

APPLICATIONS OF COMPOUND-SPECIFIC ISOTOPE ANALYSIS (CSIA) FOR
DETERMINING PERSISTENT ORGANIC POLLUTANT (POP) ORIGIN,
TRANSPORT, AND TRANSFORMATION IN THE AQUATIC ENVIRONMENT

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ABSTRACT

Since the 2001 signing of the Stockholm Convention on Persistent Organic Pollutants (POPs), scientists globally have refined the methodologies for understanding these compounds. Given that POPs can enter and move freely between diverse environmental media, the challenge has been to unravel their complex histories and determine how their sources, paths, and transformations contribute to area contamination. Owing to their connective nature and elevated burdens, riverine and estuarine systems have become focal points for POP research. Meanwhile, compound-specific isotope analysis (CSIA) has emerged as a promising tool for identifying pollutants, distinguishing molecularly similar compounds, and tracing chemical behavior based on process-associated signatures. Focused on insights into remediation, this review summarizes an existing body of research at the intersection of aquatic ecosystem monitoring of POPs and CSIA, centering on three archetypal pollutants: polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and the pesticide Σ DDT. It is found that added CSIA parameters are invaluable for source apportioning area pollution and for tracking biotransformation through its ecosystems. Several natural remediation processes are found to be detectable uniquely via CSIA of tissue samples. Nonetheless, the method remains underutilized in aquatic systems and is applied disproportionately for different pollutants, patterns attributable to its expertise demands and reliance on preceding literature. To promote its use, future work is recommended to standardize CSIA laboratory protocols and to resolve the isotopic signatures left by natural processes.

Keywords: Compound-specific isotope analysis; Persistent organic pollutants; Aquatic ecosystems

CHAPTER 1. INTRODUCTION

1.1 General overview

An improved understanding of the human and ecological toxicity of man-made environmental pollutants has led governments around the world to pursue countermeasures. International agreements to control pollutants have brought about global monitoring programs, and significant scientific interest has been taken in developing the technologies and methodologies to support these programs and inform governmental action. While mitigation strategies focus largely on regulation, either banning or limiting the production of known contaminants, governments may choose to supplement long-term regulation with remediation actions—particularly when pollutants are environmentally persistent.

Persistent organic pollutants, or POPs, are a category of organic molecules characterized by their toxicity and resistance to degradation. They tend not to form on their own in nature but are manmade for diverse applications in industry and agriculture, and they may occur as byproducts in the synthesis of other chemicals. Their propensities for long-range transport and biomagnification position POPs as uniquely problematic environmental hazards, and their study is replete with scientific challenges. A single POP ordinarily consists of dozens of congeners, or molecular variations, some of which are toxic at hardly detectable levels. Advanced instrumentation is required to separate these for evaluation, and sample concentrations are subject to multiple variables. These include transport by environmental mechanisms, metabolic transformation and abiotic degradation. In many cases, residual store leakage is believed to contribute significantly

to environmental concentrations. This may continue long after the production of POPs is halted.

Most POPs are semi-volatile. Their corresponding high boiling points and low vapor pressures permit them to exist in a given state—as a gas, liquid, or adsorbed particle—for long periods of time. Long environmental residencies are also facilitated by their overall chemical and biological inertness. Half-lives in soil and sediment can range from a few days to several years. POPs bioaccumulate easily, as their tendency to be hydrophobic prevents organisms from excreting them, while their tendency to be lipophilic results in their storage in fatty tissues. This process reiterates through subsequent predation, leading to POP biomagnification up the food chain and causing them to affect species at multiple trophic positions in an ecosystem, particularly those at the top.

Long-range transport of POPs, a further issue, may lead pollutants to deposit in jurisdictions uninvolved in their formation, presenting an ethical dilemma to nations with production legacies. Translocation occurs by several mechanisms, including atmospheric and hydrologic processes as well as diffusion; however, migrating organisms contaminated with POPs may also act as vectors. Given their widespread existence, persistence, and fluctuation, POPs are complex to trace, but the past half-century has seen a significant scientific endeavor on the subject. Frequently studied are patterns of dispersion, deposition, and biodegradation, and, particularly for regulatory and legal purposes, source apportionment.

Waterbodies have been identified as focal points in the study of POP fate and transport because they act as both collection sinks and distribution networks. The

characteristically low water solubility of POPs leads them to adsorb to sediment particles and remain at the beds of rivers or entry points to lakes and reservoirs. Inputs include contributions from industrial outfall, atmospheric deposition, and runoff spanning large areas of nonpoint source pollution. While oceans are typically considered the last destination for freely transported POPs, freshwater systems are the first major concentration point. Rivers are of keen interest, as they juxtapose industrial and agricultural pollution sources with dense human populations. They also present a convenient locus for the management of global pollution burdens, since remedial interventions initiated here can intercept ocean-bound contaminants. Given their spatial constrictions, rivers and other freshwater systems are also relatively more convenient to sample ecologically than oceans and estuaries for insight into the environmental behavior of POPs. Furthermore, for several chemicals, the dominant human exposure concern is through the consumption of contaminated fish, and this may be mitigated by freshwater interventions.

1.2 International management

The term ‘persistent organic pollutant’ has been adopted by the Stockholm Convention (UNIDO, 2017)¹, a 2001 United Nations treaty signed by 152 countries to combat the effects of these chemicals on a global scale. The United States has signed but not ratified the treaty (CHM, 2008).² The primary objectives of the Convention are (1) to eliminate the production of twelve target contaminants, (2) to clean up residual stockpiles, and (3) to inform the addition of new POPs to the focus list. The twelve chemicals that the United Nations Environmental Programme (UNEP) initially selected

are termed the ‘dirty dozen.’ These are Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Mirex, Toxaphene, Hexachlorobenzene, PCBs, Dioxins and Furans. The former eight may be grouped as organochlorine pesticides, or OCPs. Hexachlorobenzene and PCBs are industrial chemicals, while dioxins and furans are byproducts formed by chemical manufacturing. Fourteen new POPs have since been added to the focus list by annual conferences of the Convention parties, and several additional classes of compounds are widely acknowledged as persistent organic pollutants in the literature but not yet listed as target contaminants. The treaty entered into force on 17 May 2004, and a monitoring effort has ensued to establish ambient contaminant levels on a global scale. The success of this effort depends upon the inclusion of geographically diverse nations, including developing countries. Since the equipment required to separate and quantify POPs is expensive, the need exists for scientists to develop methods tailored to these monitoring programs. For methods that fall short of the so-called ‘gold standards’ employed in state of the art labs, calibration against the existing literature is possible (Muir & Sverko, 2006).³

Although the United States is not a party to the Stockholm Convention, widespread availability of gas chromatography and mass spectrometry have positioned its institutions at the vanguard of research and development on the analytical methods for POPs, and it is a committed party to two other major international agreements regarding POPs. Together with Canada, it has pledged to work towards the “Virtual Elimination of Persistent Toxic Substances in the Great Lakes” (EPA), and together with members of the United Nations Economic Commission for Europe, it has agreed to reduce atmospheric emission of POPs prone to long-range transport, signing its regional protocol for the

Convention on Long-range Transboundary Air Pollution. The Environmental Protection Agency (EPA) states the U.S. has taken “strong domestic action” motivated directly by the Stockholm Convention to reduce harmful effects of POPs. Sale and distribution of PCBs plus all organochlorine pesticides listed among the dirty dozen is strictly prohibited. Furthermore, the U.S. actively hosts one of the largest PCBs remediation projects in the world, having dredged and capped contaminated areas of the Hudson River. Monitoring of this intervention is anticipated to continue indefinitely, although the project has already cost the US over a billion dollars. (EPA, 2017)⁴

1.3 Monitoring systems

The global monitoring plan that was initiated under the Stockholm Convention focuses on collecting harmonized POP data from the core media of air, water, and human tissue. It structures both the collection of data from existing monitoring programs and the initiation of new programs to meet future monitoring needs. Individual programs submit their data to regional hubs, which issue region-specific reports. The five hubs are Africa, Asia and the Pacific, Central and Eastern Europe, Latin America and the Caribbean, and Western Europe and other states. A global coordination group with three multinational representatives from each region subsequently compiles this information to develop a global monitoring report. Reports have been issued in the years 2009 and 2015. (CHM, 2017)⁵

For a constituent entry to the regional databases of a sample taken in water, attribute sections are required describing the sampling location and POP being measured, laboratory, measurement method and lower limit of detection, and average value obtained

from a specified quantity of samples. These prescriptions seek to ensure translatability between global monitoring activities.

In the United States Code, systematic monitoring of waterbodies is prescribed under the Federal Water Pollution Control Act Amendments of 1972, also called the Clean Water Act. Air quality monitoring is similarly prescribed under the Clean Air Act of 1963. The EPA delegates the execution of each integrated program to tribal, state and local agencies (EPA, 2017).⁶ These agencies design monitoring strategies and obtain samples, but typically outsource POP quantification to an unaffiliated laboratory. Although the state agencies are responsible for monitoring, evaluating waterway status, and issuing advisories, their protocols are heavily coordinated with the EPA.

Several steps are included in an agency monitoring program. Initially, threshold environmental concentrations are established for each contaminant. These inform a set of water quality standards. If a given waterbody exceeds standards during preliminary sampling, that body is considered impaired and examined for the valuation of suitable TMDLs, or total maximum daily loads of contaminants entering the system. Regulatory action is decided based on the subsequent tendency of a waterbody to exceed these TMDLs. For impaired waterbodies consistently bearing contaminant loads above the TMDL, a required reduction in pollution including margin of error is calculated. According to this value, water discharge permits are issued and adjusted for known pollution sources. Restoration measures may also be supplemented. Sustained determination of ambient contaminant levels is necessary at each stage in this process to govern action: in the formation of water quality standards, the establishment and measure

of adherence to TMDLs, and post-action progress evaluation (EPA, 2015).⁷ Further source determination is necessary for just and effective regulation.

1.4 Analytical methods

Because POP concentrations in water are typically extremely low, analysis of biotic and sedimentary matrices is recommended. These must be measured precisely due to the potential for low-level toxicity. Since the 1970s, sample concentrations requiring low level detection have been determined using a two-part system composed of a gas chromatograph (GC) coupled to a high resolution mass spectrometer (HRMS, 2008).⁸ In this setup, the GC is responsible for separating the compounds from the sample. After vaporizing target species, it moves them through a long capillary using a flow of inert gas. The capillary is coated with a stationary phase, or a substance that interacts to varying degrees with the constituent compounds, slowing them down preferentially and emitting them in staggered fashion. Once separated, analytes enter the mass spectrometer, which finds information relevant to their mass and thus identity by ionizing them, carrying them past an electric or magnetic field, and measuring deflection—a value proportionate to the mass-to-charge ratios of the ions. High resolution mass spectrometry deviates from low resolution by its ability to generate readings with decimal places beyond the atomic mass unit integer. This allows for differentiation between compounds with the same total nucleon count.

A GC-MS or GC-IRMS process is typically preceded by sample extraction from the source matrix. In recent years, advances have been made in the extraction process, with the advent of pressurized and microwave-assisted extraction, improvements to the

cleanup process, fine-tuning of the removal of excess extraction-related solvent material, and innovated introduction of samples to the GC—popular automatic injection system now being splitless injection. Following sample injection, optimal separation is obtained by selecting the proper stationary phase, column dimensions, and carrier gas velocity. When using MS detection, stationary phase diffusion can be a problem, and phases have been idealized to minimize bleeding. Higher resolution is usually obtained from a narrower column diameter and higher carrier gas velocity. Additionally, new GC systems have been introduced that permit higher resolution separations. These include GC x GC, which employs an additional gas chromatograph connected to the first by a modulator, which freezes and then re-emits separated analytes for a second round of separation. Even with the advances that have been made to the front end of this process, however, complex POP samples may still contain too many simultaneous congeners to be separated by the available technologies.

Significant advances have also been made on the detection side. Because halogenated POPs tend to be highly electronegative, electron-capture detectors (ECDs) coupled to a GC can be very effective determinants for certain compounds. ECDs make use of the tendency of analytes to absorb free electrons, separating them by electronegativity; however, these are prone to electronegative interference and do not offer the same reliability for identification as the various forms of mass spectrometry. In mass spectrometry, analytes are ionized by either electron capture ionization (EC) or electron ionization (EI), which differ by the energies of electrons used, or by chemical ionization (CI). Owing to its use of higher energy electrons, EI tends to cause the greatest fragmentation of the analytes. CI, on the other hand, forgoes bombarding the sample with

electrons in favor of reacting it with a reagent gas. This method may cause little or no fragmentation and is implemented in cases where fragments would otherwise be too numerous to quantify. Roughly in the order of increasing expense and utility to POP-analysis, the various forms of mass spectrometry are low-resolution MS, ion-trap tandem MS, time-of-flight MS, and high-resolution MS. In a tandem mass spectrometer, ions are charged, analyzed, then fragmented and re-analyzed to provide additional insight into constituent structures. In time-of-flight MS, travel time across a broad region of magnetic field is calculated, rather than deflection due to interaction with a quadrupole or other localized field source. Although high-resolution mass spectrometry is preferred in most aspects, this method comes with high acquisition and maintenance costs.

An alternative methodology for GC-MS derived POP characterization that has received relatively less literature coverage but offers an additional avenue of analysis is compound-specific isotope-ratio mass spectrometry. Compound-specific isotope analysis (CSIA) goes a step beyond quantifying the total concentrations of a particular POP and separating its congeners by also determining the stable isotope abundances of the constituent elements of the congeners. The implied GC-IRMS (gas chromatograph isotope-ratio mass spectrometry) is accomplished by combusting the analytes after GC separation and then isolating a chosen element for MS isotope-ratio determination. Whereas typical GC-MS measures a range of masses for the ionized fragments produced by the MS, GC-IRMS measures the masses of simple gases produced through sample combustion. For a carbon isotope investigation, CO₂ is principally the combustion product of interest. Detectors are implemented to gather the abundances of three distinct isotopomers—or isomers defined by distinct masses—of CO₂. These are ¹²C¹⁶O¹⁶O

(environmentally most common), $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ (carbon-13 representative), and $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ (oxygen-18 representative), given by respective molecular weights of 44, 45, and 46 amu. Isotopomers made up of combinations of heavier isotopes, such as $^{13}\text{C}^{18}\text{O}^{16}\text{O}$, are considered sufficiently uncommon for statistically important measurement. For isotope studies of hydrogen, nitrogen, and sulfur, gases typically investigated are H_2 , N_2 , and SO_2 . Returning to the example of the ^{13}C investigation, a determination of isotopomer abundances is used to calculate an analyte ratio of ^{13}C to ^{12}C . From this ratio, a ^{13}C signature is expressed by deviation from a global reference standard, such as Pee Dee Belemnite (PDB). The consistent ratio of ^{13}C to ^{12}C for PDB, given by R_{Standard} , is utilized in the following equation to obtain the variable $\delta^{13}\text{C}$ in units of permil (‰).

$$\delta = \frac{1000(R_{\text{Sample}} - R_{\text{Standard}})}{R_{\text{Standard}}}$$

In order to keep the IRMS calibrated, the standard gas with known isotopic content may be measured either before and after, as in continuous flow IRMS, or alternated continuously with sample gas, as in dual inlet IRMS. (Schmidt et al., 2004)⁹

CSIA is preferred to a broader analysis of isotopes of the homogenized sample, even if just one POP group is extracted, because the fractionations responsible for isotopic variations take place at the level of individual congeners. Combusting a bulk sample without performing a preceding GC separation therefore risks blending together the effects of diverse, congener-specific fractionations. Indeed, due to the various fractionation processes that a specific congener may undergo throughout its history, the results of CSIA are already prone to the sort of blending described and may require further calibration against history-controlled references. Nonetheless, the method offers

researchers the unique opportunity to fingerprint persistent organic pollutants by three lines of information: (1) the identity of the POP group, (2) the abundance of congeners, and (3) the stable-isotope signatures of selected congener. Taken together, these attributes provide the most complete identification of environmental POPs that is possible by presently available methods. However, and despite initiated documentation of this method for analyzing PCBs, PAHs, and other persistent organic pollutants, regulatory agencies have not systematically utilized CSIA because of the sparse precedent for the technique in the literature, instrumentation and specialization costs, and required level of adaptation to a specific research question. Past applications of CSIA include waterway-specific research into fate and transport, environmental transformation, and source apportionment of POPs. The following sections review the literature for this existing body of work, recommending CSIA as a promising, integrable methodology for elucidating POP behavior in the aquatic environment with the potential for more widespread application.

1.5 Statement of Purpose

The focus of this thesis on CSIA applications for aquatically bound persistent organic pollutants is intended to isolate the locus of control for plausible human interventions. This occurs most distinctly in river systems and estuaries, which bind several avenues of pollutant concentration. Physically, sorbed POPs become concentrated through the hydrologic cycle. Biologically, both anadromous and land-derived inputs contribute to the composition of an ecosystem. Finally, human proximity, population density, and resource utilization centered on rivers and estuaries are drivers of

concentration burdens and the motivation for remedial action. Atmospherically deposited pollutants such as those in soils, though widely studied, are inconvenient to remedy effectively due to their diffuse extent. Accordingly, atmospheric and soil centric research is not included for review, unless such a study constitutes the nearest related work to CSIA on aquatic contaminants, sets an important methodological precedent, or produces data relevant to a selected study.

With the ultimate goal of pollutant amelioration in mind, this review is tailored to understanding not only the origination makeup of an aquatic sample, but also the transformation processes that POPs undergo naturally. This is also motivated by active human participation in local food webs through fishing. The organizational approach of the following chapters is standardized accordingly, and subchapter headings are described here. First, the pollutant is defined based on its chemical characteristics and historical relevance. Then, its toxic effects to humans are detailed, not only to define the pollutant more closely, but also to frame its typical behavior within a biological system, as this invariably includes a resistance to metabolic degradation. The following section on environmental behavior discusses existing models for degradation, revisited in the next section on monitoring practices and in the main, CSIA section, to follow that. CSIA is thus situated among allied or competing methods, and its relevance to tracking natural remedial processes is defined. Although pollutant burden alleviation can be achieved by regulation and other, earlier interventions, provided the ongoing emission, leakage, and redistribution of POPs in the environment, the later-stage concentration points given by rivers and estuaries are argued to accord to the most relevant environmental media for a progressive understanding of POPs in the environment.

CHAPTER 2. PCBS

2.1 Chemical overview

Among the most problematic of persistent organic pollutants, and the variety most classically studied in aquatic systems are polychlorinated biphenyls or PCBs, a family of 209 congeners of chlorine-substituted biphenyls. PCBs belong to a broader set of halogenated aromatic compounds. Their toxicity is directly correlated with their degree of chlorination, ranging from 2-10 chlorine atoms, which are positioned along the peripheral rings of the biphenyl, substituting hydrogens in their usual bond locations. Mono-chlorinated biphenyls bearing only one substituted chlorine may also be included in this group (NRC, 2001).¹⁰ In general, PCBs are subdivided by degree of chlorination. The members of a subset are known as isomers, the diversity of which varies according to the possible arrangements of chlorine around the phenyls. PCBs may also be categorized by this positioning of chlorines relative to the central bond, as well as by the resulting geometry of the phenyl rings. Substitution positions are classified as ortho, meta, or para—listed in ascending order of their distance from the phenyl-phenyl bond. PCBs lacking a chlorine substitution in the ortho location have both phenyl rings positioned in the same plane; thus, they are categorized as coplanar. Meanwhile, ortho-substituted, PCBs are considered ‘noncoplanar’; these have some angle between their phenyl planes. Substitution locations may also be numbered, with 2, 2', 6 and 6' corresponding to the ortho position, 3, 3', 5, and 5' corresponding to meta, and 4 and 4' corresponding to para. Specific PCB congeners are identified by listing each Cl-substitution.

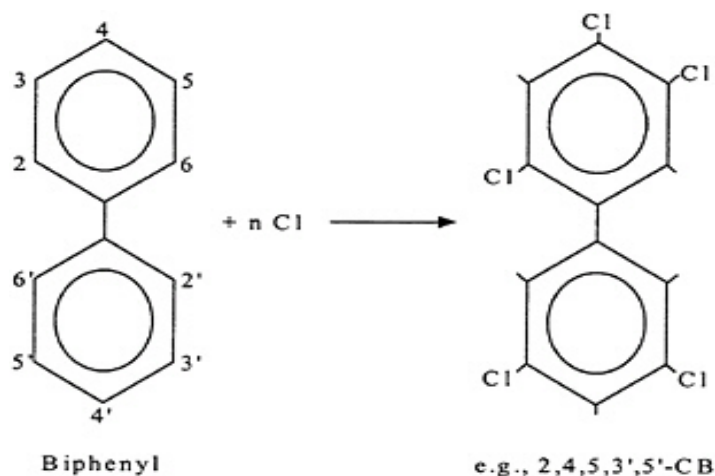


Figure 2.1 Formation of a pentachlorobiphenyl congener: 2,3',4,5,5'-CB. (NRC, 2001)

2.2 Historic production

PCBs have been manufactured around the world for industrial purposes since early in the 20th century. Due to their high dielectric constant and exceptional chemical stability, they have been applied as insulating fluids in electrical systems, as flame retardants, and as stabilizing additives in sealants, adhesives, paints, and finishes. For their versatility, PCBs were once touted as a miracle substance for industry. Various congener mixtures were manufactured in North America under the name 'Aroclor.' Products were coded with a 4-digit number, e.g. Aroclor 1242—the latter two digits of which describe average chlorination. Aroclor 1242 corresponds to a mixture of polychlorinated biphenyls that is about 42% chlorine by weight. Although these mixtures were composed of tens of distinct congeners, their compositions were determined by the conditions under which they were manufactured. Therefore, distinct mixtures of Aroclors can be source-identified by the proportions of congeners contained in them, among other methods. Following extensive documentation of their toxicity, PCBs production was banned in most of the developed world by the mid-1980s. Evidence suggests that for

several decades preceding the ban, leading historic manufacturers like Monsanto knowingly downplayed the adverse health effects they observed (Owens et al. v Monsanto Company, 2000).¹¹

2.3 Toxic effects

In high-dose exposure cases, PCBs cause abnormalities in respiration and hepatic function, as well as chloracne and skin lesions. Owing to the tendency of PCBs to accumulate in lipids, acutely exposed individuals easily pass contaminant burdens in milk on to their offspring, and this may continue for generations. Although prolonged low-level exposures best represent the risks to the general population, risk analysis for this facet remains a work in progress. In the past, agencies have routinely lowered the allowable levels for PCBs in environmental media, according to emerging research indicating the long-term carcinogenic, immunosuppressive, neurotoxic, and reproductive effects of PCBs. Furthermore, due to the persistence of PCBs, environmental concentrations have remained relatively constant throughout these amendments, leading to an increase with time in the number of waterways recognizing a PCBs problem.

According to the Agency for Toxic Substances and Disease Registry (2014),¹² the established mechanism for PCBs toxicity in humans is through the recruitment of arene oxides by activation of the aryl hydrocarbon receptor (AhR) during the attempt of the body to metabolize the compound. Arene oxides themselves are electrophilic and can interfere with DNA strand structure, while the metabolites that they subsequently form may bind to other proteins and further disrupt bodily function. Coplanar PCBs with several para and meta positions filled are considered the most efficient initiators of this

mechanism. Similar in their functionality to dioxins—which are considered exceptionally toxic among POPs—heavily substituted coplanar PCBs belong to a category of ‘dioxin-like compounds,’ which has been specially studied. Non-coplanar, ortho-substituted PCBs and coplanar versions with a lower number of overall chlorine substitutions are not believed to act as AhR agonists, though they can still cause neurotoxic and immunotoxic effects at higher doses (Safe et al., 1985).¹³

Although ample research has been conducted on the toxicology of PCBs in laboratory mammals, including rats and non-human primates (Golub et al., 1991),¹⁴ long-term data describing their effects on aquatic ecosystems is relatively lacking (NRC, 2001). Some studies, such as Schmidt et al. (2004)¹⁵ have described species-specific effects, such as alterations in the swimming behavior and diurnality of exposed carp, but often contaminated ecosystems are evaluated with an exclusive focus on human exposure risks through fish consumption. Sentinel species with heightened PCBs sensitivity have also been studied as early indicators of area contamination (Smith and Anderson, 2003).¹⁶ In severely contaminated systems like the Hudson River in New York, evidence suggests that overall ecosystem decline occurs as a direct result of PCBs presence, with several higher-trophic level species being most strongly affected. In mink, birds, fish, snapping turtles, and bullfrogs, significant reproductive impairments and juvenile mortality have been documented (DARRP, 2013).¹⁷ Another ecosystem effect observed in this region is the natural selection of fish species with PCBs immunity. The Hudson River tomcod, whose relatives in other habitats express a low frequency of immunity, is a classic example of rapid evolution by natural selection. In a large proportion of Hudson River tomcod, the AhR receptor has been deformed and is unable to be activated by PCBs,

leading to immunity despite the bioaccumulation of significant pollutant concentrations. (Berkeley, 2011)¹⁸

2.4 Environmental behavior

PCBs have entered the environment primarily through production and use. Prior to widespread bans, the hazardous wastes generated through production were routinely placed into landfills (ATSDR, 2015).¹⁹ In the present day, PCBs may still leach into the environment from poorly contained waste stores. They may also leak from old electrical equipment and residual source material that is improperly disposed. The chemical inertness, low vapor pressure, and low water solubility of PCBs allow the molecules to change phase readily and easily transition between adsorbed to pieces of industrial equipment to being suspended in the air to being dissolved in a waterbody. They exhibit an affinity for organic materials throughout their transport, partitioning preferentially to whichever environmental media has higher organic content. Coupled with their ability to adsorb to solid particles, this tendency leads to significant PCBs accumulation in fine-grained, organically rich sediment. Within sedimentary matrices, high concentrations of newly deposited PCBs can be found in layers of fine-grain sediment just below the surficial particles. In river systems, these are regularly swept up by turbulence and redeposited (NRC, 2001). PCBs that are not adsorbed to sedimentary particles may either be freely dissolved in water or adherent to molecules of dissolved organic carbon. PCBs existing in these states are most prone to long-range transport, as they can also easily volatilize back into the atmosphere. With chlorination as a key determinant for the

propensity to volatilize, PCBs have been described to have a range of half-lives in soil and sediment, anywhere between 10 days and 1.5 years. (CHM, 2008)

Like other persistent organic pollutants, PCBs are prone to environmental transformation and food web bioaccumulation. In the aquatic environment, they transform when consumed by microbes and other organisms. Certain anaerobic microbes have developed the ability to dechlorinate highly chlorinated PCBs, while certain aerobes can fully metabolize lower chlorinated PCBs. Some aerobes have even evolved to rely on PCBs as their sole source of both carbon and energy.²⁰ By several mechanisms, metabolic uptake is associated with the fractionation, or preferential separation of PCB varieties according to their physical and chemical properties. As a consequence, PCB compositions in the environment can differ significantly from those of their immediate industrial sources.

Fractionations may divide a range of congeners or some isotopically distinct groups of the same congener. Congener fractionations tend to occur because more chlorinated PCBs exhibit higher metabolic resistance and a greater affinity for organic matter. Thus over time, highly chlorinated PCBs become overrepresented in ecologically rich environments. Similar processes lead to carbon-13 enrichment with trophic level, as isotopically heavier PCBs bioaccumulate preferentially during each subsequent predator-prey exchange. This is because heavier molecules with the same chemical constituents have higher binding energies and less mobility than their lighter counterparts.²¹

2.5 Monitoring and fingerprinting

An understanding of environmental behavior on larger spatial scales, e.g. across systems such as the Great Lakes, has conventionally been approached through a combination of concentration anomaly determination and chemometric analysis of collected environmental samples. Samples for aquatic PCBs are typically collected from environmental media like water and sediment, as well as for exposed biota and passive samplers. Biota and passive samplers offer insight into bioavailability, or the proportion of a contaminant that may enter the ecosystem. Taken together with samples from non-biological media, these may be fingerprinted according to congener composition and modeled chemometrically to pinpoint controllable sources that would be candidates for regulation. Biological transformation of PCBs must be differentiated from these analyses (Benoit et al., 2016).²²

Contaminant source identification supports the emerging field of environmental forensics—the investigation of contaminant origins for regulatory and legal purposes—for which chemometric analysis spans the conventional toolset when chemical tracer mapping and on-site identification fall short. This analysis requires the presence of chemical fingerprints of the materials in a sample, e.g. PCBs congener profiles. It applies multivariate statistics to the identification and enumeration of fingerprints in a sample, determining the variability in each fingerprint and ultimately seeks to establish relative source contributions. Two major methods for fingerprinting are principal component analysis and several-receptor modeling. Each identifies the covarying components of congener-abundance profiles across multiple samples. The handful of congeners whose abundances are detected to covary are thus identified and their abundances in each covarying case are averaged for the projection of source fingerprints. This is necessary in

cases where these are not already available in Aroclor form or where some sources are yet unknown. Positive matrix factorization, a subset of several-receptor modeling, is frequently implemented.

While the operation of principal component analysis relies on an orthogonal transformation of covarying congeners to reduce their dimension or reveal independence, it can also be expressed as a factorization, like PMF. For PMF negative components are usually excluded to limit the result range, considering that congener abundances are impossibly negative. (Glenn et al., 2002)²³

2.6 Applications of CSIA

The origination and environmental transformation of PCBs may be described quantitatively by congener determination and CSIA, as fractionations of both congeners and isotopes are associated with production conditions and transformation processes of PCBs. Although the two may convolute, as effects of mixing sources blend with effects of biological transformation, a combination of congener determination and CSIA applied to both sedimentary and food-web matrices may be used to partition them. To enable such a distinction, signatures of PCB-sources must first be established, and this has been the primary goal of certain research efforts. Horii et al. (2004)²⁴ applied CSIA to determine carbon-13 signatures for commonly implemented aroclors. The group found that not only aroclor label but also geographic location of manufacture tended to influence isotope signatures, finding $\delta^{13}\text{C}$ values for whole mixtures that ranged from -34.4 to -22.0‰. The most strong correlations however were found to occur with degree of PCB chlorination, with more chlorinated PCBs corresponding to more depleted $\delta^{13}\text{C}$

signals. Such records are vital to inform source apportionment and biotransformation research, which rely on known source signatures in order to determine the magnitude of mixing or subsequent fractionations that take place.

Yanik et al. (2003)²⁵ demonstrated that carbon isotope signals overall are retained from source material through uptake into biological tissue, applying CSIA to sediment, tissue, and source Aroclors from the Housatonic River in Massachusetts. The group did find enrichment of $\delta^{13}\text{C}$ to occur through metabolic fractionation; however, this was insignificant compared to the fractionation effects associated with PCBs production, supporting the potential for source apportionment research using CSIA in biologically rich systems. The group limited themselves to just a few Aroclor varieties, including Aroclors 1242, 1254, 1260, and 1262, each of which was in turn represented by a handful of PCB congeners. Aroclor 1260 was ultimately selected, as previous research had indicated this was the only PCB variety to affect the Housatonic River area under study. Ten congeners were discovered to be shared between the tissue samples and fresh Aroclor 1260. Of these ten, four to eight—depending on the tissue matrix—agreed considerably in their isotopic signatures, varying by less than 1‰, while the majority of remaining congeners showed ^{13}C enrichment upon uptake into animal tissue, serving as evidence for the preferred excretion of lighter molecules. A small subset exhibited significant isotopic depletion in tissue samples. These were considered as metabolic products, generated from the preferred utilization of lighter PCBs. Although Yanik et al. (2003) state that existing scientific knowledge of metabolic processes in higher animals is insufficient to differentiate these effects definitively from the effects of environmental redistribution (i.e. through evaporation), their work set a key precedent for future studies.

In addition, the ecological scope of Yanik et al. (2003) was limited to the observation of three species at low trophic position—grass carp, mallard ducks, and wood ducks—and hence did not include the effects of reiterated metabolic events on $\delta^{13}\text{C}$.

Zeng et al. (2013) later filled these gaps in their own PCBs CSIA study, including tissues from snakehead (a predator species) alongside that of mud carp (a vegetation-consuming analog of the three species chosen by Yanik et al., 2013). Zeng et al. corroborated the assertion by Yanik et al. (2013) that source apportionment of PCBs using CSIA would be possible despite potential metabolic transformation, and also added insight into the trophic transfer of PCBs between the three trophic transfers: plant nutrient uptake, primary consumption, and secondary consumption. Similar to Yanik et al. (2003), Zeng et al. (2013) documented the tissue sample enrichment of congeners with high degrees of chlorination together with the depletion of congeners subject to metabolism. Exploring this, the group found the biomagnification factor (from carp to snakehead) to be generally <1 for di-CBs and tri-CBs and >1 for penta-CBs up to octa-CBs, indicating greater persistence of more highly chlorinated congeners. Each study also limited itself to only 20 or 30 congeners, based on their expected abundances, potentials for chromatographic separation, and GC-MS peak resolution. Of these, Zeng et al. (2013) recommended PCB8, PCB110, and PCB105, written by Ballschmieder number,²⁶ as promising candidates for future source apportionment studies. These three congeners are identified as uniquely resistant to metabolic fractionation, with minimal changes in $\delta^{13}\text{C}$ from sediment samples to each trophic level. In addition to recommending PCBs for CSIA-based source apportionment and discussing PCBs metabolism and trophic transfer, Zeng et al. (2013) observed $\delta^{13}\text{C}$ depletion of certain PCBs, such as PCBs 49, 48/47/75,

87/115/81, and 138, in fish relative to sedimentary sources. This was hypothesized to originate from the dechlorination of more highly chlorinated congeners—which characteristically exhibit lower $\delta^{13}\text{C}$ values. The changed identities of these PCBs to less chlorinated varieties hence lowers the average $\delta^{13}\text{C}$ of the congener groups to which they now accord. While Zeng et al. (2013) hypothesize a few pathways by which more highly chlorinated PCBs could reduce to lower chlorinated PCBs and average down $\delta^{13}\text{C}$ values, reductive dechlorination has yet to be studied extensively in fish, with existing studies focused on microbes. Relevant to this, anaerobic transformation has been documented in a number of cases, with key studies indicating decreasing concentrations of coplanar PCBs and stable concentrations of non-coplanar, ortho-substituted PCBs with sediment layer age. Evidence indicates that reductive dechlorination occurs for both legacy PCB-contaminants and for pollutants newly introduced to the sediment, and in one study, transformations were found not to occur in heat-killed control samples, indicating that the mechanisms are biological (Field and Sierra-Alvarez, 2007). Similarly, aerobic degradation of PCBs has been inferred through the detection of related metabolites, and a few aerobes such as *Burkholderia cepacia* can live on mono- or di-chlorinated congeners as their sole source of energy, converting them to chlorobenzoates, which subsequently may be mineralized. These findings are exciting as they lay out possibilities for the complete remediation of PCBs through natural microbial processes. Research has also demonstrated that the degradation process can be accelerated by introducing electron donating substrates, glucose, acetone, and methanol. In the absence of an electron-donating substrate, dechlorination is found not to occur. CSIA finds a niche here, as it can be applied to track the conversion of higher chlorinated PCBs to lower chlorinated PCBs

by linking them to their $\delta^{13}\text{C}$ values. If congener enumeration is the only method used, transformation effects on congener abundance remain indistinguishable from the effects of mixing with new system inputs. (Field and Sierra-Alvarez, 2007)

In addition to finding $\delta^{13}\text{C}$ depletion related to reductive dechlorination in certain PCBs, Zeng et al. (2013) also discovered an opposite effect, related directly to the metabolism of PCBs by fish, that enriched the $\delta^{13}\text{C}$ levels of a different set—including PCBs 17, 18, 31, 33/20, 22, 42, 44, 62, 101, and 136—in fish relative to sediment. Indeed, the majority of PCBs found in fish were isotopically enriched in $\delta^{13}\text{C}$ simply due to the preferential deposition of heavier molecules during the bioaccumulation process. Each of the PCBs listed however, showed significant enrichments of greater than 1‰ relative to sediment and were further related by their molecular structure, each having vicinal hydrogens bonded in a meta-para or ortho-meta arrangement. In support of prior research that PCBs with vicinal hydrogens at the meta-para position could be metabolized by fish (Buckman et al., 2006),²⁷ Zeng et al. (2013) attributed this enrichment to the preferred transformation of isotopically depleted PCB congeners (with lower bonding energies) during fish metabolism. Their finding that isotopic fractionations occurred similarly in each fish species further linked the metabolic pathways of PCBs in the mud carp and the snakehead, despite their difference in trophic position. This link was further supported by the methylsulfonyl-PCBs congener profiles of each fish, which were similar despite a relative enrichment of the total methylsulfonyl-PCBs to PCBs ratio in the snakehead, reflective of trophic level.

Adding an additional metric of enantiomeric and atropisomeric fraction analysis, Tang et al. (2016)²⁸ and Tang et al. (2017)²⁹ expand upon the work of Zeng et al. (2013)

and Yanik et al. (2003) by tracing biotransformation in a simple trophic uptake scenario centering on the common carp. Enantiomeric and antipisomeric fraction analysis make use of several structural patterns that exist in PCB-molecules. Enantiomers are mirror images of one another, but are not identical due to the three-dimensionality of the PCB-molecule, while antipisomers are exact images of one another, but with differing three-dimensional bond angles between phenyls. Stereoisomers typically remain intertwined by conventional analytical methods, but are separable by the introduction of a Chiralsil-Dex column into the GC, as applied by Tang et al. (2016) and Tang et al. (2017).

Supplementing stereoisomer ratio analysis with CSIA, Tang et al. (2016) sought to differentiate between the signatures of fish food, fish tissue, and fish feces in a controlled experiment. Before initiation of the experiment, fish in two separate groups were fed a diet spiked with PCBs and not spiked with PCBs. Subsequently, PCBs concentration decay in the tissues were measured. In agreement with the literature, the group found that higher chlorination was correlated with greater uptake into tissue. Similarly, they found regular reductions of overall PCBs concentration in fish tissue over the 30-day study period. While key PCB-metabolites historically found in fish and used prior to Zeng et al. (2013) as evidence for the ability of fish to metabolize PCBs were not found in this study, pronounced enantiomer fractionation and isotopic fractionation were documented.

Stereoselective metabolism of PCBs had been known to occur in fish prior to this experiment, and Tang et al. (2016) present the first instance in which this knowledge has been implemented as a preliminary screening for the CSIA of biotransformed PCBs.

Three chiral PCB congeners were discovered to be preferentially metabolized by the carp, in agreement with findings from Zeng et al. (2013). CSIA also identified PCBs 5/8,

18, and 20/33 as most $\delta^{13}\text{C}$ enriched upon uptake. Each had vicinal hydrogen bonds, and were indicated as bioaccumulative in agreement with Buckman et al. (2006), as well as Yanik et al. (2003) and Zeng et al. (2013). Concluding their work, Tang et al. (2016) recommend future work into the biotransformation of PCBs in fish to make use of stereoselectivity evaluation in conjunction with state of the art CSIA. An interesting, untried avenue to which the group alluded was a direct combination of CSIA and enantiomer selection, whereby known enantiomers are themselves tested for $\delta^{13}\text{C}$. Unfortunately, in Tang et al. (2016), this was not possible due to the prohibitively small concentrations of chiral PCBs. However, Tang et al. (2017) filled this gap by extracting sufficient quantities of chiral PCBs for CSIA to take place. Observing biotransformation fractionations in carp, the group identified three candidate PCB congeners—45, 91, and 95—that might undergo stereoselective metabolic processes. For enantiomer 1 of PCB 45, written as E1-PCB 45, isotopic signatures remained similar throughout the biotransformation process. Meanwhile for PCBs 91 and 95, one enantiomer was observed to undergo a metabolic fractionation associated with the carbon bond, while the other remained unaltered.

2.7 Conclusions

The conclusion than can be drawn from Yanik et al. (2003), Zeng et al. (2013), Tang et al. (2016), and Tang et al. (2017), who together have authored the most ecologically integrated works on compound-specific isotope analysis of PCBs in aquatic ecosystems, are numerous and open-ended. Firstly, the chemometric fingerprinting of PCBs in aquatic systems offered by CSIA can be more detailed than that offered by

another method, save perhaps by one integrated with CSIA. Notably, in the most recent of these studies, Tang et al. (2016) and Tang et al. (2017) couple CSIA with enantiomer fraction evaluation, a combination that will likely prove useful for future research in this field, provided the strong stereoselectivity of PCBs metabolism in various organisms.

In general, the explanatory power of CSIA stems from its assumption of several layers of complexity in the PCB-character of a polluted site and its sources. As a compound-selective method, it naturally links covariations in congener abundance with covariations in their isotopic signal. For example, congener fingerprints with non-matching carbon isotope signatures across samples may be called into question as statistical errors, or in the desired case, may be considered as evidence for the biotransformation of PCBs within a system. As Yanik et al. (2003) discuss, $\delta^{13}\text{C}$ differences due to metabolic fractionations in fish are typically small compared to $\delta^{13}\text{C}$ differences across sources; therefore, as demonstrated, source apportionment relying on principal component analysis or positive matrix factorization is still possible using CSIA and without necessarily adjusting for the fractionation effects of uptake. Nonetheless, this is untrue for certain preferentially metabolized congeners, and Zeng et al. (2013) build on prior knowledge by listing a few resistant congeners for which this problem is diminished, and which thus may be useful for source apportionment.

Additionally, Zeng et al. (2013) describe CSIA as a potential method for linking species on a trophic chain, i.e. the mud carp and snakehead, who exhibited common congener signatures with one another and the sediment as well as trophic $\delta^{13}\text{C}$ enrichment. They also demonstrate the application of CSIA to understanding the biotransformations predicted to occur during the transference of PCBs through the

ecosystem. Both $\delta^{13}\text{C}$ enrichment and depletion can act as indicators of these processes, the former for direct metabolism of lower chlorinated PCBs by fish, and the latter for dechlorination of more highly chlorinated varieties. Both PCBs metabolism and dechlorination are understudied topics in freshwater ecosystems, despite that these transformations take place in immediate proximity to human PCBs exposure through fish consumption. In the future, CSIA may thus offer a shortcut to mapping the prevalence of PCBs biotransformation due to either fish or microbes throughout large bodies of water, and may even provide additional clues to finding more efficient remediation strategies.

Excerpted results of the PCBs portion of the study by Zeng et al. (2013) are copied below for reference.

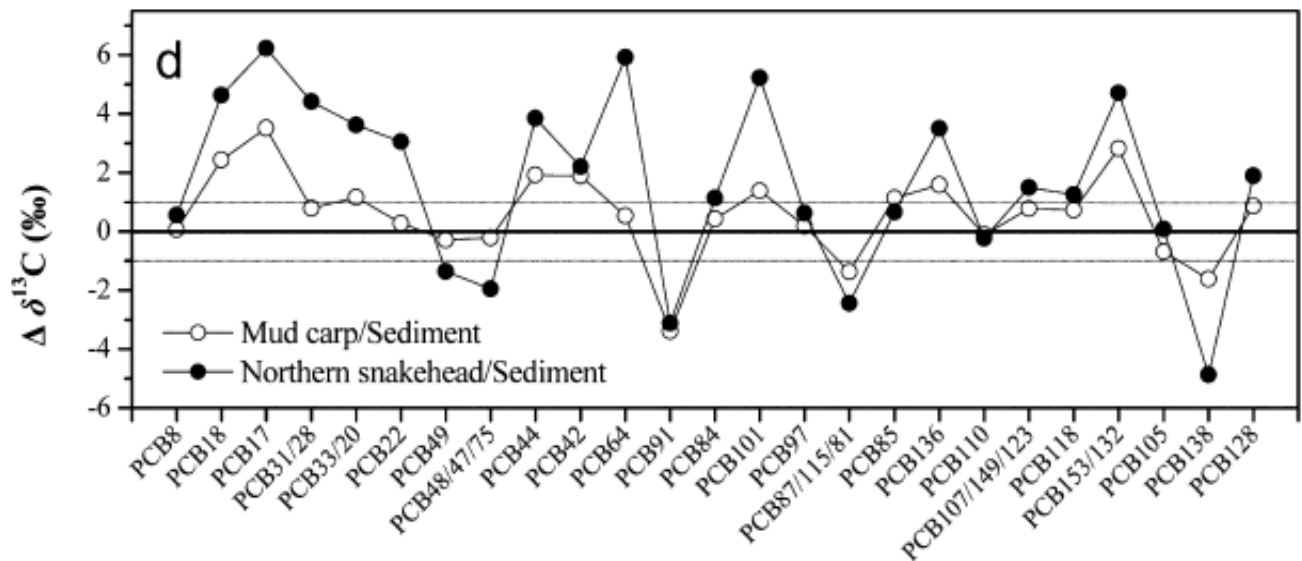


Figure 2.2 Compound-specific $\delta^{13}\text{C}$ results from Zeng et al. (2013). 25 PCBs extracted from samples of sediment and tissue (mud carp/snakehead) are included, with differences (given by Δ) plotted between $\delta^{13}\text{C}$ measured for fish and for sediment. Similar values correspond to PCBs resistant to predator-prey trophic biotransformation, staggered distances from zero correspond to biotransformation across all trophic levels, and values close to zero evidence an absence of metabolic PCB fractionations.

CHAPTER 3. PAHS

3.1 Chemical overview

Polycyclic aromatic hydrocarbons, or PAHs, have been studied intensively through the lens of CSIA to determine and distinguish sources. They encompass a set of molecules characterized by their fused aromatic rings and consisting of many arrangements. In contrast with PCBs, these hydrocarbons by definition do not include any noteworthy substitutions along their peripheral hydrogen bonds, although halogenated derivatives, i.e. Cl-PAHs, do exist, and these are similar in structure and toxicity to PCBs and other halogenated hydrocarbons. Nonetheless, ordinary PAHs are known carcinogens, and many have indicated the potential for immunosuppressive and harmful effects on reproduction in humans. Owing to the large variety of congeners that may theoretically exist, a few with known toxicities, carcinogenicities, or environmental presence are commonly isolated for research and regulation. (Abdel-Shafy & Mansour, 2016)³⁰

The aromaticity, or the delocalization of electrons within cyclic hydrocarbons, of PAHs is a key factor determining chemical character. Individual PAHs are defined not only by their unique numbers of aromatic rings, but also by their unique aromaticity, which is given by the quantity of rings with delocalized electrons. The latter quantity determines isomer reactivity, increasing with aromaticity. According the aromatic π -sextet rule of Erich Clar (1972),³¹ for an isomer having two resonance structures with diverging electron delocalization patterns, the one with higher aromaticity is considered for characterization purposes. In general, PAHs may be subdivided as ‘small’ or ‘large’

chains, depending on whether they contain less than or equal to six, or more than six aromatic rings. Naphthalene, a two-ring aromatic hydrocarbon, is occasionally considered only a bicyclic aromatic hydrocarbon and separate from PAHs. Three benzene molecules listed by the International Agency for Research on Cancer (IARC)³² include phenanthrene and anthracene. Although ‘small’ PAHs are more frequently studied due to their abundance, ‘large’ ones tend to be more environmentally persistent and more toxic. PAHs in each size category may be further categorized by bonding arrangement, which will be either linear, clustered, or angular as shown below.

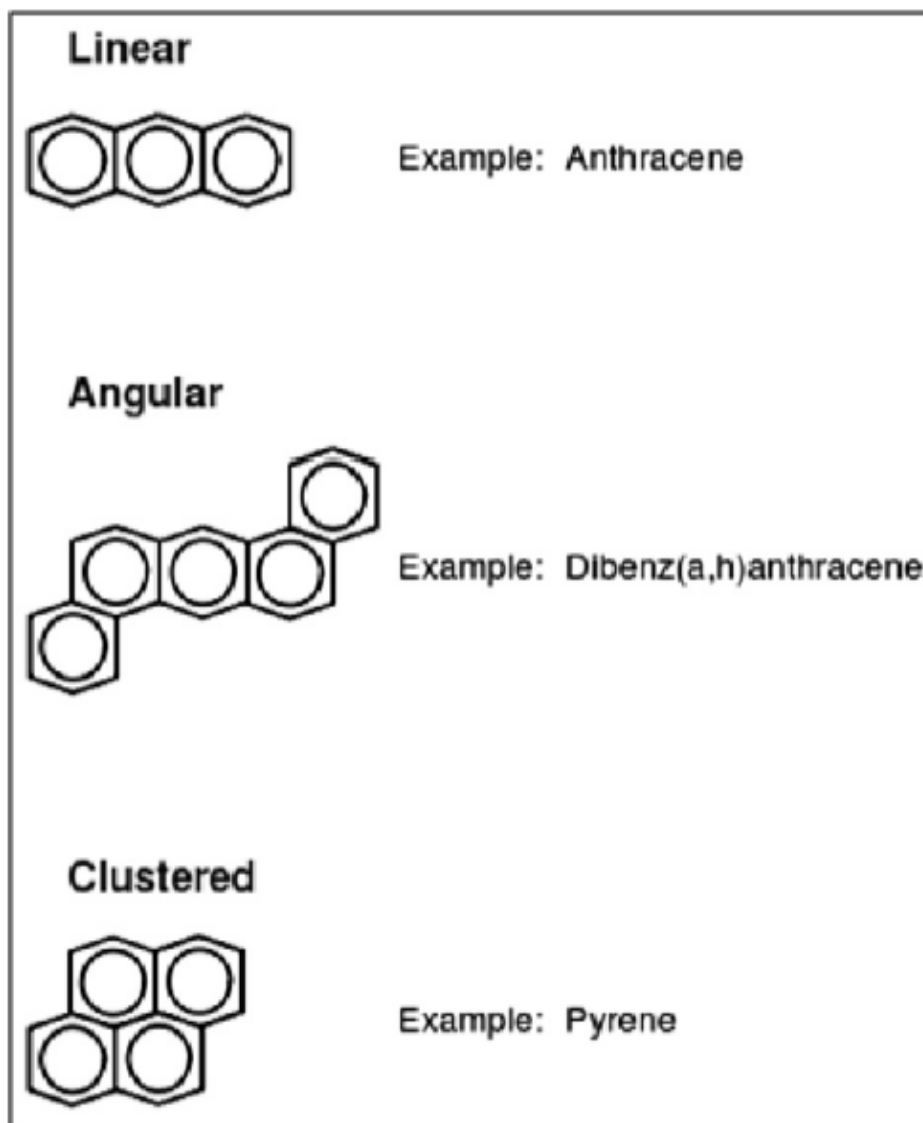


Figure 3.1 Various arrangements of polycyclic aromatic hydrocarbons. Types are not dependent on the number of aromatic rings present, although a minimum of three may be required for consideration among PAHs. (Abdel-Shafy & Mansour, 2016)

3.2 Historic production

Although PAHs are an exception among POPs, given that they occur naturally, humans are largely responsible for currently elevated environmental concentrations, as a number of anthropogenic processes can accelerate their occurrence (Obiakor et al., 2014).³³ PAHs are known broadly to form through three distinct mechanisms: the heating or combustion of organic material, the maturation of crude oil, and the biological transformation of organic matter. These sources are termed pyrogenic, petrogenic, and biological, respectively. Among these, the major source is the combustion of fossil fuels and wood. While complete combustion generally destroys and thus remediates PAHs, incomplete combustion or exposure to high heat in the presence of minimal oxygen is more conducive to pyrolytic PAH production. Incomplete combustion of fuels by vehicle motors and heating systems are key contributors to this, and therefore pyrogenically sourced PAHs—thus PAHs in general—are concentrated in urban areas. Meanwhile, significant concentrations of petrogenic PAHs may stem from crude oil spills or store leakage into groundwater or the ocean, as well as the integral of unintended, non-point source emissions of fuel into the environment from human activities. Principal component analysis (PCA) and positive matrix factorization (PMF) have demonstrated seasonal variations of PAH concentrations in some parts of the world, with higher concentrations found in winter than summer (Ma et al., 2010).³⁴ This variation is attributed to the relatively higher use of heating systems in winter.

Because PAHs are among the earliest persistent organic pollutants studied, and because these various sources have mechanistic signatures—including, for example, the shorter chains of PAHs produced under lower temperatures in a power plant stack—source identification of PAHs has developed into a mature science. Chemical fingerprinting techniques exist to differentiate between coal-based, oil-based, and wood-based groups, and also between sources originally formed petrogenically, over geologic time-scales, and pyrogenically. This latter distinction is vital, since pyrogenically produced PAHs are much more likely to be recent and correspond to anthropogenic actions. Meanwhile, biological formation of PAHs is less frequently acknowledged or studied due to its relatively insignificant contributions to overall PAH contamination levels. Biological and other natural sources of PAHs include bacterial and algal synthesis, volcanoes and forest fires, petroleum seeps and erosion, and the decomposition of vegetation. (Abdel-Shafy & Mansour, 2016)

A key concern associated with PAHs is their disproportionate effect on developing nations, stemming from poor regulation of industrial effluent and fossil fuel combustion. In contrast with PCBs, PAHs have not been synthesized for industrial purposes, although a few commercial uses do exist in the manufacture of pigments, dyes, resins, plastics, pesticides, and pharmaceuticals (Abdel-Shafy & Mansour, 2016). Less fortunately, but also in contrast with PCBs, PAH-concentrations in the environment continue to increase with time, spurred on by both their environmental persistence and continued inadvertent production through the incomplete burning of fossil fuels.

3.3 Toxic effects

As of 2014, the World Health Organization (WHO) and U.S. Environmental Protection Agency (EPA) have classified sixteen PAHs—including the commonly studied benzo(c)pyrene, 7,12-dimethylbenz(a)anthracene, and chrysene—as priority pollutants (Abdel-Shafy & Mansour, 2016)(Obiakor et al., 2014). Many more have been identified as carcinogenic, and several are known as potential mutagens and teratogens. Given the abundance of PAHs in the atmosphere, one of the primary health effects on humans is lung cancer, the risk for which may be compounded by the inhalation of tobacco smoke or by occupational exposures to combustion. Humans and other mammals may take up PAHs through inhalation, ingestion, and dermal contact. Food processing procedures including smoking, drying, and grilling are also thought to be major routes of PAH exposure. Although PAHs are not soluble in water, notable amounts may be transferred through water if a pollution event is recent. The lipophilicity of PAHs, meanwhile, is responsible for their significant bioavailability and storage in internal organs. Multiple metabolic steps are required to effectively detoxify PAHs, including the epoxidation of the PAH and the conjugation of epoxides with glutathione. Often, PAH epoxides will remain unconjugated. Following their subsequent conversion into phenols and diols, these metabolites may yet require further conjugation to be excreted, and in the meantime can exercise carcinogenic effects, disrupting the membranes of nearby cells or binding directly into DNA strands. (Abdel-Shafy & Mansour, 2016)

Since longer-chain PAHs pose greater challenges to the metabolic system and are associated with large numbers of metabolites, they present a greater toxicity and carcinogenicity burden to humans than lighter varieties. Toxicity is dependent largely on exposure route, effecting various bodily systems accordingly, and on pollutant

concentrations, as may be expected. However, toxicity is also correlated with the diversity of PAHs to which one is exposed, which is associated in turn with the variety of metabolic transformations undergone. Metabolic transformations that form electrophilic derivatives are ultimately most likely to produce genotoxic effects. Meanwhile, embryotoxic effects have been described in rodents but not humans, though high prenatal PAH exposure has been associated with developmental impairments and carcinogenically link DNA damage (Edwards et al., 2010).³⁵

Toxicity to aquatic organisms has been studied to a lesser extent; however, acute toxicity as well as reproductive, developmental, immune, and carcinogenic effects have all been observed to affect birds and fish. Additionally, PAH bioaccumulation in the aquatic ecosystems is shown to be significant in both fish and shellfish. (Abdel-Shafy & Mansour, 2016)

3.4 Environmental behavior

PAHs are ubiquitous in the environment, occurring in diverse environmental matrices like water, soil, sediment, and biological tissue. The majority of them exist in the atmosphere, either freely in the vapor phase or sorbed to organic particulate matter in the solid phase. Congeners with lower vapor pressure, such as benzo(a)pyrene, tend to adhere to atmospheric particles more easily, and vapor pressure has been shown to vary inversely with molecular weight. As a result, concentrations of heavier PAHs tend to be diminished in the vapor phase as compared to the particle phase, though the total concentration of PAHs in the vapor phase is often higher due to the prevalence of light congeners (Abdel-Shafy & Mansour, 2016). Precipitation may remove atmospheric

PAHs and transfer them into aquatic systems. Notably, sorbed PAHs are more easily removed from the atmosphere by precipitation than those in the vapor phase, leading to the preferred wet deposition of heavier molecules. The same tendency exists for dry deposition, with lighter varieties settling more slowly.

Similarly to its relationship with vapor pressure, PAH solubility in water varies inversely with molecular weight, resulting in the overrepresentation of lighter varieties in the water column and the overrepresentation of heavier varieties in the benthic zone. Due to their hydrophobicity, PAHs have short residence times in the water column. Their presence here therefore points to recent deposition (Obiakor et al., 2014). Heavier PAHs and especially halogenated varieties tend to be highly persistent in the aquatic environment. Stability of solid-phase PAHs as well as their resistance to photo-chemically induced transformation processes increase upon adsorption to organic particles. As a result, PAH concentrations in the benthic zone are usually much higher than those in the water column. Meanwhile, the propensity of many species of fish and other aquatic organisms to metabolize PAHs may significantly reduce their overall burden in biological matrices. For example, Obiakor et al. (2014) found that PAH concentrations in the Anambra River of Nigeria were greater in sediment than in fish tissues—although lowest in the water column, as is to be expected. Citing variations in the abundance of contaminants according to trophic level, Obiakor et al. (2014) state that the PAH biotransformations occurring in fish are a key determinant of overall PAH toxicity and distribution in an area. While the potential exists for the biomagnification of PAHs with trophic level—given their hydrophobicity and lipophilicity—the opposite effect, known as trophic dilution, has been observed in certain aquatic food webs (Wan et

al., 2007).³⁶ In this particular case, dilution is attributed to a combination of low assimilation efficiency and high metabolic efficiency of PAHs at high trophic levels. In many aquatic species, biotransformation is known to be dependent on various factors, including the lipid content, exposure route, species, sex, age and health conditions of the organism. Despite these potential benefits of biotic PAH uptake, Obiakor et al. (2014) also found fish tissue concentrations of benzo(a)pyrene in their study area to be elevated beyond safe consumption levels.

Bacterial biodegradation of PAHs may occur aerobically or anaerobically. PAHs must exist in either the dissolved phase or the liquid phase in order to be bioavailable to these organisms because sorbed PAHs are usually already bound at the binding locations preferred by the enzymes implied in the bacterial utilization. PAHs may desorb and thus become bioavailable again at various rates. These rates are dependent on a variety of factors, including PAH age and surrounding aqueous solubility levels. In addition to being biodegraded, PAHs may also be photochemically degraded, through oxidation reactions initiated by sunlight, or simply chemically oxidized by adsorbing particles. Predominate degradation mechanisms depend largely upon the properties of unique PAHs. (Obiakor et al., 2014)

3.5 Monitoring and fingerprinting

Research samples of ambient PAH concentrations are often derived from the atmosphere, via filters or sorbents, although they may also be obtained directly from sediment, soil, or biological tissue. These are then extracted by organic solvents and prepared for detection and measurement in a GC-MS. As for other POPs, source

identification and apportionment is of keen importance for regulatory decisions and litigation. As discussed in section 3.2, various source distinctions can be made for PAHs by traditional isomer-ratio fingerprinting methods, i.e. by quantifying the abundances of PAHs by benzene ring-count or isomer type, though even overall concentration measurements can provide crude insights.

In general, fractionations of isomers by weight, identity, and isotopic content are of interest, and these are associated with formation conditions, such as temperature and pressure. For example, five-member hydrocarbons tend to be overrepresented in petroleum-based hydrocarbons than in pyrogenic hydrocarbons, which more readily convert source material into six-member rings. Furthermore, each PAH source carries certain indicators; petrogenic PAHs, for example, exhibit higher concentrations of alkyl-substituted PAHs (Yanik et al., 2003)³⁷, which are readily detected by GC-MS. As discussed in section 3.2, longer-chain PAHs also tend to be formed in higher abundance under higher temperatures. In more refined cases, where PAHs are sought to be differentiated between two pyrolytic sources with unknown combustion conditions, reference fingerprints must first be established from source samples. Alternatively, PCA and PMF can be applied to a mixed sample to determine the covarying fingerprints, though this alone will not provide identification.

Significant work has been done to establish detailed fingerprints for a variety of environmental sources and combustion processes, and this is still very much a work in progress with disagreements existing among researchers that pertain to the viability of certain signature distinctions. For example, Guillon et al. (2013)³⁸ performed both molecular and isotopic characterizations of PAHs originating from the burning of several

species of wood largely from the Mediterranean Basin in France. They found that softwoods and hardwoods from this region were not readily distinguishable in molecular profile, in disagreement with Gonçalves et al. (2010)³⁹, who reported differences between these types. While Guillon et al. (2013) were able to locate some distinctions between the molecular profiles of tree samples originating in the Mediterranean region and those of North America, these indications were limited relative to those discoverable by isotope-ratio analysis.

Similarly to Guillon et al (2013), Walker et al. (2005)⁴⁰ were able to distinguish isomer-isotope signatures between PAHs sourced from coal from those stemming from coal gasification. Walker et al. (2005) found this was not possible solely looking at isomer ratios. While isomer-ratio distinctions may be readily made between sources with significantly diverging origins, such as atmospherically deposited pyrogenic PAHs from a forest fire and tanker-leaked petrogenic PAHs from crude oil, finer distinctions often require additional information, which may be achieved by CSIA.

3.6 Applications of CSIA

The parent body of work for all recent advancements in compound-specific isotope-ratio research of PAHs was performed in the mid 1990s, concurrently by two groups of scientists. Recognizing the need to understand the impact of PAHs as combustion products, Turekian et al. (1996)⁴¹ compared bulk $\delta^{13}\text{C}$ variations with compound-specific $\delta^{13}\text{C}$ variations between samples undergoing a burn. Significantly, the group found that in controlled trials of high intensity burns, which most closely mimic natural events, CSIA of individual fatty acids emitted from the burn showed greater

variability between the vegetation before and after the burning process than did bulk isotopic analysis. The kinetic isotope effect that they hoped to find was easier to isolate by CSIA than by bulk isotope analysis, since sample dilution can more easily occur in the bulk isotope analysis. For CSIA to be influenced by dilution, an identical fatty acid from another source would need to deposit on the filter during the time of collection. Finally, the group determined that overall C3 and C4 plant signatures tended to be maintained throughout the burn, though C4 plants exhibited more pronounced changes from before to after the burn.

An overlapping set of authors, Ballentine et al. (1996),⁴² confirm much of the work of Turekian et al. (1996) by measuring fatty acid ^{13}C and bulk ^{13}C compositions before and after two burns of sugar cane, varying by temperature. This study selected sugar cane, a C4 variety, informed by the findings of the previous study. Adding to the previous understanding, Ballentine et al. (1996) also identified three useful PAHs for future research: phenanthrene, fluoranthrene, and pyrene. They confirmed that higher intensity burns were associated with significant (1‰ to 6‰) depletions in fatty acid-specific $\delta^{13}\text{C}$ measurements before and after the burn, whereas low intensity burns were insufficient to alter the isotopic composition of fatty acids significantly, deviating from the prior finding.

The second group to establish significant precedents in the CSIA of burned material and PAHs was McRae et al., (1996).⁴³ Together with the two studies already covered, this work set an important precedent for future, CSIA-based PAHs source apportionment of field samples. McRae et al., (1996) compare three distinct sources found to be the most dominant in the environment: coal, biomass, and diesel. Heating

these, similar to Turekian et al. (1996) and Ballentine et al. (1996), McRae et al. (1996) found that two and three ring PAHs exhibited the greatest kinetic isotope effect in terms of $\delta^{13}\text{C}$ fractionation. Using selected PAHs within this subgroup, the three distinct sources could be differentiated with significance. McRae et al., (1999) followed this controlled research, which had focused on coal gasification, and applied it to field samples of vegetation and soil influenced by carbonization and combustion, as well as gasification. Doing so, they were able to differentiate and determine the inputs of PAHs from transport fuels, high temperature carbonization, and coal combustion in the vicinity of a low temperature carbonization plant.

While CSIA evaluations of PAHs in the aquatic environment have typically entailed such an examination of carbon-13 ratios, both for purposes of determining sources (Turekian et al., 1996; Ballentine et al., 1996; Walker et al., 2005; Buczyńska et al., 2013;⁴⁴ Fabbri et al., 2003;⁴⁵ Okuda et al., 2002;⁴⁶ Stark et al., 2003;⁴⁷ Kim et al., 2008;⁴⁸ Petrišič et al., 2013)⁴⁹ and for monitoring biotransformation processes (Yanik et al., 2003; Mazeas et al., 2002),⁵⁰ hydrogen/deuterium ratios have also recently proven useful. In a recent work, Kümmel et al. (2016)⁵¹ found carbon isotope fractionations associated with the anaerobic activation of naphthalene by Naph6S to be minimal, while hydrogen isotope fractionations were significant. However, within the same study, $\delta^{13}\text{C}$ values were found to track aerobic activation of other PAHs, and carbon-13 investigation remains the standard.

The majority of CSIA studies into environmental PAHs have focused on elucidating the isotope signatures that may indicate sources. Studies tracing the transformation processes of PAHs using CSIA are comparably rare (Buczyńska et al.,

2013). As a consequence, the bulk of the existing work dedicated to the CSIA of PAHs is focused on the media of soil, sediment, and air, with no significant studies to date existing for tissues of aquatic macro-organisms. Unfortunately—and although a search returns various studies applying radioisotopes as PAHs tracers in fish-tissue—CSIA of PAHs has to date been confined to more inert media. This may be because isotope signatures for biodegraded PAHs are often indistinguishable from their parents, owing to the relative strength of the isotope signal associated with compound generation (Buczyńska et al., 2013).

Mazeas et al. (2002) tested this hypothesis for $\delta^{13}\text{C}$ in a biodegradation experiment that applied marine bacteria to crude oil. The group found that source-specific $\delta^{13}\text{C}$ signatures were maintained throughout the biodegradation process, rendering future isotope analyses to trace this route of degradation practically useless, while strongly encouraging the future use of CSIA to determine sources. While degradation processes for a variety of POPs have been shown to exhibit regular, predictable fractionations, this finding is rather unique. Meanwhile Mazeas et al. (2002) found in their experiment that significant and regular molecular fractionations did occur, making molecular profile enumeration the default method for tracing aerobic biotransformation of PAHs.

This standard has since been challenged by both Yanik et al. (2003) and Meckenstock et al. (2004),⁵² each of whom found evidence for biotransformation-associated carbon isotope fractionations in PAHs. The latter group of Meckenstock et al. (2004) focused on fractionations associated with anaerobic biotransformation in aquifers. Their significant finding was that one congener in particular, toluene, exhibited a pronounced fractionation effect of up to 10%. However, the shifts observed for others

were relatively small, usually only 1‰ or 2‰, which are each common cutoffs for in-situ statistical significance. Meanwhile, Yanik et al. (2003) discovered ^{13}C enrichments in the range of 2‰ to 8‰ for various PAH congeners in crude oil exposed to microbial degradation under aerobic conditions. This was explained as resulting from the preferred utilization of light compounds by soil microbes, although Yanik et al. (2003) acknowledged a possible contribution from evaporative fractionation as well. Nonetheless, the authors theorized that microbial transformation was the key factor, citing that past research has shown evaporative fractionation effects to amount to less than 1‰ enrichment for chlorinated hydrocarbons, but without mention of a known effect for PAHs. Furthermore, the unexpected result that nutrient application—in the form of nitrogen fertilizer—did not amplify the fractionation effect indicated that the wetland site used in the experiment did not have a previous nitrogen limitation. Another interpretation is that this lack of variability with nitrogen application serves as evidence against the microbial role in fractionation. Additional experimentation in a nitrogen-limited system would help to solidify these conclusions. Concluding statements made by Yanik et al. (2003) highlight the importance of CSIA as a source-apportionment tool, and they caution against discounting biotransformative fractionations in future source apportionment studies, as these may alter source signatures. Finally, an important caveat of this research study is that fractionation occurrence and direction were typically predicted by exposure to biotransformative mechanism. Magnitude of fractionations remained unresolved, as these appeared irregular in the data, illustrated below in the figure excerpted from Yanik et al (2013).

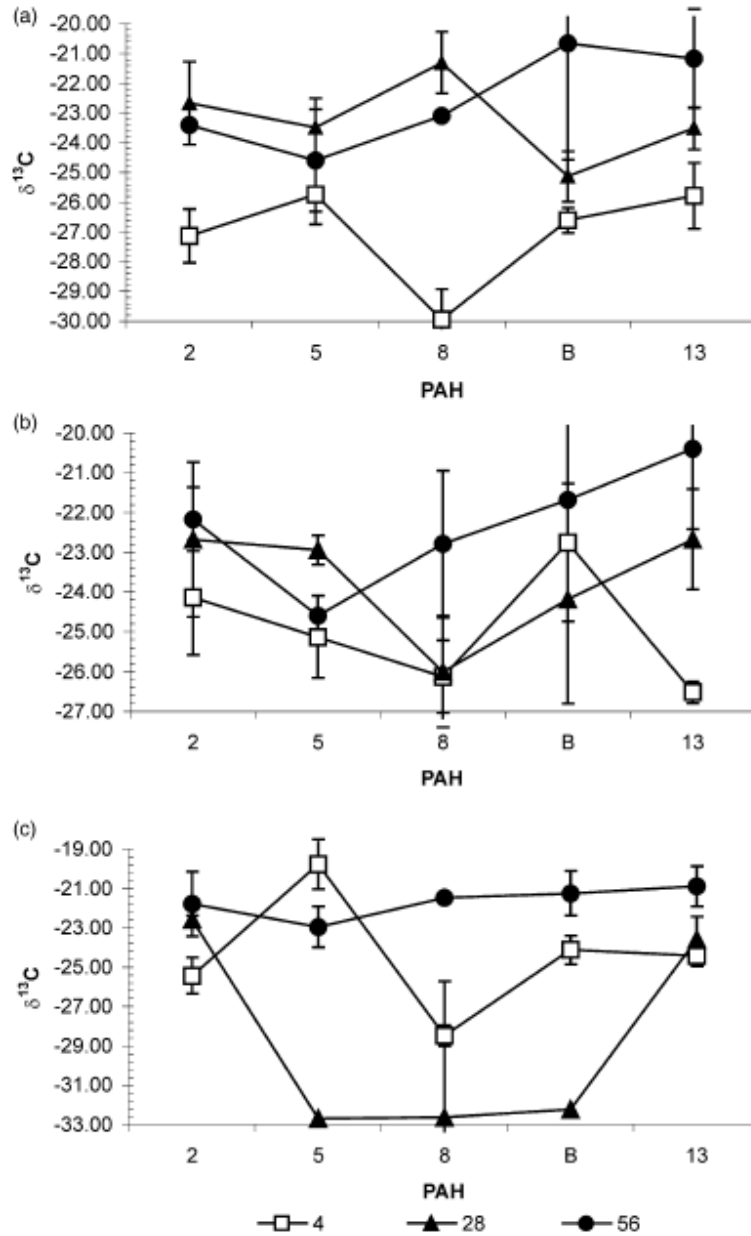


Figure 3.2 Progression of carbon-13 signatures from day 4 (outline square) to day 28 (full triangle) to day 56 (full circle) for a. nutrient-treated, b. control, and c. microbe-amended soils. In each case, final values correspond to greater carbon-13 enrichment than initial values, though intermediate signatures and enrichment magnitudes are highly variable.

As mentioned, source apportionment of PAHs using CSIA is a more mature science, with ample precedent in the literature for waterway contaminants, although CSIA typically serves as a supplement to the yet more established method of molecular signature evaluation. In the Elizabeth River of southeastern Virginia, PAHs have been

source identified using this combination (Walker et al., 2005). This study, introduced at the end of section 3.5, is archetypal for this topic. The Elizabeth River is situated in a highly polluted region of the Chesapeake Bay, where numerous pollutant sources are known or suspected as inputs, and where urban influxes likely continue to the present day. The group used principal component analysis, isomer ratios, and compound-specific isotope ratios to differentiate between sources of PAHs found in 119 sediment samples. In particular, the group measured the relative influences of four point sources, two retired wood treatment facilities known to be sources of subsurface creosote in the waterbody, a coal-fire power plant, and a suspected subsurface petroleum storage area. Correlation matrices found useful data in the ratios between low and high molecular weight PAH isomers, with a dominance of low molecular weight (LMW) PAHs in the vicinity of one suspected pollution source. This served as evidence for the recent or continuous release of creosote, which tends to be abundant in light PAHs, from the adjacent facility. Meanwhile, in main-stem regions of the river, higher molecular weight PAHs contributed more significantly to the overall picture, pointing to a dominant pyrogenic source. Petrogenic sources were found to dominate for only a few sporadic samples, and each case was spatially correlated with a creosote-implied location. To further test this prediction, CSIA was enlisted. Evaluating the petrogenically linked samples, one key outlier was discovered near a coal-gasification plant. Although the findings were ultimately a product of several methods, including principal component analysis of the isomer ratios and simple PAH weight-abundance ratios, CSIA offered the final confirmation for a link between three of the petrogenic PAH samples, while one was demonstrated to be different. The anomalous isotopic signature, of $-22.7\text{‰ } \delta^{13}\text{C}$ versus

the expected 25.1‰ $\delta^{13}\text{C}$ for a theoretical site sample including 50% coal inputs, was attributed to the high temperatures used during coal gasification, and relatively stronger kinetic isotope effect associated with this process. (Walker et al., 2005)

Additional source apportionment studies centering on waterbodies include the work of Fabbri et al. (2003) with sediment cores in the Adriatic Sea, a similar case study by Petrišič et al. (2013) in Lake Bled, Slovenia, a study of various surface waters in the northwestern United States by Kim et al. (2008), and the research by Stark et al. (2003) on the St. Lawrence River in the Great Lakes region. Similar to Walker et al. (2005), each of these groups implement isomer ratio analysis as their primary tool, and supplement CSIA for remaining questions not answered by the former method. In each case, the implementation of CSIA provided novel findings.

Fabbri et al. (2003) found overall ^{13}C depletion in sampled sediment cores from a lagoon, relative to bulk samples of soil or other environmental media, as well as subsurface ^{13}C minima. They interpreted these signatures, in conjunction with associated tracer molecules (PVC and mercury), as evidence for PVC production-related contamination. Many of the isomer ratios conventionally used for source apportionment returned inconclusive results for this study. Medium values of close to 1 evidenced either a mixture of pyrogenic and petrogenic sources, or a biogenic source. Meanwhile, a virtual absence of alkylated PAHs as well as an abundance of heavier isomers initially pointed to a likely high-temperature combustion source. Using CSIA, and observing the significance of natural gas as a source of energy in their study area, Fabbri et al. (2003) concluded that natural gas and methane-related manufacture of PVC were primary contamination sources. One congener in particular, cyclopenta[cd]pyrene, was found to indicate this

source, with $\delta^{13}\text{C}$ values in the range of -63‰ to -36‰. This finding is significant because past work on PAH signatures of natural gas is scarce, even given the recent usage spike of the resource. Given the presumption that natural gas is a cleaner fuel source than coal and other fossil fuels, related PAH contaminations are important to study, especially in light of the prevalence of cyclopenta[cd]pyrene—a keenly toxic congener—in the sediments of this lagoon. (Fabbri et al., 2003)

The utility of sampling distinct layers of sediment is to determine inputs during different periods of time, and to compare or model changes in concentration from anthropogenic behaviors over time. Petrišič et al. (2013) outline this capability, using molecular and stable isotope analysis to pinpoint pyrolytic inputs from the 1950s, largely related to coal combustion, and even indicating differences throughout a ten-year period, which indicated significant introductions from vehicular exhaust and wood burning. Petrišič et al. (2013) performed this study by focusing on only two PAHs, retene and perylene.

Studying the sediments associated with various surface water types, Kim et al. (2008) mimicked the assumed workflow, initially applying isomer evaluations and filling the knowledge gaps from this method with CSIA. Notably, the group remarks on a limitation to CSIA in their research—a lack of knowledge of the endmember isotope ratios implied in their research. Indeed, this is a significant limitation not only of CSIA but also of isomer evaluation, for in situ studies. Nonetheless, Kim et al. (2008) make one important finding using CSIA, namely the ‘uniqueness’ of PAH signatures in a remote lake, as compared with the harbor and shipping waterway also studied and influenced by the high levels of contamination present in the lake. Following the research, which found

$\delta^{13}\text{C}$ levels in line with those of automotive exhaust, Kim et al. (2008) determined that a nearby coal gasification plant was the likely culprit. Although automotive exhaust shared the isotopic signature of the CG plant, its ambient effects would be inconsistent with the exceptionally high levels of contamination in the lake. Isotope ratios were highlighted as useful by the authors, due to their robustness across physical and chemical transformation relative to molecular indicators. (Kim et al., 2008)

Finally, the work of Stark et al. (2003) is interesting as it applies CSIA to differentiate between sedimentary PAHs on the north and south banks of the St. Lawrence River. Again, CSIA is not the leading method for PAH source identification, with observations of PAH molecular weight distribution and parent/alkylated PAH ratios primarily utilized. Nonetheless, CSIA was able to connect three-ring PAHs found on the southern bank of the river with a nearby aluminum smelting process, via $\delta^{13}\text{C}$ enrichments. Meanwhile, PAHs profiles on the northern bank showed strong petrogenic signatures, and in areas upstream of the aluminum smelting plant were more closely associated with pyrogenic sources, with larger and more $\delta^{13}\text{C}$ enriched PAHs dominating. Stark et al. (2008) demonstrate an interesting case in which waterbody-scale hydrologic and geomorphic processes are shown through CSIA, since their findings link only one bank of the river to the high-contamination aluminum smelting operation.

Between Walker et al. (2005), Fabbri et al., (2003), Petrišič et al. (2013), Kim et al. (2008), and Stark et al. (2003), various findings are highlighted that are made possible exclusively by CSIA. So too are various challenges associated with the method. Buczyńska et al. (2013) review the current challenges facing CSIA investigations of $\delta^{13}\text{C}$ into PAHs in the laboratory setting. They discuss the importance of understanding

isotope effects associated with the analytical treatment of PAH samples, i.e. the extraction and separation of target analytes. This advice can be applied broadly to the CSIA of other POPs as well, as any lab-induced fractionations have the potential to distort or skew environmentally originating signals. In the example of PAHs, such fractionations tend to affect the resolution of IRMS peaks, which as a result of the faster elution of isotopically heavier molecules will skew towards ^{13}C enrichment at the start of a peak and towards depletion at the end. In cases where peaks are not well resolved, i.e. where gas chromatography has not effectively sieved out all mass-associated impurities with a given PAH in a complex mixture, subsequent compound peaks may appear unduly ^{13}C depleted and preceding compounds may register as enriched. This problem is particularly relevant for PAHs because of the variety of comparable compounds that must be considered for residual mixing, and because of the variety of PAHs that may exhibit this effect. (Buczyńska et al., 2013)

3.7 Conclusions

While PAHs likely receive the most advanced CSIA treatment of the reviewed pollutants and of POPs in general, several gaps still exist in method applications for aquatic ecosystems. Most importantly, there is a lack of preceding literature on the isotopic signatures associated with the metabolism of PAHs by macro-organisms, especially as compared to the research that exists for isotope fractionations of PCBs biotransformation in fish, reviewed in the preceding chapter. Even for microorganisms, divergent accounts exist of the fractionation effects of both anaerobic and aerobic degradation. Although Meckenstock et al. (2004) and Yanik et al. (2003) have

demonstrated fractionation effects for each of these mechanisms respectively, their research has served primarily to caution others conducting source apportionment research against ignorance of biotransformation fractionations. For the strict purpose of tracing PAH transformation through an ecosystem, research using either CSIA, isomer enumeration, or PCA is scarce, likely because PAHs are not deemed an important health hazard in food, at least as compared to the inputs from PAHs incidentally produced through food preparation. Nonetheless, fractionations of both isomers and isotopes should be expected for PAHs, given the relative stability of heavier PAHs and the general preference of organisms to metabolize lighter molecules. Additionally, while the mentioned studies have focused on determining $\delta^{13}\text{C}$ effects, recent research has highlighted the utility of other isotope ratios, such as deuterium (Kümmel et al., 2016).

Source apportionment of PAHs has been performed primarily using tracer molecules, the relative contributions of large and small PAHs, and the abundances of certain isomers associated with either pyrogenic or petrogenic sources. CSIA has been implemented in several cases to distinguish between sources that are unresolved by these methods, for example displaying inconclusive or atypical isomer abundances for a sample region. Interesting findings in aquatic systems to which CSIA has contributed include (in two cases) the discovery that coal gasification is a prevalent input of PAHs to a waterway, the tracing of natural gas combustion as an important input to lagoon sediments, and the resolution of PAH input sources to opposite banks of a river—found to diverge due to fluvial transport processes. Given these findings, as well as the observation by Kim et al. (2008) that such research is often limited by the lack of knowledge of endmember signatures, future work should emphasize the determination of

source signatures. This is particularly important for understudied inputs, like natural gas, that are likely contributing more significantly to environmental PAH concentrations in recent times. Finally, attention must be paid to the isotope effects associated with various extraction procedures, and authors of future PAH-CSIA studies should provide detailed accounts of their laboratory protocols as well as the measures taken to normalize any isotopic fractionations that may have taken place during sample extraction and separation.

CHAPTER 4. DDT

4.1 Chemical overview

One persistent organic pollutant that has seemingly been at the center of controversy more than any other is DDT, or dichlorodiphenyltrichloroethane, an organochlorine molecule initially hailed as a lifesaver for its insecticidal properties (Hellou et al., 2013).⁵³ Part of the broader groups of organohalogen (OH) and organochlorine (OC) pesticides, DDT itself is comprised of a significantly narrower set of molecular forms than either PCBs or PAHs, despite that the label DDT, or Σ DDT, also includes its metabolites: dichlorodiphenyldichloroethane (DDD), -dichloroethylene (DDE), -chloroethylidene (DDMU), -chloroethylidene (DDMS), -chlorophenylethylene (DDNU), chlorophenylethanol (DDOH), and chlorophenyl-acetic acid (DDA). Of these, the former two metabolites, DDD and DDE, are most commonly researched. Each of these molecules has two common isomer variants, producing a total of six isomers of DDT that are conventionally considered. The isomer types—para-para (p,p') and ortho-para (o,p')—accord to the arrangements of chlorines on the phenyl rings, one chlorine corresponding to each ring. Although extant, the meta-para (m,p') variant is far less common and rarely considered for study. While toxicity to humans and other organisms is generally maximized for DDT as opposed to its metabolites, DDD and DDE also exhibit toxicities with modes of action overlapping that of their parent (Beckvar & Lotufo, 2011).⁵⁴

Scientists have identified DDT as an endocrine disruptor in fish and other aquatic organisms. This behavior is explained by the similarity in structure of the toxin to

synthetic estrogen. Deconstructing its name, the parent DDT molecule is composed of two singly chlorinated phenyl rings bound together through an outside carbon, which comprises one end of an ethane molecule. The second carbon comprising the ethane in turn is bound to a trio of peripheral chlorines (Rogers et al., 1953).⁵⁵ Two diagrams of p,p'-DDT are given below.

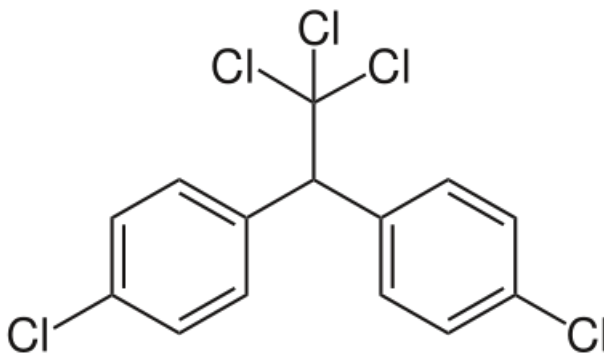


Figure 4.1 Chemical structure of p,p'-DDT ($C_{14}H_9Cl_5$), the most common congener present in commercial DDT mixtures. Note that the dimension has been reduced for the purpose of representation, nor is this an accurate cross-section. In reality, larger, tetrahedral bond angles exist between the three chlorines bound to the ethane (top), and planar rotations into the page actually exist for each of the phenyls (bottom) (Leyo, 2010).⁵⁶

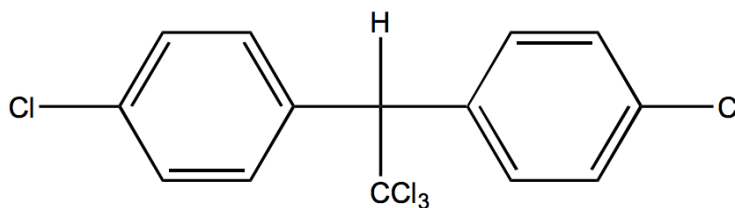


Figure 4.2 Top down schematic, relative to Fig. 4.1, of the structure of p,p'-DDT ($C_{14}H_9Cl_5$). Note this is not an accurate cross-section either, but instead is meant to

supplement Fig. 4.1. A true cross-section from above would depict the chlorinated phenyls rotated into the page relative to the central C. Also, the CCl_3 structure (top of the ethane in Fig. 4.1) would overlay the central C, while the remaining H (not pictured in Fig. 4.1) would be overlain by it. (ATSDR)⁵⁷

4.2 Historic production

Although initially synthesized in 1874, DDT did not become a ubiquitous environmental hazard until after its implementation as a pesticide in the 1940s. Low purported acute toxicity to humans and other mammals combined with high acute toxicity to insects made DDT an ideal candidate for large-scale agricultural implementation. During this time period, cotton growing regions of the southeastern United States were among the areas most heavily supplied with aeriually-sprayed OH pesticides, including DDT and DDD. Inadvertent toxicities to other non-human organisms were observed very early in this implementation period, in some cases within the first 5 years. (Beckvar & Lotufo, 2011)

Additionally, DDT was implemented heavily by U.S. forces in Europe during World War II, in a largely successful effort to eradicate diseases like typhus in the region. Meanwhile, implementation in the Southern Pacific was also a largely successful measure against malaria. The opposing harms and benefits of DDT have since sparked controversy, with an environmental movement of those concerned with the long-term ecosystem effects of DDT receiving stark criticism for the opportunity costs of the disease burden that could potentially be eliminated. The 1962 publication of *Silent Spring* by Rachel Carson, a documentation of the deleterious ecosystem effects of DDT, proved

to be a turning point in the United States' approach to environmental issues surrounding organohalogen pesticides and persistent organic pollutants. The book delineated many environmentally toxic effects of DDT, such as those on birds, and it inspired a wave of environmental activism that eventually led to the creation of the U.S. Environmental Protection Agency in 1970. Thus, it was not until 1972 that the United States issued a ban on widespread agricultural uses of DDT, following in the footsteps of Canada and Sweden, where such bans had already been implemented, and preceding bans of other OH pesticides such as aldrin and dieldrin. According to long-term contamination data maintained by the U.S. Fish and Wildlife Service (USFWS) and later by the U.S. Geological Survey (USGS), the overall trend in contamination levels has been downward since the implementation of the ban. However, the resistance of DDT to biodegrade and the tendency to bioaccumulate have led to its persistence around the United States and the world, with significant concentrations still to be found in a variety of environmental media, including water, soil, sediment, and tissues of humans and other organisms. (Beckvar & Lotufo, 2011)

DDT and DDD mixtures have been manufactured under a variety of names, including Zerdane, Pentachlorin, Neocidol, Neocid, Ixodex, Gyron, Guesarl, Guesapon, Gesarol, Genitox, Dinocide, Detoxan, Chlorophenothane, Cezarex, Anofex, Dilene, Rothane, and Lysodren (ATSDR)(WHO, 1979).⁵⁸ Meanwhile, DDE exists exclusively as a metabolite. Technical mixtures consist of approximately 65% to 80% p,p'-DDT, considered to be the active ingredient in all but the latter three mixtures listed above. For these, the other 20% to 35% are comprised of DDT variants and metabolites. Although technical mixtures of DDT are now out of historical use, indoor spraying of DDT to

control malaria epidemics continues in much of Africa and Asia, and has been supported there by the WHO since 2006. Additionally, DDT continues to be used in China as an antifouling agent in paints. In the last 20 years, per year estimates peg global DDT production at around 4000 to 5000 metric tons, with manufacture occurring in India, China, and South Korea. By comparison, DDT application in the United States alone for the year 1959—at the height of widespread agricultural implementation—was estimated at 450,000 metric tons. (Beckvar & Lotufo, 2011)

4.3 Toxic effects

Toxic effects of DDT in nontarget species were discovered in the early 1940s, shortly after the 1939 discovery of its insecticidal properties. Among other effects, these include the observation that the eggshells of many bird of prey species were thinning in areas where DDT treatment had taken place (Hellou et al., 2013). Effects on humans were not investigated until several years later. Faroon et al. (2002)⁵⁹ of the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) review the toxic effects of the primary isomers (p,p') of DDT, DDE, and DDD on humans. Owing to the representation of each in technical mixtures, exposure typically takes place to a combination of the three, with (o,p')-DDT also heavily represented. The document is intended for the management of human exposure, and is divided by exposure route: inhalation, oral, and dermal. Acute respiratory effects associated with DDT inhalation are reported to be minimal. On longer time-scales however, occupational exposure to DDT has been associated with an elevated risk of lung cancer. A wider variety of studies have documented adverse reproductive, hepatic, endocrine, and nervous system effects for oral

exposure vectors, while similar trends have been documented for dermally applied DDT. In general, the greatest risks to humans posed by DDT exposure are endocrine system effects and neurological effects. (Faroon et al., 2002)

Neurotoxic mechanisms for DDT effects in both humans and non-human organisms occur by interference with the normal movement of ions through neural membranes (Hellou et al., 2012). By several actions, the introduction of DDT to the body may prevent the normal repolarization of the neural membrane, allowing for releases of copious transmitters, which in turn can cause the central nervous system to overexcite. In extreme cases, such as in the only recorded human fatality related to acute exposure by ingestion, this excitation of the central nervous system can lead to seizures, tremors, and convulsions. (Faroon et al., 2002)

Endocrine effects, meanwhile, disproportionately affect males and are thus believed to occur through the interactions of DDT with steroid hormone receptors. Binding predominately to estrogen receptors, DDT and its metabolites are believed to be responsible for the feminization of fish in ecosystems around the world, inhibiting the secretion of gonadotropins, which are responsible for sexual development. In these ecosystems, sexual differentiation may be limited; thus the number and health of offspring is likely to be compromised (Beckvar & Lotufo, 2011). While the carcinogenicity of DDT in humans is still technically undetermined, carcinogenicity has been demonstrated in laboratory animals. Dominant mechanisms are believed to be mutagenicity due to direct binding with the DNA structure of electrophilic DDT metabolites, or toxicity to individual cells themselves, resulting in their subsequent overproduction. (Faroon et al., 2002)

While the endocrine disrupting effects of DDT in aquatic species have only recently begun to receive attention, many studies since the 1940s have reported on the acute toxicity of DDT through neurological action. For aquatic species, the majority of observed mortalities are caused by an overabundance of spontaneous nerve discharges. Due to the effectiveness of this mode of action, DDT is lethal to many organisms at levels far below the lethal doses of other organohalogen pesticides. In addition, while this mode of neurotoxicity is prominent for both DDT and DDD, it has not been associated with the metabolic product DDE. Furthermore, DDT and DDD themselves are neurotoxic to varying degrees depending on the species. In general, for all toxic effects, regulatory revisions are most likely to arise from the discovery of longer-term or less apparent but statistically important features of DDT toxicity. In both humans and wildlife, these include the recently emphasized endocrine system effects as well as developmental effects. (Beckvar & Lotufo, 2011)

4.4 Environmental behavior

In the environment, DDT exhibits all of the usual properties of a persistent organic pollutant. Half-lives in soil have been estimated at 10-15 years (CHM, 2008). DDT is insoluble in water and overrepresented in sediments and tissues of aquatic organisms due to its affinity for other organic substances and tendency for persistence. Long-range transport of DDT is enabled due to these properties and because it may readily volatilize and redeposit between various media. Biomagnifying effects have been observed across multiple species, and microbial transformations have been widely documented. HCl removal by oxidation, as well as Cl removal by reductive

dechlorination are known to occur under aerobic and anaerobic conditions, respectively. The transformation product DDE is normally produced through aerobic degradation, while the product DDD tends to be produced through anaerobic degradation. Generally, DDE is the most readily transportable of the three, followed by DDT and then DDD. Notably, DDE has also been observed as a degradation product of DDD. (Hellou et al., 2013)

Among persistent organic pollutants, the environmental prevalence of Σ DDT in remote locations is second only to that of PCBs, with unusually high concentrations found in Arctic food webs, thousands of miles from application loci. Determination of sources becomes particularly relevant in these areas. The potential to biotransform DDT varies by species, with mammals exhibiting less enzymatic action than birds, and marine mammals exhibiting less than terrestrial mammals. Tissue burdens are determined by a variety of additional factors, including species, sex, and age of the individual. For example, in birds, it has been discovered that maximum burdens are present in eggs (Norstrom et al., 2007).⁶⁰ In fact, maternal transfer has been found to be a critical mechanism for toxic exposure in various organisms, juxtaposing significant concentrations of DDT with the sensitivity of early life stages. Studies in fish have found that bioaccumulation occurs most rapidly during this time because of the relatively high metabolic capacity of juvenile fish. Furthermore, the enzymatic activity associated with DDT transformation may develop later in life, resulting in the exposure of juveniles to more toxic compounds (Beckvar & Lotufo, 2011).

Various behavioral phenomena have been discovered from investigations into DDT in surface soil. These have been many, given the importance of soil as a proximal

media to the initial application of DDT. These are summarized briefly, since the focus of this review remains on water, sediment, and tissues of aquatic organisms. Firstly, it has been found that soils can act as a net source for DDT in the remaining environmental matrices, tending to volatilize significant concentrations, although the half-life for DDT in the top 5cm of soil has been estimated at 200 years. Additionally, tropical soils have been found to accelerate DDT metabolism, with observed half-lives of only three years, compared to approximate 20 years for temperate soils. Finally, half-lives are dependent on the concentrations of p,p'- and o,p'-DDT, with o,p'-DDT half-lives being shorter; o,p'-DDT has also been observed to mobilize preferentially along the soil-air boundary, compared to p,p'-DDT. (Ricking & Schwarzbauer, 2012)⁶¹

4.5 Monitoring and fingerprinting

A significant body of work has been dedicated to the analysis of DDT in environmental matrices since the 1950s. Extensive monitoring and fingerprinting of the contaminant, however, has been a phenomenon of the past 20 years. Fingerprinting of DDT to apportion sources establish culpability for contamination has been since performed by a variety of techniques, including simple concentration analysis, isomer abundance ratio determination, and compound-specific isotope analysis. These fingerprints are sensitive to biotransformation, photo-transformation, reaction, and transport history, as well as to source production conditions (Hellou et al., 2013). Ratios of p,p'-DDT to p,p'-DDE, for example, can provide insight into the relative contribution of atmospherically transported DDT to a sample. Because the lighter isomer, p,p'-DDE is preferentially volatilized, it tends to be overrepresented in samples that have accumulated

atmospherically transported DDT (Hellou et al., 2013). In addition, because the ratios for insecticidal, technical DDT mixtures are known to be approximately 77% p,p'-DDT, 15% o,p'-DDT, 4% p,p'-DDE, and 0.4% p,p' DDD, significant deviations from these known values are typically indicative of non-insecticidal sources. This conclusion is particularly strong if the enrichment observed is for o,p'-DDT, ruling out the possibility that a transformation process has converted some amount of p,p'-DDT into either of its metabolic products.

Ratios of o,p'- to p,p'-DDT have also been used to differentiate between DDT and dicofol emission sources. Dicofol is an organochlorine pesticide with an unsettlingly similar structure as DDT. Although dicofol is classed by the WHO as a less hazardous chemical and is thus less regulated, it is in fact synthesized directly from technical DDT itself and may contain traces of any DDT variant as unintended impurities. Dicofol is currently being used in Europe, Asia, and North America (Ricking & Schwarzbauer, 2012).

Source apportionment studies applying several receptor modeling to a matrix of isomer-abundances have been successful for evaluating DDT in a variety of media, including aquacultured fish. For Fang et al. (2015),⁶² these methods allowed for the determination that food sources and contact exposure through ambient seawater were approximately equal contributors to DDT levels in ocean-farmed fish. The understanding that seawater and sediment were significant contributors led the authors to recommend a reduction in the use of anti-fouling paint, which was a dominant contributor to sea-water contamination in the area. Fortunately for this study, dietary compositions of the fish were known and could be fingerprinted for comparison. (Fang et al., 2015)

4.6 Applications of CSIA

Although the majority of the fingerprinting of DDT and its metabolites has been performed using isomer ratios, a handful of studies have added in CSIA parameters, supported by the effectiveness of this method for other POPs and by the work of groups like Ivdra et al. (2014)⁶³ to establish extraction methods for field samples. Incorrect field extraction methods may be a fatal flaw to CSIA research, if they extract some isotopomers preferentially or otherwise alter environmentally indicative isotope signatures. Investigations that apply CSIA to understand environmental DDT origins and behavior have been scarce, given the convenience and forensic power of simpler parameters of DDT (i.e. isomer type and metabolite type) that are conventionally measured and paired for analyses of variance. Through these, DDT-investigations have a distinct advantage over those done for other POPs, for which difficult target selections from a long list of congeners must often be made. Although a search for stable isotope studies of DDT will heavily return bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ food-web investigations paired with molecular concentration analysis, several studies have been performed to date on isomer-specific stable-isotope ratios of DDT and its metabolites in the environment. While these fall short in range compared to those for PAHs, and in specificity to freshwater ecosystem trophic transformations compared to those for PCBs, conclusions are consistent. Compound-specific isotope analysis adds an extra line of information that may be applied to differentiate between the effects of distinct production histories and transformation processes on the identity of sample pollutants. Several examples of this advantage are given.

Niu et al. (2016)⁶⁴ coupled isomeric signatures with compound-specific carbon stable isotope signatures of DDT in a comprehensive study of soils in China. Using a combination of these methods, Niu et al., (2016) were able to infer the sourcing of DDT contaminations in eastern versus western China, and they also established several important fractionations relevant to the joining of these methods. CSIA was used to model the degradation pathway of DDT in these areas, while isomeric signatures were applied primarily to differentiate between difocol and technical sources of DDT. Because lighter isotopes preferentially partition between media and preferentially react in chemical processes, soil $\delta^{13}\text{C}$ enrichment for a particular isomer was seen as a product of both degradation and volatilization, although $\delta^{13}\text{C}$ depletion could have been a product of dilution from the inputs of parent molecule degradation. As predicted, warmer sites displayed greater soil $\delta^{13}\text{C}$ enrichments due to their enhancement of volatilization to the atmosphere, but also of microbial degradation processes. Ultimately, $\delta^{13}\text{C}$ proved to be more sensitive to biodegradation processes than to atmospheric fluxes, accounting for an observed enrichment of $\delta^{13}\text{C}$ for p,p'-DDE in cooler western China, where influxes of the biotransformed parent p,p'-DDT (high preference for ^{12}C) were minimized compared to escaping ^{12}C by subdued atmospheric processes. A forerunner of its kind, this study concluded that further controlled experimentation would be required to differentiate between the convoluted isotope effects. Nonetheless, valuable clues are offered as to the likely prevalence of biotransformation processes on DDT isotope abundances in a variety of media. (Niu et al., 2016)

Prominent transformation-related fractionation effects had previously been documented not only for $\delta^{13}\text{C}$, but also for $\delta^{37}\text{Cl}$ and $\delta^2\text{H}$, two other interesting

parameters for chlorinated organic pesticides. Ehlers et al. (2014),⁶⁵ performed CSIA using deuterium fractionations associated with the DDT to DDD conversion. Finding that specific formation conditions lead to the $\delta^2\text{H}$ enrichment at just a single position in a DDD congener, Ehlers et al. (2014) were able to differentiate between the contributions of degrading DDT and originally synthesized DDD to the total DDD of a sample. CSIA is the only known method that can isolate this signature.

Looking at $\delta^{37}\text{Cl}$, Reddy et al. (2002)⁶⁶ found that significant compound-level fractionations took place while converting DDT to DDE via controlled HCl removal. These effects were found to be mostly temperature-independent. Although chlorine-based CSIA has yet to be applied to a significant number of environmental DDT studies, documentation of this kinetic effect lays the groundwork for such work in the future, boding for its prospective sensitivity to fractionation effects while screening out the noise from temperature-dependent variables. One example of such work, implementing $\delta^{37}\text{Cl}$ measurement, and the most relevant compound-specific DDT study to this review, was performed by Holmstrand et al. (2007).⁶⁷ This group took samples of bioaccumulated DDT directly from the fatty tissues of marine mammals and measured these for evidence of environmental degradation. Blubber specimens were collected at the Swedish Museum of Natural History, from the bodies of two stranded grey seals. As a top predator in its environment, the grey seal acts as an excellent representation of contaminant concentration across its feeding grounds and ecosystem. Holmstrand et al. (2007) cite literature $\delta^{37}\text{Cl}$ values of roughly -3.5‰ to -5.5‰ for pristine DDT, implied to be the source material for the ecosystem under study. Meanwhile the measured, blubber DDT registered a $\delta^{37}\text{Cl}$ value of about -0.7‰. The enrichment observed in the blubber, which

was mirrored by the $\delta^{37}\text{Cl}$ signatures for DDE, serves as evidence for an environmental degradation process associated with a kinetic isotope effect. Although a potential dehydrochlorination reaction was known to convert sample DDT to DDE, such a reaction has not been found to correspond to any chlorine isotope effect, allowing the researchers to disregard it as a potential confounding variable in this study.

Several studies have approached better understandings of DDT in aquatic systems through the lens of isotope analysis. However, much of this is encompassed by a body of research applying radioisotopes to trace the behavior of the pollutant. This research, some of which dates back to the 1960s, has generally taken the form of controlled, before-after experimentation. Dindal (1970)⁶⁸ applied Cl^{36} -marked DDT to an exposure area, and subsequently introduced several species of ducks to elucidate the metabolic and bioaccumulative tendencies of DDT in aquatic life. This technique is referred to as radio-labeling. While radio-isotope analysis applies an entirely different set of principles to the investigation of POPs, it is worth mentioning that controlled introductions of labeled DDT have generally been favored for experimental purposes (Bao et al., 2013).⁶⁹ Actual applications of stable-isotope principles to the determination of ambient sample DDT origins have been severely limited, with none performed to date with a focus on freshwater CSIA. Meanwhile, strong historical preference has been given to the established methods of isomer abundance ratio analysis for these purposes.

4.7 Conclusions

The combination of $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{37}\text{Cl}$ investigations that have been reported here paint a clear picture of the potential for compound-specific isotope analysis to be

used in a wide range of environmental DDT studies. However, while several isotope- and stable-isotope-focused studies have been undertaken, the majority have followed tangential paths, either applying known experimental inputs to an environmental system and then observing the effects, or isolating a single chemically controlled transformation. In fact, one of the only two studies to take advantage of the explanatory power of CSIA in a distinctly environmental situation was published only a year ago (Niu et al., 2016) and was focused on fractionation effects between the soil and atmosphere, thus removed from the aquatic systems this review has sought to encapsulate. The other, Holmstrand et al. (2007), makes a compelling case for CSIA as a tool to track biotransformation in the environment, though findings were limited to establish the probable existence of—rather than fully quantifying or pinpointing—a biotransformation process in a marine ecosystem in Sweden.

The need exists for quality fingerprinting and transformation monitoring research to be conducted for DDT, as it continues to be used around the world in disease vector control and persists from historical applications on large agricultural scales. Particularly in China, the results of concentration studies for biota have been worrisome. Grung et al. (2015)⁷⁰ posit that passive sampling of biota coupled with source determination will be key for bringing about a viable, regulatory-informing state of knowledge of DDT and other pesticides in the environment. Knowledge gaps are also reported to exist in remote environments and in developing countries, where DDT is still applied within human dwellings. Meanwhile, recently popularized organophosphate pesticides will require equal treatment as probable POPs, whereas Σ DDT has received generous attention by comparison.

In conclusion, an unanticipated gap exists in the CSIA of ambient DDT, as compared to the CSIA of other organohalogen pollutants. Although a simple explanation for this discrepancy is not readily deduced from the literature, it is likely a product of several factors. CSIA is a relatively new technology; meanwhile DDT has been researched intensively since inception of its insecticidal properties, circa 1939. The framework that has been established for the source apportionment and determination of the environmental history of DDT is thorough and heavily skewed to isomeric methods, which are both powerful and relatively simple. Finally, CSIA requires a GC-IRMS—or NMR technology in the case of the $\delta^2\text{H}$ study reviewed (Ehlers et al., 2014). Such instrumentation requires extra expertise and financing to operate, as compared to a simple GC-setup. All disadvantages considered, compound-specific isotope analysis continues to offer the key benefit of an additional layer of forensic information. Isotope fractionations are seemingly ubiquitous for the environmental processes undergone, and DDT may be a model chemical with which to continue pioneering this field of research, given the existing understanding of the fractionations of its isomers.

CHAPTER 5. CONCLUSIONS

The primary purpose of this thesis has been to encapsulate the state of aquatic contaminants research performed by means of compound-specific isotopic analyses of hydrogen, carbon, and chlorine. It argues that CSIA provides an indispensable advantage to source apportioning POPs that have undergone transformations since their emission into the environment. To this end, and according to integrated literature findings, it recommends strategies for CSIA implementation in source apportionment and aquatic biotransformation research that will enable better future understandings of pollutant remediation. The secondary purpose of this thesis been to give a collective voice to the commentary and cautions of environmental chemists versed in the discipline, bearing in mind that numerous barriers to entry exist for agencies and individuals interested in applying state of the art methods to monitor local concentrations of aquatic persistent organic pollutants. Together, the aquatic CSIA stories of PCBs, PAHs, and Σ DDT reported here capture the variability that exists for applications on different persistent organic pollutants, although scientists continue to explore many additional chemical species, including newly discovered varieties (Elsner and Gwenaël, 2016).⁷¹

Because PCBs and PAHs have been two of the most intensively studied pollutants through this lens, the accounted research reflects the scope of the applicability of CSIA. Meanwhile, perhaps the most interesting finding of this review has been the relative absence of compound-specific isotopic data taken for Σ DDT, relative to the abundance of available literature covering its study via molecular fingerprinting. This mirrors two other discovered literature gaps, (1) that for the CSIA-based source apportionment of PCBs and

(2) that for the CSIA-based study of trophic transformation of PAHs. The large research gap for Σ DDT is attributable to the relative simplicity and versatility of already established methods, while those for the source apportionment of PCBs and evaluation of trophic transformation in PAHs are better explained by a combination of barriers to entry and research trends.

Since a pioneering effort by Yanik et al. (2003), aquatic PCBs research involving CSIA has enjoyed a recent surge from overlapping groups in China (Zeng et al., 2013)(Tang et al., 2016). While the former work was intended as a framework for future source apportionment research, outlining promising and problematic congener candidates for this application, it has instead served as a starting point for the aquatic ecosystem PCBs biotransformation research that followed. Including environmental source and tissue samples of various species exposed through their diet, these authors have expanded on a list of PCBs—bearing vicinally bound hydrogens—which were previously known to be metabolized by fish. Furthermore, this research amends a plausible mechanism for the complete remediation of PCBs through biological degradation, with reductive dechlorination by either aquatic organisms or anaerobes preceding aerobic microbial utilization and mineralization. Since PCBs account for a significant portion of currently issued fish consumption advisories, this understanding of pollutant behavior *in vivo* is a vital complement to our understanding of its environmental toxicity and overall character. Meanwhile, actual source apportionment research conducted by means of CSIA, as outlined by Yanik et al. (2003), has not yet been popularized for aquatic media. Overall, research on the source apportionment of PCBs has not been scarce, yet it has been limited to certain methods of investigation, including isotopic and molecular tracer analysis

(Mechlinska et al., 2010),⁷² congener determination (Benoit et al., 2016), and congener fingerprinting by a combination of determination and statistical tools like principal component analysis and positive matrix factorization (Johnson & Ehrlich, 2002). Bulk and compound-specific isotopic analyses of PCBs are also documented in various tangents to this field, while in situ research remains largely untried. In the nearest example, Reddy et al. (2000)⁷³ pioneered chlorine stable isotope analysis for source identifying PCBs from fresh Aroclor to sediment, finding insignificant $\delta^{37}\text{Cl}$ variations between congeners and recommending the adoption of a bulk-analytical technique, whereby congener variations are disregarded. Given the tendency of this method to forego sensitivity to significant effects, such as the dechlorination of PCB molecules and resulting isotopic enrichment or depletion of associated congener pools, further research is required to determine the advisability of this recommendation. Indeed, subsequent bulk analyses of $\delta^{37}\text{Cl}$ have yet to be performed for PCBs extracted from environmental samples. Meanwhile, the more commonly pursued method of compound-specific $\delta^{13}\text{C}$ analysis has routinely been applied to characterize Aroclors and other commercial mixtures (Mandalakis, 2008;)⁷⁴ therefore the stage is set for future PCBs source apportionment to take place using carbon-13 based CSIA. Fortunately for prospective endeavors, Yanik et al. (2003), Zeng et al. (2013), and Tang et al. (2016) have already converged on several PCB congeners whose signatures are sufficiently resilient across transformations to serve as starting points for such research.

Rather on the opposite end of the spectrum, PAHs have received liberal coverage by source apportionment research in a variety of aquatically-related media, nonetheless largely excluding biological matrices. Several factors account for this bias. Provided that

ongoing human practices are continuously producing or reintroducing PAHs to the environment, the significance of input vectors for this POP are heightened compared to those for others. PAH exposures via contaminated tissue consumption—a dominant concern for PCBs—has been a relatively muted issue given that PAHs are easily absorbed through inhalation, and ordinary food preparation processes can produce significant exposures. Because of these factors, and because PAHs in their common form are less environmentally persistent than other POPs, CSIA-based biotransformation research has remained unexplored for aquatic species. This will be an important gap to consider in future work, given that halogenated PAHs tend to be similarly persistent and exhibit similar toxicities as other organohalogen pesticides and pollutants. In the meantime, PAHs have served as a model for the pollution-sourcing insights attainable through CSIA. In several cases (Walker et al., 2005)(Fabbri et al., 2003)(Okuda et al., 2002) (Stark et al., 2003)(Kim et al., 2008) CSIA has been applied as a supplemental line of evidence to source apportion aquatic PAHs. In no cases, however, has CSIA been implemented as the sole or leading method for such research. This clearly illustrates its role as a method to fill in the gaps left by leading methodologies, such as heavy to light isomer ratio analysis, congener abundance analysis, PCA, and combinations of these methods. This faculty also highlights its empirical strength, to succeed in differentiating highly similar sources like coal combustion and coal gasification (Walker et al., 2005), which other methods have failed to distinguish, as well as its relative impracticality and cost, to be applied so sparsely despite its clear advantage.

Although CSIA comes with high costs and demands specialized knowledge, several of these examples may justify its implementation versus exclusion in

environmental pollution studies. In addition to the conscientiousness of implementing the most state-of-the-art method and investigating the most fine-tuned signatures reliably produced through physical processes, benefits of application are considered when CSIA offers statistically significant answers to otherwise unanswered questions. In Walker et al. (2005), it is explicitly noted that CSIA accomplishes what a combination of PCA and isomer ratios could not. Source isomer signatures of coal were uninfluenced by the gasification process, while compound-specific isotope signatures were sensitive to the process. Similarly, in Fabbri et al. (2003) molecular signatures were constant between sediment layers, pointing to a pattern of deposition that did not vary with time. However, isotope profiles across these same layers contained wide ranges of $\delta^{13}\text{C}$, warranting an alternative explanation. Although Fabbri et al. (2003) did not converge on an answer to this question—the anomalous signatures were unlikely to be explained only by biotransformation—the curtailment of a potential alternative, erroneous conclusion that all sediments had a common origin and uneventful history following deposition is scientifically important. In addition to these examples of the indispensability of CSIA for source apportionment research without a necessary role of biotransformation, several examples can also be cited of the indispensability of CSIA for controlling source apportionment studies for biotransformation. These include the study by Niu et al. (2016) of soils in China, in which the degradation pathway of DDT, modeled by CSIA, was used to elucidate original signatures and thus establish source influences. Finally, in Zeng et al. (2013), Tang et al. (2016) and Tang et al. (2017), CSIA is applied exclusively to understand biotransformation, where other methods fall short. Totaling these examples,

for PCBs, PAHs, and DDT, compound-specific isotope analysis enables unique conclusions, justifying its costs relative to other methods.

CSIA has been applied to other POPs in similar fashion to the three selected for review. As an example, PBDEs or polybrominated diphenyl ethers were coupled into the trophic biotransformation project of Zeng et al. (2013). Other POPs belonging to the Stockholm Convention ‘dirty dozen’ have also been studied according to various CSIA objectives. This review has focused upon and recommended future work in the field of aquatic POP transformation, the logic for which is not only that waterway systems act as pollution arteries, but also that the tissue burdens of species exposed at these loci are probably analogous to patterns of human exposure risk in the surrounding area.

Sedimentary, soil, atmospheric, and water column POPs research is merely a means to the end of understanding contaminant effects on biological life—the contaminants do no intrinsic harm, after all, to the former matrices themselves. In light of international agreements and the yet-optimistic ideal of equipping our domestic monitoring programs in the United States with the most cutting-edge technologies available, CSIA is a logical target for mass integration to problem areas specified by the TMDL program in coming years. In the experience of most any other industry, technological development is the promise of survival. There can be no exception to this for progress in the environmental sciences that promises to grow societies toward a cleaner future, in which aquatic resources and wildlife may exchange harmoniously with human practice, and in which the ideals of environmental stewardship are responsibly upheld.

CHAPTER 6. REFERENCES

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