ECOLOGY

# Isotopic and Molecular Methods

## Sourcing Environmental PAHs: A Review

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Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants that accumulate in the environment as a result of both natural and human processes. The molecular and isotopic signatures of these compounds vary depending on production conditions, and can be exploited to trace PAH contaminants in the environment to a particular source or responsible party. Environmental forensics investigations relating to PAHs are often motivated by environmental remediation or litigation efforts and depend heavily on geochemical principles. The field has recently benefitted from the application of compound-specific stable isotope analysis (CSIA) to the source apportionment of PAHs. Although many advances have been made with this strategy since it was introduced sixteen years ago, further research is needed to overcome deficiencies in the database of isotopic signatures, technological limitations, and a lack of standardized methods.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are planar, high molecular weight organic compounds of environmental concern due to their suspected mutagenic and carcinogenic properties. PAHs are composed of two or more fused aromatic rings, formed during the incomplete combustion of biomass and fossil fuels or during the slow conversion of organic matter into petroleum (1, 2). PAHs formed from these two processes can be described as pyrogenic or petrogenic, respectively (1,3,4). Although natural processes occasionally deposit these contaminants, the deposition rate of PAHs into environmental reservoirs has been greatly accelerated in recent years due to human industrial activities and fossil fuel consumption (5).

A variety of environmental forensics and geochemistry techniques have emerged for studying organic pollutants over the past few decades in response to growing concerns about human impact. These techniques exploit the unique molecular or isotopic compositions of PAHs that arise from different production processes in order to provide insight into the sources of contaminants on a local or larger scale. Source apportionment techniques are of particular interest because they can be used for the purposes of environmental remediation, prevention of future contamination, and evidence in litigation (6,7).

Molecular compositions of PAH mixtures are frequently determined using gas chromatography mass spectrometry (GC-MS) and gas chromatography with a flame ionization detector (GC-FID), in addition to other techniques (8). These analytical methods are capable of quantifying concentrations and ratios of particular PAH compounds. Molecular signatures can be inconclusive in the absence of other information, however, so molecular analyses are often paired with a newer technique called compound specific stable isotope analysis (CSIA). CSIA has become an increasingly common and trusted analytical method for PAH source apportionment over the past sixteen years. It exploits the isotopic rather than the molecular signature of PAH compounds, a signature which tends to be less subject to interference by weathering processes (9).

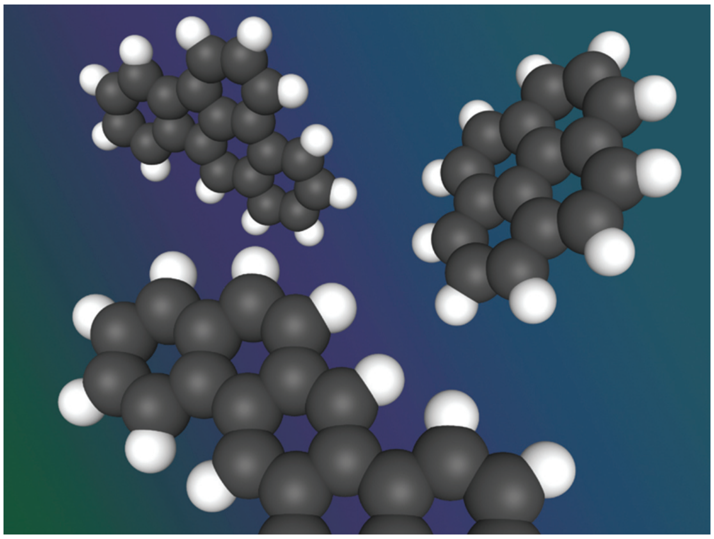

Image retrieved from http://upload.wikimedia.org/wikipedia/commons/c/c0/Polycyclic_Aromatic_Hydrocarbons.png (Accessed 29 Jan 2011).

Three examples of Polycyclic aromatic hydrocarbons: benz[e]acephenanthrylene, pyrene, and dibenz[a,h]anthracene.

The aim of this review is to provide an overview and analysis of the current state of source apportionment techniques as they relate to PAHs from a broad range of sources. The focus will be on the geochemical principles and methods employed in CSIA, as well as its complementary molecular methods. Both strategies will then be analyzed in terms of known applications and limitations. Future research should be directed toward overcoming these limitations, which include deficiencies in the database of known isotopic fingerprints, and technological and methodological limitations.

## Isotope Geochemistry Principles Employed

CSIA is a technique which generates isotope ratio data. The main ratio of interest is that of $^{13}C$ to $^{12}C$. This ratio is reported using delta notation, (Eq. (1)), which gives the permil (‰) deviation of the isotope ratio of a sample from that of a standard:

$$\delta^{13}C_{sample} = [(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{standard} - 1] \times 10^{3} \qquad (‰, \text{VPDB}) \qquad (1)$$

The Vienna Peedee belemnite standard (VPDB) is the most commonly used standard for this type of analysis, defining 0‰ on the δ-scale (5, 10).

The primary geochemical concept underlying CSIA involves kinetic isotope effects. These effects determine which isotopes are preferentially incorporated into PAHs during formation or into their organic precursors during photosynthesis. Kinetic effects alter the isotope ratios of the resulting PAHs at each major stage and pathway to formation, as described below. These situations collectively demonstrate that the isotopic and molecular signatures of PAHs are determined both by the isotopic composition of the precursor compounds and by formation conditions (3).

### Assimilation of $CO_2$

A kinetic isotope effect alters the isotopic composition of precursor organic materials as a consequence of $CO_2$ assimilation by autotrophs through either a $C_3$ or $C_4$ photosynthetic pathway. Both photosynthetic pathways discriminate against $^{13}C$, but to different extents: $C_3$ plants assimilate heavier isotopes slower than $C_4$ plants. Thus, $C_3$ plants have isotope values ranging from -22 to -30‰, while $C_4$ plant values range from -10 to -18‰ (10). The resulting isotope ratios are reflected to varying degrees in petrogenic PAHs made from plant matter and pyrogenic PAHs formed from the incomplete combustion of plant-derived fuels.

### Petrogenic PAHs

Crude oil and coal are produced by the thermal maturation of organic material of marine or terrestrial origin (3). PAHs also form during these processes and can be found in crude oil and refined petroleum products. Crude oil typically contains between 0.2 and 7% total PAHs; refined petroleum products such as diesel fuels and gasoline contain a combination of these parent crude oil PAHs and trace amounts of PAHs formed during refining processes (4). The isotopic compositions of different petroleum products will vary from one another because they originate from different sources of crude oil (8).

### Pyrogenic PAHs

Pyrogenic PAHs are formed during the incomplete combustion of fuels. Organic compounds are first cracked into smaller, unstable hydrocarbon fragments during pyrolysis and then undergo a series of radical reaction pathways involving carbon-carbon bond formation, cyclisation, and ring fusion to form more stable aromatic compounds (3, 5). As these radical reactions occur, $^{12}C$ is preferentially incorporated into bonds over $^{13}C$ in accordance with a normal kinetic isotope effect. Thus, PAHs get progressively depleted in $^{13}C$ as the number of rings in the molecular structure increases (11). Kinetic isotope effects, rather than equilibrium isotope effects, predominate in PAH formation because these processes occur rapidly and do not achieve equilibrium.

### Degradation

In contrast to formation pathways that generate pyrogenic and petrogenic PAHs, degradation pathways through weathering and other natural processes do not significantly affect the isotopic signature of PAHs. O'Malley *et al.* found that the isotope ratios of PAHs are preserved during processes such as volatilization, photolytic, and microbial degradation reactions (i.e., the isotopic signature of PAHs is conservative) (9). These conditions make it possible to use isotopic fingerprints to implicate a source in the creation of PAHs because the fingerprint can be assumed to remain constant.

## Molecular Characteristics Exploited

Petrogenic compounds can be distinguished from pyrogenic compounds based on different molecular "fingerprints." The aromatic rings of petrogenic PAHs frequently contain alkyl substituents. Alkylated PAHs are more abundant than the parent PAH compounds in petrogenic mixtures, whereas alkylated PAHs are far less abundant than the unalkylated parent compounds in pyrogenic mixtures (4). Additionally, low molecular weight compounds are more common in petrogenic PAHs while high molecular weight compounds are more common in pyrogenic PAHs (2). Examples of commonly analyzed PAH compounds for molecular analyses are depicted in Fig. 1.

## Methods

### Isotopic Methods

Compound specific stable isotope analysis pairs a gas chromatography separation method with an isotope-ratio mass spectrometer (GC-C-IRMS, Fig. 2) to yield the isotopic ratios of individual compounds in a heterogeneous sample. The gas chromatograph separates organic components from one another in complex mixtures and is attached to a combustion furnace which combusts the organic components into $CO_2$ (5). The $CO_2$ will have a mass of 45 or 44 depending on whether it contains $^{13}C$ or $^{12}C$. The $CO_2$ then passes continuously through an isotope ratio mass spectrometer where the isotope ratios of the compounds are determined by comparison with the 45:44 mass to charge ratio of reference $CO_2$ (5, 10, 12). Purification procedures vary from laboratory to laboratory but usually include an extraction step and a column chromatography separation procedure. These procedures must maintain the isotopic integrity of the PAH samples in order to be useful for source apportionment. Dichloromethane is often used for the extraction, and a silica gel column is typically used for the column chromatography step (11, 13). The column purification step is necessary to separate the aliphatic fraction from the PAH fraction because the two would otherwise coelute during GC-C-IRMS analysis (7). Kim *et al.* recommended that additional high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) purification steps be employed as well, as detailed in Fig. 3. The authors reported no isotopic fractionation as a consequence of the purification procedures, even in cases of low yields (7). O'Malley *et al.* also reported a sample processing strategy involving extraction and column purification that did not alter the isotopic signature of standard compounds, even when less than 50% of the starting material was recovered (9).

In addition to the $\delta^{13}C$ analysis, hydrogen stable isotopes can be used to further elucidate the sources of PAHs.

Sun *et al.* reported δD values in conjunction with $\delta^{13}C$ values, and found that the combination allowed a much greater ability to differentiate among PAHs derived from petrol, jet fuel, and different coal conversion processes than using $\delta^{13}C$ alone. Sun *et al.* further noted that deuterium enrichment takes place simultaneously with $^{13}C$ depletion. This deuterium enrichment is consistent with expectations based on PAH formation mechanisms which typically involve dehydration steps. Dehydration allows lighter hydrogen isotopes to preferentially leave the molecular structure because C-H bonds are weaker than C-D bonds (11).

### Molecular Methods

Two other gas-chromatography inlet techniques predate the GC-C-IRMS technology and are useful for source apportionment of PAHs. GC-MS and GC-FID can be used to determine concentrations and patterns of particular PAH compounds in a mixture. GC-FID reveals the relative amounts and presence of PAH compounds; GC-MS provides similar information and also includes a mass spectrum (8). Ratios of fluoaranthene/pyrene and anthracene/phenanthrene are two commonly examined ratios in environmental forensics investigations (14). Ratios such as these can be compared to extensive chemical fingerprint databases to provide a preliminary test as to whether PAHs are pyrogenic or petrogenic. Typical analyses examine the relative numbers of alkylated versus parent PAHs and low versus high molecular weight PAHs in a mixture ( 4, 8 ).

## Investigated Sources and Applications of CSIA

CSIA, often used in conjunction with molecular methods, has been successfully used to allocate a broad range of sources and production conditions for PAHs from air, water, sediment, and soil samples. O'Malley *et al.* first demonstrated that CSIA could be used for source apportionment by showing that PAHs emitted by wood burning exhibit a different isotopic signature than those found in car soot (9). O'Malley *et al.* noted that low molecular weight PAHs enriched in $^{13}C$ are characteristic of pyrogenic mixtures, and high molecular weight PAHs depleted in $^{13}C$ are characteristic of petrogenic mixtures, providing a precedent for using isotopic data to source environmental PAHs (9, 15).

McRae *et al.* further demonstrated the utility of CSIA by reporting that PAHs generated by coal and biomass pyrolysis, and in diesel particulates, contained substantially different $\delta^{13}C$ values (5). These three sources are irresolvable without CSIA, as demonstrated by high performance liquid chromatography (HPLC) data reprinted in Fig. 4, followed by a more successful isotope analysis in Fig. 5. A separate study found differences in $\delta^{13}C$ of atmospheric PAHs which allowed the authors to conclude that automotive exhaust contributed the most to atmospheric PAHs in Beijing, while coal combustion was the major contributor to air in Chongqing and Hangzhou (16). CSIA has also been applied to tar identification (11).

Environmental PAHs with extremely low, variable $^{13}C$ compositions were linked to biodegradation, suggesting that PAHs from microbially generated PAHs tend to be more depleted in $^{13}C$ than those derived from other processes (13). Analysis of $\delta^{13}C$ has also been proposed as a means of studying paleo-fire activity to learn about climate-biosphere interactions because it has been demonstrated that $C_3$ and $C_4$-derived PAHs formed by combustion are isotopically distinct. O'Malley *et al.* discovered that PAHs formed from biomass burning during forest fires largely retain the isotopic composition of the original plant material (17).

In addition to research investigating which types of sources can be distinguished using isotope ratios, many successful investigations relating to particular environmental sites are published. For example, CSIA was used to allocate PAHs in sediments from St. John's Harbor in Newfoundland to a primarily woodburning source, rather than from crankcase oil or other petroleum products (9). In another study, molecular methods attributed PAHs in an urban estuary in Virginia to wood-treatment facilities, while CSIA revealed an additional contribution by coal transport, a source that had not been revealed by previous techniques or been anticipated by the authors (2).

$\delta^{13}C$ measurements have also been used to analyze product versus source PAHs in order to learn about the mechanisms of PAH formation. These mechanisms can provide insight into sources that utilize different reaction conditions. For example, it was demonstrated that PAHs formed from different coal conversion processes could be differentiated due to a progressive enrichment of $^{12}C$ accompanying higher temperatures of formation (18). This research indicated that the isotopic values of PAHs from coal are likely a function of the extent of ring growth required to form PAHs during processing, meaning that mild processes such as low temperature carbonization yield two or three ring PAHs with alkyl substituents and isotopic signatures similar to the signatures of the parent coals, while high temperature carbonization, gasification, and combustion exhibit distinct ranges of -25 to -27‰, -27 to -29‰, and -29 to 31‰, respectively, as ring condensation increases (18).

## Limitations

As CSIA becomes an increasingly common source apportionment tool, several considerations need to be addressed. There is currently a lack of standardized methods for CSIA with respect to PAHs. Purification procedures are continuously being modified and are inconsistent across studies. For example, differences in purification procedures were recently listed as a possible explanation for disagreements between two studies seeking to source PAHs derived from creosote wood preservatives (2).

Furthermore, although GC-C-IRMS is a fairly sensitive instrument, it requires at least 10 mg/L of an individual PAH for each injected sample, which is a relatively high concentration for natural samples. New techniques need to be developed to improve CSIA for the analysis of environmental samples with low concentrations of organic pollutants, such as particulate matter for air pollution studies. One promising advance within the past year to circumvent this problem was the development of a large volume temperature-programmable injector technique for GC-C-IRMS analysis of PAHs, to be used in place of the more common splitless injector method. This technique was demonstrated to measure samples with concentrations as low as 0.07 mg/L (19).

Although a vast number of $\delta^{13}C$ values for pyrogenic compounds have been published over the past sixteen years, two other source apportionment ratios of potential utility have been neglected. $\delta^{13}C$ values for petrogenic PAHs are relatively limited in the literature (3). Furthermore, although Sun *et al.* revealed that analyzing PAH $\delta D$ values in combination with $\delta^{13}C$ values appears to be a promising strategy for differentiating similar sources, $\delta D$ data are not yet commonly measured or published (11). This is an area where future research should be directed to expand the capability of CSIA as an environmental forensics device.

CSIA of PAHs can be inconclusive on its own when isotope ranges from different sources are similar. To overcome this limitation, many studies combine carbon CSIA with other molecular analyses of chemical fingerprints, including alkylated ratios, isomer ratios, low molecular weight to high molecular weight ratios, or a statistical analysis called principal component analysis (2, 8, 9). Source apportionment using chemical fingerprints faces even greater limitations than CSIA, however, and is not always reliable on its own. Molecular signatures are far more subject to interference from weathering than isotope ratios. For example, one study found that after only eighty days of weathering, parent PAHs predominate over alkylated species meaning that the two types of compounds weather at different rates (13). This change could lead to an incorrect conclusion in some cases that a mixture of PAHs was pyrogenic rather than petrogenic. Moreover, the molecular characteristics for many potential sources are not unique, further limiting the value of chemical fingerprinting independent of CSIA (17). The reliability of GC-FID and GC-MS methods for measuring molecular signatures also decreases when PAHs originate from multiple sources (8).

## Conclusion

PAHs can be found virtually everywhere in the environment and often demonstrate human pollution. Source apportionment of PAHs using molecular methods has been common practice for many years, used for the identification of responsible parties in mystery oil spills or environmental remediation efforts, among other applications. The field of environmental forensics was revolutionized sixteen years ago by the application of compound specific stable isotope analysis to these investigations, using relatively new GC-C-IRMS technology. CSIA has proven valuable in yielding PAH source apportionment information because isotope ratios tend to remain more constant over time and provide more information than molecular techniques alone. $\delta^{13}C$ data have already been reported for a wide variety of PAH sources and reaction conditions, but certain isotope ratios of potential use have been neglected. These include $\delta^{13}C$ values of petrogenic sources and $\delta D$ values for PAHs from all sources. Furthermore, standardized methods still need to be established for the field, and sensitivity and concentration limits could benefit from future technology research. Moving forward, it appears that a combination of molecular and isotopic techniques rather than sole reliance on one over the other provides the greatest assurance of apportioning the correct source.

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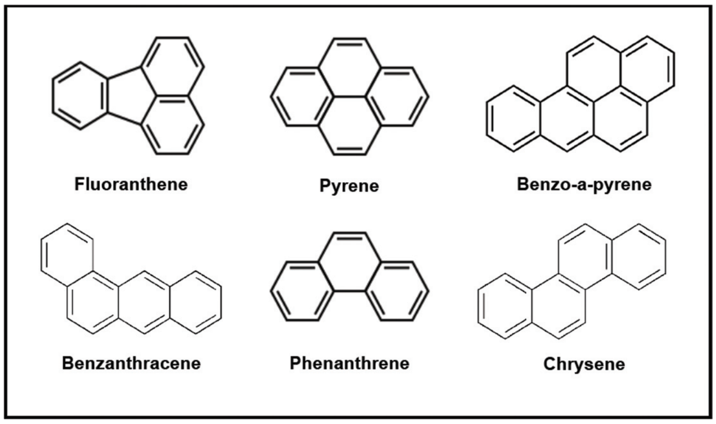

Figure 1: Examples of common PAHs.

**For all other figures, please refer to the following:**

**Figure 2:** T.C. Schmidt, et al. Anal. Bioanal. Chem. 378, 283-300 (2004).
**Figure 3:** M. Kim, M.C. Kennicutt II, Y. Qian, Environ. Sci. Technol. 39, 6770-6776 (2005).
**Figures 4&5:** C. McRae, et al. Anal. Commun. 33, 331-333 (1996).

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