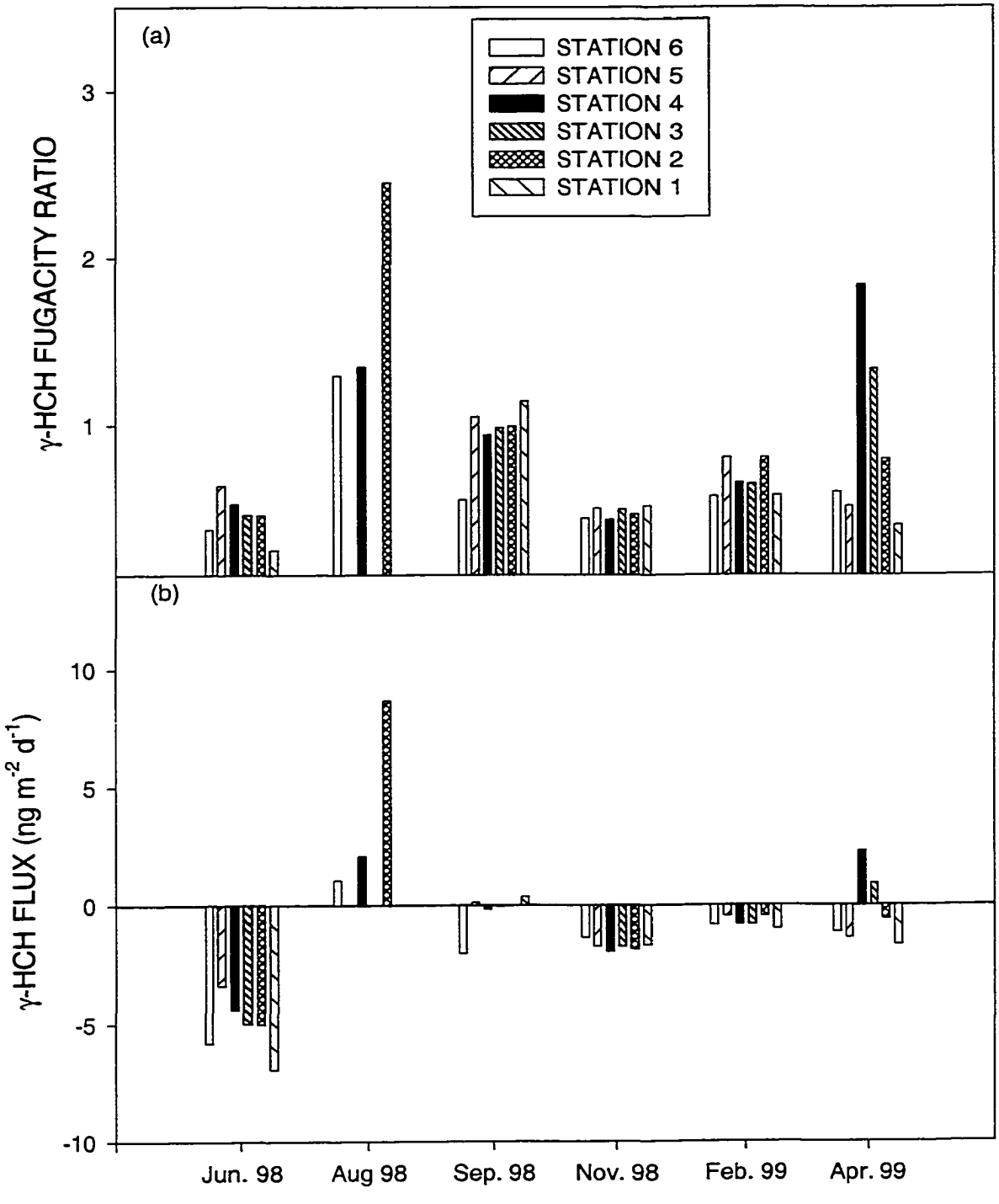


Figure 4-8. Monthly rainfall at Gloucester Point, VA during the sampling period. Arrows indicate sampling times.

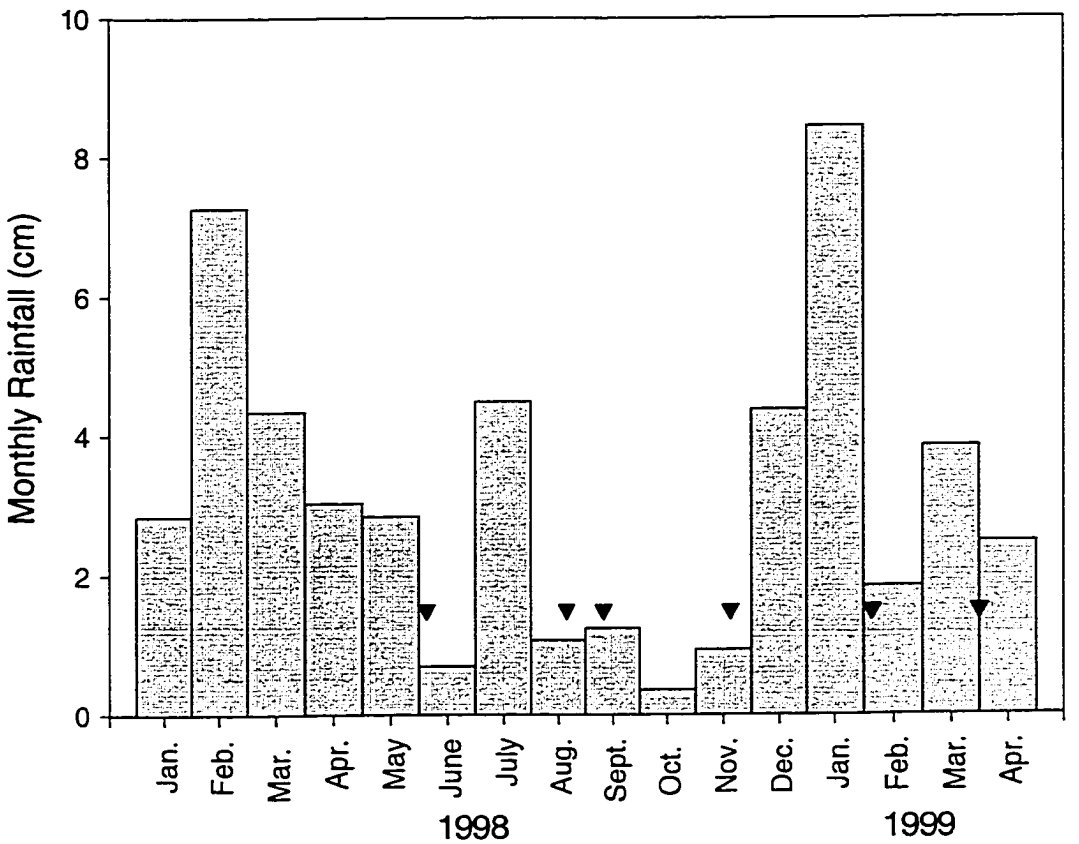


Figure 4-9. Gas washout of α- and γ-HCH at Gloucester Point, VA during the sampling period.

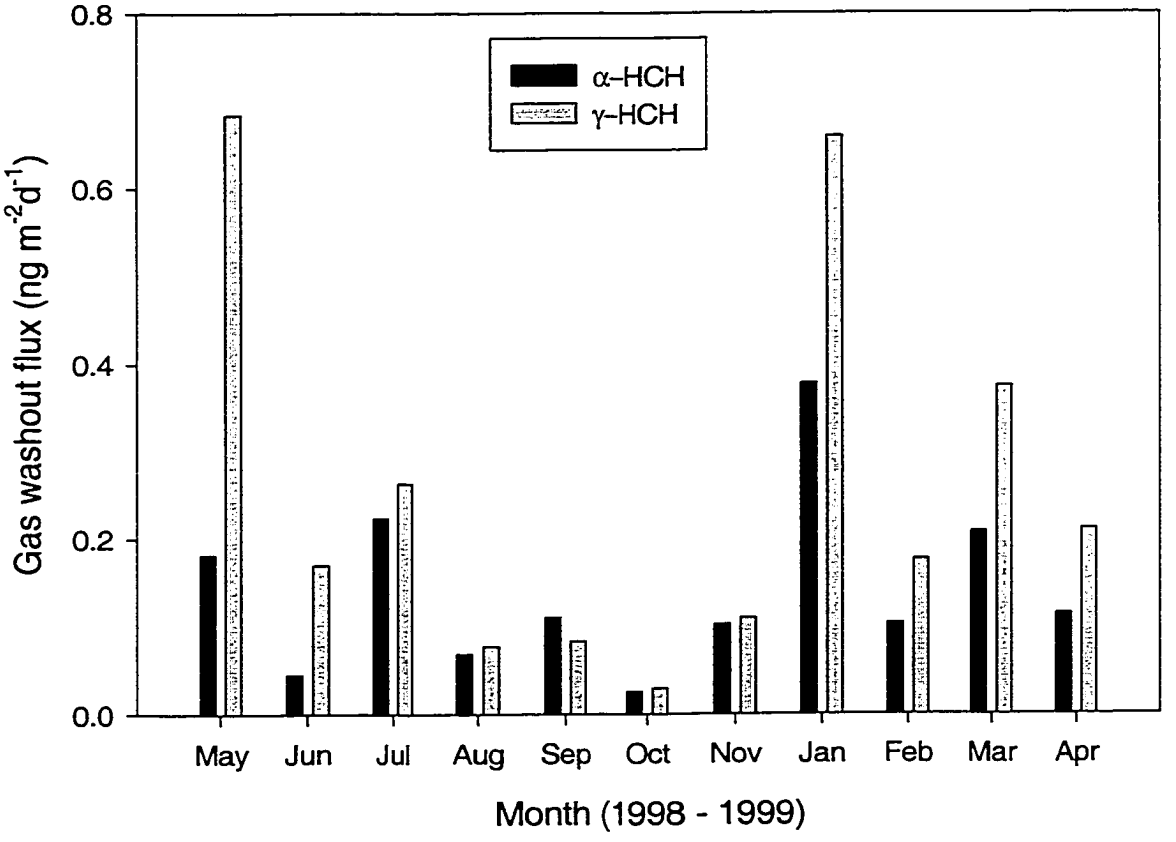


Figure 4-10. Bacterioplankton abundance in the York River estuary between September 1998 and February 1999. The dotted line indicates how bacterial abundance would change if abundance was a factor of dilution in the estuary alone (conservative mixing).

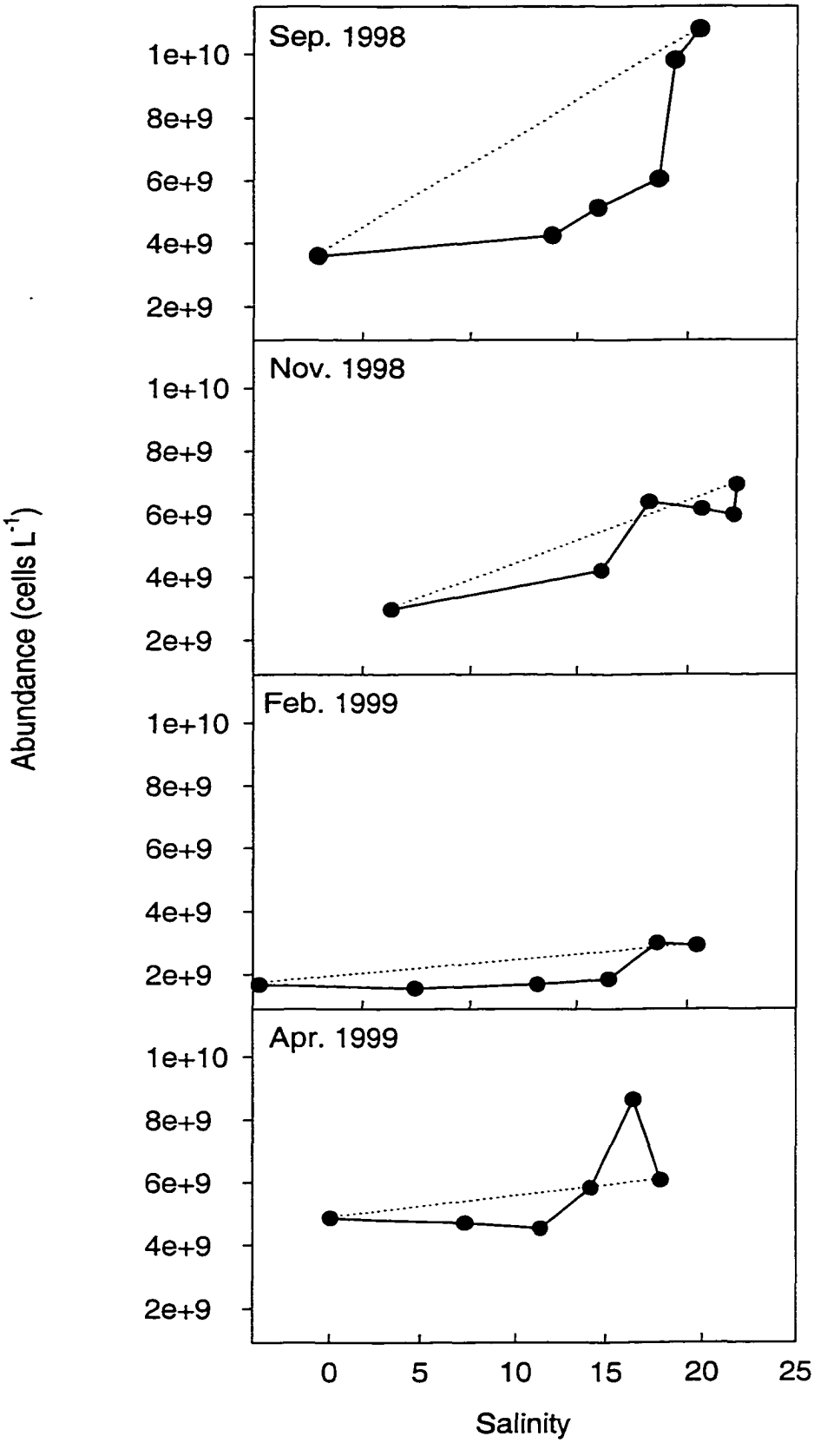


Figure 4-11. Bacterioplankton activity in the York River estuary between September 1998 and February 1999. The coefficient of variation within the triplicate samples collected for ^{3}H-TdR activity and ^{3}H- Leu activity at each station was usually lower than 10% and always lower than 20%.

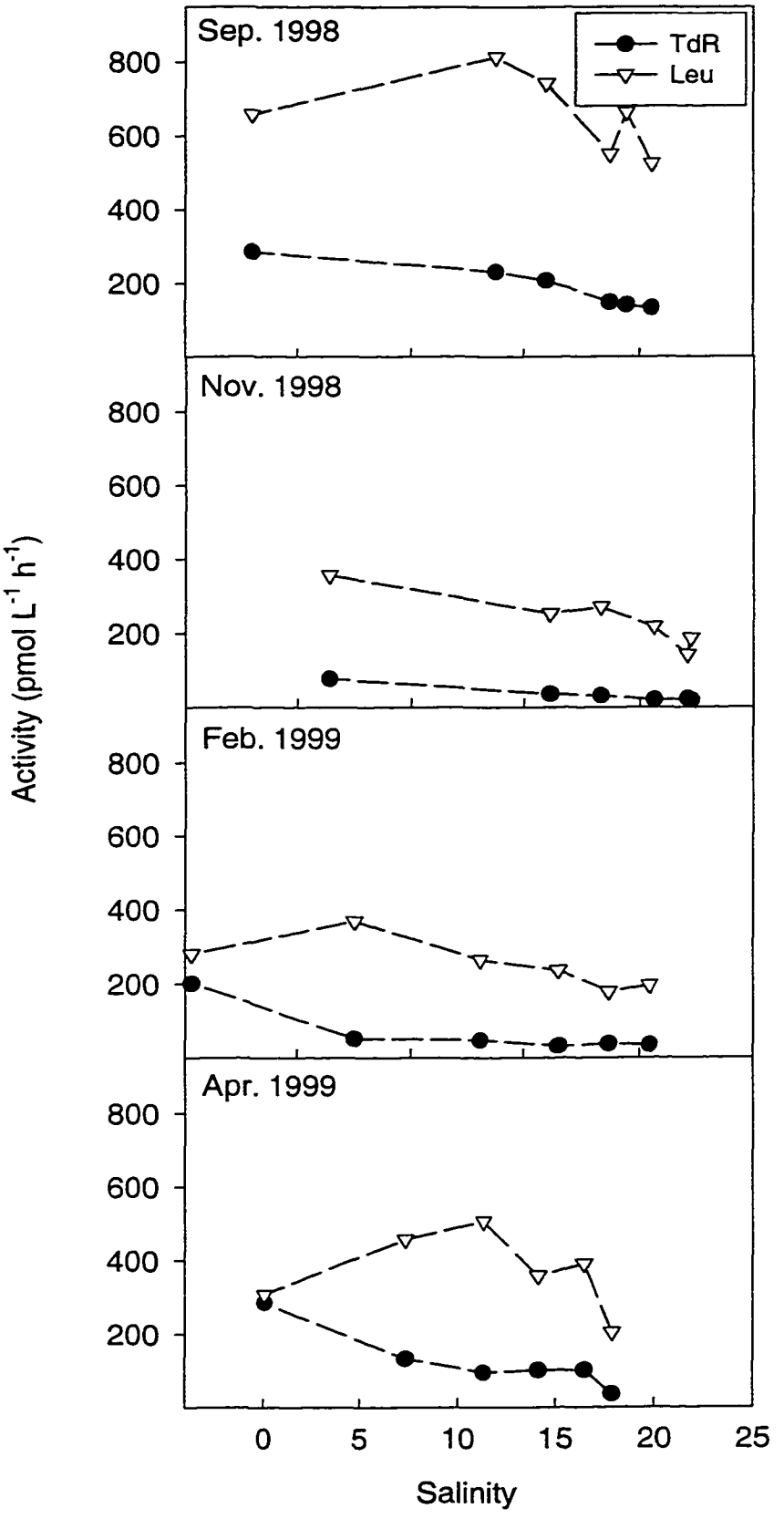


Figure 4-12. Positive correlation between HCH concentrations and bacterial abundance in the York River estuary during fall 1998.

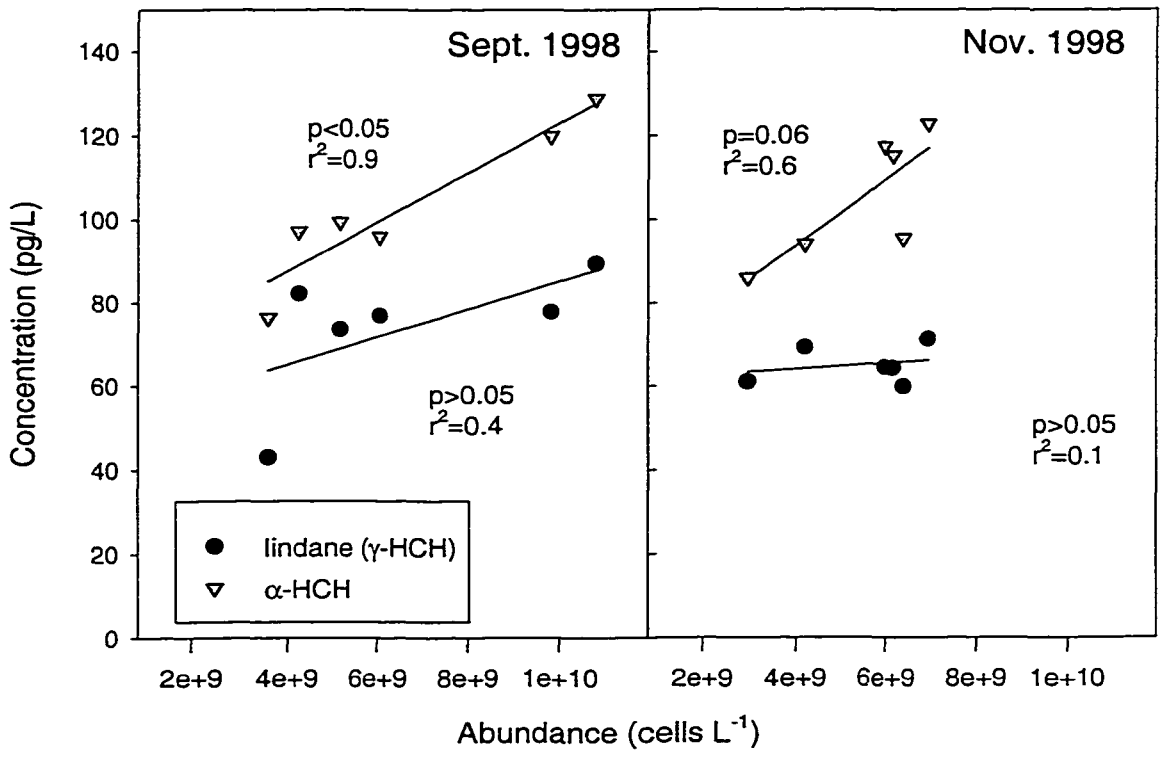
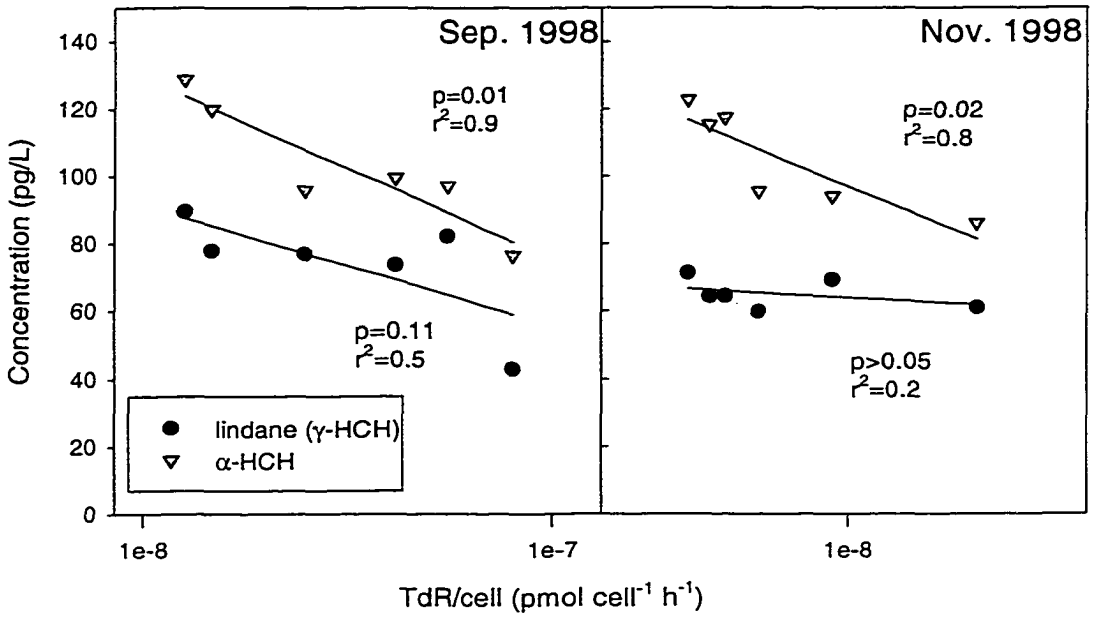


Figure 4-13. Negative correlation between HCH concentrations and microbial activity normalized to cell abundance (plotted on a logarithmic scale) in the York River estuary during fall 1998. All regressions were significant for α-HCH in both months.



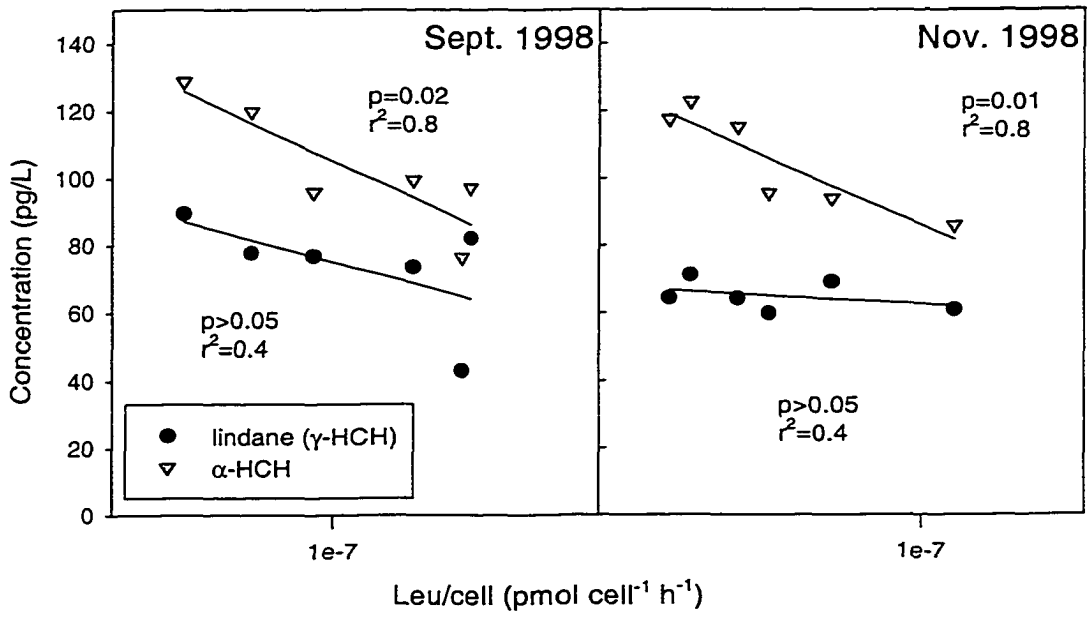
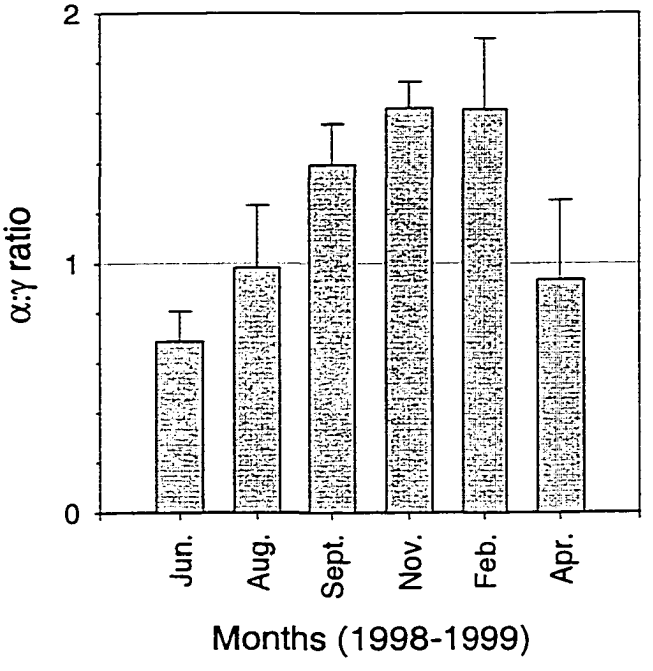


Figure 4-14. Average α/γ-HCH concentrations in the York River estuary from June 1998 to April 1999. Error bars indicate the standard error of the quotient for n = 6 measurements along the salinity gradient except during Aug. 1998 when replicate samples were collected from 3 sites.



Chapter 5.

VARIATIONS IN α-HEXACHLOROCYCLOHEXANE ENANTIOMER RATIOS IN RELATION TO MICROBIAL ACTIVITY IN A TEMPERATE ESTUARY

ABSTRACT

Changes in the enantiomer ratios (ERs) of chiral pollutants in the environment are often considered evidence of biological alteration despite the lack of data on causal or mechanistic relationships between microbial parameters and ER values. ERs that deviate from 1:1 in the environment provide evidence for the preferential microbial degradation of one enantiomer, whereas ER values equal to 1 provide no evidence for microbial degradation and may mistakenly be interpreted as evidence that biodegradation is not important. In an attempt to link biological and geochemical information related to enantioselective processes, I measured the ERs of the chiral pesticide α-hexachlorocyclohexane (α-HCH) and bacterial abundance and activity in surface waters of the York River estuary bimonthly throughout one year. Despite lower overall α-HCH concentrations, α-HCH ER values were unexpectedly close to 1:1 in the freshwater region of the estuary with the highest bacterial activity. In contrast, ER values were non-racemic (ER ≠ 1) and α-HCH concentrations were significantly higher in the higher salinity region of the estuary, where bacterial activity was lower. These data may indicate that racemic environmental ER values are not necessarily reflective of a lack of biodegradation or recent input into the environment, and that non-enantioselective biodegradation may be important in certain areas.

INTRODUCTION

Microbial degradation of organic compounds is an important biogeochemical process that may lower environmental concentrations of xenobiotics. Since biodegradation is often stereo-selective (Airens *et al.*, 1988), enantiomers (non-superimposable mirror image isomers) of chiral contaminants may be degraded at significantly different rates leading to increased environmental persistence of the non-degradable isomer. Consequently, isomeric persistence may be important since most commercial pesticide formulations are marketed as racemic mixtures (containing equal concentrations of both enantiomers), and up to 25% of pesticides manufactured may be chiral (Williams, 1996).

After Faller *et al.* (1991) achieved the first successful enantioselective analytical separation of pesticide isomers, there has been increasing evidence of non-racemic pesticide concentrations resulting from biodegradation (Buser and Muller, 1995; Ulrich and Hites, 1998; Aigner *et al.*, 1998). For example, enantioselective pesticide degradation has been observed in laboratory experiments and in the environment (Ludwig *et al.*, 1992, Lewis *et al.*, 1999). Since physicochemical processes such as volatilization, hydrolysis, and partitioning to organic matter are not enantioselective, the degree of change in a pesticides' enantiomer ratio (ER) from the original 1:1 relationship should be related to the extent to which the pesticide has been biodegraded (Ludwig *et al.*, 1992, Schwarzenbach *et al.* 1993, Aigner *et al.*, 1998). Consequently, it is often assumed that

ERs ≠ 1, indicate the significance of microbial modification of a pesticide mixture compared with other physicochemical processes. However, environmental changes such as nutrient enrichment may cause changes in enantioselectivity by bacteria sometimes leading to increased degradation of the less toxic enantiomer (Lewis *et al.*, 1999). Thus, the influence of environmental factors, particularly those that influence microbial activity, abundance, and community composition, on enantioselective degradation of chiral pollutants warrants further investigation.

Over the past decade, α-hexachlorocyclohexane (α-HCH) was among the most prevalent organochlorine contaminants in Northern hemisphere water and air (Hinckley *et al.*, 1991; Iwata *et al.*, 1993). Simonich and Hites (1995) also found that α-HCH was among the most prevalent organochlorine residues in vegetation samples collected from around the world. α-HCH is the only chiral HCH isomer, and changes in α-HCH ERs have been well documented in seawater and arctic lake water (Bidleman *et al.* 1989, Falconer *et al.* 1995), as well as in the North Sea (Huehnerfuss *et al.*, 1993) and the Great Lakes (Ridal *et al.*, 1997). Moreover, there are few published studies on α-HCH levels and ERs in mid-latitude estuaries although based on their physical-chemical properties (such as volatility), HCHs are considered likely to deposit in these areas during global atmospheric transport (Wania and Mackay, 1996).

The objective of my study was to examine links between α-HCH ER values and bacterioplankton activity in the York River, a tributary of the Chesapeake Bay in Virginia, USA. Microbial degradation rates are often related to measures of bacterial activity and abundance. For example, microbial activity is often stimulated as a pollutant

undergoes biodegradation, and this increased activity in turn, is related to higher rates of degradation (King *et al.*, 2001; Shi *et al.*, 1999; Lee *et al.*, 1996). The results presented here relate isomeric changes in α-HCH to microbial activity in a temperate estuary, which exhibits a consistent gradient of decreasing microbial activity from the head to the mouth of the estuary (Schultz and Ducklow, 2000; Chapter, 4). This effort should aid in development of the use of enantiomer ratios as a geochemical tracer for the transport and fate of chiral pollutants in the environment.

MATERIALS AND METHODS

Surface water samples (~35 L) were collected bimonthly from June 1998 through April 1999 into clean stainless steel tanks from a depth of 1m using a high volume peristaltic pump at each of six sites (except in August, when replicate samples were collected at stations 2, 4, and 6) along the York River (Figure 5-1). In June 1998, bottom waters (1 m above the bottom) were also sampled at the same six stations twenty days after the June surface samples were collected. Samples were processed immediately after return to the laboratory (within 10 hours of collection). The water samples were filtered through pre-combusted Gelman type A/E glass fiber filters and suspended particle samples consisted of compounds retained by the filter. The particle samples were extracted using a modification of the extraction procedure first described by Bligh and Dyer in 1959 (Countway, 1999), after addition of PCB 65 as a surrogate standard. The filtrate was passed over Amberlite XAD-2 resin (Rohm and Haas, Co.) to isolate

dissolved HCHs (materials that passed through the 1 μm pore size filter) and processed as described below.

Air was sampled at stations 2 and 6 using a high volume air sampler (General Metal Works model GPYN 1123) according to the methods described by Gustafson and Dickhut (1997), for approximately ten hours concurrent with surface water sampling dates. Briefly, particulate atmospheric samples were collected onto a pre-weighed, pre-combusted glass fiber filter (8 in. x 10 in., Gelman type A/E), while gas phase atmospheric samples were collected on two pre-cleaned polyurethane foam (PUF) plugs (Dickhut and Gustafson, 1995).

The XAD-2 and PUF plugs were Soxhlet extracted for 48 h with acetone followed by hexane, and acetone followed by petroleum ether, respectively, after addition of PCB 65 as a surrogate standard. The acetone fraction from the surface water samples was then back-extracted with equal amounts of hexane and hexane-extracted water. The hexane fractions were then combined and reduced in volume using rotary evaporation. After sample clean-up using silica column chromatography (Dickhut and Gustafson, 1995) and further solvent reduction, the samples were analyzed using a gas chromatograph equipped with an electron capture detector (GC-ECD). Compound identifications were also verified for a third of the samples using negative chemical ionization mass spectrometry, as described in detail elsewhere (Chapter 4). A β-dex 120 chiral column (Supelco) and the temperature program described by Falconer *et al.* (1995) were used to achieve baseline separation of the α-HCH enantiomers. Enantio-pure (99%) α-HCH standards (Ehrenstorfer Laboratories) were injected daily to determine the order of elution of the (+)

and (-) α-HCH enantiomers. GC-ECD performance was also checked daily by monitoring the relative retention times and areas of standards at concentrations bracketing those in the samples. Each α-HCH enantiomer ((+) α-HCH and (-) α-HCH) was quanitified relative to the surrogate standard. Results for the enantiomer ratios (i.e. (+) α-HCH/(-) α-HCH) are reported here.

Concurrent measurements of bacterioplankton abundance and activity in surface waters were also taken from September 1998 until April 1999. Samples were processed in the manner described by Shiah and Ducklow (1997). Bacterioplankton numbers were estimated by acridine orange direct counts using epifluoresence microscopy (Hobbie *et al.*, 1977). Bacterioplankton activity was estimated based on bacterioplankton incorporation of tritiated methyl thymidine (^{3}H-TdR) and tritiated (4,5-^{3}H)-leucine (Leu) substrates. Uptake of TdR, which is a DNA precursor provides a measure of DNA synthesis, while Leu, which is an amino acid, provides information about protein synthesis by the bacteria. Samples were processed according to Schultz (1999).

RESULTS AND DISCUSSION

In all months sampled, total α-HCH concentrations were significantly lower ($p < 0.05$, paired t-test) in freshwater (station 6) than in higher salinity water (station 1) (Figure 5-2a). α-HCH ERs were also significantly different between endmember stations (paired t test, $p<0.05$); however, the ER was closer to racemic at station 6 where α-HCH concentrations were significantly lower than at station 1 (Figure 5-2b). Overall, at nearly

all sites and in all months sampled, I found α-HCH ER values to be less than 1 (i. e. enriched in (-) α-HCH). Depletions in the relative abundance of the (+) α-HCH versus the (-) α-HCH enantiomer (ERs < 1), have also been observed in environmental samples of water, snow and air from ecosystems such as Lake Ontario, Amituk Lake and Resolute Bay, as well as in seawater (Falconer *et al.*, 1995; Jantunen and Bidleman, 1996; Ridal *et al.*, 1997). In contrast, preferential degradation of the (-) α-HCH enantiomer has been observed in certain locations (Faller *et al.*, 1991; Mueller *et al.*, 1992, Huehnerfuss *et al.*, 1993).

Detailed analysis of HCH surface water concentrations in the York River estuary relative to air-water flux, wet deposition, and particle deposition/concentrations suggested that during periods of increased precipitation (winter-spring) HCHs were added to the estuary via wet deposition and associated runoff and/or groundwater discharge, whereas during periods of low freshwater flow (late summer and fall; August-November) biodegradation is likely to be an important sink process for HCHs in the estuary (Chapter 4). During the low freshwater flow period (August-November, 1998), concentrations of α-HCH showed negative deviations from (i.e. fell below) the conservative mixing line indicative of removal within the estuary (Chapter 4). ERs for α-HCH were also non-conservative at these times, but with positive deviations from conservative mixing as modeled using the equation presented by Bidleman and Falconer (1999) (Figure 5-3). Moreover, α-HCH concentrations in York River surface waters were negatively correlated with microbial activity, suggesting increased biodegradation of α-HCH as microbial activity increases (Chapter 4). However, α-HCH ER became more racemic and

was significantly correlated with microbial activity as measured by thymidine and leucine incorporation rates (Figure 5-4).

In general, the ER of α-HCH in surface waters of the York River increased, becoming more racemic, upstream along the estuary in all seasons, despite increases in bacterial activity (Schultz and Ducklow, 2000; Chapter 4.) and decreases in overall α-HCH concentrations (Chapter 4). Based on these opposite trends in α-HCH concentration and ERs, α-HCH degradation appears to be less enantioselective (resulting in ERs closer to racemic) in the freshwater compared to the higher salinity region of the estuary.

An alternative explanation for the more racemic ER observed in freshwater relative to marine water is related to residence time. For instance, α-HCH entering the estuary at the marine station may reside in the Bay (or ocean) for longer periods compared to the α-HCH entering the estuary from freshwater. Thus, differences between the ERs observed at either end of the estuary might, in part, be a result of longer residence times at the marine end-member enabling greater enantioselective biodegradation, whereas there is less time for enantioselective degradation processes at the freshwater endmember. However, although the difference in residence time may contribute to the differences between end-member ERs, I observed deviations from expected (predicted) mixing of the ERs within the estuary, which can only be attributed to biodegradation, as explained below.

During late summer and fall, the (-) α-HCH enantiomer was preferentially degraded within the York River estuary compared to predicted distributions based on conservative mixing (Figure 5-3). In contrast, during the high flow period (winter-spring)

when HCHs were input into the estuary via atmospheric deposition, no consistent preference for degradation of either enantiomer was observed, and at these times α-HCH ERs varied almost conservatively (Figure 5-5). However, in the high flow months, interpretation of the data is complicated by the fact that α-HCH is input to the estuary from external sources (Chapter 4). Thus it is not possible to ascertain to what extent the deviations in ERs from predicted conservative mixing during the winter-spring months are caused by biodegradation versus inputs of non-racemic mixtures via groundwater or watershed runoff. However, since mixing curves for α-HCH indicate that there was overall depletion, rather than input, to the estuary during late summer and early fall (Chapter 4), the observed deviations in ERs relative to the conservative mixing line in the late summer and fall months (Figure 5-3) are not likely due to inputs from non-racemic mixtures. Hence biodegradation, which is a major sink process during these months most likely results in observed ER values that are different from those expected.

In order to explain the observed trends in α-HCH concentrations and ER values in the York River Estuary, I propose that when bacterioplankton activity is high, such as in the freshwater region of the estuary, microbes are not enantioselective. In this case, assuming first order kinetics:

$$dC / dt = -kC$$

the rate constants for biodegradation of both enantiomers of α-HCH are equal ($k_+ = k_-$). In contrast, when microbial activity is low such as at the marine end of the estuary, the (+)α-HCH enantiomer is selectively degraded ($k_+ > k_-$). Therefore, overall α-HCH

degradation is expected to be higher in the freshwater region of the estuary resulting in the observed lower concentrations of this pesticide (Figure 5-2). Moreover, since marine water, which provides an additional source of α-HCH to the estuary (Figure 5-2), is depleted in (+)α-HCH and enriched in the (-)α-HCH enantiomer ($C_+ < C_-$), the (-)α-HCH enantiomer is degraded more rapidly. This will result in more racemic ERs from the mouth to the head of the estuary, as well as positive deviations for ERs from predicted conservative mixing along the estuarine gradient if bacteria become less enantioselective with decreasing salinity (Figure 5-3). Alternatively, the microbial communities in the York River estuary may switch preferences from the (+)α-HCH enantiomer in the saline regions to the (-)α-HCH enantiomer in the freshwater regions of the estuary. This scenario would likewise result in preferential depletion of the (-)α-HCH enantiomer towards the head of the estuary.

Finally, if bacterial activity alone were responsible for enantioselectivity (i.e. assuming constant community composition), then no seasonal difference in the ER-microbial activity relationship would be observed. However, the ER for α-HCH was less racemic at a given microbial activity during September, when water temperatures were warmer, compared with November, when water temperatures were cooler (Figure 5-4). If more racemic ER values are due to less enantioselectivity, then this implies that bacteria become less enantioselective with time, or at cooler temperatures. However, ER values were not significantly correlated with temperature. Hence, it seems likely that spatial and seasonal bacterial community differences may account for the observed differences in the ER-microbial activity relationship. Indeed, microbial communities in the York River

estuary are differentiated by salinity, as well as temperature (Schultz and Ducklow, 2000).

In conclusion, opposing gradients in α-HCH concentrations and ER values were observed in the York River estuary in all seasons sampled, such that surface water ERs were inversely related to α-HCH concentrations (Figure 5-6). Unexpectedly, ER values were more racemic at the head of the estuary, where there was high bacterioplankton activity and low α-HCH concentrations, than at the mouth of the estuary where the microbial activity was lower. An important implication of the inverse relationship between enantioselectivity and overall bacterial degradation is that it may not always be possible to use ER values as tracers of pollutant transport or biodegradation. Specifically, racemic ratios of chiral pollutants may not be indicative of a lack of biodegradation. Attempts have been made to use ER values as tracers of transport distance and microbial degradation rates (e.g. Harner *et al.*,1999) based on the assumption that a minimal departure from the racemic ratio indicates that a compound has not had time to undergo significant biodegradation, and therefore, must have been recently released into the environment or else have undergone relatively rapid and small-scale transport. In light of my study, this assumption may not always be tenable.

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Figure 5-1. Location of sampling sites in the York River estuary. Station 1 was at the high salinity end and station 6 was at the upstream freshwater end of the estuary. The area shown is bounded by approximately 37.2°N to 37.5°N latitudes and 77°W to 76.3°W longitudes.

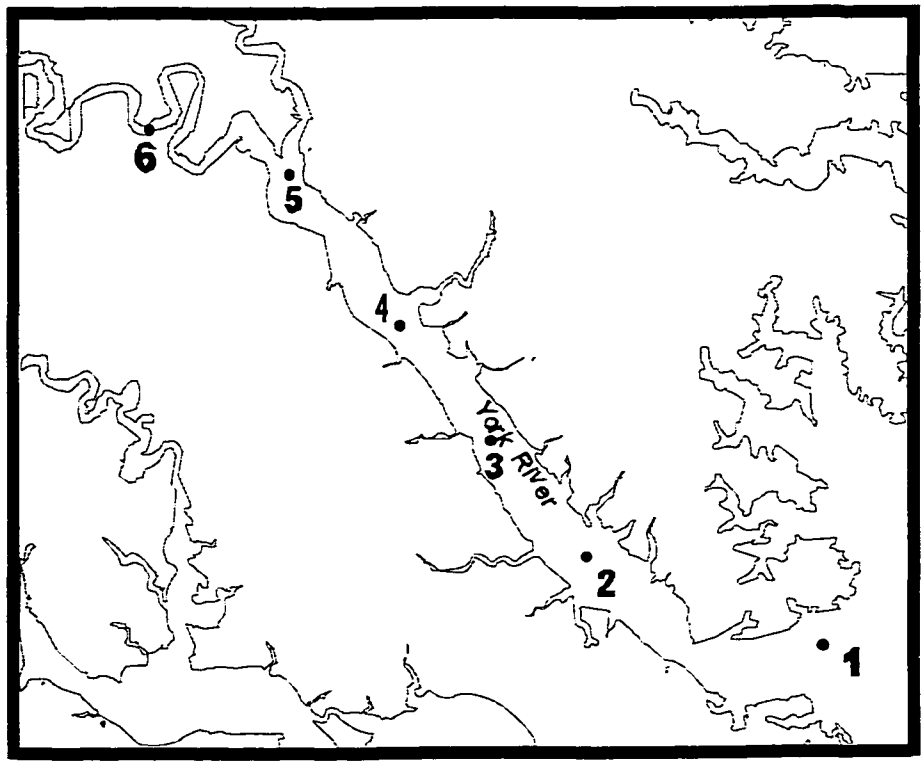


Figure 5-2 a. Total α-HCH concentrations at marine and freshwater sites. Station 1 coincides with the highest salinity sample and station 6 coincides with the lowest salinity sample at all times except in Aug. 1998 when only stations 2, 4, and 6 were sampled. Differences between Station 1 and Station 6 concentrations were significant (t-test, $p<0.05$). Error bars illustrate the pooled estimate of variance, based on duplicate samples measured in August.

Figure 5-2 b. α-HCH ER at marine and freshwater sites, showing a consistent but opposite trend compared to concentration values at the same sites. Differences between Station 1 and Station 6 ER values were significant (t-test, $p<0.05$). Error bars illustrate the pooled estimate of variance, based on duplicate samples measured in August.

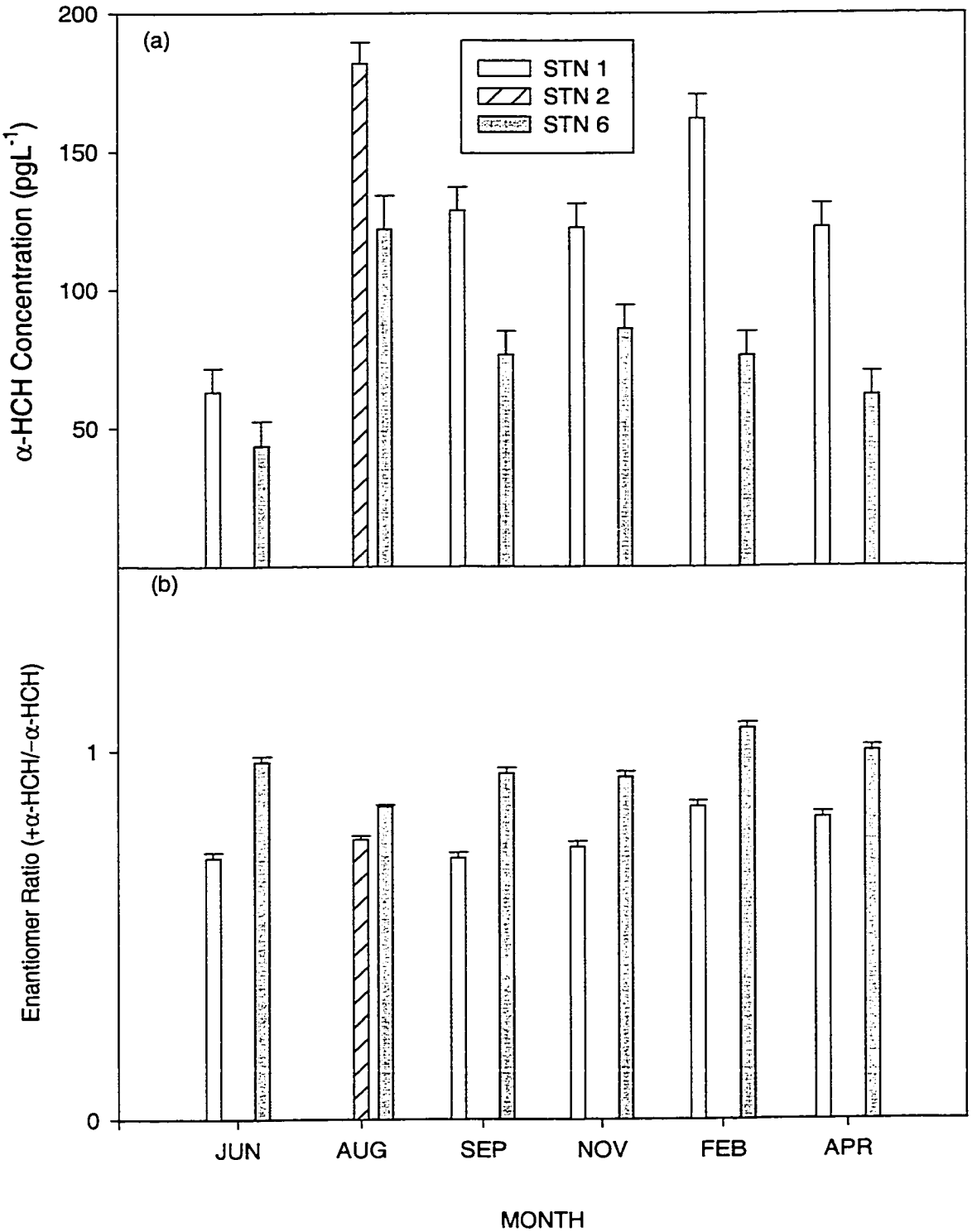


Figure 5-3. ER distributions versus salinity in the York River Estuary during months of low water flow, when there were depletions in overall α-HCH concentrations in the estuary. Dotted lines connecting ER values measured at highest and lowest salinity are not linear despite their appearance, and may be estimated using the equation described by Bidleman and Falconer (1999). Solid lines connecting measured ER values at each station represent the observed ER values. Error bars illustrate the pooled estimate of variance, based on duplicate samples measured in August.

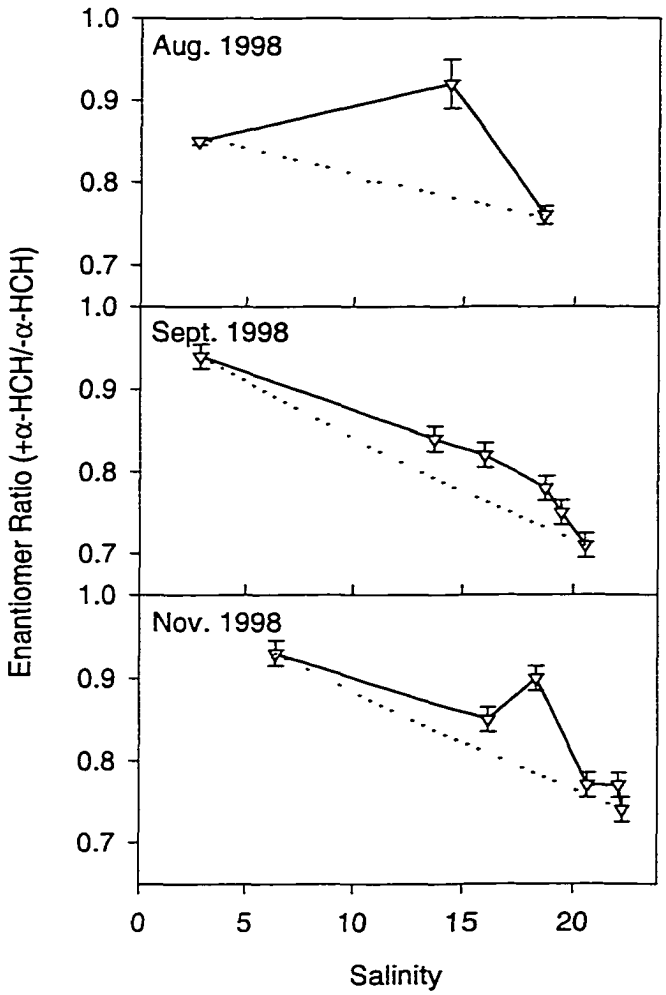
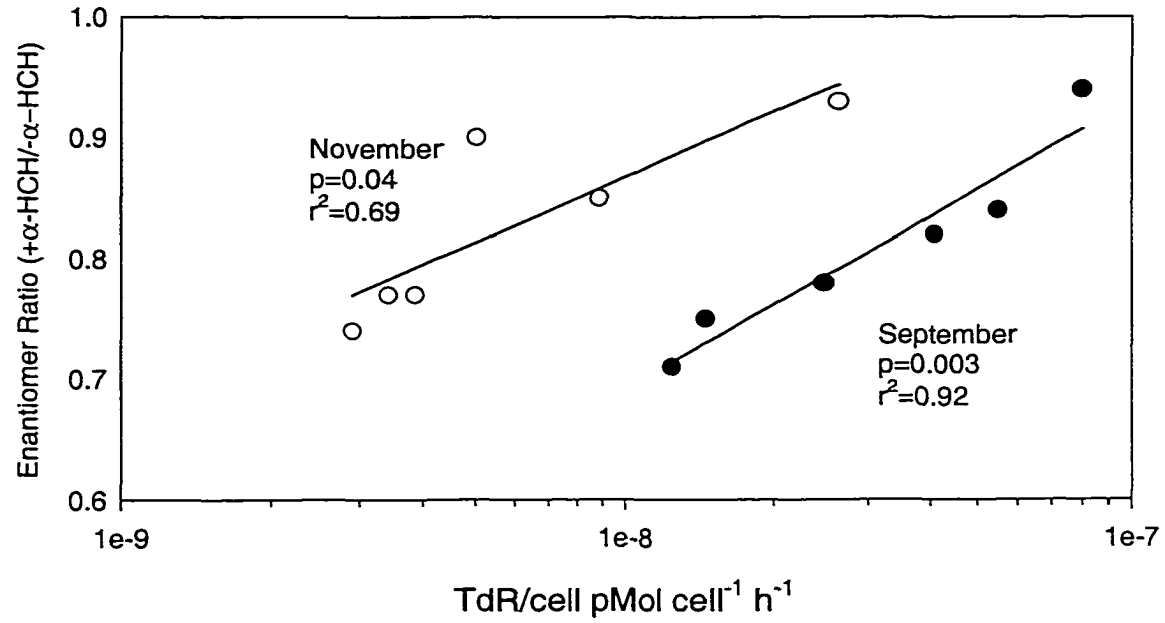


Figure 5-4. Correlations between the logarithm of bacterioplankton thymidine incoporation (a) and leucine incorporation (b) normalized to cell abundance, and α-HCH ER values during fall, when bacterioplankton activity is thought to be the main sink process controlling α-HCH concentrations in surface waters. The coefficient of variation within the triplicate samples collected at each station was usually lower than 10% and always lower than 20%. Open circles represent the data for November and black shaded circles represent the data for September.



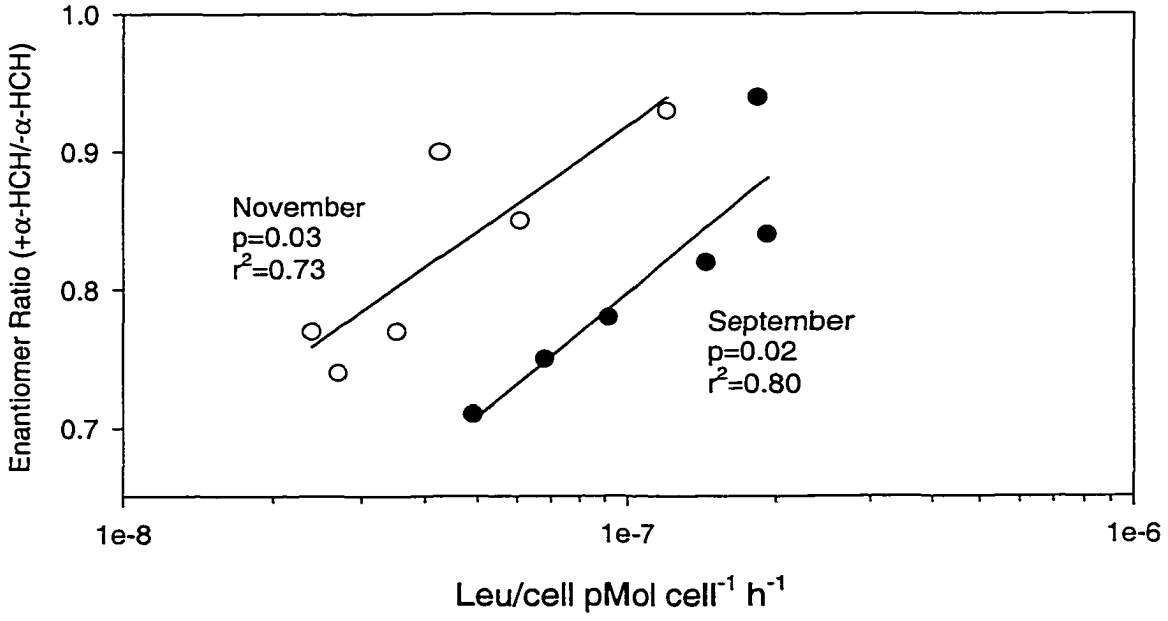


Figure 5-5. ER distributions versus salinity in the York River Estuary during months when there was high water flow and input of HCH to the estuary. In June, surface as well as bottom waters were sampled about three weeks apart. Dotted lines connecting ER values measured at highest and lowest salinity are not linear despite their appearance, and may be estimated using the equation described by Bidleman and Falconer (1999). Solid lines connecting measured ER values at each station represent the observed ER values. Error bars illustrate the pooled estimate of variance, based on duplicate samples measured in August.

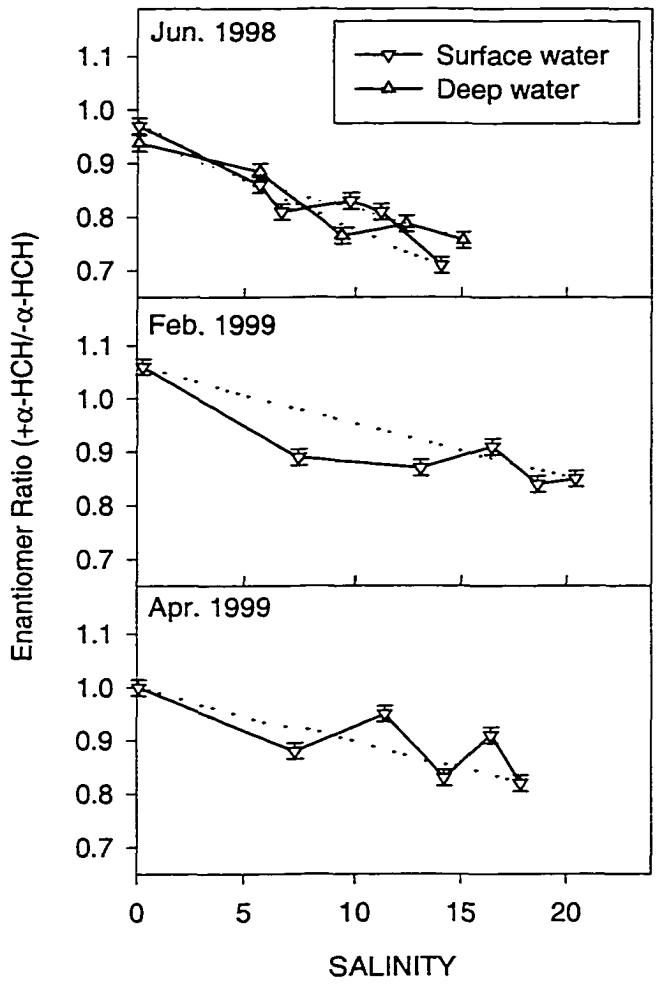
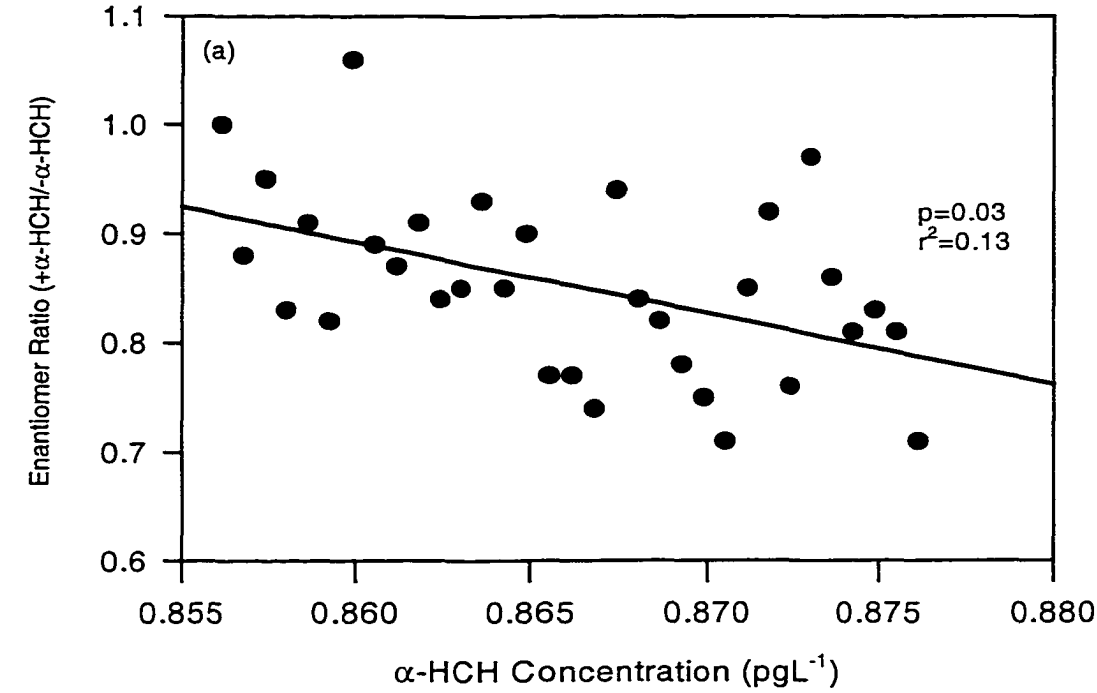
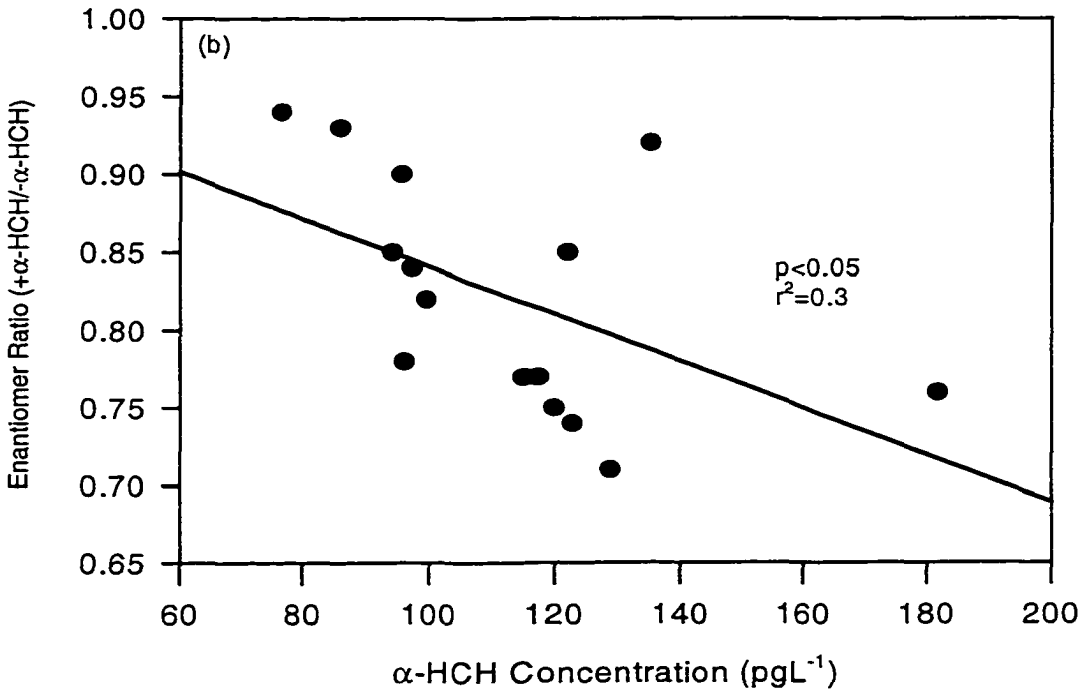


Figure 5-6. Correlation between ER values and α-HCH concentrations for all data in all months (a), as well as for all data during the months of August, September and November, when biodegradation was a major sink process within the estuary (b).





Chapter 6

SUMMARY AND CONCLUSIONS

α-HCH (α–hexachlorocylcohexane) is a persistent organic pollutant (POP) due to its toxicity and ubiquitous distribution in the environment. Although α-HCH is banned in industrialized nations including the United States, it continues to flux into the U.S. via atmospheric transport from tropical nations where it was extensively used until recently. In general, α-HCH is subjected to global transport, migrating from lower latitude source areas to polar regions while depositing in temperate latitudes during this process. However, prior to this study, there was little information on the dynamics of HCH isomers including α-HCH in temperate estuarine waters, especially in the context of enantioselective biodegradation. To gain insights into the physicochemical transport and biological transformation processes to which α-HCH and similar POPs may be subjected during global transport, and to further the development and use of biogeochemical tracers in POP fate and transport studies, I used compound specific stable isotope ratios (CSIRs) and enantiomer ratios (ERs) to gain insights into the air-water transfer and biodegradation, respectively, of α-HCH.

The first portion of this work consisted of a laboratory study to investigate the feasibility of using compound specific stable isotope ratios (CSIRs) to track the transport of α-HCH, as well as phenanthrene (another POP of similar mobility), in a laboratory simulation of global distillation. The objective of this set of experiments was to compare the fractionation of perdeuterated and ^{13}C stable isotope labeled POPs during air-water

transfer along a strong temperature gradient. The underlying hypothesis was that for a given POP, measurable fractionation of heavy (labeled) and light (unlabeled) POPs would occur due to faster movement of the lighter POP through the system. This hypothesis was validated within the confines of the experimental set up. Isotopically heavy POPs labeled with deuterium moved at a slower rate through the laboratory system compared with unlabeled compounds, leading to fractionation in which deuterium labeled contaminants accumulated close to the source (at warmer temperatures), whereas isotopically light compounds were preferentially transported away from the source. In contrast, ^{13}C-labeled POPs did not demonstrate significant fractionation relative to their isotopically light isomers, with the exception of phenanthrene at very cold temperatures.

Thus, these experiments support the idea that CSIR fractionation is likely to occur during the global distillation of POPs via air-water and air-earth exchange, and suggest that CSIRs may be useful as tracers for the long-range atmospheric transport of POPs. If the results hold true for larger scales, it seems likely that the deuterium isotopes of POPs will fractionate during volatilization and atmospheric transport in the environment, but that the ^{13}C-label of POPs will not. If so, it may be possible to establish a relationship between isotopic depletion and transport distance of deuterium isotopes, and thus use deuterium fractionation to measure a POP's transport distance in the environment. On the other hand, carbon isotopes could be a potentially powerful tool for tracing source material, since no significant fractionation of these isotopes occurs during transport. This hypothesis could be tested in a controlled tracer release of perdeuterated, ^{13}C-labeled, and unlabeled POPs on a local or regional scale. After this, isotope fractionation in unlabeled POPs (natural abundances) could be investigated.

The second portion of my work focused on the use of another biogeochemical

tracer, enantiomer ratios (ERs), to gain information on the biodegradation of α-HCH in the York River estuary. Although there are numerous reports of enantioselective degradation of α-HCH (the only chiral HCH isomer) in the environment, only a few studies have attempted to link specific microbial factors with the rate or extent of enantioselective degradation. Such studies, which combine microbiological information with data on a pollutant's fate and transport, are vital both in order to study chiral pesticide fate (particularly the possibility of persistence of non-degradable enantiomers) and to examine the potential use of ERs as geochemical tracers of transport and indicators of biodegradation. Therefore, I conducted an interdisciplinary study in the York River estuary in which I explored the spatial and temporal variation in HCH concentrations, as well as α-HCH ERs in air and water. The rationale for this portion of the study was that since α-HCH (and other chiral pesticides) are released into the environment as racemic mixtures (ER = 1), and enzymatic processes are often enantioselective while physical processes are not, measured deviations in ER from 1 imply biodegradation. I hypothesized that overall α-HCH concentrations in surface waters would be nearly equal along the estuary, since the primary source of α-HCH would be atmospheric deposition from remote sources. I also hypothesized that there would be a greater change in surface water ERs from 1:1 at sites with high bacterial activity, due to greater biodegradation.

Microbial abundance, activity, concentrations of HCH isomers, α-HCH ERs, and various physical parameters were measured along the salinity gradient in the York River estuary several times over the course of a year. In regard to the first hypothesis, concentrations of α-HCH were greater at the mouth of the estuary than in freshwater, suggesting that atmospheric input from remote areas was not the only source of α-HCH

to the estuary and that atmospheric inputs built up over time in the Chesapeake Bay or the Atlantic Ocean might provide additional inputs. Both α-HCH concentration and ER in surface waters of the York River were significantly correlated with microbial activity as measured using thymidine incorporation rate. Moreover, the results suggested that the extent of microbial degradation of HCH isomers was greatest in the freshwater regions of the York River, where thymidine incorporation rate was high, and lowest in the marine regions of the estuary, where thymidine incorporation was low. However, in regard to the second hypothesis, ERs became more racemic as bacterioplankton activity increased, suggesting that the more active freshwater community was less enantioselective. This implies that biodegradation of chiral pollutants need not always be enantioselective, and may not always cause a shift from the racemic ratio (ER = 1). In addition, differences in bacterioplankton community structure, as well as microbial activity may affect enantioselectivity. Hence, if a racemic ER is measured in the environment, it may not necessarily imply recent release, short travel or lack of retention by water and soil reservoirs in which biodegradation can occur.

A major conclusion of my research is that the influence of microbial factors on the ERs of chiral pollutants needs to be further investigated in laboratory as well as field studies under different environmental conditions before ERs can be used to track POP transport and fate. For example, since a POP's ER is altered by biological processes alone (due to the stereo-specificity of enzyme function), and since physico-chemical processes such as volatilization, hydrolysis and photodegradation are not enantio-selective, it could be assumed that the degree of change from racemic in a POP's ER, is directly related to increasing retention by terrestrial or aquatic reservoirs (and thus also to decreasing vapor pressure, volatility and gas-phase residence times). This rationale could

be extended to assume that measurement of environmental ER values close to 1:1 for a particular POP indicate that the POP has either been recently released into the environment or that it has not traveled far from its source. However, due to the existence of non-enantioselective biodegradation, as proposed here, the relationship between ERs and biodegradation is much more complex, and measured ER deviations (from original 1:1 ratio) may not necessarily indicate the age or distance a POP is transported over global scales.

Despite this, my research demonstrates that ER values within a given system indeed do provide unique information on the relative persistence of enantiomers of a chiral compound and on in-situ biodegradation that cannot be provided by studies in which chirality is overlooked. Thus, although measurement of ERs may not provide a direct or unambiguous indication of the significance of microbial breakdown processes in POP fate on large scales, ER values are useful in local and regional studies of processes influencing chiral compounds, including various pesticides, pharmaceuticals, and food additives.

On the global scale, it might be possible in the future to obtain detailed information on the significance of microbial degradation in the overall transport of a POP by combining CSIR and ER techniques, and measuring the CSIR separately for each enantiomer, as well as any degradation products resulting from biological processes. For example, since microbial breakdown is likely to favor lighter isotopes (i.e. H as opposed to D), and since light isotopes also travel faster across biological membranes compared to heavy isotopes, the parent form of the more degraded enantiomer of a chiral POP is likely to become enriched in the heavy isotope. Conversely, the degradation product of the more susceptible enantiomer will be more depleted (contain more of the light isotope),

and will exhibit a more negative $\delta^{13}D$ signature. Experiments could be conducted in the laboratory, as well as the field, to test this hypothesis.

Overall, my work indicates that both CSIRs and ERs are potentially powerful tools that could provide unique information on the fate and transport of α-HCH, as well as other chiral and/or relatively mobile POPs. This study also adds significantly to the existing knowledge on the fate and transport of α-HCH within temperate estuaries.

APPENDIX I: Data from air-water stable isotope fractionation experiments including a detailed summary table listing each experiment in chronological order

	1-EXPERIMENT 1	2	3	4	5	6
1	TIME @ 40 (h)	ug/g phe	std dev phe	ug/g dphe	std dev dphe	RATIO
2	0.0000	0.2582	0.0152	0.0662	3.4000e-3	0.2570
3	24.0000	0.1008	7.8000e-3	0.0292	1.6148e-3	0.2900
4	48.0000	0.0442	6.2780e-4	0.0144	2.3060e-5	0.3260
5	72.0000	0.0266	4.9060e-4	9.0000e-3	1.6754e-4	0.3410
6	96.0000	0.0162	1.4736e-5	5.6000e-3	1.8028e-4	0.3410
7	120.0000	0.0132	5.4400e-5	4.6000e-3	6.9540e-5	0.3550
8	144.0000	0.0108	3.8600e-5	3.6000e-3	1.9676e-6	0.3340
9	168.0000	0.0100	2.0640e-5	3.4000e-3	1.9380e-8	0.3500
10	192.0000	0.0128	5.0240e-4	4.2000e-3	1.0806e-4	0.3340
11	TIME @ 20	ug/g phe	std dev phe	ug/g dphe	std dev dphe	RATIO
12	0.0000	0.0000	0.0000	0.0000	0.0000	
13	24.0000	0.0648	7.2000e-3	0.0154	1.7576e-3	0.2380
14	48.0000	0.0738	1.2958e-3	0.0190	1.5446e-4	0.2590
15	72.0000	0.0750	1.3298e-3	0.0208	3.6340e-5	0.2770
16	96.0000	0.0662	6.7140e-4	0.0208	1.5944e-4	0.3130
17	120.0000	0.0634	2.6000e-3	0.0196	3.0500e-5	0.3100
18	144.0000	0.0598	9.3320e-4	0.0186	3.6720e-4	0.3120
19	168.0000	0.0554	1.5072e-3	0.0184	3.2100e-4	0.3330
20	192.0000	0.0606	1.1272e-3	0.0188	3.2360e-4	0.3120
21	TIME @ 0	ug/g phe	std dev phe	ug/g dphe	std dev dphe	RATIO
22	0.0000	0.0000	0.0000	0.0000	0.0000	
23	24.0000	1.2720e-3	1.2070e-5	3.3120e-4	1.2482e-5	0.2600
24	48.0000	7.2000e-3	9.3240e-4	2.0000e-3	4.3600e-4	0.2880
25	72.0000	0.0128	7.1940e-6	3.2000e-3	1.0052e-4	0.2540
26	96.0000	0.0178	2.3700e-4	6.2000e-3	6.9260e-4	0.3530
27	120.0000	0.0198	6.1960e-5	5.4000e-3	1.7164e-4	0.2750
28	144.0000	0.0274	1.0564e-3	7.0000e-3	1.1244e-4	0.2570
29	168.0000	0.0328	6.0960e-4	9.2000e-3	1.9054e-4	0.2830
30	192.0000	0.0358	4.2440e-4	9.2000e-3	4.2440e-4	0.2590

	8-EXPERIMENT 2	9	10	11	12	13
1	TIME @ 40 (h)	ug/g phe	ug/g dphe	std dev phe	stdevdphe	ratio
2	0.0000	0.3250	0.0800			0.4262
3	24.0000	0.3030	0.0650	0.0344	0.0257	0.2141
4	48.0000	0.1300	0.0150	0.0000	0.0000	0.1165
5	72.0000	0.0560	0.0140	8.8180e-4	2.2782e-4	0.2413
6	96.0000	0.0260	7.0000e-3	0.0000	0.0000	0.2672
7	120.0000	0.0160	4.0000e-3	6.6685e-4	2.0765e-4	0.2561
8	144.0000	0.0120	3.0000e-3	3.4104e-4	1.5602e-4	0.2586
9	168.0000	9.0000e-3	2.0000e-3	2.3091e-4	3.6604e-5	0.2667
10	192.0000	8.0000e-3	2.0000e-3	3.5313e-4	1.0737e-4	0.2500
11	216.0000	0.0100	2.0000e-3	5.7479e-4	1.1751e-4	0.2353
12	240.0000	7.0000e-3	2.0000e-3	8.9436e-4	1.2619e-4	0.2368
13	264.0000	6.0000e-3	1.0000e-3	4.4374e-4	6.8067e-5	0.2333
14	288.0000	6.0000e-3	1.0000e-3	3.6403e-4	6.6072e-5	0.2500
15	312.0000	5.0000e-3	1.0000e-3	4.4018e-4	2.6186e-4	0.2083
16	TIME @ 20	ug/g phe	ug/g dphe	stdev phe	stdevdphe	ratio
17	24.0000	1.0000e-3	0.0000	7.3419e-5	8.3378e-6	0.2500
18	48.0000	9.0000e-3	2.0000e-3	1.6591e-3	3.4383e-4	0.1837
19	72.0000	0.0120	2.0000e-3	1.8834e-4	3.0773e-5	0.1935
20	96.0000	0.0130	3.0000e-3	1.1060e-3	2.1713e-4	0.1940
21	120.0000	0.0200	4.0000e-3	8.2067e-3	1.6476e-3	0.1981
22	144.0000	0.0170	3.0000e-3	7.4863e-4	2.0129e-4	0.2000
23	168.0000	0.0170	3.0000e-3	2.8380e-4	7.7822e-5	0.1954
24	192.0000	0.0180	4.0000e-3	0.0000	0.0000	0.1935
25	216.0000	0.0180	4.0000e-3	4.0116e-3	5.0431e-4	0.2111
26	240.0000	0.0190	4.0000e-3	7.1093e-3	8.6462e-4	0.2083
27	264.0000	0.0180	4.0000e-3	6.9966e-3	9.2385e-4	0.2174
28	288.0000	0.0180	4.0000e-3	9.0029e-3	1.0688e-3	0.2174
29	312.0000	0.0180	4.0000e-3	8.6246e-3	1.3010e-3	0.2308

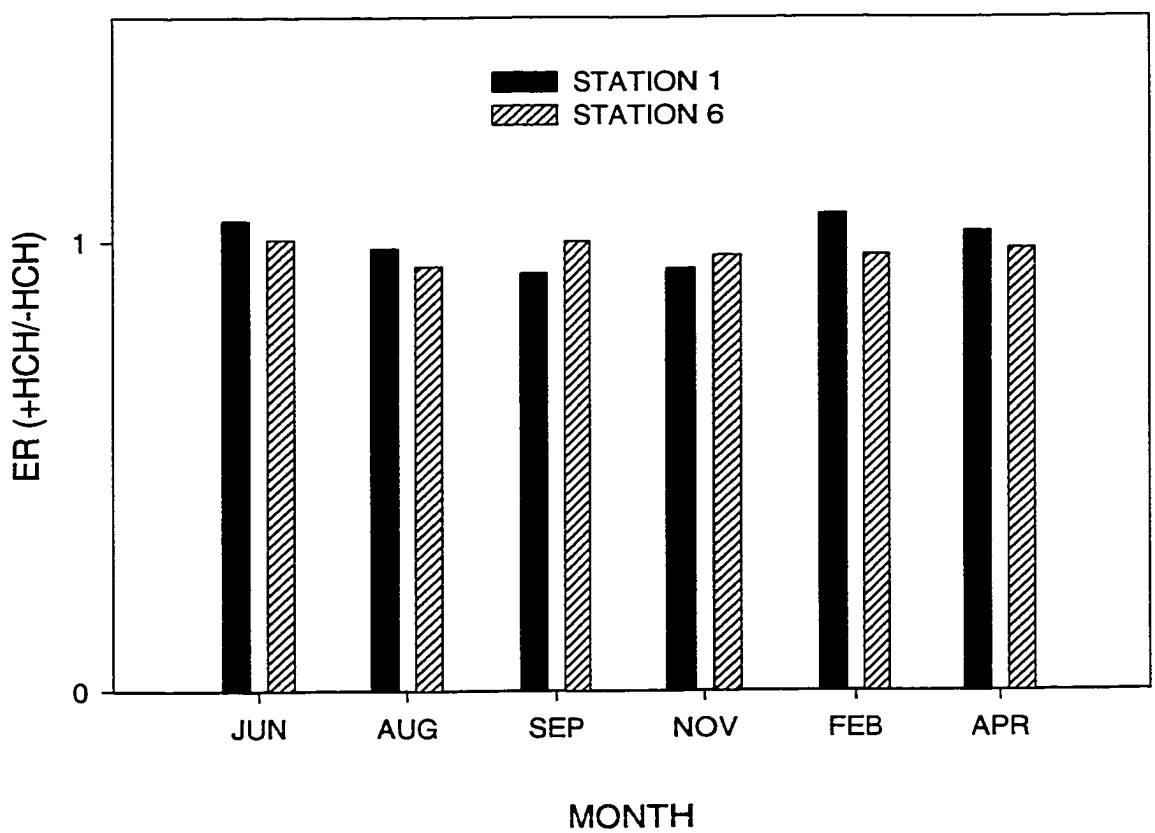
	14
1	std dev ratio
2	
3	0.0704
4	0.0000
5	2.4496e-4
6	0.0000
7	0.0231
8	0.0208
9	2.6047e-3
10	0.0244
11	0.0251
12	0.0115
13	0.0297
14	0.0278
15	0.0733
16	std dev ratio
17	8.8646e-3
18	3.0348e-3
19	5.0280e-4
20	2.2123e-5
21	1.9806e-3
22	3.1795e-3
23	1.3029e-3
24	0.0000
25	0.0192
26	0.0355
27	0.0349
28	0.0538
29	0.0420

	EXPERIMENT 2 (Cor	16	17	18	19	20
1	TIME @ 0	ug/g phe	ug/g dphe	stdev phe	stdevdphe	ratio
2	24.0000	1.9449e-4	0.0000	2.9295e-4	0.0000	0.0000
3	48.0000	7.8994e-4	0.0000	3.0168e-5	0.0000	0.0000
4	72.0000	9.8104e-4	0.0000	2.6317e-4	0.0000	0.0000
5	96.0000	7.8797e-4	0.0000	5.5358e-5	0.0000	0.0000
6	120.0000	9.4721e-4	0.0000	2.7651e-5	0.0000	0.0000
7	144.0000	1.1933e-3	1.9888e-4	2.8474e-6	1.8572e-4	0.1667
8	168.0000	1.3755e-3	1.9650e-4	8.3749e-5	1.2360e-5	0.1429
9	192.0000	1.7709e-3	1.9677e-4	3.7581e-4	8.6982e-6	0.1111
10	216.0000	1.7991e-3	3.9981e-4	9.7029e-5	2.0151e-5	0.2222
11	240.0000	1.9775e-3	3.9549e-4	2.4985e-4	1.1331e-5	0.2000
12	264.0000	2.1781e-3	3.9602e-4	1.6070e-4	8.8240e-5	0.1818
13	288.0000	2.0315e-3	3.6937e-4	1.0078e-4	4.0023e-4	0.1818
14	312.0000	2.1749e-3	3.9544e-4	9.3227e-4	1.6184e-4	0.1818

APPENDIX II

ENANTIOMER RATIOS IN AIR

ER in air were always close to 1 indicating that atmospheric ER was not greatly affected by ER in water, even when the flux was from water into the air. Atmospheric ER values were always close to racemic at both sites even during periods of net volatilization. This suggests that atmospheric ER values in estuarine systems may not be influenced by the ER of water even when net flux is from water into the air. This is in contrast to previous studies over large lake systems in which atmospheric ER resembled water ER when volatilization occurred (Ridal *et al.* 1997). This is probably due to the small surface area of estuaries compared to large lakes and ocean systems.



PROCEDURE FOR ANALYSIS OF AIR SAMPLES

Some of the air samples (collected on PUF plugs) needed a few additional clean up steps to remove interfering compounds before good peak shape could be achieved for ECD analysis. An acid cleanup was performed as follows: Prior to GC analysis, the sample (1-2 mL) was vortexed (on a mechanical mixer) with 0.5-1 mL 18 M (concentrated) sulfuric acid for a minute. The two layers were allowed to separate and the organic layer (hexane) which was on top, was pipetted off into another tube(being careful not to get any of the bottom acid layer). 1-2 mL clean hexane (petroleum ether or any organic except acetone, methanol and DCM because of their water solubility may also be used) to the acid layer. The sample was vortexed again and the organic layer pipetted off again. The organic layers were passed over a sodium sulphate column, reduced in volume and then analyzed using the GC.

APPENDIX III: HCH concentrations and microbial data

	1	2	3	4	5	6
1	SAL	LINDANE/L		ALPHA HCH TOTA	ALPHA HCH ER	TdR act/abd
2	14.0310	68.7534	J	63.0427	0.71	
3	11.1825	127.0650	J	107.6200	0.81	
4	9.7970	127.9990	J	103.6270	0.83	
5	6.7355	146.5030	J	96.7597	0.8100	
6	5.7265	177.2850	J	101.2280	0.8600	
7	0.0650	103.3860	J	43.9061	0.9700	
8	18.5975	214.9440	A	181.6850	0.76	
9	14.4320	118.0680	A	135.1970	0.92	
10	2.7825	113.5650	A	121.9990	0.85	
11	20.5315	89.5074	S	128.7870	0.71	1.2526e-8
12	19.4350	77.8361	S	119.8890	0.75	1.4598e-8
13	18.7065	76.9371	S	95.9402	0.78	2.4813e-8
14	15.9700	73.6142	S	99.4877	0.82	4.0766e-8
15	13.7560	82.1658	S	97.1815	0.84	5.4765e-8
16	2.8795	43.0519	S	76.4630	0.94	7.9965e-8
17	22.2625	71.2980	N	122.7430	0.74	2.8873e-9
18	22.1040	64.4088	N	117.4550	0.77	3.8533e-9
19	20.6580	64.2909	N	115.2580	0.77	3.4071e-9
20	18.3000	59.8143	N	95.5043	0.90	5.0058e-9
21	16.1385	69.2805	N	94.0993	0.85	8.8365e-9
22	6.4060	60.9628	N	85.9626	0.93	2.6371e-8
23	20.4500	61.3590	F	161.8830	0.85	1.1997e-8
24	18.6515	85.8357	F	173.7760	0.84	1.2729e-8
25	16.4885	68.6543	F	46.5886	0.91	1.7324e-8
26	13.0650	69.4410	F	135.2040	0.87	2.8584e-8
27	7.5060	85.7347	F	142.4200	0.89	3.3395e-8
28	0.2735	85.2980	F	76.2294	1.06	1.2024e-7
29	17.8340	40.6243	A	122.6520	0.82	6.0074e-9
30	16.4415	81.3059	A	39.3392	0.91	1.1762e-8
31	14.1865	82.8817	A	25.4029	0.83	1.7418e-8
32	11.3515	188.6550	A	105.1840	0.95	2.0891e-8
33	7.3435	52.3848	A	119.8300	0.88	2.8195e-8
34	0.0620	61.2011	A	61.7251	1.00	5.8722e-8

VITA

Born in Chennai India. Graduated with a state rank (52^{nd} out of 22,000 students) and a triple major in Chemistry, Botany and Environmental Science from St. Joseph's college, (Bangalore, India) at age 19. Worked at Brockwood park school, UK prior to entering the Master's program at SMS, College of William and Mary, USA in fall, 1990. Conducted research for the Andaman and Nicobar Islands Environmental Trust and the M.S. Swaminathan Research Foundation, India after completing the master's degree. Supervised research cruises on the Baltic sea, as a Research Scientist BAT II A ½ of the IfM an der Universitat Kiel, Germany, for a year prior to entering the doctoral program at the College of William and Mary in spring 1996. Plans to begin post-doctoral research at the Department of Geography and Environmental Engineering, Johns Hopkins University in September, 2001.