ABSTRACT

THORSEN, WAVERLY ANNE. Bioavailability of Particulate-Sorbed Polycyclic Aromatic Hydrocarbons. (Under the direction of Drs. Damian Shea and W. Gregory Cope).

Understanding the behavior of contaminants in the environment is essential for an adequate assessment of a chemicals' fate, subsequent exposure to organisms, and potential for toxic effects. However, contaminant behavior can be complex, involving multiple interactions such as sorption to, and desorption from, particles present in the water column and sediment phase, competition for binding sites, and sequestration deep within particle pores, altering the chemical and biological availability of the contaminants. Therefore, one way to understand a contaminants' behavior in the environment is to assess its bioavailability, or fraction of contaminant available for uptake by organisms. This is generally accomplished by measuring the concentration of a contaminant present in organism tissue, and comparing it to concentrations in different environmental compartments (water, sediment).

In this study, numerous toxicokinetic parameters, bioconcentration factors and biota-sediment accumulation factors for 46 polycyclic aromatic hydrocarbons in freshwater mussels were measured. Elimination rates ranged from 0.04 (perylene) to 0.26/day (2,6-dimethylnapthalene), half-lives ranged from 2.6 to 16.5 days, and times to reach 95% of steady-state ranged from 11.3 to 71.3 days. The ranges and individual values compare well to available published literature values.

Bioconcentration factors ranged from 1.54 (naphthalene) to 5.20 (coronene), depending on individual analyte and study conditions, but generally increased with increasing PAH hydrophobicity. Bioconcentration factors exhibited sensitivity to

concentrations of dissolved and particulate organic carbon present in the water column, and to partition coefficients used to account for these parameters. Additionally, biota-sediment accumulation factors demonstrated that pyrogenic PAH (associated with incomplete-combustion) have much lower bioavailability than petrogenic PAH (associated with petroleum), with average values ranging from 0.55 +/-0.049 to 2.44 +/- 1.15, respectively. Moreover, PAH bioavailability was dependent on concentrations of soot carbon in the environment as well as the specific source of the PAH (petrogenic vs pyrogenic). This is the first study to publish these measured values for a full suite of PAH analytes.

BIOAVAILABILITY OF PARTICULATE-SORBED POLYCYCLIC AROMATIC HYDROCARBONS

By

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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

DEPARTMENT OF ENVIRONMENTAL AND MOLECULAR TOXICOLOGY

Raleigh

2003

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This dissertation is dedicated to my husband, Corbyn, and to my parents, Karen and Don, without whom the completion of this project would not have been possible.

BIOGRAPHY

Waverly Anne Thorsen was born Waverly Anne Watson and raised in Hanover, New Hampshire. Her interest in science began in high school and continued at Bowdoin College in Brunswick, ME, where she majored in chemistry. Throughout college she pursued her love of science and the outdoors, both in the classroom and through summer jobs. This included time as a hiking guide on the Appalachian Trail (for the Appalachian Mountain Club), as well as work as a summer student/laboratory technician at Los Alamos National Laboratory in New Mexico. Waverly's senior thesis at Bowdoin involved work with marine mussels as indicator organisms for measurement of hydrocarbon residues at the site of an oil spill off the coast of Maine. This work led directly to her enrollment at North Carolina State University in the Department of Environmental and Molecular Toxicology. At NCSU, Waverly focused on studying the bioavailability of certain classes of environmental organic contaminants to aquatic organisms, such as freshwater mussels. She was particularly interested in understanding interactions between organic contaminants and various sediment components such as organic carbon and soot carbon, and the ultimate effects they have on contaminant bioavailability. Apart from her academic goals, Waverly enjoys multiple outdoor pursuits including trail running, hiking and skiing.

ACKNOWLEDGEMENTS

I would like to thank the many people that aided me in completing this dissertation: to my fellow Chemical Exposure Assessment laboratory members for their support, encouragement and advice, particularly Peter R. Lazaro; to the administrative staff who helped with the numerous purchase orders, curriculum requirements, exam scheduling, and always offered friendly concern and care; and to my advisors, committee members and professors for their support, constructive criticism, and many scientific discussions.

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LIST OF ABBREVIATIONS

AC	acenapthene (PAH) (Chapter 2 for full list of PAH)
AF	accumulation factor
BAF	bioaccumulation factor
BaP	benzo(a)pyrene (PAH)
BCF	bioconcentration factor
BDL	below detection limit
BkF	benzo(k)flouranthene (PAH)
BF	benzofluoranthene (PAH)
BghiF	benzo(g,h,i)fluoranthene (PAH)
BSAF	biota-sediment accumulation factor
Chem. Class	chemical class
CNF	chlornitrofen (pesticide)
CO	coronene (PAH)
C0	chrysene (PAH)
C4	c4-chrysenes (PAH)
26DMN0	2,6-dimethylnapthalene (PAH)
DA	dibenz(a,h)anthracene (PAH)
D0	dibenzothiophene (PAH)
D1	c1-dibenzothiophenes (PAH)
D2	c2-dibenzothiophenes (PAH)
D3	c3-dibenzothiophenes (PAH)
dep	depuration
DOC	dissolved organic carbon
Ffd	fraction of contaminant freely dissolved
F0	flourene (PAH)
FL	fluoranthene (PAH)
HCB	hexachlorobenzene
HCBP	hexachlorobiphenyl (PCB)
HOC	hydrophobic organic contaminant
K_{AC}	activated carbon-water partition coefficient
K_{DOC}	DOC-water partition coefficient
K_{POC}	POC-water partition coefficient
Koc	organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
Ksc	soot carbon-water partition coefficient
K_1	uptake rate constant
K_2	elimination rate constant
MFO	mixed-function oxidase system
MP0	methylphenanthrene (PAH)
MPY	methylpyrene (PAH)
MT	metallothionein protein
N0	napthalene (PAH)
ng	nanogram (10^{-9})

OC	organochlorine (Chapter 1)
OC	organic carbon (Chapter 4)
OCS	octachlorostyrene
OX	oxadiazon (pesticide)
PAH	polycyclic aromatic hydrocarbon
PB	physiologically based
PCP	pentachlorophenol
PCB	polychlorinated biphenyl
Petro	petrogenic PAH
PE	perylene (PAH)
P0	phenanthrene (PAH)
POC	particulate organic carbon
pg	picogram (10^{-12})
pyro	pyrogenic PAH
PY	pyrene (PAH)
RIS	recovery internal standard
SC	soot carbon
sed	sediment
SIS	surrogate internal standard
TBC	thiobencarb (pesticide)
TCBT	tetrachlorobenxyltoluene
$T_{1/2}$	half-life
T_{95}	time to 95% steady-state
ug	microgram (10^{-6})

CHAPTER 1

TOXICOKINETICS OF ENVIRONMENTAL CONTAMINANTS IN FRESHWATER MUSSELS

INTRODUCTION

For decades, mussels have been used as a sentinel species to monitor pollution in the aquatic environment (Foster 1978, Farrington 1983, Colombo 1995, Peven 1996, Blackmore 2003). Many different classes of chemicals have been studied in this way including hydrophobic organic contaminants (HOCs), such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCB's), and organochlorine (OC) pesticides, as well as inorganic contaminants such as heavy metals (Cd, Pb, Hg) and radionuclides ($^{239, 240}$Pu, ^{137}Cs). The application of mussels for indicators of environmental pollution originally stemmed from difficulties associated with determining aqueous contaminant concentrations (Farrington 1983). Many hydrophobic organic contaminants exhibit very low water solubilities (i.e: coronene: 1.4×10^{-4} mg/L, at 25°C), which require large sample sizes for adequate instrumental analysis. Moreover, trace metals require 'ultraclean' techniques and are also frequently found in very low concentrations in the aqueous phase, sometimes at levels close to instrument detection limits (i.e: pg/L). Additionally, random water sampling may not capture real trends in pollutant concentrations over an integrated time scale.

In an attempt to overcome these obstacles, native mussels frequently are collected worldwide, extracted, and analyzed for pollutant tissue burdens to provide preliminary information at sites suspected of contamination, or to monitor chemical/waste discharge effluents. However, in order to effectively understand and correlate the relationship between concentrations of pollutants in the aquatic environment to concentrations in mussel tissue and potential toxic effects, it is best to have an understanding of the kinetics

involved in the uptake, distribution, and elimination of pollutants by/from mussel tissues. Additionally, this information is required in order to utilize data and to predict concentrations in other environmental compartments, such as predicting aqueous or sediment exposure concentrations from mussel tissue burdens (Neff 1996).

Traditionally, marine mussels such as the blue mussel, *Mytilus edulis*, have been used for environmental monitoring due to concern for pollution in coastal and estuarine areas (Farrington 1983, Salanki 1989, Beliaeff 2002,). However, more recently (1970's) freshwater mussels have been increasingly utilized in order to assess the quality of lakes, rivers, and streams of concern, not only for the protection of human health, but also to better explain recent major declines of many North American freshwater mussel populations (Keller 1991, Naimo 1995, Jacobson 1997). Generally, information gleaned from freshwater mussels has demonstrated similarities to marine mussels; however, physiologies can vary greatly between species, age, body size, ingestion rate, reproductive state, stress, and location, among other factors (Landrum 1994, Naimo 1995, Morrison 1996). Therefore, in an attempt to better evaluate pollutant fate and to effectively protect and remediate the natural environment, it would be most beneficial to understand the toxicokinetics of both marine and freshwater mussels. The intent of this chapter is to present background information and to assess the toxicokinetic information available for freshwater mussels. Where data are limited, information on marine mussels will be presented, and in some cases will be presented in tandem with freshwater mussel information in a comparative context. This chapter is not meant to be an exhaustive review of the literature pertaining to these issues, but rather is meant to aid researchers,

managers, and others, in understanding the bioaccumulation of organic and inorganic contaminants in freshwater mussels.

UPTAKE and ELIMINATION

Mussels are exposed to and take up pollutants in tandem with their primary breathing and feeding mechanisms: chemicals enter mussels passively as they filter water through their gills for respiration and feeding (dietary exposure), or in the case of inorganic contaminants such as metals, through facilitated diffusion, active transport or endocytosis (Marigomez 2002). Additionally, some mussel species are exposed to pollutants through pedal feeding or gut ingestion of sediment (McMahon 2001). Therefore, chemical uptake can occur in a direct fashion when mussels draw large quantities of water (up to 20L/mussel/day (Naimo 1995)) into their gills, or, in an indirect fashion when ingestion of sediment occurs and chemicals desorb (passively or through facilitated desorption) from the sediment particles into the mussel gut and become assimilated. Once chemicals enter the mussel, they partition into or associate with mussel tissues. For example, heavy metals will accumulate primarily in muscles and organ (soft) tissues (Plette 1999, Markich 2001, Marigomez 2002) and organic pollutants will accumulate in lipid (Farrington 1983, DiToro 1991). Generally, uptake is very rapid when the mussel is first exposed, and then levels off, sometimes requiring extensive time periods for an equilibrium state to be reached. A similar trend is observed for the elimination process, which may be rapid at first and then level off, some compounds never being fully eliminated (i.e: some compounds with half-lives of 20 years) (see figure 1a,b).

Uptake and elimination rates for both HOCs and metals can be determined through field and/or laboratory studies. A typical uptake/elimination experiment consists of 'clean' mussels (reference, or depurated prior to commencement of the study) exposed to a constant chemical concentration in water, and collected at increasing time intervals, in order to determine the chemical concentrations in mussel tissue over time. For example, mussels can be collected from a relatively clean field reference site, and deployed at a contaminated field site, or brought back to the laboratory for contaminant exposure. After sufficient exposure time, mussels are removed and placed in clean water for measurement of the elimination (depuration) rate of the compound(s). In the natural environment, elimination of certain chemicals might require extensive time periods. In locations where exposure levels are constant or increasing, mussels may not eliminate the chemical(s). In many instances, mussels will accumulate contaminants to levels significantly higher than those in the water column. This can pose toxicity risks to predatory animals, and can result in biomagnification- or subsequent increases in contaminant concentrations progressively up the food chain. A well known example of a compound that is persistent in the environment, possesses the potential to bioconcentrate to very high levels, and biomagnifies through the food chain is 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, or DDT. Many long-lived organisms (such as certain freshwater mussels that can live to greater than 60 years old (McMahon, 2001)) will never sufficiently eliminate DDT (or its primary metabolites, DDE and DDD), potentially resulting in subacute toxic effects to the organism, and remaining a continual 'source' to predators, including humans.

BIOCONCENTRATION

The accumulation of contaminants from the water column by mussels is referred to as 'bioconcentration'. Bioconcentration is defined as the partitioning of a contaminant from an aqueous phase into an organism, and this will occur when the contaminant uptake rate is much greater than that for elimination. Typically, this leads to high concentrations of chemicals in mussel tissues. For HOC's, partitioning generally occurs between the dissolved phase of the water and the mussel lipid. The most basic example of partitioning is defined as the octanol-water partition coefficient, or K_{ow}:

$$K_{ow}=[\text{contaminant}]_{octanol}/[\text{contaminant}]_{water}$$

The K_{ow} is a measurement of a chemical's affinity for octanol vs water. In many cases, octanol is used as a surrogate for organism lipid. A chemical with a smaller K_{ow} value (<100) will partition less into lipid than a chemical with a larger K_{ow} (>1000). This type of partitioning will occur between the aqueous phase and mussel lipid until a steady-state condition has been reached: that is, the concentration in the mussel relative to the exposure system is unchanging with time. Once steady-state or equilibrium has been reached, this can be referred to as 'equilibrium partitioning'. In a simple system, equilibrium partitioning can be modeled by comparing the affinities (i.e: solubilities, fugacities) of a chemical for mussel lipid vs water (Figure 2). In order to determine the extent of bioconcentration of a chemical in mussel tissues, one can calculate a 'bioconcentration factor' or BCF. The BCF is defined as the pollutant concentration in the mussel tissue (C_{mussel}) divided by the dissolved aqueous pollutant concentration (C_{water}) at steady-state:

$$BCF = C_{mussel}/C_{water}$$

The BCF can also be determined by dividing the empirically derived contaminant uptake rate (K_1) by the empirically derived elimination rate (K_2):

$$BCF=K_1/K_2$$

Generally, the greater the hydrophobic character of the contaminant, the greater the BCF. In this way, BCF values typically correlate in a linear fashion to $K_{ow's}$ (Geyer 1982, Makay 1982, Pruell 1986, Hawker 1986, Schuurmann 1988, Thorsen 2003) (figure 3).

In many cases, a steady state bioconcentration regression equation can be developed by linearly regressing a log BCF vs log K_{ow} plot. The resulting equation for the line takes the form of :

$$\log BCF = m*\log K_{ow} + b$$

where m and b are the slope and y-intercept of the line, respectively. This equation models the bioconcentration of hydrophobic organic pollutants by mussels, and can be used to predict aqueous exposure concentrations.

The 'partitioning' of metals however, generally refers to the adsorption of metals onto active sites in/on target mussel tissues, such as anionic sites on mussel gills (Kramer 1996, Marigomez 2002), rather than absorption into mussel lipid. A bioconcentration factor, though slightly less utilitarian than for HOCs due to very slow uptake rate constants, can similarly be computed by:

$$BCF_{metal}=C_{mussel}/C_{water}$$

where C_{mussel} is the moles of metal/g soft weight mussel and C_{water} is the moles of metal dissolved per mL (or L) of water. This BCF value must also be calculated when the system has reached steady-state. More complex equations exist for predicting bioconcentration (and uptake, elimination rates) when a system is not at steady state, and

are discussed elsewhere (Russell 1989, Butte 1991). The bioconcentration of metals is affected by many factors, including water pH, hardness, alkalinity, conductivity, and dissolved organic and inorganic matter, which will be discussed in following sections.

BIOACCUMULATION- BAF and BSAF

While bioconcentration refers only to the uptake of chemicals directly from the water, the term bioaccumulation does not differentiate between uptake media and includes chemical accumulation into organisms from both abiotic (i.e: water, sediment) and biotic (i.e: food) sources. For example, mussels can bioaccumulate chemicals and metals from the water column and the sediment phase in the natural environment. Typically, scientists may model this relationship by calculating either a bioaccumulation factor (BAF) or a biota-sediment accumulation factor, or BSAF. The BAF includes exposure due to water and food sources, while the BSAF (only used for HOCs) models the partitioning/association of a chemical between the lipid phases in the organism (mussel) and the sediment, where the sediment 'lipid' phase is considered to be organic carbon. The BAF is represented by:

$$BAF = C_{mussel}/C_{food}+C_{water}+C_{other\ exposures}$$

while the BSAF is mathematically defined as:

$$BSAF = (C_{mussel}/\text{lipid fraction})/(C_{sediment}/\text{organic carbon fraction})$$

where the chemical concentration in the mussel (C_{mussel}) and sediment ($C_{sediment}$) are normalized to the mass fraction of mussel lipid and sediment organic carbon, respectively. Similar to the BCF calculation, a BSAF value is calculated when the chemical has reached a steady-state within the study system. Theoretically, BSAF values

will equal unity, or one. However, BSAF values may be less than one if the mussel metabolizes the chemical or the system has not reached steady-state (chemicals may not be fully available to the mussels due to very slow desorption, or very strong binding). BSAF values can also be greater than one because organic carbon is generally less 'lipid-like' than organism lipid due to hydrophilic components of natural organic matter (DiToro 1991). The calculation of BSAF values can lend information about a particular chemical's bioavailability (See Bioavailability section).

Metals do not interact with organisms in the environment in the same way that hydrophobic organic contaminants do. As previously mentioned, while hydrophobic organic contaminants generally partition (absorb) into the lipid phase of a mussel, metals adsorb to the gill and other anionic sites on tissue surfaces, or are actively transported via membrane pumps. For example, metals such as cadmium can enter a mussel by binding to membrane transport ligands. Bioaccumulation of metals, including filtration of water and ingestion of food particles, in mussels can be similarly measured through the use of a BAF:

$$BAF = C_{mussel}/C_{water,\ dissolved}$$

Bioaccumulation factors for metals are more difficult to interpret in that the interactions between a target site (biological organism) and the metal are complicated by competition for binding sites and many more environmental parameters than simply dissolved or particulate organic carbon. For all chemicals and metals, bioaccumulation is the balance between all means of chemical uptake and all means of elimination.

METABOLISM and BIOTRANSFORMATION

For those contaminants that mussels are capable of metabolizing, BCF, BAF, and BSAF values will be decreased. In general, it is the lack of metabolic capacities in mussels that makes them adequate indicators of aquatic environmental pollution (James 1989), however, mussels have been shown to metabolize certain classes of compounds better than others. For example, mussels possess only minimal abilities to biotransform polycyclic aromatic hydrocarbons (PAHs), and therefore are good indicators of the accumulation of PAHs, but some marine mussels (*Mytilus edulis*) have been shown to metabolize the PCB, hexachlorobiphenyl (HCBP) (Bauer 1989), and therefore will exhibit lower BCF values. Additionally, mussel have been shown to possess detoxification systems including low molecular weight metallothionein (MT) proteins and lysosomal granules that complex and chelate metals, thus resulting in alterations in the metal uptake/distribution/elimination kinetics (Naimo 1995, Tessier 1996, Vesk 1999, Byrne 2000, Baudrimont 2002).

BIOAVAILABILITY and BIOTIC-LIGAND MODEL (metals)

Underlying all of the above concepts is the notion of bioavailability. Bioavailability can be defined as the percentage of a chemical fully available for uptake by an organism. Different chemicals and inorganic contaminants have unique bioavailabilites, which will depend on many factors including water conditions such as hardness, pH, temperature and turbidity, as well as the physical-chemical characteristics of the compound such as water solubility, vapor pressure, and speciation (ionic state). For

example, chemicals that exhibit very low water solubilities readily sorb to organic carbon phases in the water column, such as particulate or dissolved organic carbon (POC, DOC). The rate of desorption and co-occurrence of the mussel with the particle(s) partially determines the chemical's bioavailability. If the rate of desorption is rapid relative to the co-occurrence of the particle and the organism, the chemical may be fully bioavailable. However, if the rate of desorption is very slow, the chemical may not be readily available. Hydrophobic organic contaminants may frequently become associated with natural organic matter in the aqueous and sediment phases, while metals may become complexed to various organic (DOC) and inorganic compounds present in the water such as calcium and potassium carbonates ($CaCO_3$, KCO_3).

The bioavailability of a chemical is important to understand both to ensure the protection of aquatic organisms, and to implement effective and cost-efficient remediation techniques. This is particularly important because underpredictions of toxicity can result in unacceptable risks to organisms, while overpredictions of toxicity can require costly practices for clean-up. For instance, mussel tissue burdens are traditionally compared directly to total aqueous or sediment contaminant concentrations, without regard for the bioavailable fraction. This method can overpredict the actual exposure concentrations mussels (and other aquatic organisms) receive, and may result in costly, yet ineffective remediation of a site. Moreover, sediment concentrations of total metal do not always correlate well with mussel tissue burdens. Rather, it may be the speciation of the metal (i.e.: Hg^{+} vs Hg^{++} vs Hg^{0}), or ratio of metal concentration to amount of acid-volatile sulfate in the sediment (DiToro 1992), that best determines the metal concentration in and subsequent toxicity to, the mussel. One can see the problems

that may arise when regulatory and remediation techniques are based on incorrect assessments of chemical bioavailability.

CHEMICAL CLASSES

HYDROPHOBIC ORGANIC CONTAMINANTS

Uptake

As previously stated, hydrophobic organic contaminants (HOC) primarily partition into mussel lipid, which is considered essentially an 'infinite sink' whereby saturation of the pool does not occur. The uptake of a hydrophobic organic chemical into mussel tissues can be defined mathematically as:

$$dC_{mussel}/dt=K_1*C_{water}-K_2*C_{mussel}$$

where dC_{mussel}/dt is the change in mussel contaminant concentration over change in time (t), K_1 is the uptake rate of the chemical, C_{water} is the aqueous chemical concentration, K_2 is the elimination rate (see Elimination section) and C_{mussel} is the concentration of chemical in mussel. If the concentration of the pollutant in the water column changes, this change will be mirrored in the mussel over several days to weeks. This process is considered first-order on a log (ln) basis. By integration, the above equation becomes

$$C_{mussel}=(K_1/K_2)*C_{water}*(1-e^{(-K_2 t)}).$$

Mussels primarily take up HOCs directly from the water column (Thomann 1999, Birdsall 2001) through their gills, although some studies have suggested additional chemical inputs from dietary exposure (Brieger 1993, Gossiaux 1996, Bjork 1997, Raikow 2001,) and direct sediment ingestion via pedal feeding mechanisms (McMahon 2001, Raikow 2001). There is debate in the literature over the relative contribution of

each of these uptake routes, however it should be noted that once the system has attained steady-state (dC/dt=0), the route of contaminant exposure is irrelevant (DiToro 1991). Because of their minimal metabolic capabilities for metabolizing the majority of HOC's (Farrington 1986, James 1989), mussels accumulate these contaminants to high levels in their lipid tissues which can often reach many orders of magnitude greater than the corresponding concentrations in water or sediment phases. Despite the common use of freshwater mussels for monitoring aquatic environments, relatively little information is known for freshwater mussels in terms of HOC uptake rate constants, compared with that for marine mussels. Moreover, much of both the freshwater and marine data represent only a few species. For instance, the majority of the freshwater uptake studies focus on *Dreissena polymorpha*, while the majority of marine uptake studies use *Mytilus edulis*.

There are various ranges in reported K_1 values for freshwater mussels depending on mussel species, and study parameters such as temperature variables, exposure environment, mussel size, and lipid content (Table 1a,b; Table 2a,b for study summaries). (Fisher 1993, Bruner 1994, Gossiaux 1996, Fisher 1999). However, based on the available data, most K_1 values compare fairly well, with a few exceptions (Table 1a). Many studies demonstrate initial rapid uptake during preliminary exposure for both freshwater and marine species (Lee 1972, Obana 1983, Bjork 1997, Birdsall 2001). For example, Birdsall et al. (2001) reported rapid uptake of the PAHs naphthalene (N0), anthracene (AN) and chrysene (C0) by *Elliptio complanata* gills. Their data demonstrated that the average uptake of AN and C0 was similar, and both were greater than that for naphthalene which was explained by its lower lipid affinity (log K_{ow}=3.37, vs 4.54 and 5.86 for AN and C0).

Differences in K_1 can be observed when comparing the same analyte across different studies, as well as when comparing different analytes with similar physico-chemical parameters. However, with a few exceptions, the differences appear to be relatively small, considering the many variables that can exist between studies. For example, K_1 values measured for BaP and HCBP in both the field and laboratory over the course of three years and at different temperatures (5-24°C) in *D. polymorpha* compare well (Table 1a). Specifically, for BaP the range of uptake rates is from 9,960 to 32,736 mL/g*d, a factor of 3 difference. The differences between highest and lowest K_1's for HCBP, PCP and PY are even less, at factors of 2, 2.6 and 2, respectively. Data from two collection timepoints have been omitted for this comparison due to very low K_1's which the authors believed was from over-wintered mussels experiencing stress (both occurred for mussels collected at 4°C in the field, however when mussels were fed while being acclimated to 4°C in the laboratory, these effects were not observed (Gossiaux 1996)). Therefore, it is important to consider that larger differences can occur based on the physiological state of the organism. Laboratory derived K_1's for pentachlorophenol (PCP) increased from 3960 mL/g*d at 4° C to 5928 mL/g*d at 15°C, while field derived K_1's showed even less difference with a more dramatic temperature increase from 4 to 24°C (3240 vs 2640 mL/g*d, respectively) (Gossiaux 1996). These authors noted that others (Reeders 1989) have documented this lack of substantial change in *D. polymorpha* filtration activity over a temperature range of 5 to 20°C, which helps to explain their data (Gossiaux 1996). While K_1's for some of the compounds in this study increased proportionally with increasing temperature in the field (i.e.: BaP and HCBP), this was not consistently exhibited over the three year time frame, and lead the authors to suggest

that uptake kinetics do not change in a proportional manner with temperature (Gossiaux 1996), at least across the range tested. Although Reeders et al. (1989) reported no significant change in uptake rates in *D. polymorpha* within a season, a significant change between seasons was documented.

Variations in uptake rates with *D. polymorpha* body size and lipid content were reported by Bruner et al. (1994) for HCBP, tetrachlorobiphenyl (TCBP), BaP and PY. The average uptake rate for HCBP over varying mussel lipid and size was 23,680 mL/g*d (Bruner 1994) which compares remarkably well with K_1's reported by Gossiaux et al. (1996) for *D. polymorpha* over varying temperatures, averaging 18,624 mL/g*d in the laboratory and 21,000 mL/g*d in the field. When varying pH is considered in combination with changing temperatures, differences in K_1's increase, but are still within a factor of less than 5 on average, which translates into about an order of magnitude difference in BCF values. The reported field and laboratory K_1's in *D. polymorpha* for PCP (log K_{ow} 5.12) are 2760 and 4120 mL/g*d (Gossiaux 1996), while those reported for varying pH (and averaged over temperature) are lower: 1,657 (pH 6.5), 1,218 (pH 7.5) and 868 (pH 8.5) (Fisher 1999). The lowered K_1's may be due to a combination of effects caused by changing pH and temperature on mussel filtration rates and subsequent uptake rates. When individual values are compared, rather than averages, the variation in K_1 is increased. For instance, the smaller the mussel size (measured in length), the faster the uptake rate (Bruner 1994). Twenty-one mm ('large') Zebra mussels with high lipid content had TCBP uptake rates of 10,080 mL/g*d, while smaller 15 mm but also high lipid mussels, had TCBP uptake rates more than double that of the larger mussels at 23,760 mL/g*d (Bruner 1994).

In general, uptake rates were directly proportional to compound K_{ow}: as K_{ow} increased, K_1 increased as well. For example, as log K_{ow} values increased from 5.18 for PY to 6.90 for HCBP, the average uptake rates increased from 10,480 to 23,680 mL/g*d, respectively. An additional study reported K_1's ranging from 2,976 to 25,752 mL/g*d in *D. polymorpha* for PAHs, PCBs and OCs (DDT) spanning a similar log K_{ow} range of 5.2 to 6.7 (Fisher 1993). This range compares well to other K_1's listed above, when values for DDT are omitted (lowest values). Moreover, K_1 values reported for HCB (hexachlorobenzene) and OCS (octachlorostyrene) in *E. complanata*, also increased with increasing log K_{ow}: from 650/day for HCB (log K_{ow}: 5.45) to 1010/day for OCS (log K_{ow}: 6.29) (Russell 1989). However these values are substantially lower than those reported for *D. polymorpha*.

In contrast to the linear relationship between K_1 and K_{ow} reported by some (Russell 1989, Bruner 1994, Gossiaux 1996), uptake rates for 8 different TCBT congeners in *D. polymorpha* were independent of K_{ow} (van Haelst 1996). As log K_{ow} increased from 6.73 (TCBT # 28) to 7.54 (TCBT # 25), K_1's varied little, from 772 to 803 mL/g*d (van Haelst 1996), respectively. However, when all TCBT congeners are included across this log K_{ow} range, the K_1 values demonstrated larger variability and range from 683.3 to 848.8. This may be partially explained by the high K_{ow} values, or the decreased ability of highly hydrophobic compounds to permeate membranes (van Haelst 1996). Moreover, the uptake rates reported for *D. polymorpha* for TCBT congeners are lower than those for PAHs or PCBs with similar hydrophobicity (see above). Uptake rate constants for PCB congener 153 (Bruner 1994) and TCBT (tetrachorobenzyltoluene) congener 28 (van Haelst 1996), which have similar log K_{ow} values (6.92 and 6.73,

respectively) differ by as much as a factor of 50, from as low as 771 mL/g*d for TCBT congener 28 (van Haelst 1996), to between 9,120-38,592 mL/g*d for congener 153 (Bruner 1994), both for *D. polymorpha*.

Bjork et al. (1997) reported K_1's for 3 PCB congeners (31, 49, 153) using the marine mussel, *M. edulis*, ranging from 2160 (153) to 168,000 mL/g*d (153). While the upper range is quite large, and is about 4 times greater than the upper range reported for *D. polymorpha*, the freshwater mussel K_1's are still within these limits. The larger K_1 values in *M. edulis* are probably due to the addition of contaminated food in the study conducted by Bjork (1997). In contrast, Ogata et al. (1984) reported K_1's for parent and various alkylated dibenzothiophenes in a marine short-necked clam, which were significantly smaller ranging from 33 for dibenzothiophene to 66/day for dialkylated dibenzothiophene. It should be noted that some authors (Ogata 1984, Russell 1989) report K_1 values in reciprocal days, which is assumed to be equivalent to mL/g*d, where 1mL=1g. However, this assumption may not always be valid, which may explain some of the differences observed in K_1 values.

Uptake rates for various pesticides in *C. leana* (Uno 1997) are much lower than those reported in *D. polymorpha* for compounds with similar K_{ow}'s. While the log K_{ow} for the pesticides thiobencarb, oxadiazon and chlornitrofen are lower than for the HOCs, the uptake rates are more than proportionally lower, ranging from 24.2 for thiobencarb to 626 mL/g*d for chlornitrofen in the field, and 140 for thiobencarb to 338 mL/g*d for chlornitrofen in the laboratory (Uno 1997). The authors attributed the low uptake rate(s) for thiobencarb to a temperature decrease of 2 degrees over the course of a year causing slower ventilation rates in the mussels. This is in contrast to reports on *D. polymorpha*

that a temperature range of 20 degrees does not cause substantial changes in uptake rates (Reeders 1989, Gossiaux 1996). The large differences in uptake rates for *C. leana* vs *D. polymorpha* and *M. edulis* are probably due to a combination of species and chemical differences.

In summary, uptake rate constants were remarkably similar across temperature, seasonal, pH, chemical, and study parameters, although some differences were observed, particularly when comparing chemicals of similar log K_{ow} (TCBTs vs PCBs), low vs high lipid mussels of different size, and species. Large variation in K_1 was demonstrated for stressed mussels (Gossiaux 1996), suggesting one must consider mussel physiology when measuring empirical uptake rates, or BCFs under adverse conditions such as very low temperatures. Moreover, K_1's were greater for combined food and water exposures (Bjork 1997). The uptake rates reported in this chapter represent only those for a few freshwater mussel species, which demonstrates the need for further research in this area. For instance, while *D. polymorpha* uptake rate constants may not vary substantially with increases or decreases in temperature (over a 20°C range-Gossiaux 1996), this may not be the case for other freshwater mussel species (i.e: *Corbicula*- Uno 1997).

Bioconcentration

Gossiaux et al. (1996) reported bioconcentration factors in *D. polymorpha* for BaP (log K_{ow}= 6.04) that ranged from 4.38 (10°C) to 5.28 (4°C) in field exposures at temperatures from 4 to 24°C. The BaP log BCF values had a similar range in the laboratory for temperatures from 4 to 20 °C (4.6 (4°C) to 5.43 (15°C)) (Table 1b). The log BCF values for PY (log K_{ow}=5.18) in both the field and laboratory ranged from 4.34 to 4.89, over a similar temperature range. However, the authors were not convinced that

steady-state had been reached due to a factor of 100 difference between BCF values calculated from C_{mussel}/C_{water} and those calculated from K_1/K_2. This implies BCF values in reality would be larger than those reported. In comparison, Bruner et al. (1994) reported similar log BCF values also in *D. polymorpha* for both BaP, ranging from 4.61 to 4.92 and PY, ranging from 4.11 to 4.54, depending on mussel lipid and size. These values compare fairly well, especially when considering the variation in temperature, lipid content and mussel size.

In contrast, log BCF values reported by Thorsen et al. (2003) for *E. complanata* are lower for both PAHs, ranging from 3.50-4.66 for BaP, and 2.29 to 3.79 for PY, depending on exposure source (water-only vs sediment). The discrepancies between these data may be partially explained by differences in lipid content between *D. polymorpha* and *E. complanata*, as *D. polymorpha* are generally 7-15% lipid on a dry wt. basis (Gossiaux 1996) and *E. complanata* are much lower, typically 3-4% lipid (Thorsen 2003). This can be partly confirmed by results from Bruner et al. (1994) who reported an increase in BCF values with subsequent increase in mussel lipid content. However, this effect was only observed for the higher K_{ow} compounds (HCBP and BaP), and not for the lower K_{ow} compounds (TCBP and PY). Furthermore, log BCF values determined for HCB and OCS in *E. complanata* (log K_{ow}s 5.49 and 6.29, respectively) compare well with those for PAHs of similar hydrophobicity, ranging from 3.56 to 4.16 (i.e.: 3.58 and 3.64 for C2-dibenzothiophenes with log K_{ow}=5.50, and 4.23 and 4.54 for benzo(e)pyrene with log K_{ow}=6.20) (Thorsen 2003). Additional variations in BCFs may be further explained by physiological differences between *E. complanata* and *D. polymorpha*, differences in study design, or a combination of environmental and physiological factors.

In a further comparison between freshwater mussel species, Makela et al. (1995) reported BCF values for PCP for two freshwater mussels, *Anodonta anatina* and *Pseudanadonta complanata*: ranging from 1.9 to 2.1 and 1.8 to 1.9, respectively. These BCF values are much lower than those reported by Gossiaux et al. (1996) for PCP in *D. polymorpha*, which ranged from 4.0 to 5.27, depending on study temperature. In contrast, log BCF values reported for PCP in a different study for *D. polymorpha* with varying temperature and pH are mid range between those reported for *A. anatina*, and *P. complanata* (with a range of 2.60 to 3.13 (Fisher, 1999)) and *D. polymorpha* (4.0 to 5.27 (Gossiaux 1996)) (Table 1b).

The log BCF values for HCBP determined in two separate studies on *D. polymorpha* compare well, ranging from 4.79 to 5.38 in one study (Bruner 1994) and 5.24 to 5.74 in the second (Gossiaux 1996). Brieger et al. (1993) reported log BAF values for *D. polymorpha* of 4.02 and 4.45 for 2 PCB congeners, 77 (log K_{ow}=6.36) and 169 (log K_{ow}=7.42) which are lower relative to their K_{ow} values than those reported for similar log K_{ow} compounds, TCBT congener 28 (log Kow=6.73, log BCF=4.83 (van Haelst 1996)), HCBP (log K_{ow}=6.9, log BCF range=4.8-5.7 (Bruner 1994, Gossiaux 1996)), and TCBT congener 22 (log Kow=7.43, log BCF= 4.71 (van Haelst 1996)). This discrepancy may simply suggest a lack of steady state, as BAF values would be expected to be larger than BCF values from increased exposure to contaminated food.

Log BCF values for various pesticides including chloronitrofen, thiobencarb and oxadiazon have been reported for *C. leana* ranging from 2.34 for oxadiazon (log K_{ow}=3.89) to 4.14 for chlornitrofen in the field, and from 3.79 for chlornitrofen to 3.45 for thiobencarb (log K_{ow}=4.22) in the laboratory (Uno 1997). It should be noted that the

log BCF values for oxadiazon and thiobencarb increase with corresponding increases in hydrophobicity.

Bioconcentration factors determined for PAHs for *Mytilus edulis* (Pruell 1986) and a marine short-necked clam, oyster and mussel (Ogata 1984) compare well to those for *E. complanata* (Thorsen 2003), but are lower than those reported for *D. polymorpha* (see above comparison between *E. complanata* and *D. polymorpha*). For example, across a log K_{ow} range of 3.9 to 6.1, log BCF values for *M. edulis* range from 2.0-4.4 (Pruell 1986), while across a similar log K_{ow} range of 3.37 to 7.6 for *E. complanata*, log BCF values range from 1.5-5.2 (Thorsen 2003). Moreover, the log BCFs reported for dibenzothiophene (D0) in marine clam, oyster and mussel are 2.17, 3.12, and 3.13 (Ogata 1984), close to the range reported for *E. complanata* of 2.69-2.93 (Thorsen 2003), and similar to those reported for thiobencarb (of similar log K_{ow} to D0: 4.22 vs 4.49) in *C. leana* of 3.25 to 3.48 (Uno 1997) (Table 3).

Similar to uptake rate constant data, empirically derived BCF values generally increase with increasing K_{ow} of the compound (Pruell 1986, Brieger 1993, Bruner 1994, Gossiaux 1996, Thorsen 2003). For example, as the log K_{ow} is increased from 5.18 (PY) to 6.90 (HCBP), the average log BCF values for *D. polymorpha* increase from 4.28 to 5.14 (Bruner 1994). However, exceptions to this are observed. The BCFs for compounds with log K_{ow} values greater than 6-7, tend to level off due to factors such as steric hinderance (reduction of membrane permeation), lack of steady state (very long times required to reach equilibrium), and growth dilution. Van Haelst et al. (1996) found no correlation between log BCF values for 8 TCBT congeners and log K_{ow}. These authors suggested this was due to the small range of log K_{ow} (6.73-7.54) compounds used as well

as the fact that the TCBT congeners all have log K_{ow}'s >6 (i.e: may be in the linear part of the curve).

Log BCF values for PCBs of similar hydrophobicity reported for *M. edulis* were significantly higher than those for PAHs: ranging from approximately 5.0 to 5.7 for a corresponding PCB log K_{ow} range of approximately 6.0 to 7.0 (Pruell 1986). This log BCF range fits within those reported for *D. polymorpha* (Bruner 1994, Gossiaux 1996) for various PCBs over the same log K_{ow} range: 4.0 to 6.9, however, the differences between PAH and PCBs for freshwater mussels appear to be less pronounced (Bruner 1994, Gossiaux 1996). Moreover, a linear relationship between log K_{ow} and log BCF was observed for both PAHs and PCBs in *M. edulis* (Pruell 1986), *E.complanata* (Thorsen 2003), and *D.polymorpha* (Bruner 1994). Comparisons of steady-state bioconcentration regression equations (Table 4) generally show good agreement in PAH accumulation, with some exceptions. For example, Pruell et al. (1986) reported a slope of 0.965 and a y-intercept of -1.41 for *M. edulis*, while Thorsen et al. (2003) reported a slope of 0.895 and a y-intercept of -1.21 (r^2=0.8325) for *E. complanata*. However, Ogata et al. (1984) reported regression equations with slopes much less than one (0.16 (short-necked clam), 0.49 (oyster) and 0.31 (mussel)), and positive y-intercepts (1.54, 1,03, 1.63, respectively). The differences may be due to the fact that Ogata's regression equations are based on the parent and alkyated homologues of dibenzothiophene only, whereas Pruell's and Thorsen's are based on larger data sets. These data suggest a good correlation between marine and freshwater BCF values, for *M. edulis*, *E.complanata*, and *M. arenaria*.

Elimination

The elimination rate (K_2) can be calculated from an elimination plot of the lipid normalized, natural log (ln) of the contaminant concentration in mussel vs time. In a first-order, one-compartment kinetic model, K_2 is the absolute value of the slope of this line, based on the equation

$$\ln C_{mussel} = -K_2 * t + \ln C_{mussel,0}$$

where $C_{mussel,0}$ is the mussel chemical concentration at elimination time zero (figure 4).

Elimination rates in mussels are also fairly consistent, depending on compound, study, and mussel species (Table 1a). Elimination rates for HOC's are generally much lower than their counterpart uptake rates, but similarly are dependent upon the hydrophobic character of the compounds (Dunn 1976, Bruner 1994, Morrison 1995, Gewurtz 2002, Thorsen 2003). In a study conducted by Gewurtz et al. (2002), K_2's for nine PAH were calculated for *E. complanata*. The K_2's varied from 0.037/day for benzo(k)fluoranthene (BkF) to 0.217/day for fluoranthene (FL). An inverse linear relationship was observed between analyte elimination rate constant and corresponding log K_{ow}, which the authors noted suggested a passive elimination of PAHs (Gewurtz 2002). This is characteristic of monophasic, first-order elimination also reported for *D. polymorpha* for lower K_{ow} compounds (Gossiaux 1996). Additional K_2 values reported for 45 PAHs in *E. complanata* from sediment exposure uptakes range from 0.04 to 0.22/day (Thorsen 2003). The authors noted that elimination rate constants were lower in a water only exposure study, and suggested this was due to increased stress on the mussels from excessive fungal growth and subsequent increased handling (Thorsen

2003). The K_2 values for OCS and HCBS also in *E. complanata* range from 0.16 to 0.41/day and are slightly higher when compared to similar log K_{ow} PAHs (Russell 1989).

Moreover, Gossiaux et al. (1996) demonstrated slow elimination rates for *D. polymorpha*, ranging from, for example, 0.024/day to 0.096/day for HCBP in field studies, and 0.024/day to 0.384/day for BaP. For the lower hydrophobic compounds in this study (PCP and PY), elimination was rapid during the first 24 hrs, and then leveled off, while elimination of HCBP and BaP was minimal over the first 24 hrs, increased during the following 48-168 hrs, and then slowed, suggesting a biphasic, two-compartment model [however these authors classify the elimination as solely monophasic].

Furthermore, K_2's from Gossiaux et al. (1996) and Gewurtz et al. (2002), determined for *D. polymorpha* and *E. complanata* respectively, compare well when HOC's of similar log K_{ow} are compared: Gewurtz's reported K_2 for PY is 0.144/day, which is within the range also reported for PY by Gossiaux of 0.048-0.312/day. Moreover, a comparison of dibenzo(a,h)anthracene (DA, log K_{ow}=6.8) (Thorsen 2003) and HCBP (log K_{ow}=6.9) (Gossiaux 1996) results in similar K_2 values as well: DA, 0.068/day ; HCBP, 0.024-0.096/day. The K_2 values reported by Bruner et al. (1994) are generally higher than those from Gerwurtz's and Gossiaux's. Bruner's HCBP K_2 for *D. polymorpha* ranges from 0.12 to 0.168/day, the lower range of 0.12 close to the upper range from Gossiaux. Overall, however, Bruner's values are still less than twice those of Gewurtz and Gossiaux. The K_2's for PCP in *D. polymorpha* are about 2-3 times less than those reported for C. *fluminea*, ranging from 0.14 to 0.192/day in *D. polymorpha* (Bruner 1994) to 0.39 to 0.40/day in *C. fluminea* (calculated using data from Basack 1997).

However, both are less than other K_2s for PCP reported for *D. polymorpha* ranging from 0.86 to 1.56/day (Fisher 1999), which may be greater due to the combination of changing pH and temperature in that study. However, the overall consistency in K_2 values across species and study further suggests that elimination of HOCs is highly dependent upon compound hydrophobicity, rather than other factors such as the physiology of the organism, etc. and can be described (generally) by a first order, one-compartment kinetic model (Morrison 1995).

Much smaller K_2 values are observed for HOC's with log K_{ow} values greater than 7. Van Haelst et al. (1996) reported a range of 0.005-0.037/day in *D. polymorpha* for TCBT congeners ranging in log K_{ow} from 6.73-7.54, which are approximately 2 to 3 times lower than other literature K_2's. For the same K_{ow} range, Morrison et al. (1995) demonstrated a K_2 range of 0.042-0.098, also for *D. polymorpha*, while Breiger et al. (1993) reported an elimination rate constant of 0.034 for PCB congener 169 (log K_{ow}=7.42) in *D. polymorpha*. There appears to be a plateauing effect of K_2 values observed for HOCs with log K_{ow}'s greater than approximately 6 (Morrison 1995, Gewurtz 2002, Thorsen 2003).

Smaller K_2 rates are also reported in *C. leana* for chlornitrofen and thiobencarb. These rates vary little between laboratory and field studies, ranging from 0.045 (field) to 0.054/day (lab) for chlornitrofen and 0.049 (lab) to 0.060/day (field) for thiobencarb (Uno 1997). However, these K_2 values do not follow the trend seen with K_{ow}, as the pesticide K_{ow}'s are much less than those for the TCBT congeners. These compounds may be metabolized to some extent by the mussels.

Elimination of HOC's in marine mussels are also found to be moderately variable, ranging from rapid elimination (<4 days) (Pittinger 1985) to no measureable depuration in 45 days (Tanacredi 1991). However, elimination rate constants reported for *M. edulis* (Pruell) a short-necked clam (species not identified: Ogata 1984) and *C. virginica* (Sericano 1996) exhibit similar ranges for PAHs and PCBs as those reported for freshwater mussels. For instance, over a log K_{ow} range of 3.9-6.1 (PAHs), K_2's for *M. edulis* range from 0.023 to 0.058/day, within the range reported for *D. polymorpha* by Gossiaux et al. (1996), but lower than those also reported by Bruner et al. (1994) (Table 1a). Gewurtz et al. (2002) present a compilation of K_2 vs K_{ow} regression equations for multiple species and chemical classes which for the most part show good correlation with slopes varying from 0.21 to 0.60 and y-intercepts ranging from 0.02 to 0.11. What is remarkable is the consistency in the regression equations across variations in freshwater vs marine mussels, PAH vs PCB classes and field vs laboratory studies. A wider range in slopes and y-intercepts are observed however, if all data are included as there are conflicting data for *C. virginica* (Eastern oyster), and *M. mercenaria* (Hard Clam), which demonstrate negative slopes in some instances, and no significant depuration (Tanacredi 1991) in other studies. Further research is required for a more robust comparison of freshwater and marine mussels and for a more complete understanding of why these differences occur.

Attainment of Steady-State

It should be noted that while most mussel uptake kinetics are generally rapid for HOC's, there is a broad range of time required to reach steady-state (Table 2a,b). For example, Thorsen et al. (2003) reported steady-state was reached between the water

column and mussel tissue for most PAH within the first 24-48 hours of exposure, and that all PAHs reached steady-state within 10 days. Moreover, the uptake kinetics of *D. polymorpha* are rapid (Bruner 1994, Morrison 1995, Gossiaux 1996), which enables them to reach steady-state quickly (within a few hours, for lower hydrophobicity chemicals). In contrast, Pruell et al. (1986) reported a slower time frame to steady-state of 20 days for *M. edulis*, which may have been due to slow contaminant desorption from the sediment slurry source into the water. Moreover, Brieger (1993) reported that a steady-state of hexachlorobenzene in *D. polymorpha* required 20 days, while PCB congener 77 did not appear to reach steady-state, even after 30 days of exposure. The attainment of steady-state will depend on mussel lipid content, metabolic capabilities, physical-chemical characteristics of the compound (highly hydrophobic compounds may require longer time periods for equilibration), and the availability of the chemical. In studies where sediment serves as the primary exposure system, slow chemical desorption from sediment particles may influence the time required to reach steady state (i.e: Pruell 1986).

Bioaccumulation and Bioavailability

Many studies demonstrate mussel uptake of HOCs not only from water, but from contaminated food and sediment as well (Augenfield 1982, Pruell 1993, Brieger 1993, Bjork 1997, Gossiaux 1998). The uptake rates of PCB 77 in *D. polymorpha* were found to increase when the exposure environment was altered to include food and sediment, in the following increasing order: water>food>sediment (Brieger 1993). In contrast, Thorsen et al. (2003) did not observe consistent differences in PAH BCF/BAF values determined for *E. complanata* in water-only (BCF) vs sediment (BAF) exposure studies, implying that the sediment PAH concentrations were simply driving the water

concentrations, which *E. complanata* were directly exposed to. This was also the case when *E. complanata* were allowed to burrow into the sediment phase in both field and laboratory studies (Thorsen 2003). Moreover, with the exception of benz(a)anthracene, no statistically significant differences were observed in tissue PAH burdens between *D. polymorpha* placed in the upper water column vs the sediment surface at a confined disposal facility (Roper 1997). However, this may not be the case for deposit-feeding mussels, or for mussels exposed to PCBs which have generally been shown to be more bioavailable than PAHs of similar physical-chemical characteristics (Lamoureux 1999, Kraaij 2002).

While the addition of food and sediment in exposure environments may result in increases in accumulation, these factors can also result in the decreased bioavailability of HOCs by serving as binding agents that sequester them (Kraaij 2002). Many factors can affect HOC bioavailability including the feeding and digestion mechanisms of the mussel, as well as the rates of sorption and desorption between the HOC and particle/sediment (Kraaij 2002), concentrations of dissolved and particulate organic carbon and soot carbon, and aging of contaminants and sediments (Schrap 1990, Readman 1984, Gustaffson 1997, Bucheli 2000, Accardi-Dey 2002, Alexander 2000, respectively). Sediments may often act as a 'sink' for HOCs and modulate the corresponding water concentrations (Spacie 1994), and thus the observation of high total HOC sediment concentrations may not correspond to high exposure levels to organisms because of decreased bioavailability. For instance, HOCs that are bound to sediment or particulate matter in the water column can exhibit slow desorption rates, rendering them essentially unavailable to mussels. Thorsen et al. (2002) noted BSAF values in *E.*

complanata of less than one for pyrogenic PAHs (PAHs of combustion origin). These authors observed that the greater the concentration of soot carbon, the lower the PAH bioavailability, depending on PAH source (petroleum vs combustion origin). In a study conducted at a creosote contaminated site (expected to contain relatively bioavailable PAHs), BSAF values for *A. anatina* ranged from 0.79 to 1.45 for 6 PAHs, acenapthene, phenanthrene, anthracene, fluorene, pyrene and benz(a)anthracene. Biota-sediment accumulation factors for phenanthrene were close to one, ranging from 0.80 to 0.96 (Hyotylainen 1996). In comparison, BSAF values have been reported for P0 in *M. arenaria* (marine) and *E. complanata* ranging from 0.17 to 1.8, depending on environmental location (PAH source) (Thorsen 2002).

Other studies have calculated assimilation efficiencies, rather than BSAF values in order to measure the bioavailable fraction of HOCs to mussels (Morrison 1996, Gossiaux 1998). For example, *D. polymorpha* assimilation efficiencies (AE) were measured for contaminants sorbed to algal (food) particles vs those sorbed to suspended sediment, demonstrating the availability of PY, BaP, C0 and HCBP which were nearly 100% assimilated from algae, but only 45 to 58% assimilated from suspended sediment particles (Gossiaux 1998). Even lower AE's from sediment particles (21%) were observed for BaP in *D. polymorpha* and a positive correlation was noted between AE and log K_{ow} (Gossiaux 1998). However, the AE from each source will vary based on algal lipid content, particulate organic carbon, particle size, etc. Although systems are not always at equilibrium, route of exposure is unimportant when a system is at equilibrium (or pseudo/apparent steady-state), and still serves as a simple model for predicting bioavailability of HOCs to mussels and other benthic invertebrates. The incorporation of

the many differences in physiology of the mussels and interactions with sources of contamination can become complicated quickly. However, the difference in log BFC/BAFs of only approximately two orders of magnitude (4.4-6.8) between HOCs with similar log K_{ow} values (Table 1b) across studies with exposures of water only, water and food, and the inclusion of sediment, temperature changes, mussel species, size and lipid content changes suggests that simplification in modeling through the use of equilibrium partitioning theory is appropriate (i.e: DiToro 1991). Additional toxicological data would aid in the comparison and summary of results.

Implications and Potential for HOC Toxicity to Freshwater Mussels

The demonstration that mussels bioaccumulate HOC's from various environmental compartments demonstrates the importance of understanding the details underlying the toxicokinetics in order to assess and predict the potential for subsequent toxicity. The bioaccumulation that occurs at environmentally relevant concentrations (ng-ug/L aqueous concentrations or ng/g sediment concentrations) typically manifests in chronic, subacute effects, rather than acute consequences such as mortality. It can be difficult to tease out specific consequences associated with contaminant exposure because other types of stresses can induce adverse effects, as well. However, information can still be gleaned by monitoring biomarker effects such as alterations of reproductive health, changes in filtration and/or ventilation rate and lipid content, reattachment success, DNA damage, lipid peroxidation, and EROD or metallothionein induction.

METALS

Uptake

Metal uptake into mussels is much more complex than that of HOCs due to the increased number of interactions that can occur between metals and environmental ligands such as dissolved organic and inorganic matter, and other complexing and competing components (Table 5). Metal uptake primarily involves interaction with active sites, usually on mussel gills, and therefore follows isotherms such as the Fruendlich or Langmuir isotherms (Spacie 1994, Churchill 1995). However, simpler models have been used to describe metal uptake into a mussel based on the dissolved water concentration (C_{water}) and the metal influx rate (I):

$$I = K_1 * C_{water}$$

(Wang 2001), where K_1 is the uptake rate constant, and C_{water} is the metal concentration in the dissolved phase. The bioenergetic based kinetic model is also used to assess and predict bioaccumulation of toxic metals in mussels (and other aquatic organisms). This model requires knowledge of uptake efficiencies from water and food, as well as depuration, filtration, ingestion, assimilation, and growth rates (Roditi 1999).

The amount of metal taken up by mussels will depend on characteristics of the metal, the physiology of the mussel, as well as environmental conditions of the water column and sediment phase. For example, metal speciation will depend on whether it is freely dissolved or bound to inorganic (OH-, Cl-) or organic (DOC, oxalate) matter which subsequently affects its bioavailability. Additionally, competition between the metal and other cationic ligands for anionic ligands or anionic biological targets may occur. In terms of water conditions, factors such as hardness, alkalinity, pH, DOC,

salinity/conductivity, total dissolved solids, and anthropogenic inputs can influence metal behavior. Metal uptake in mussels is affected by numerous factors including the reproductive state of the organism, feeding strategy (filter feeder vs. deposit feeder), length and size of the organism, the source(s) of food, as well as assimilation and absorption efficiencies (Roditi 1999, Stewart 1999, Plette 1999, Roditi 2000, Wang 2001).

Mussels bioconcentrate and bioaccumulate both essential (i.e.: Ca, Na) and non-essential (i.e.: Hg, Cd, Pb) metals to high levels in their tissues. This can frequently result in concentrations that are significantly higher than the concentration present in the water column, and can cause toxicity to the mussels as well as to predatory organisms, such as aquatic birds or terrestrial mammals (Naimo 1995, Stuijfzand 1995). Metals are taken up by mussels via facilitated diffusion, active transport or endocytosis (Marigomez 2002). Some non-essential metals such as cadmium can become incorporated into calcium and sodium active pump channels designed for essential metal uptake, and regulation of ionic/osmotic balance (Stewart 1999). Because of these active ion pumps required for normal homeostasis, mussels possess the ability to regulate certain essential metals (Zn, Cu) to a point. This results in aqueous metal concentrations that are not directly proportional to the corresponding tissue burden (Kraak 1994, Blackmore 2003). For example, Zn and Cu were not accumulated by *D. polymorpha* exposed to low concentrations (<28 ug Zn/L and <191 ug Cu/L). The authors suggested that this inferred a homeostatic regulation of these metals (Tessier 1996). Homeostatic regulation can modulate metal accumulation to a threshold level. Once this threshold level is surpassed, bioaccumulation and potential toxicity, will occur.

Metal uptake takes place primarily on active sites in mussel gills, although additional uptake can occur in the digestive tract from ingested food particles, as well as in the mantle, kidney, foot and hepatopancreas (Inza 1998, Plette 1999). The percentage of the metal that is absorbed from the total amount of water pumped through is termed the absorption efficiency (α). The percentage of ingested metal that crosses the gut lining is termed the assimilation efficiency (AE) (Roditi 1999), and will depend on many factors including the length of time required for processing, the particle type, the metal concentration associated with the particles, and the organisms' ingestion rate (Fan 2002). For some mollusks, assimilation efficiencies of Cd associated with different food sources, have been reported to be greater than 20% (Fan 2002). Uptake can vary significantly based on the individual metal or mixtures of metals studied. Frequently, metal concentrations are reported in content (i.e.: total ng = Concentration (ng/g) x Tissue weight (g)) to minimize effects of size differences and/or growth during a study, and for cross-study comparisons (Naimo 1995, Beckvar 2000).

TOXIC, NON-ESSENTIAL ELEMENTS

Cadmium

Uptake and Accumulation

Mussels accumulate cadmium (Cd) across their gills by active uptake (binding to membrane transported ligands or incorporation of Cd into a major ion active pump (Stewart 1999)) as well as phagocytosis by cytosomes (Roseman 1994, Marigomez 2002). Roditi et al. (2000) reported that Cd uptake in *D. polymorpha* primarily resulted from ingested particles rather than the dissolved phase (For a summary of Cd studies see

Table 6). Moreover, Cd uptake by *L. margnialis* was shown to be primarily from the water column through mussel gills, rather than from the sediment phase (Jana 1997). Certain freshwater mussels accumulate Cd to such an extent that some have proposed their use as biofilters in an attempt to remediate moderately contaminated sites (Jana 1997). Cadmium uptake by freshwater mussels has been shown to be highly dependent on dose, time of exposure (Das 1999), mussel length (Roseman 1994), and detoxification mechanisms, and therefore great variation is observed. For instance, Cd accumulation in mollusks ranged from 5.8 to 600 ug/g depending on species, exposure time and Cd concentration (Jana 1997). Specifically, *E. complanata* accumulated Cd to 65ug/g after a 60 day exposure to 50 ppb Cd, while *M. edulis* accumulated 900ug Cd/g during the course of a 51 day exposure to 50 ug/g Cd (Jana 1997). Moreover, maximum uptake for *L. marginalis* exposed to low (10 and 20 ppm) Cd concentrations occurred in the liver, while at high Cd concentrations (30 ppm), the gill was the primary site of uptake (Das 1999). This was also the case for Cd concentrations in *C. fluminea*, which exhibited gill burdens as high as 1500 ng/g wet wt from exposure to 68 mg Cd/L aqueous concentrations (Inza 1998). The next highest concentrations occurred in the visceral mass, mantle and foot, in descending order (Inza 1998). Accumulation of Cd in *U. pictorum* was rapid, and demonstrated that the kidney was the primary target organ of Cd accumulation (Jenner 1991). Roseman et al. (1994) reported the Cd absorption rate was directly related to *D. polymorpha* and *D. rostriformis bugensis* mussel length, while others noted that *E. complanata* mussel length had no significant effect on Cd body burdens (Tessier 1996).

Uptake rates of Cd reported in *D. polymorpha* and *D. rostriformis bugensis*, 20 to 30 mm long, ranged from 0.22 to 0.45 ug/mussel/hr and 0.29 to 0.49 ug/mussel/hr over a 24 hour exposure period, and measureable decreases in Cd water concentration upon addition of mussels to the exposure system were reported (Roseman 1994). It was noted that no uptake occurred in the smaller, 10mm long mussels for either of these species, possibly due to inhibition of filtration by Cd, as fifty percent decreases in filtration rates of *D. polymorpha* (16 to 22mm long) have been previously reported (Roseman 1994). Cadmium uptake has been shown to decrease in the presence of other metals such as Cu, Zn, Pb and Ni where, although measured aqueous Cd concentrations increased, *P. grandis* accumulated less Cd (Stewart 1999). Moreover, Cd filtration times of *A. cygnea* decreased from 20 to 8 hours in the presence of 10 ug Cu/L for 240 hours exposure, and from 30 to 60 down to 7 hours with 50 ug Pb/L, also for a 240 hour exposure (Stewart 1999).

Steady-State and Bioconcentration

Various times required to reach steady-state have been noted for Cd ranging from 4 days for *C. fluminea* (Inza 1998) to greater than 40 days for *L. marginalis* (Das 1999). This is probably due to differences in mussel species as well as exposure environment: the 4 day steady-state occurred in a water exposure, while the other occurred in a sediment and water exposure (Das 1999). The authors suggested that the steady state was observed following 4 days Cd exposure in *C. fluminea* because of the saturation of binding sites in the mussel gut, an increase in elimination rate, or a physiological modification of filtration activity (Inza 1998). In another study where *D. polymorpha* were exposed to Cd concentrations ranging from 0.30 to 44 ug/L steady-state was not

reached after 27 days of exposure (Mersch 1993). Steady-state was noted for Cd in the kidney of *U. pictorum* within 3 weeks of exposure in field and laboratory experiments with and without substratum (Jenner 1991).

Tissue concentration factors (BCFs) have also been shown to be affected by metal concentration (Das 1999). Bioconcentration factors for Cd in *L. marginalis* ranged from 35 to 280, depending on Cd water concentration and tissue (gill, liver, shell, mantle), and varied greatly when sediment exposure was included: BAF's were much lower ranging from 1.5 to 5 (Das 1999). These values are most likely significantly underpredicting actual equilibrium bioaccumulation because in all cases, the uptake curves were linear, even after 40 days (Das 1999). In comparison, accumulation factors for Cd in *D. polymorpha* ranged from 3,000 in whole body to as high as 70,000 in the periostracum (Roseman 1994), and from 150,000 to 640,000 at different field sites (Roditi 2000). However, Jenner et al. (1991) reported a much lower BCF value of approximately 660 (6,000 dry wt converted to wet wt) for *U. pictorum* kidneys. Similar BCF values were reported for *Anodonta spp* collected in the field, of 981. Bioaccumulation factors comparing *Anodonta spp* tissue Cd concentrations to sediment Cd concentrations were significantly lower at 0.06, suggesting a decreased bioavailability of the Cd associated with the sediment phase (Dobrowoski 2002). Winter reported BAFs in different freshwater mussels (ranging from 1300 to 19000 dry weight, approximately log BAF 2.16-3.32 wet weight), and demonstrated higher Cd concentrations in *D. polymorpha* than *A. anatina* (Winter 1996). It is somewhat impractical to compare accumulation not only across species, but also across studies because of the complexity of the interactions with Cd, and other metals in the environment, which makes it difficult to compare water

concentrations (or food, sediment and water) to corresponding tissue burdens. For instance, poor correlations between organism Cd concentrations and Cd water concentrations have been observed (Campbell 1991).

Elimination

Elimination of Cd has also been shown to be dose and time dependent, as well as highly variable. Following a 14 day depuration phase, *D. polymorpha* exhibited only 5% Cd loss, while no loss of Cd was reported in *C. fluminea* after 30 days depuration. Even following 120 days of depuration, only 25% of the original Cd in *C. fluminea* had been eliminated (Inza 1998), and 22% of the Cd loss occurred from the gills (Inza 1998). It was also reported that changes in temperature (12 vs 24°C) and pH (6 vs 8) had no effect on Cd elimination, on both an organism and individual organ level (Inza 1998). However, it cannot be ruled out that greater changes in temperature and pH could influence Cd elimination. Elimination of Cd in *U. pictorum* was also found to be very slow, where during a 29 week elimination phase an initially rapid loss of approximately 33% of Cd was observed, however no further elimination was observed (Jenner 1991).

Mercury

Uptake and Accumulation

Both methyl mercury (CH_3Hg) and inorganic mercury (Hg(II)) have been shown to accumulate in mussels (Beckvar 2000, Malley 1996), though freshwater mussels are generally thought to be poor indicators of Hg contamination because of highly variable uptake rates (Naimo 1992) (summary, table 6). Similar to Cd, length of exposure and aqueous Hg concentrations affect mussel body burdens (Tessier 1996). Additionally, Hg concentrations were shown to increase with increasing temperature in *E. complanata*

(Beckvar 2000), and methyl Hg was demonstrated to be more bioavailable to *E. complanata* than inorganic Hg (Beckvar 2000). Linear uptake over the course of 14 days was observed in all organs and tissues (mantle, foot and adductor, gills and visceral mass) in *C. fluminea* for both inorganic Hg and methyl Hg with the exception of the gills, where uptake appeared to plateau following 4 days of exposure (Inza 1998). Moreover, concentrations of methyl Hg (average 5750 ng/g dw in all tissues) were nearly seven times those for inorganic Hg (average 850 ng/g dw in all tissues), and demonstrated differences in distribution (Inza 1998). Concentrations were highest in the foot and adductor muscles and gills for methyl Hg, but in the gill and visceral mass for inorganic Hg. In comparison, methyl Hg concentrations measured in *P. grandis* were highest in the foot or kidney, and lower in the gills and visceral remains (Malley 1996). In studies containing both water and sediment, differences in Hg phase distribution were noted. For inorganic Hg, 26% associated with the sediment, and only 2.7% accumulated in *C. fluminea* tissues, vs 14% of methyl Hg distributed to the sediment phase, and 31.2% partitioned into *C. fluminea tissues* (Inza 1998). The differences in partitioning behavior are quite obvious, and are due to the lower aqueous solubility of methyl Hg over the inorganic form. Bioaccumulation factors for Hg in *D. polymorpha* have been reported that range from 14,000 to 250,000, depending on field location (Roditi 2000). Moreover, it was demonstrated through the calculation of particulate: aqueous partition coefficients (K_d's) (81% of Hg determined to be on particles) that Hg is primarily accumulated in *D. polymorpha* through ingested particles (Roditi 2000), and 4 to 40% is assimilated from food (Roditi 1999).

Elimination

Elimination of Hg from mussel tissues appears to be generally very slow. After 30 days of depuration, no significant loss of inorganic or methyl Hg was observed in *C. fluminea*, yet after 120 days, 30% of inorganic Hg was eliminated from mussel tissues, while 40% of methyl Hg was eliminated (Inza 1998). Inorganic Hg has been shown to be depurated more rapidly than methyl Hg by *E. complanata* (Beckvar 2000). Changes in temperature (12 vs 24°C) and pH (6 vs 8) were shown to have no effect on Hg elimination (Inza 1998), but again, it should be noted that greater changes in temperature and pH could influence Hg elimination. Mercury efflux (depuration) rates determined after uptake from food of four different types (algae, bacteria, seston) ranged from 0.043 to 0.056/day, corresponding to biological half-lives of 13 to 16 days (Roditi 1999).

Lead

Uptake and Accumulation

Lead (Pb) uptake by mussels appears to be slow (Riget 1997), and primarily associated with the gill, at least soon after initial exposure has occurred (summary, table 6). For example, in native *Anodonta spp*., and *U. pictorum*, the gills contained the highest Pb concentrations, across all field sites examined, while the lowest average Pb concentrations for both mussels were found in the shells (Gundacker 2000). These authors also noted that while other metal concentrations exceeded those in the environment, this was not the case for Pb, suggesting a decreased bioavailability (Gundacker 2000). This is consistent with other reports demonstrating significant binding of Pb to humic acids and subsequent decreased bioavailability to *E. complanata*

(Campbell 1987). In contrast to highest Pb accumulation observed in the gill, Boisson et al. (1998) described nearly equivalent distribution in the marine mussel, *M. galloprovincialis* of 49% of Pb in soft tissues and 46% in the shell, which they speculated meant similar uptake rates for both over the course of a 21 day exposure. Pb has been documented at the electron microscopy level in digestive cells in *M. galloprovincialis* and in hemocytes in *D. polymorpha* (Marigomez 2002). However, Gundacker et al. (1999) reported significantly higher concentrations of Pb in *D. polymorpha* byssal threads, as opposed to soft body parts. The authors suggested the allocation of Pb to the bysall threads was a mechanism for detoxification. The BCF value for Pb was much greater than the BAF value reported for *Anondonta spp*. in the field, where the BCF was 250, and the BAF (comparing *Anodonta spp*. tissue Pb concentrations to those in sediment) was 0.10 (Jenner 1991). A BAF of 211 was reported for *M. galloprovincialis* in Pb based on exposure in seawater (Boisson 1998). These authors noted that this BAF is 2 orders of magnitude lower than other BAFs for Pb, although it compares well to that reported for *Anodonta spp* in the field, and suggested this may be due to inefficient accumulation by *M. galloprovincialis* from water (Boisson 1998).

Elimination

Elimination of Pb from mussels is typically very slow (Riget 1997). For instance, elimination of Pb in *M. edulis* transplanted from a contaminated field site to a clean reference site eliminated only ½ of the original Pb contamination in 2 to 3 years, after which elimination appeared to cease (Riget 1997). However, Pb elimination in *M. galloprovinicalis* was described as biphasic, with short-term compartments exhibiting a biological half-life of 1.4 days, and a much slower secondary release of 2.5 months

(Boisson 1998). Despite the differences in these results, it is clear is that Pb kinetics in mussels are very slow, and need to be considered when mussels are used as indicators of Pb pollution in the environment.

Silver

Uptake and Accumulation

Although Ag concentrations are usually fairly low in invertebrates, it is frequently detected in organism tissues and BCF/BAF values can be quite high (Moore 1991) (table 6). Roditi et al. (2000) reported an uptake rate for Ag in *D. polymorpha* of 3670 mL/g*d, determined in a laboratory radiotracer study. Presumably uptake rates would be quite variable, depending on water chemistry, and individual organism variation, among other factors. The uptake rates of Ag relative to other heavy metals in this study including Cd, Cr and Hg, are quite high (approximately 1.9, 6.1, and 1.6 times higher, respectively). In comparison, high Ag accumulation and toxicity variability were reported for marine mussels, and Ag concentrations in *M. galloprovincialis* did not reach steady-state in 4 weeks of exposure (Baud 1990). It was also demonstrated in *M. galloprovincialis* that Ag uptake was greatest with exposure in food and water, lower with exposure in only water, and lowest with exposure only to food (Baud 1990). In contrast, Roditi et al. (2000) predicted using a bioaccumulation model that *D. polymorpha* primarily accumulates Ag from ingested particles, where anywhere from 64 to 90% of bioaccumulated Ag originated from food, depending on field characteristics/location. Obviously differences in mussel species, feeding mechanisms, routes of exposure, and water chemistry will influence Ag accumulation. For instance, log BAFs reported for Ag in *D. polymorpha*

ranged from 4.3 to 5.1, depending on field location (Roditi 2000), and in this study were lower than BAFs reported for Cd and Hg.

Elimination

Elimination of Ag from *D. polymorpha* is very slow relative to uptake. For example, reported elimination rates range from 0.07/day to 0.09/day, depending on prior uptake from food or water, respectively (Roditi 2000). In a separate study by the same authors, elimination rates in *D. polymorpha* for Ag were 0.067 after exposure to food, and 0.084 after exposure to Ag contaminated water. Apparently, differences in sources of Ag during uptake influence the rates of subsequent metal elimination, though it is questionable how significant these differences are.

Biological half-lives reported for Ag in *D. polymorpha* are 8 to 9 days in the literature, significantly shorter than those reported for other heavy metals such as Cd (i.e.: 51 d) (Roditi 2000).

Nickel

Although Nickel (Ni) is present fairly consistently in the environment (Moore 1991, Stuijfzand 1995), it is generally less toxic than other heavy metals (Cd, Hg) (Keller 1991), and subsequently relatively little information exists on toxicokinetic information in freshwater mussels (table 6). It has been demonstrated, however, that Ni concentrations in both freshwater (*D. polymorpha* (Stuijfzand 1995)) and marine (*M. edulis* (Friedrich 1976)) mussels increase with increasing Ni concentrations in water. Nickel was reported to accumulate in various mussel tissues in *L. marginalis* including the hepatopancreas, mantle, adductor muscle and foot, when these mussels were exposed

to both acute and subacute Ni concentrations over the course of 15 days (Sreedevi 1992). Lee et al. (1994) reported a BCF value in *M. edulis* for Ni of 432, much lower than that reported for Cd (2,814) and Zn (2,900), about half that reported for Cu (807), but higher than those reported for Cr (228) and Pb (127). Additional research to determine uptake, distribution and elimination of Ni in freshwater mussels is required in order to provide a comparison/assessment of these values.

Tin

Uptake and Accumulation

The uptake of Tin (Sn) by mussels is generally assessed as total Sn, inorganic Sn, or organo-Sn, such as tributyl- or dibutyl-Sn (table 6). The organotins are readily accumulated by mussels, as a result of the hydrophobic character of the organic methyl or other alkyl group. In general Sn concentrations in mussels are reported to be highest in the gills, viscera, adductor and mantle (Laughlin 1986, Moore 1991), and uptake is concentration dependent (Moore 1991). Van Slooten et al. (1994) reported rapid uptake (K_1 = 25,000/day) of tributyltin by *D. polymorpha* in a field environment (freshwater marina), such that tissue concentrations were 63 ug/g after 35 days of exposure (steady-state), resulting in a log BCF value of approximately 6 based on dry weight and approximately 5 based on wet weight. Additionally, dibutyltin also reached steady-state in *D. polymorpha* after 35 days of exposure, but the accumulation was not as great as that for tributyltin (van Slooten 1994). In contrast, Laughlin et al. (1986) reported steady-state was not reached in *M. edulis* after 47 days exposure to tributyltin in water and 30 days exposure to tributyltin in food.

In comparison to the log BCF values reported for tributyltin listed above (approximately 5 based on tissue wet wt.), other values have been reported in *D. polymorpha* which are much lower, ranging from 4 to 4.6 based on wet wt (Fent 1991). It was suggested by van Slooten et al. (1994) that the BCF variations are due to lower aqueous tributyltin concentrations, that is, the lower the water concentrations, the greater the BCF values. In comparison, log BCF values reported for organic and total tin in *M. edulis* ranged from 3.7 to 4.8 (Zuolian 1989). These values were calculated following a 51 day exposure. It was also noted by these authors that the BCF values were much higher than others reported in similar laboratory studies (Zuolian 1989). Additional log BCF values in marine mussels (species not identified) for tributyltin were reported as approximately 3.7 based on wet weight. and 4.6 based on dry weight. (Shawky 1998), and log BCF values were reported to range from 3 to 4 in the laboratory, and predicted to be at least two orders of magnitude greater in the field (Laughlin 1986). However, log BAF values reported for tributyltin in *M. edulis* as a result of exposure to contaminated food were less than 0.3 (Laughlin 1986), suggesting the predominant source of accumulation is from the dissolved phase, although uptake of tributyltin was more rapid and resulted in increased tissue concentrations with food exposure (Laughlin 1986). There appears to be a wide range of BCF/BAF values documented for tributyltin, depending on mussel species and exposure environment (aqueous Sn concentrations).

Elimination

Elimination rates for Sn and organoSn are generally small, reported as 0.027/day for tributyltin in *D. polymorpha* (van Slooten 1994), and from 0.17 to 0.36/day for *M. edulis* (Laughlin 1986). Both studies reported no return to background levels in mussel

tissue over the course of 105 days to 6 months, respectively, and in the case of the 105 day depuration, *D. polymorpha* tributyltin tissue concentrations were still twice those of the background mussels (van Slooten 1994). Moreover, the elimination of tributyltin in *D. polymorpha* was faster over the first 63 days than compared to the following timepoints (van Slooten 1994). Biological half-lives reported for organotin range from 14 days (tribuytltin, *M. edulis*) (Laughlin 1986) to 25.7 d (tributyltin, *D. polymorpha*) (van Slooten 1994) to 40 days (organotin, *M. edulis*) (Zuolian 1989). A biological half-life for total Sn in *M. edulis* was reported as 25 days, similar to that for organotin (Zuolian 1989).

Metal Mixtures and Effects on Toxicokinetics

Various reports have discussed the effects of mixtures of metals on mussel uptake, distribution, and elimination. Understanding the effects of metal mixtures is particularly important as metals are not usually singly present in the environment, and the effects on toxicokinetics and toxicity on mussels of a combination of metals is seldom related to those for individual mussels (Keller 1991, Kraak 1994, Stewart 1999, Kraak 1999). Many reports focusing on metal mixtures discuss their effects on mussel filtration rates (Kraak 1993). A constant theme continually demonstrated is the lack of accurate prediction of effects of individual metals from short-term tests to effects of mixtures of metals in both short and long term tests (Kraak 1993, Kraak 1994). For example, Kraak et al. (1994) reported that the additive effects of certain metals (Cu+Zn+Cd) result in EC_{50} (effective concentration required to elicit some observed effect (i.e.: change in filtration rate) in 50% of organisms studied, over a specified time interval) values that are below the NOECs (no observable effects concentration) for the representative individual

metals. Specifically, Kraak et al. (1994) observed less than concentration additive effects on filtration rates in *D. polymorpha* for Cu+Zn, additive effects for Zn+Cd, greater than concentration additive effects for Cu+Cd, and additive effects for the combination of all three metals. Stewart observed Cd loss from the water column was slower with the addition of a metal mixture containing Cu+Zn+Pb+Ni, such that half-lives in the water increased from 11 days (Cd only) to 22-34 days (Cd+Metal mixture at different concentrations), but accumulation of Cd in *P. grandis* decreased, even with increases in aqueous Cd concentrations (Stewart 1999). Other authors' discusses similar results observed for *A. cygnea* (Hemelraad 1987) and *M. edulis* (Elliott 1986). While these data demonstrate a clear effect of metal mixtures on mussel toxicity and accumulation, much more research is needed in order to thoroughly assess the implications. One must be cautious when interpreting single metal toxicity data as well as toxicokinetic data, as environmental contaminants are generally represented by mixtures rather than individual metals, while the bulk of research focuses on individual contaminants.

Platinum Group Metals

Recently, novel research has begun to focus on the platinum (Pt) group metals, including Pt, palladium (Pd), and rhodium (Rh) to determine whether these elements are bioavailable to mussels and how readily they might be taken up (Sures 2002, Zimmermann 2002). Concentrations of Pt, Pd and Rh determined in *D. polymorpha* are low compared to those reported for other metals, ranging from 0.10 to 0.50 ng/g for Rh to 0.90 to 6.2 ng/g for Pd, depending on exposure environment (Zimmerman 2002). However, a relatively high bioavailability of Pd was documented for *D. polymorpha*

exposed to road dust for 26 days, on par with that for Ag, Cd and Cu, with BAF values ranging from 0.80 to 1.80 depending on water characteristics (dechlorinated tap water vs lake water containing humic material) (Zimmerman 2002). Bioaccumulation factors for Pt and Rh were substantially lower, ranging from 0.60 (Pt) to between 0.04 to 0.20 for Rh (Zimmerman 2002). It was noted by others (Sures 2002) that bioaccumulation of these metals occurs in *D. polymorpha* even when water concentrations are below 0.1 ng/L (Sures 2002). Thus, the observations that mussels bioaccumulate these metals demonstrates the need for further study in this area, in terms of understanding the fate and effects of these ubiquitous and potentially hazardous contaminants in the environment.

ESSENTIAL ELEMENTS

Zinc, Calcium, Copper

Environmental Interactions

Zinc (Zn), calcium (Ca) and copper (Cu) toxicokinetics are unique because they represent essential elements required for many biological processes including proper enzyme function, active membrane ion pumps, and normal metabolic homeostasis. Because these metals are actively taken up from the surrounding water and regulated by mussels, they typically are not toxic at low levels, and therefore uptake is not directly proportional to environmental concentrations (Kraak 1994, Mersch 1996, Blackmore 2003, Camusso 1994). These metals can also interact to impede the uptake of non-essential metals such as Cd (Holwerda 1991). For example, co-exposure can result in uptake inhibition of one of the metals, competition for unique binding sites, or the physical effect of decreased filtration rate of the mussels (Holwerda 1991).

Zinc reportedly can be regulated by freshwater and marine mussels at higher environmental concentrations than Cu (Kraak 1994). For example, Tessier et al. (1996) reported that *D. polymorpha* do not accumulate Zn and Cu at low (Zn <28 ug Zn/L and Cu <191 ug Cu/L) concentrations. In contrast, these authors reported accumulation of Cd in *D. polymorpha* with water concentrations of 9 ug Cd/L (Tessier 1996). Further evidence for Zn regulation in mussels is the consistency/similarity exhibited in Zn concentrations (Blackmore 2003). Moreover, in *M. edulis*, Cu uptake was reported to displace other metals such as aluminum (Al) and molybdenum (Mo) within the course of 4 hours (Sutherland 1981). Holwerda demonstrated a significant decrease (90% reduction) in the accumulation factor of Cd when *A. cygnea* were simultaneously exposed to Cu over a 6.5 week exposure period (Holwerda 1991). Copper accumulation was also decreased by simultaneous Cd exposure, however the effect was much lower, decreasing the Cu uptake by only a factor of 2 (vs a factor of 12 for Cu effect on Cd uptake) (Holwerda 1991).

It has also been suggested that certain non-essential metals can share binding sites with essential metals. For instance, Cd is believed to compete for binding sites also used by Zn (Roditi 1999) because of their affinity for sulfur (S) ligands (Fan 2002) and both Ca and Mg have been shown to minimize the particulate adsorption of Cd (Roditi 1999). Fan et al. (2002) reported similar findings for two marine bivalves (*P. viridis* and *R. philippinarum*), where Cd assimilation efficiencies decreased with increasing Zn concentrations. Inverse relationships have been reported between Ca and Cd, and Zn and Pb implying that mussels will compensate for metal accumulation by actively taking up Ca and Zn from the surrounding water, in an attempt to contain toxic metal

concentrations in interstitial tissues (Jana 1997). These observations correlate with certain freshwater mussels such as *E. complanata* incorporating toxic metals into calcium-phosphate granules (Jana 1997). [See Detoxification Mechanism Section]

Bioavailability

Metal bioavailability can depend on many different environmental factors. Based on the knowledge that simple aqueous and sediment metal measurements generally do not adequately predict metal availability and toxicity, sometimes resulting in expensive and inefficient site remediation, the concept of a water-effects ratio (WER) was developed. The WER attempts to incorporate differences in water characteristics at different locations when assessing metal toxicity (Pendergast 1997). More recently, the biotic-ligand model has been used (Paquin 2002, Bianchini 2002) in an attempt to further understand and assess the bioavailability and potential toxicity of metals in the environment. This model attempts to simplify the complex interactions between a metal and different environmental sorbents/complexing agents and directly relate metal exposure to metal toxicity. This is accomplished by measuring water parameters including hardness (Ca), alkalinity (CO3), dissolved organic carbon, chloride and pH. In this model, the metal can be freely dissolved in the aqueous phase, or it can be associated with multiple ligands, including biological ligands (for example the gill surface on a mussel), sediment ligands (acid-volatile sulfides, natural organic matter), dissolved aqueous ligands (dissolved organic matter, or dissolved inorganics, Cl^-, or other anions), and particulate ligands (particulate organic or inorganic carbon). The complexing agents can compete with each other to bind the metal, which can result in protective effects,

where the metal is bound and unavailable to associate with the target ligand, or other anions can outcompete the metal for association with the biological ligand. For example, dissolved organic carbon, and other organic or inorganic ligands can bind the metal, eliciting a protective effect, or cations can bind the metal such as Na^{2+}, H^{+}, or Ca^{2+}, thus inhibiting the metal from binding to the target tissue (Kramer 1991). Therefore, metal speciation will depend on the nature of the water chemistry in different environments. However, metal bioavailability can also be influenced by the condition and/physiology of the organism, including such factors as species, assimilation efficiency, clearance rate, ingestion rate, and stress level (Table 5). All of these factors must be considered to most adequately examine metal bioavailability.

Metal Detoxification Mechanisms

Mussels possess multiple detoxification mechanisms that can influence the uptake, distribution and elimination of metals which range from simple mechanisms like avoidance (shell closure, or decreased ventilation and filtration rate) to induction of low molecular-weight metallothionein proteins (Kadar 2001, Naimo 1995, Marigomez 2002, Tessier 1996, Baudrimont 2002). The classification of the metals into two groups (A and B) is helpful in assessing specific detoxification mechanisms involved. Group A metals include barium (Ba), magnesium (Mg), aluminum (Al), and strontium (Sr), which exhibit high affinities towards O and P ligands, such as those found in calcium-phosphate granules (i.e: *H. depressa*) (Byrne 2000). In contrast, Group B metals, Hg, silver (Ag), and less frequently, Cd, Cu, iron (Fe), manganese (Mn), Pb and Zn, demonstrate affinities for S and N ligands, found in proteins like metallothionein (Byrne 2000). However, Group B metals will bind to calcium-phosphate granules in the absence of

metallothioneins (Byrne 2000). Metallothionein (MT) activity was observed in *C. fluminea* following exposure to Cd and Zn, in which MT concentrations were induced 3.5 times those of reference levels (Baudrimont 2002). During depuration of Cd, MT concentrations were shown to decrease nearly 40%, particularly early on in the elimination phase (Baudrimont 2002). These authors reported that 40% of accumulated Cd was bound to MT, while Tessier et al. (1996) observed that 85% of Cd measured in *D. polymorpha* was bound. Thus, there may be species differences associated with detoxification mechanisms. Other mechanisms that have been observed in mussel include incorporation of metals into lysosomal granules as well as histidine rich glycoproteins.

Implications and Potential for Metal Toxicity

The ultimate effects of metal pollution in the environment on freshwater mussels have yet to be completely assessed. While it is clear that mussels possess the ability to accumulate metals to very high levels, it is more difficult to determine specific chronic adverse effects that culminate with the exposure and subsequent uptake. The complexity of interactions between metals and the environment makes it difficult to classify behavior into simple models, however, further research in this field should include correlations between tissue burdens and physiological parameters, as well as measurements of uptake and elimination rates and the combined effects of metal mixtures on toxicokinetics and mussel health.

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Table 1a. Various toxicokinetic parameters across mussel species, chemical class, and study

Study	Species	Chem. Class	log K_{ow}	K_1 (mL/g*day)	K_2 (day^{-1})
Thorsen 2003	*E. complanata*	PAH (34)	3.37(N0)-7.64(CO)		
		PAH (38)	3.37(N0)-7.64(CO)		
		PAH (45)	3.37(N0)-7.64(CO)		0.04(PE)-0.26(26DMN0)
Gewurtz 2002	*E. complanata*	PAH (14)	3.92(AC)-6.75(DA)		0.037(BkF)-0.0217(F0)
Fisher 1999	*D. polymorpha*	PCP	5.12	369-2133	0.86-1.56
Basack 1997	*C. fluminea*	PCP	5.12		0.39-0.40
Uno 1997	*C. leana*	Pesticides (3)	3.22(OX)-4.22(TBC)	24.2(TBC)-338(CNF)	0.045(CNF)-0.060(TBC)
Gossiaux 1996	*D. polymorpha*	PAH (2)	5.18(PY)-6.04(BaP)	672(BaP)-32737(BaP)	0.024-0.384(BaP)
		PCB (2)	5.9(PCP)-6.9(HCBP)	2280(PCP)-26448(HCBP)	0.024(HCBP)-0.192(PCP)
van Haelst 1996	*D. polymorpha*	TCBT (8)	6.73(28)-7.54(25)	683.3(52)-848.7(80)	0.0052(27)-0.0226(21)
Morrison 1995	*D. polymorpha*	PCB (36)	5.6(42)-7.36(180)		0.042(183)-0.172(64)
Makela 1995	*A. anatina*	PCP	5.12		
	P. complanata	PCP	5.12		
Bruner 1994	*D. polymorpha*	PAH (2)	5.18(PY)-6.04(BaP)	7680(PY)-31200(BaP)	0.192(BaP)-0.576(PY)
		PCB (2)	5.9(TCBP)-6.9(HCBP)	9120-40320(HCBP)	0.12(HCBP)-0.504(TCBP)
Fisher 1993	*D. polymorpha*	PAH (2)	5.18(PY)-6.04(BaP)	10272(PY)-20112(BaP)	0.009(PY, BaP)
		PCB (2)	5.9(TCBP)-6.9(HCBP)	4008-25752(HCBP)	0.004(HCBP)-0.017(TCBP)
		OC (1)	6.19(DDT)	2976-17664(DDT)	0.007-0.008(DDT)
Brieger 1993	*D. polymorpha*	PCB (2)	6.36(77)-7.42(169)	551(77)-1480(169)	0.034(169)-0.035(77)
Russell 1989	*E. complanata*	HCB, OCS	5.45(HCB)-6.29(OCS)	650(HCB)-1010(OCS)	0.41(HCB)-0.16(OCS)
Bjork 1997	*M. edulis*	PCB (3)	5.67(31)-6.92(153)	2160-168,000(153)	0.0288(153)-0.1368(31)
Sericano 1996	*C. virginica*	PAH (7)	4.57(P0)-7.0(IP)		0.02(FL)-0.077(BaP)
		PCB (9)			0.0053(149)-0.0154(110)
Tanacredi 1991	*M. mercenaria*	PAH (9)	3.37(N0)-6.04(BaP)		ND over 45 d
Bender 1988	*C. virginica*	PAH (14)	4.57(P0)-6.50(BghiF)	330(P0)-2365(MPY)	0.009(BF)-0.118(FL)
	M. mercenaria	PAH (14)	4.57(P0)-6.50(BghiF)	187(MP0)-2842(BaA)	0.087(BaP)-0.213(FL)
Pruell 1986	*M. edulis*	PAH (6)	3.9-6.1		0.0231(FL)-0.0578(BkF)
		PCB (4)	5.0-6.6		0.015(HCBP)-0.042(TCBP)
Ogata 1984	Short-necked clam	PAH (4)	4.42(D0)-5.89(D3)		0.10(D3)-0.24(D2)
	Oyster	PAH (4)	4.42(D0)-5.89(D3)		
	Mussel	PAH (4)	4.42(D0)-5.89(D3)		

Table 1b. Various toxicokinetic parameters across mussel species, chemical class, and study.

Study	Species	Chem. Class	log K_{ow}	log BCF	$T_{1/2}$ (days)
Thorsen 2003	*E. complanata*	PAH (34)	3.37(N0)-7.64(CO)	1.54(N0)-4.66(PE)	
		PAH (38)	3.37(N0)-7.64(CO)	1.90(N0)-5.20(CO)	
		PAH (45)	3.37(N0)-7.64(CO)	1.60(AN)-5.51(C4)	2.6(26DMN0)-16.5(PE)
Gewurtz 2002	*E. complanata*	PAH (14)	3.92(AC)-6.75(DA)		3.2(F0)-18.7(BkF)
Fisher 1999	*D. polymorpha*	PCP	5.12	2.6-3.1	0.44-0.81
Basack 1997	*C. fluminea*	PCP	5.12		1.73-1.78
Uno 1997	*C. leana*	Pesticides (3)	3.22(OX)-4.22(TBC)	2.34(OX)-4.14(CNF)	11.6(TBC)-15.4(CNF)
Gossiaux 1996	*D. polymorpha*	PAH (2)	5.18(PY)-6.04(BaP)	4.34(PY)-5.43(BaP)	1.75(BaP)-28.8(BaP)
		PCB (2)	5.9(PCP)-6.9(HCBP)	4.0(PCP)-5.74(HCBP)	3.6(PCP)-28.8(HCBP)
van Haelst 1996	*D. polymorpha*	TCBT (8)	6.73(28)-7.54(25)	4.43(80)-5.19(27)	18.6(80)-71.8(22)
Morrison 1995	*D. polymorpha*	PCB (36)	5.6(42)-7.36(180)		4.0(64)-16.5(183)
Makela 1995	*A. anatina*	PCP	5.12	1.9-2.1	
	P. complanata	PCP	5.12	1.8-1.9	
Bruner 1994	*D. polymorpha*	PAH (2)	5.18(PY)-6.04(BaP)	4.11(PY)-4.92(BaP)	1.2(PY)-3.6(BaP)
		PCB (2)	5.9(TCBP)-6.9(HCBP)	4.32(TCBP)-5.38(HCBP)	1.4(TCBP)-5.8(HCBP)
Fisher 1993	*D. polymorpha*	PAH (2)	5.18(PY)-6.04(BaP)	4.65(PY)-4.88(BaP)	2.6(BaP)-3.0(PY)
		PCB (2)	5.9(TCBP)-6.9(HCBP)	4.62(HCBP)-5.43(HCBP)	1.7(TCBP)-7.2(HCBP)
		OC (1)	6.19(DDT)	4.72-5.03(DDT)	3.6-4.3(DDT)
Brieger 1993	*D. polymorpha*	PCB (2)	6.36(77)-7.42(169)	4.02(77)-4.45(169)	19.8(77)-20.4(169)
Russell 1989	*E. complanata*	HCB, OCS	5.45(HCB)-6.29(OCS)	3.56(HCB)-4.16(OCS)	1.7(HCB)-4.3(OCS)
Bjork 1997	*M. edulis*	PCB (3)	5.67(31)-6.92(153)	4.7(49)-6.8(153) *BAFs	5(31)-24.2(153)
Sericano 1996	*C. virginica*	PAH (7)	4.57(P0)-7.0(IP)		9(BaP)-26(FL)
		PCB (9)			22(26)-130(149)
Bergen 1996	*M. edulis*	PCB (21)	5.07(8)-7.42(169)	approx. 5.3-7.1	
Bender 1988	*C. virginica*	PAH (14)	4.57(P0)-6.50(BghiF)	3.2(P0)-4.9(BF)	5.9(FL)-77(BF)
	M. mercenaria	PAH (14)	4.57(P0)-6.50(BghiF)	3.2(MP0)-4.4(BghiF)	3.3(FL)-8.0(BaP)
Pruell 1986	*M. edulis*	PAH (6)	3.9-6.1	2.0-4.4	11.9-29.8
		PCB (4)	5.0-6.6	4.5-6.6	16.3-45.6
Ogata 1984	Short-necked clam	PAH (4)	4.42(D0)-5.89(D3)	2.17(D0)-2.58(D3)	2.9(D2)-6.9(D3)
	Oyster	PAH (4)	4.42(D0)-5.89(D3)	3.12(D0)-4.45(D3)	
	Mussel	PAH (4)	4.42(D0)-5.89(D3)	2.87(D1)-3.62(D3)	

Table 2a. Literature list of toxicokinetic studies with different HOC classes and mussel species: exposure and study duration

Year	Author	Species	Exposure	Class	Duration
1989	Reeders	*D. polymorpha*			
1989	Russell	*E. complanata*		HCB, OCS	
1993	Fisher	*D. polymorpha*		PAH, PCB	
1993	Breiger	*D. polymorpha*	water, sediment, food	PCB, 3	21-100d exposure, rapid dep
1994	Bruner	*D. polymorpha*	water only	PAH, PCB	6 hr uptake
1995	Makela	*A. anatina, P. complanata*	water only	PCP	steady-state in 16 hrs
1995	Morrison	*D. polymorpha*	water and field	PCB, 36	2 d. exposure,16d elimination
1996	Gossiaux	*D. polymorpha*	water only	PAH, PCB	6 hr uptake, 15d elimination
1996	Chevreuil	*D. polymorpha*	water only	PCBs, Ocs	
1996	van Haelst	*D. polymorpha*			
1996	van Halest	*D. polymorpha*	water only	TCBTs, 8	21d uptake, no steady-state
1997	Basack	*C. flumina*	water only	PCP	96hr uptake,72hr elimination
1997	Uno	*C. leana*	river water	Pesticides	14d uptake,15d elimination
1999	Fisher	*D. polymorpha*	water only	PAH, PCB	
2001	Birdsall	*E. complanata*	water only	PAH, pestic.	used excised gills
2002	Gewurtz	*E. complanata*	water only	PAH, 14	5d uptake, 32d elimination
2003	Thorsen	*E. complanata*	water only and sediment	PAH, 34-48	20d exposure,20d elimination
1970	Blumer	oysters		No.2 Fuel oil	60d uptake, 180d elimination
1972	Lee	*M.edulis*	water only	PAH	
1973	Stegman	oysters		No.2 Fuel oil	49d uptake,28d elimination
1975	Clark	*M.edulis*		PAH	
1976	Dunn	mussels		PAH, BaP	
1977	Boehm	clams		chronic pollut.	120d depuration
1978	Hansen	*M.edulis*		PAHs	
1981	Riley	*O. edulis*	flow-through system	PAH, N0	
1983	Obana	*T. japonica*	water and field	PAH, 9	7-14d exposure
1984	Ogata	clam, oyster, mussel	water only	PAH, D0-D3	
1985	Pittinger	oysters		PAH	15d uptake
1986	Pruell	*M. edulis*	sediment dosed	PAH, PCB	40d uptake, 40d elimination
1986	Broman	*M.edulis*		PAHs	
1986	Hawker	mutiple aq. Org		multiple HOCs	
1987	Tanabe	*P. viridis*	field	PCB, 54	17d uptake, 32d elimination
1988	Bender	*C. virginica, M. mercenaria*	field, lab; 15 + 25 deg C	PAH, 14	28d uptake, 28d elimination
1991	Tanacredi	clams		PAH	2 d uptake, 45d elimination
1992	Sericano	oysters	water only	PCB, 77	
1996	Bergen	*M.edulis*	field	PCBs	28d exposure
1996	Gilek	*M.edulis*	water and food	PCBs	
1996	Bjork	*M.edulis*	water and algae	PAH, P0	20d exposure,14d elimination
1996	Sericano	*C. virginica*	field	PAH, PCB	28-50d uptake,50d depuration
1997	Bjork	*M. edulis*	water and food	PBCs	

Table 2b. Literature list of toxicokinetic studies with different HOC classes and mussel species: miscellaneous and parameters

Year	Author	Species	Miscellaneous Information	Parameters Measured
1989	Reeders	*D. polymorpha*	no change in K_1 w/in season, but change between seasons	
1989	Russell	*E. complanata*		BCF, K_2
1992	Fisher	*D. polymorpha*		K_1
1993	Breiger	*D. polymorpha*		K_1, K_2, BCF/BAF
1994	Bruner	*D. polymorpha*	K_2 depends on lipophilicity of chem	K_1, K_2, BCF, $T_{1/2}$
1995	Makela	*A. anatina, P. complanata*		BCF
1995	Morrison	*D. polymorpha*		K_2, T_{95}
1996	Gossiaux	*D. polymorpha*	temperature effects, monophasic elimination	K_1,K_2, BCF, $T_{1/2}$
1996	Chevreuil	*D. polymorpha*	responses to change in [OC] in water w/in 7 d	
1996	van Haelst	*D. polymorpha*	bivalves: have MFO but capabilities << fish	
1996	van Halest	*D. polymorpha*	log K_{ow} vs K_2 independent + mussel lipid decrease over time	K_1, K_2, BCF, $T_{1/2}$
1997	Basack	*C. flumina*	no extensive phase I metabolism.	K_2 ,
1997	Uno	*C. leana*	[pesticide] in mussels in rice patties	K_1, K_2, BCF
1999	Fisher	*D. polymorpha*	temperature and pH effects	K_1,K_2, BCF, $T_{1/2}$
1999	Thomann		PAH uptake due to partitioning from water to animal across gill surface	
2001	Birdsall	*E. complanata*	average uptake of AN=CO > N0	
2002	Gewurtz	*E. complanata*	linear relationship between log K_{ow} and K_2	K_2
2003	Thorsen	*E. complanata*	stressed mussels: lower K_2s	K_2, BCF, $T_{1/2}$
1970	Blumer	oysters	little elimination after 180 d	
1972	Lee	*M.edulis*	rapid N0, BaP uptake but no metabolism, K_2 depends on lipophilicity of chemical	
1973	Stegman	oysters	elimination nearly complete after 28 d	
1975	Clark	*M.edulis*	K_2 depends on lipophilicity of chem	
1976	Dunn	mussels	K_2 depends on lipophilicity of chem	
1977	Boehm	clams	slight depuration after 120 d	
1978	Hansen	*M.edulis*	hi lipid tissues=rapid elimination vs low lipid tissues=slower elimination: biphasic	
1981	Riley	*O. edulis*	gill: primary site: uptake + accumulation	
1983	Obana	*T. japonica*	rapid PAH accumulation	
1984	Ogata	clam, oyster, mussel		K_1, K_2, BCF
1985	Pittinger	oysters	analytes BDL w/in 4 d	
1986	Pruell	*M. edulis*	slow elimination observed and K_2 depends on liophilicity of chemical	K_2, BCF, $T_{1/2}$
1986	Broman	*M.edulis*	hi lipid tissues=rapid elimination vs low lipid tissues=slower elimination: biphasic	
1986	Hawker	mutiple aq. Org	log BCF vs log K_{ow} relationship, K_2 depends on lipophilicity of chemical	
1987	Tanabe	P. viridis	rapid uptake + release of lower K_{ow} PCBs	K_2, $T_{1/2}$, T_{90}
1988	Bender	C. virginica, M. mercenaria	clams K_2>>oyster K_2	K_1, K_2, BCF
1991	Tanacredi	clams	no depuration observed w/in 45 days	
1992	Serciano	oysters	Equilibrium attained in 30 d	K_2?
1996	Bergen	*M.edulis*	Coplanar PCBs reach steady-state faster (7 d) than nonplanar PCBs (14-28d)	BCF
1996	Gilek	*M.edulis*	body size affects bioaccumulation because of influences on K_1s	K_2
1996	Bjork	*M.edulis*	K_2 unaffect by [POC], and initial uptake rapid	K_2
1996	Sericano	*C. virginica*		K_2, $T_{1/2}$
1997	Bjork	*M. edulis*	PB model of bioaccumulation, food ration affected K_1, but not K_2	K_1, K_2, BAF, $T_{1/2}$

Table 3. List of numerous individual HOCs across studies for comparison of toxicokinetic parameters

Study	Analyte	log K_{ow}	*Species*	log BCF/BAF	$K_{1\ (mL/g*d)}$	$K_{2\ (day^{-1})}$	Exposure
Thorsen 2003	P0	4.54	*E. complanata*	3.02			water, lab
Thorsen 2003	P0	4.54	*E. complanata*	3.44			sed, lab
Thorsen 2003	P0	4.57	*E. complanata*	3.06			sed, field
Bjork 1997	P0	4.57	*M. edulis*	2.90			water, food
Bender 1988	P0	4.57	*C. virginica*	3.21	330*	0.206	sediment
Bender 1988	P0	4.57	*M. mercenaria*	3.29	224*	0.114	sediment
Thorsen 2003	D0	4.49	*E. complanata*	2.86			water, lab
Thorsen 2003	D0	4.49	*E. complanata*	2.69			sed, lab
Thorsen 2003	D0	4.49	*E. complanata*	2.93			sed, field
Ogata 1984	D0	4.49	marine clam	2.17			water
Ogata 1984	D0	4.49	marine oyster	3.12			water
Ogata 1984	D0	4.49	marine mussel	3.13			water
Thorsen 2003	D1	4.86	*E. complanata*	3.43			water, lab
Thorsen 2003	D1	4.86	*E. complanata*	3.38			sed, lab
Thorsen 2003	D1	4.86	*E. complanata*	3.07			sed, field
Ogata 1984	D1	4.86	marine clam	2.38			water
Ogata 1984	D1	4.86	marine oyster	3.40			water
Ogata 1984	D1	4.86	marine mussel	3.12			water
Basack 1997	PCP	5.2	*C. fluminea*			0.39-0.40	water
Fisher 1993	PCP	5.2	*D. polymorpha*	2.6-3.2	8856-51192	0.86-1.56	water
Gossiaux 1996	PCP	5.2	*D. polymorpha*	4-4.6	2280-5928	0.14-0.19	water
Makela 1995	PCP	5.2	*A. anatina*	1.9-2.1			water
Makela 1995	PCP	5.2	*P. complanata*	1.8-1.9			water
Gossiaux 1996	BaP	6.04	*D. polymorpha*	4.4-5.4	9960-32736	0.02-0.38	water
Bruner 1994	BaP	6.04	*D. polymorpha*	4.6-4.9	7920-18240	0.19-0.41	water
Thorsen 2003	BaP	6.04	*E. complanata*	3.5-4.7			water, sed
Pruell 1986	BaP	6.04	*M. edulis*	4.50		0.045	sediment
Bender 1988	Bap	6.04	*C. virginica*	4.29	639*	0.032	sediment
Bender 1988	BaP	6.04	*M. mercenaria*	3.62	361*	0.087	sediment
Gossiaux 1996	HCBP	6.9	*D. polymorpha*	5.2-5.7	13536-26448	0.024-0.96	water
Bruner 1994	HCBP	6.9	*D. polymorpha*	4.8-5.4	9120-38592	0.12-0.68	water
Bjork 1997	HCBP/153	6.9	*M. edulis*	4.9-6.8	2160-168000	0.029	water, food
Thorsen 2003	IP	7	*E. complanata*	4.4-4.56			water,sed
Thorsen 2003	DA	6.75	*E. complanata*	4.8-5.2			water, sed
Brieger 1993	PCB 169	7.42	*D. polymorpha*	4.50			water, food
van Haelst 1996	TCBT 28	6.73	*D. polymorpha*	4.80			water
van Haelst 1996	TCBT 52	7.26	*D. polymorpha*	4.50			water
Pruell 1986	PCB		*M. edulis*	5.70			sediment

* units not specified in reference

Table 4. Comparison of steady-state bioconcentration regression equations for organic contaminants in mussels (freshwater and marine)

Study	Species	Chemical Class	Exposure	Slope	y-intercept	r^2
Thorsen 2003	*E. complanata*	PAH (34)	water, lab	0.895	-1.21	0.83
Thorsen 2003	*E. complanata*	PAH (35)	sediment, field	0.786	-0.98	0.78
Thorsen 2003	*E. complanata*	PAH (45)	sediment, lab	0.807	-1.12	0.73
Ogata 1984	marine clam	PAH (4, all D0)	water, lab	0.163	1.52	0.71
Ogata 1984	marine oyster	PAH (4, all D0)	water, lab	0.494	1.03	0.62
Ogata 1984	marine mussel	PAH (4, all D0)	water, lab	0.311	1.63	0.64
Pruell 1986	*M. edulis*	PAH (6)	sediment, lab	0.965	-1.4	
Geyer 1982	*M. edulis*	multiple HOCs	water, lab	0.858	-0.81	0.96
Hawker 1986	multiple marine	multiple HOCs		0.844	-1.23	0.83
Thorsen 2003	*M.arenaria*	PAH	water, field	1.097	-1.54	0.85
Thorsen 2003	*M. arenaria*	PAH	sediment, field	1.042	-1.28	0.85

Table 5. List of complex interactions between mussel, water and sediment that influence behavior, speciation, and accumulation of metals

Mussel	Water	Sediment	Metal
gut retention time	dissolved organic carbon concentrations	acid-volatile sulfide concentrations	bound vs unbound
feeding mechanism	particulate organic carbon concentrations	total organic carbon	easily labile vs not easily exchangeable
ingestion rate	minerals, other metals present	total inorganic carbon	distribution coefficients under various conditions
assimilation rate	total dissolved solids	minerals, other complexing reagents such as Fe, Al, etc.	
absorption efficiency	hardness, alkalinity, pH		
site saturation	other anionic/cationic ligands present		
misc. physiology	ionic strength/salinity		

Table 6. Comparison of toxicokinetic parameters for metals across studies and mussel species

Study	Species	Metal	Measurement(s)	Miscellaneous Information
Roditi 2000	*D. polymorpha*	Cd		uptake primarily from ingested particles
Jana 1997	*M. edulis*	Cd	900ug/g after 51d exposure to 50 ug/g	
	L. marginalis	Cd	5.8-600ug/g	uptake primarily via gills from water column
	E. complanata	Cd	65ug/g after 60d exposure to 50ppb	rather than from sediment phase
Das 1999	*L. marginalis*	Cd	BCF:35-280	uptake dependent on dose + time of exposure, steady-state requires > 40d
			BAF:1.1-5 (sediment exposure)	10-20ppm Cd, accumulation in liver highest, 30ppm accumulation in gills
Roseman 1994	*D. polymorpha*	Cd	AF:3000-640,000	
			K_1:0.22-0.45 ug/muss/hr (20-30mm mussels)	uptake dependent on mussel length, no uptake in smaller (10mm) mussels
Inza 1998	*C. fluminea*	Cd	gill: 1500ng/g from exposure to 68 mg/L	
			30d depuration, no loss; 120d depuration, 25% loss (22% gills)	highest [Cd]=visceral mass, mantle, foot; 4d to steady-state; change in T and pH, no effect on elimination
Jenner 1991	*U. pictorum*	Cd	BCF:660	rapid uptake; steady-state (kidney) >3 weeks; 29 week elimination:33% loss
Tessier 1996	*E. complanata*	Cd		mussel length: no significant effect on body burdens
Mersch 1993	*D. polymorpha*	Cd		exposure concentrations: 0.30-44ug/L; no steady-state after 27d exposure
Dobrowoski 2002	*Anodonta spp.*	Cd	BCF:981; BAF:0.06 (sediment)	field collected mussels; low Cd bioavailability
Winter 1996	*D. polymorpha*	Cd	BAF:144.5-2089.3	
	A. anatina	Cd		
Campbell 1991		Cd		poor correlation between water and mussel concentrations
Backvar 2000	*E. complanata*	Hg	elimination Hg(II)>>CH_3Hg	concentrations increase with temperature; CH_3Hg bioavailability>>Hg(II)
Tessier 1996		Hg		mussel concentrations dose and time dependent
Inza 1998	*C. fluminea*	Hg	CH_3Hg:5750ng/g dw all tissues; Hg:850ng/g dw all tissues	linear uptake over 14d in all organs except gills (steady-state 4d). CH_3Hg uptake>> Hg(II): differences in distributions
Malley 1996	*P. grandis*	CH_3Hg		CH_3Hg highest in foot/kidney; lowest in gills/viscera
Roditi 2000	*D. polymorpha*	Hg	BAF:14,000-250,000, depending on field location; K_ds reported	81% Hg on particles; accumulation primarily through ingested particles
Roditi 1999	*D. polymorpha*	Hg	K_2:0.043-0.056/d; $T_{1/2}$:13-16d	4-40% assimilation from food; K_2s determined after uptake of 4 different food types

Table 6(Cont'd). Comparison of toxicokinetic parameters for metals across studies and mussel species

Study	Species	Metal	Measurement(s)	Miscellaneous Information
Riget 1997	*M. edulis*	Pb		slow uptake; very slow elimination: 33% eliminated after 2-3 yr
Gundacker 2000	*Anodonta spp*	Pb		highest [Pb]:gills, across field locations; lowest [Pb]:shells; low bioavailability
	U. pictorum	Pb		
Boisson 1998	*M.galloprovincialis*	Pb	BAF:211 (seawater); biphasic elimination	21d exposure; $T_{1/2}$:1.4d, 2.5mo (biphasic); 46% distribution, shell;49% distribution, soft tissues
Marigomez 2002	*M.galloprovincialis*	Pb		distribution in digestive granules
	D. polymorpha	Pb		distribution in hemocytes
Gundacker 1999	*D. polymorpha*	Pb		significantly higher concentrations in bysall threads than soft tissues: mechanism for detoxification
Jenner 1991	*Anondonta spp.*	Pb	BCF:250>>BAF:0.1 (sediment)	
Roditi 2000	*D. polymorpha*	Ag	K_1:3670 mL/g*d; K_2:0.07-0.09/d, $T_{1/2}$:8-9d; BAF:4.3-5.1	variations by field location; K_1's higher than for other metals; primary accumulation from ingested particles: 64-90% accumulation from food
Baud 1990	*M.galloprovincialis*	Ag		no loss in 4 wks exposure; uptake: food+water>>water only>>food only
Stuijfzand 1995	*D. polymorpha*	Ni		body burdens increase with increasing [Ni] in water
Freidrich 1976	*M. edulis*	Ni		body burdens increase with increasing [Ni] in water
Sreedevi 1992	*L. marginalis*	Ni		accumulation occurs in hepatopancreas, mantle, adductor muscle and foot when exposed to acute + subacute [Ni] over 15d
Lee 1994	*M. edulis*	Ni	BCF:432	BCF Ni>Cr, Pb but <BCF for Cd, Zn, Cu
Moore 1991		Sn		uptake greatest in gills, viscera, adductor + mantle; uptake is concentration dependent
van Slooten 1994	*D. polymorpha*	TBSn	K_1:25,000/d; K_2:0.027/d; log BCF:5; $T_{1/2}$:26d	field exposure; slow elimination (105d); DBSn steady-state in 35d, but accumulation<<TBSn
Laughlin 1986	*M. edulis*	TBSn	log BCF:3-4; log BAF<0.3 (contaminated food);K_2:0.17-0.36/d (6mo elim);$T_{1/2}$:14d	accumulation greatest in gills, viscera, adductor + mantle; no steady-state in 47d (water), 30d (food)
Fent 1991	*D. polymorpha*	TBSn	log BCF:4-4.6	
Zuolian 1989	*M. edulis*	Sn	total Sn/TBSn: log BCF:3.7/4.8; $T_{1/2}$:25d/40d	51d uptake/exposure
Shawkey 1998	marine mussels	TBSn	log BCF:3.7	
Sures 2002	*D. polymorpha*	PtGM	0.1-0.5ngRh/g;0.9-6.2ngPd/g	bioaccumulation low, but occurs even at very low aqueous concentrations, i.e: <0.1ng/L
Zimmerman 2002	*D. polymorpha*	PtGM	BAF(Pd):0.8-2;BAF(Pt):0.6;BAF(Rh):0.04-0.2	26d exposure to road dust;BAFs vary with exposure water characteristics

Chapter 1: Figure Legends

Figure 1: Example of uptake curve (a) and elimination curve (b) in freshwater mussel, *Elliptio complanata*. Note in this example, the rapid uptake that initially occurs, followed by a leveling off of the concentration of the contaminant in mussel tissue. The leveling off is considered steady-state, and in this example is reached following approximately 100 hours of exposure. The rate of elimination is also rapid in this example, and is essentially the reverse of the uptake curve. When placed in clean water, the mussels initially depurate the contaminant rapidly from their tissues, and then reach a plateau, where no further elimination occurs on this time scale.

Figure 2: Simple equilibrium partitioning diagram. The contaminant partitions between the dissolved phase in the water column, the particulate phase in the water column and the mussel lipid/tissues. According to Le Chatelier's principle, when a system at equilibrium is disrupted (i.e: removal of contaminant from particulate phase by mussel), it will shift to re-establish equilibrium (i.e: system responds to change by contaminant from dissolved phase binding to particulate phase). This assumes all rates are relatively rapid.

Figure 3: An example of a plot of log BCF vs log K_{ow}. Linear regression has been performed and the resultant regression equation takes the form: log BCF = m*log K_{ow} +b. This regression equation (through simple mathematical procedures) can be used to predict aqueous exposure concentrations based on mussel tissue residues.

Figure 4: An example plot of the natural log (ln) of the lipid normalized mussel tissue concentrations vs time. The absolute value of the slope of the linear regression of the data points represents the elimination rate constant, K_2, for this particular compound. The equation for the line follows the form: $\ln C_{mt} = (K2)*t + \ln C_{m0}$, where C_{mt} is the contaminant concentration in mussel tissue at time, t, and C_{m0} is the contaminant concentration in mussel tissue at time t=0 (initial).

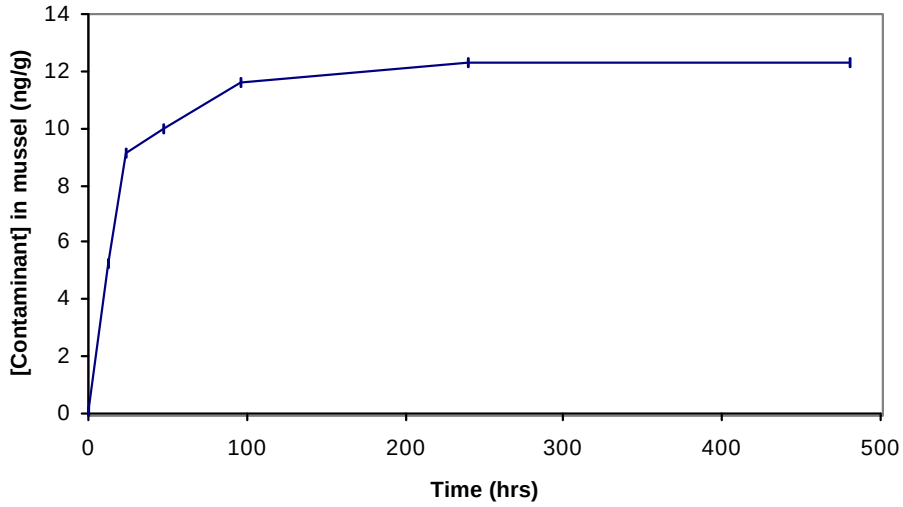

Figure 1a: Uptake in mussel over time

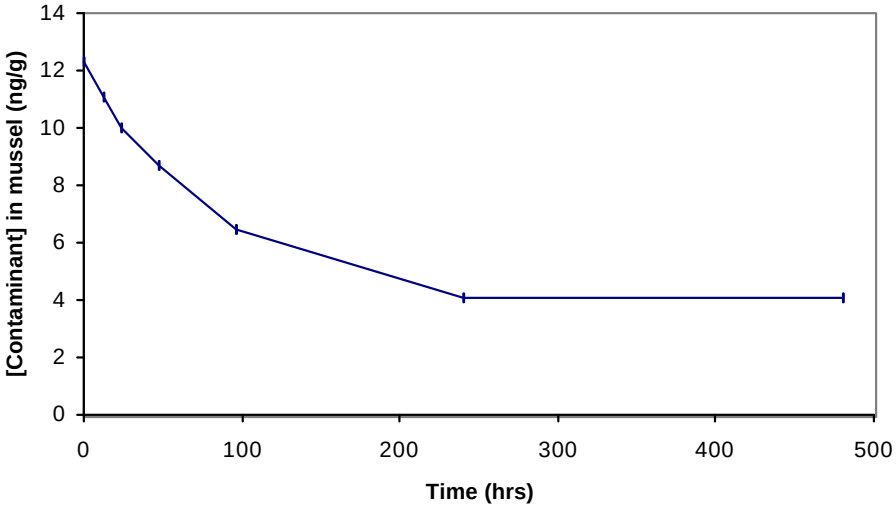

Figure 1b: Elimination in mussel over time

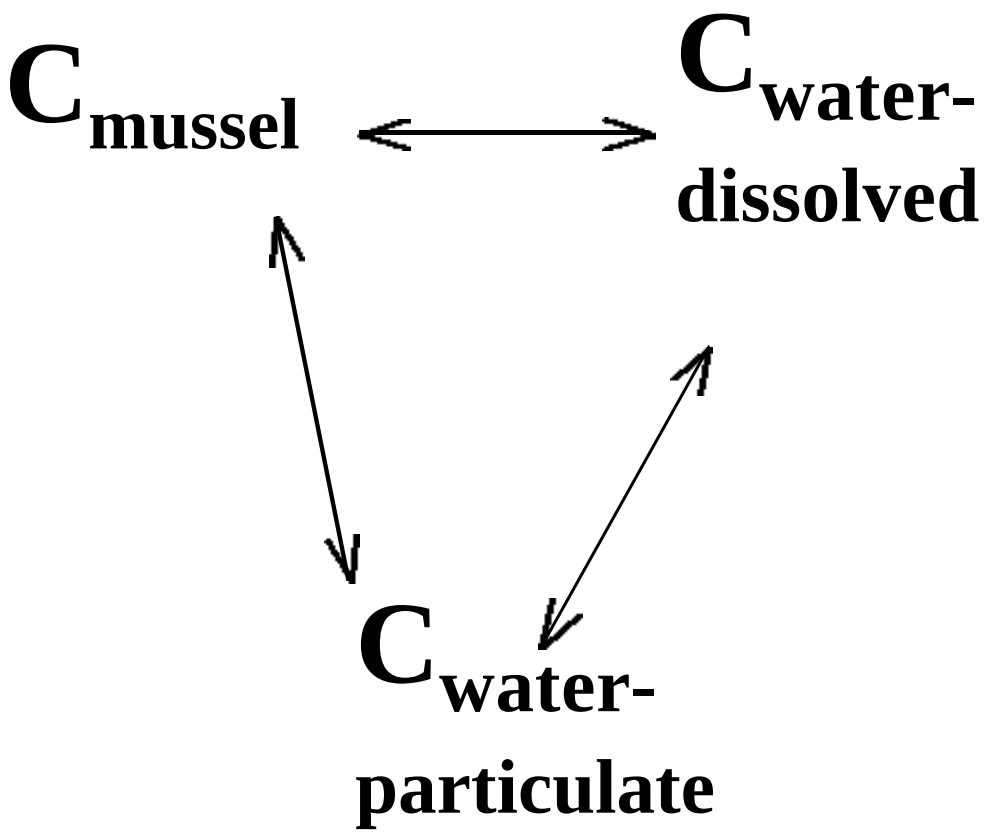

Figure 2: Simple equilibrium partitioning diagram

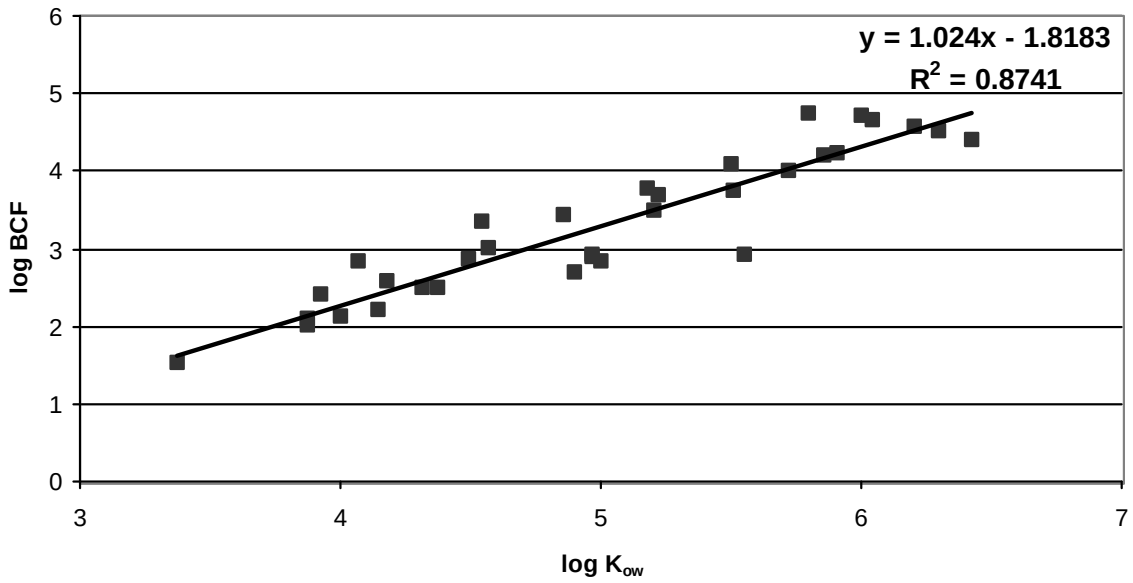

Figure 3: Example of log BCF vs log Kow: linear regression

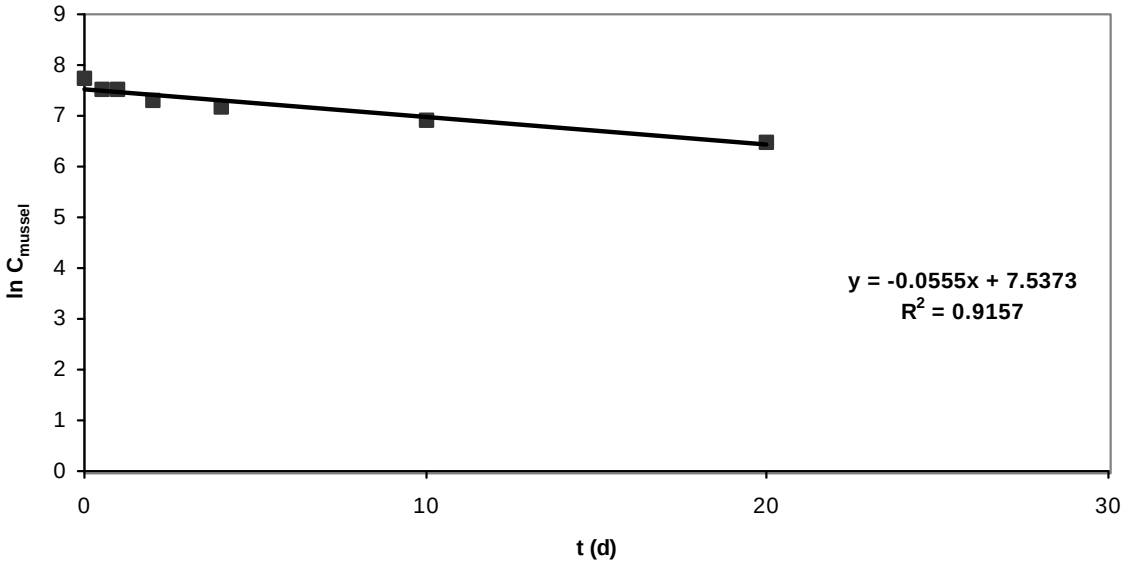

Figure 4: ln Cm vs t: determination of K_2

CHAPTER 2

ELIMINATION RATE CONSTANTS FOR 46 PAHs IN *Elliptio complanata*

ABSTRACT. Elimination rate constants (K_2), biological half-lives ($T_{1/2}$) and the time required to reach 95% of steady-state (T_{95}) are reported for 46 individual polycyclic aromatic hydrocarbons (PAHs) including both parent and alkyl homologues, for the freshwater mussel, *Elliptio complanata*. Elimination rate constants generally follow first-order kinetics and range from 0.04/day for perylene to 0.26/day for 2,6-dimethylnapthalene; half-lives range from 2.6 to 14.2 days; and T_{95} values range from 11.3 to 71.3 days. These values compare well with other K_2, $T_{1/2}$ and T_{95} values reported in the literature for PAHs as well as other classes of hydrophobic organic contaminants. A linear regression of K_2 vs log K_{ow} demonstrates dependence of PAH elimination on hydrophobicity, as measured by an r^2 value of 0.8344, and produces the following regression equation: $K_2 = -0.056 (\log K_{ow}) + 0.44$. This paper provides evidence that mussels that experience different forms of physiological stress (handling stress and fungal growth) can exhibit large variation in toxicokinetic parameters. This is particularly relevant when data from laboratory studies may be applied to field experiments.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment resulting primarily from anthropogenic inputs including the production, transport and use of petroleum and its refined products (Lima et al. 2003, van Metre et al. 2000, Harvey 1997). Some PAHs including benzo(a)pyrene and benzo(b)fluoranthene (Neff 1979, Varanasi 1989) demonstrate carcinogenic and mutagenic properties, and therefore it is important to understand their fate in the environment. The environmental fate of PAHs can be complex, particularly in the aquatic environment, involving interactions with

various forms of organic carbon in both the water column (dissolved and particulate organic carbon) (Gobas et al. 1994) and sediment phase (total organic carbon and soot carbon) (Gustafsson et al. 1999). These numerous forms of organic carbon can influence the bioavailability of PAHs, rendering traditional measurements of total PAH concentrations in sediment and water poor predictors of the fractions truly available to organisms. One way to monitor the fate of the bioavailable fraction of PAHs in the environment is through the use of an indicator species such as the mussel, which possesses the capability to bioaccumulate organic contaminants to very high concentrations in their tissues (Farrington et al. 1983). This high bioaccumulation potential is due to numerous factors including a minimal ability to metabolize hydrophobic organic contaminants, particularly PAHs (James 1989, Neff 1979), as well as the large quantity of water mussels are capable of filtering through their gills (Naimo 1995). For many years, mussels have been used as sentinel species to monitor the overall health of various ecosystems including coastal and marine environments (Farrington et al. 1983), and, more recently freshwater lakes, rivers, and streams (Kauss et al. 1991, Roper et al. 1997). However, in order to adequately acquire and interpret bioaccumulation data, it is necessary to understand the toxicokinetic parameters that influence ultimate PAH bioaccumulation, such as uptake rates (K_1), elimination rates (K_2), biological half-lives ($T_{1/2}$) and times required to reach steady-state (T_{95}).

Traditionally, the easiest kinetic parameter to measure is the elimination rate, from which biological half-lives and steady-state time requirements can be calculated. Elimination is easiest to measure because of the simplicity of placing contaminated mussels in clean water and measuring tissue burdens over a designated time period. This

avoids confounding factors such as the difficulty in directly measuring uptake rates because of the simultaneous elimination processes. The elimination of a contaminant from mussel tissue is generally considered to follow first-order kinetics on a natural log scale and can be described mathematically as:

$$dC_m/dt = -K_2*C_m$$

where dC_m/dt is the change in PAH concentration in mussel tissue over the change in time. This equation can be integrated to allow for a direct calculation of K_2:

$$\ln C_{m,t} = -K_2*t + \ln C_{m,0}$$

where $\ln C_m$ is the lipid normalized PAH concentration in the mussel tissue at time, t, and at time initial (t=0). Subsequently, in a plot of $\ln C_m$ vs t, the absolute value of the slope is the elimination rate constant. Furthermore, K_2 values can be used to calculate biological half-lives: $T_{1/2} = \ln 2/K_2$, as well as the time required to reach 95 % of steady-state: $T_{95} = -\ln 0.05/K_2$. All of these parameters can be used to provide information on how long mussels should be deployed in order to reach steady-state and calculate bioconcentration factors, as well as determine which contaminants can be expected to reach steady-state in a reasonable time frame. One must use caution when simply deploying mussels without regard for specific contaminant kinetics, as bioconcentration can be underestimated (i.e: long time required for steady-state, such as very high log K_{ow} compounds), or significant pulses of contamination may be missed (i.e: for those compounds that require a very short time frame for steady-state, and therefore only 2 to 3 days duration for changing water concentrations to be reflected in mussel tissues burdens). Mussel tissue contaminant concentrations can serve as time integrated measurements; however, one must define the ‘time’ component carefully with a focus on

empirical data and specific physical-chemical measurements (i.e: K_{ow}) of the compounds of interest.

Generally, kinetic data gleaned in the laboratory are applied to field environments as well. However, consideration of different variables present in the laboratory vs field is important as different stressors in each setting can influence results. For example, others (Bruggeman et al. 1984, Gossiaux et al. 1996) have reported changes in elimination rates and decreases in mussel filtration rates with various stressors including the presence of contaminants, very high or low temperatures, and changes in food supply. Consideration must also be applied to handling stress and lack of feeding in laboratory toxicokinetic studies.

In this paper, we present K_2, $T_{1/2}$, and T_{95} values, and K_2 vs log K_{ow} regression analyses for 46 individual PAHs. We also compare these data to other values in the literature, and discuss any differences. Additionally, we present evidence for decreased K_2 values and increased variability as a result of laboratory stressors on mussels during one of two elimination studies.

METHODS

Field collection and experimental design

Two different laboratory elimination studies were conducted in order to determine elimination rate constants and bioconcentration factors for individual PAHs. Study A was a water only uptake study, while Study B used sediment as the PAH exposure source. For both studies, mussels (*E. complanata*) were collected from a reference creek in a relatively rural area of central North Carolina. Background contamination of PAHs in

mussels was minimal. Any preexisting tissue PAH concentrations were further depleted in Study A due to holding of mussels in a clean water tank for 3 days for gradual temperature acclimation (<3°C increase per day) prior to commencement of the uptake study. Sediment PAH concentrations in Study B were high enough that any preexisting background PAH concentrations would have been negligible as a result of the initial PAH uptake.

The flow-through uptake portion of Study A consisted of three, 70L tanks, which held 23 mussels each. The mussels were placed individually in hand-made polyethylene crates designed to simulate 'burrowing' behavior. Water and PAH stock solution containing all 46 PAHs (spiked with Alaskan North Slope crude oil (Battelle, Duxbury, MA) and creosote (AccuStandard Inc, New Haven, CT)) was pumped into a mixing chamber that fed each 70L exposure tank through a water splitter. The stock solution was made by first dissolving the crude oil and creosote in a mixture of acetone:ether:reagent alcohol (1:1:1) (v:v:v), and mixing continually for 24 hours prior to the commencement of the experiment to allow for the volatilization of the solvents. Water was replenished in a flow-through manner approximately every 5 hours during the course of the 8 day uptake study. Originally, the uptake study had been designed for a 10 day duration; however, the growth of a filamentous-type fungus in the tanks and the concern for mussel survival required a shorter duration of uptake. As a result, the mussels were removed from the tanks at uptake day 8, gently, manually cleaned of fungus, and placed in clean tanks with clean water for the depuration portion of the study. During the depuration portion of the study, the water in each tank was again renewed approximately 5 times per

day, and mussels were collected in triplicate from each tank at 0, 12, 24, 48, 96, 240 and 480 hour intervals.

Study B represented a 10 day, static, sediment exposure study in which contaminated (creosote) sediment (Elizabeth River, VA) provided the PAH exposure. Six to seven mussels were placed in 20L jars containing contaminated sediment and water. The sediment-water interface was allowed to equilibrate and settle for 24 hours prior to mussels being placed in the system. Mussels were allowed to burrow into the sediment. Following a 10 day PAH exposure, mussels were placed in 70L tanks with clean water. The water in each tank was renewed approximately 6 times per day. PAH concentrations were not measured in the water during the elimination phase in either study. However, we believe PAH concentrations as a result of elimination from mussels in the water were minimal, and other studies have reported minimal PAH recycling as a result of mussel depuration (Gewurtz et al. 2002).

Mussels were not fed during the course of both the uptake and depuration phases in Studies A and B. There was no statistically significant different in lipid content in mussels over the course of the studies. Nominal water concentrations of PAH in study A ranged from 1 to 144 ng/L, and in study B from 14 to 5100 ng/L, depending on individual analyte.

Monitoring of water quality parameters

Water quality parameters including alkalinity (20 to 25mg/L), hardness (40 to 48mg/L), pH (7.40 to 8.07), temperature (20.5 to 21.5°C), and dissolved oxygen (8.0 to 9.6mg/L) were monitored daily in each study in order to assess the living conditions of

the mussels. Additionally, ammonia was monitored in study B. Parameters were constant (with minimal variation as listed above) throughout both the uptake and depuration portion of the studies.

Tissue PAH extraction and analysis

Immediately following collection at various time points, mussels were wrapped as composites in pre-combusted aluminum foil and frozen. Shucked, weighed, and homogenized mussels (either wet (Study A) or dry (Study B) wt.) were spiked with surrogate internal standards, and extracted with dichloromethane (DCM) by maceration (Tekmar Polytron Tissuemizer®) or by 24 hour automated shaker extraction. Sample extracts were concentrated using either rotary evaporation under reduced pressure (40°C) and/or a gentle stream of nitrogen. Concentrated samples were fractionated and cleaned-up using gel permeation chromatography and silica gel column chromatography. Recovery internal standards were added and the extracts were analyzed using an HP 5890 series II GC coupled to an HP 5970 MSD using an RTX-5 MS (Restek, 30mmx0.25mmx0.25um film thickness) with a 5m Integra-Guard column. Analysis was run in the SIM mode using the following temperature program: Injection port: 300 ° C, Transfer line: 280 ° C, Initial temp: 40° C, Initial Hold: 1 min., Ramp rate: 6°C/min, Final temp: 290°C, Final hold: 30 min. One uL of sample was injected. Method detection limits varied for individual PAH analytes, but were approximately 0.2-0.5 ng/g.

Quality control and quality assurance protocol

Quality control and quality assurance procedures included the addition of a surrogate internal standard solution (Napthalene-d8, Acenapthene-d10, Chrysene-d12 and Perylene-d12, Accustandard Inc, New Haven, CT) to all samples (excluding POC, DOC measurements and water condition analyses) prior to experimental procedures. A matrix spike and matrix spike duplicate consisting of all individual PAH analytes except the alkylated homologues were run once for each matrix analysis (water and mussel tissue samples). Procedural blanks were run with each sample batch and recovery internal standards (Phenanthrene-d10 and Benzo[a]pyrene-d12, Accustandard Inc, New Haven, CT) were added immediately prior to sample analysis on the GC-MS for analyte peak quantification. Recoveries were within acceptable ranges (40 to 120%) and were uncorrected for SIS loss. Dissolved oxygen and pH meters were calibrated daily, prior to testing.

RESULTS

Elimination rate constants (K_2), half-lives ($T_{1/2}$) and time required to reach 95% steady-state (T_{95}) are reported for a large suite of PAH compounds from study B (Table 1 + 2). Elimination rate constants (Table 1 + 3) ranged from 0.04 (PE) to 0.26/day (26DMN0). The elimination rate constants for study A are not reported due to a number of factors including complications during the uptake portion of the study with fungal growth and resultant second-order elimination kinetics which require detailed analysis (Figure 1a,b). However, when study A data were forced into first-order kinetics (i.e: simple linear regression of ln [lipid normalized PAH in mussel tissue] vs time) (Figure

1a,b), the K_2 values were much less than those in study B, and ranged from 0.01 (BaP) to 0.17 (235TMN0). On average, K_2s from study B ranged from 2 to 4 times greater than K_2s from study A, with a few exceptions, most noteably F3 (7 times greater in study A) and PY (53 times greater in study B).

In contrast to the second-order kinetics in study A, all analytes in study B demonstrated first-order elimination kinetics (similar to Figure 1d: BaP) with the exception of 1MN0 (Figure 1c). Note the differences between figures 1c + d, where in figure 1c, rapid elimination initially occurs from day 0 to about day 2 (48 hrs) but then levels off. This effect is much more dramatic in figure 1a. However, in figure 1d, the elimination rate appears relatively consistent through the 15 d time course.

Biological half-lives (Table 2) ranged from 2.6 to 14.2 days in study B. Similar comparisons between half-lives were observed as were for elimination rate constants. The shortest half-life was for 26DMN0, while the longest was for PE. The time required for individual PAHs to reach 95% steady-state was also calculated (Table 2), and ranges from 11.3 (26DMN0) to 71.3 days (PE). However, these values may be somewhat misleading, as steady-state for all PAH analytes was observed in similar studies by this laboratory with much shorter exposures of 7 to 10 days.

For the most part, K_2s decrease with increasing PAH hydrophobicity. Figure 2a depicts the linear regression analysis of the K_2 vs log K_{ow} plot, demonstrating the dependence of elimination on PAH hydrophobicity, as measured by an r^2 value of 0.8344. The regression equation for study B is: $K_2 = -0.0561(\log K_{ow}) +0.4356$. Moreover, an apparent plateau in elimination rate constants occurs at log K_{ow} values greater than approximately 6, where the K_2 values level off at approximately 0.05/day.

DISCUSSION

Comparison of K_2s and other kinetic parameters to literature values

The K_2 values reported in this study generally compare well to others reported in the literature for PAHs in *E. complanata* (Gewurtz et al. 2002) (Table 1). For example, K_2 values for P0 compare very well, at 0.171/day for this study vs 0.177/day for Gewurtz et al (2002). Additional comparisons between other analytes are good, with exceptions for AY (0.187 vs 0.046, respectively), AC (0.238 vs 0.095) and IP (0.047 vs 0.162). Elimination rate constants for AY and AC are larger in this study by approximately 4x and 2.5x those of Gerwutz et al. (2002); however, the K_2 value for IP is larger for Gewurtz by a factor of 3.4. The reasons for the discrepancy between these three analytes is unclear, and most likely is due to differences in experimental design, and/or general mussel variation.

The good correlation between the K_2 values for this study and Gerwutz et al. (2002) is also demonstrated for $T_{1/2}$ (calculated from Gerwutz's data) and T_{95} values (Table 2). Because $T_{1/2}$ and T_{95} values are calculated using K_2 values, AC, AY and IP are again exceptions to the overall good comparison. For instance, the half-life of AY in this study is 3.8 days, while it is 15.1 days in Gewurtz et al. (2002). Moreover, the T_{95} for AY in this study is 16.2 days, whereas it is 64.7 days in Gewurtz et al. Again, the reasons for these distinct differences are unclear, as the other 11 of 14 PAH analytes reported by Gewurtz compare very well.

First-order vs second-order elimination kinetics

With the exception of 1MN0, all PAH exhibited first-order elimination kinetics in study B. First-order elimination of PAHs in *E. complanata* (Gewurtz et al. 2002), and *M. edulis* (Pruell et al. 1986), and of PCBs in *D. polymorpha* (Morrison et al. 1995) has also been reported. In contrast, elimination in study A demonstrated apparent second-order kinetics for the majority of PAH analytes, with only a few exceptions where first-order elimination was observed (C0, FP1). Reasons for this discrepancy are most likely due to unusual experimental conditions during the uptake portion of study A. During the uptake portion of the study, fungal growth, resembling white, fluffy, cobweb-type material began to occur 4 days into the exposure. The fungal growth increased substantially over the following 4 days, covering large surface areas of the exposure tanks, mussel crates, and mussel shells, and appeared to be hindering the siphoning capacity of the mussels (no quantitative analysis of this available, but visual decrease in time inhalant siphons were open was observed by WAT). At day 8 of the exposure, the mussels were removed so the tanks could be cleaned, to avoid mussel mortality. Following cleaning, mussels were placed back into the tanks and the depuration portion of the study was commenced, cutting short the uptake phase by 2 days. The second-order elimination kinetics could have been caused by an increase in mussel siphoning during the initial portion of the depuration study in response to improved water conditions (i.e.: specifically lack of fungal growth inhibiting siphoning activity, as water quality parameters such as alkalinity, hardness and dissolved oxygen were monitored daily and did not change throughout the course of the study), or simply being able to siphon normally again. Once the mussels were placed in water without fungal growth, they

might have increased filtration in an attempt to re-establish normal homeostasis (initial rapid rates of depuration), and then dropped to normal levels after the first few days (lower rates of depuration following initial rapid decline). This is consistent with K_2 values from study A calculated with the last two time points removed (days 10 and 20), that are nearly all equivalent to or greater than those from study B. Higher average K_2 values are also apparent in the K_2 vs log K_{ow} regression, where the y-intercept is 0.68 vs 0.44 in study B (Figure 2a,b). Moreover, the scatter is greater in study A, suggesting greater differences in individual mussel elimination rates, and/or less dependence on PAH hydrophobicity when mussels are experiencing excessive stress. Another option is that mussel health simply declined to a greater extent through the course of the depuration study, (i.e.: due to continued lack of food, increased handling, and post-stress from the fungal growth), and this resulted in a decrease in elimination rates, thus creating the biphasic nature of the elimination kinetics. However, without quantitative assessment data, we are unable to draw a solid conclusion on the reasons behind the apparent second-order kinetics.

Relationship between K_2 and log K_{ow}

Elimination rate constants decrease with increasing PAH hydrophobicity (Figure 2a). The largest K_2 values generally correspond to the lowest log K_{ow} values, ranging from for example, 0.24 for N0 (log K_{ow}=3.37) to 0.21 for N2 (log K_{ow}=4.37), while the smallest K_2 values correspond to the highest log K_{ow} values: 0.059 for BkF (log K_{ow}=6.0), and 0.042 for PE (log K_{ow}=6.3). A simple linear regression of the K_2 vs log K_{ow} plot yields the following regression equation: $K_2 = -0.0561 \log K_{ow} + 0.4356$, with an

r^2 of 0.83. The linear relationship between K_2 and log K_{ow} is similar to that reported by Gewurtz et al. (2002) and Russel et al. (1989) (Table 3), with slopes of -0.05 and -0.04, and y-intercepts of 0.39 and 0.34, respectively. The r^2 value is similar as well for Gewurtz et al. (0.71), however, is much lower for Russel et al. (0.44). Further comparisons can be made between regression equations from an excellent comprehensive table in Gewurtz et al. (2002). With some exceptions (i.e: *C. virginica* field studies and *M. mercenaria* laboratory study (see Gewurtz et al. 2002)), nearly all of the regression equations (8) reported are similar to the one presented in this study.

Reasoning behind apparent plateau in K_2 values at log K_{ow}>6

An apparent plateau in K_2 values is observed for log K_{ow} values >6 (Figure 2a). The same factors responsible for the plateau in accumulation observed for bioconcentration factors for hydrophobic organic contaminants with log K_{ow}>6 are likely to explain these results. Higher log K_{ow} contaminants likely have the same limiting factors controlling their release from membranes as they do controlling contaminant uptake into membranes, namely, steric hinderance and other physical restraints. A similar leveling off in K_2 values is apparent for PCBs with log K_{ow}>6.5 in *D. polymorpha* (Morrison et al. 1995). This effect is much more distinct when a larger suite of compounds is used (i.e.: 46 PAHs and 36 PCBs). For instance, the leveling off is not as noticeable for the 14 PAHs in Gewurtz et al. (2002), but still does appear to occur for PAHs with log K_{ow}> approximately 5.6.

The plateau effect was also observed, although it was less dramatic, for data from study A. Interestingly, this effect was only observed when the last two mussel

concentration timepoints (10 and 20d) were removed when calculating K_2 values to plot against log K_{ow} (Figure 2b). This might suggest that the mussels response to the fungal growth and stress associated with the cleaning procedures did not manifest itself until the latter timepoints had been reached in the depuration study, or the overall health began to decline late into the elimination portion of the study.

Effects of stress on kinetics

Certainly stress in various forms can alter the normal homeostasis of living organisms. However, what is more difficult to conclude is the exact nature and mechanism of the process(es). Although our quantitative data are limited regarding overall mussel health (aside from lipid content), our data suggest that mussels exposed to excessive fungal growth that potentially impedes their siphoning capabilities, and that experience increased handling, exhibit lower than average elimination rate constants and increased individual variability. These speculations should be considered, particularly when interpreting field data, or extrapolating laboratory data to field experiments, as over or under-estimations of uptake, accumulation and elimination could result, with potential for toxicological implications.

CONCLUSIONS

Elimination rate constants, $T_{1/2}$ and T_{95} values reported for PAH in the freshwater mussel, *E. complanata* compared well to other values available in the literature. Moreover, a linear relationship was exhibited between K_2 and log K_{ow}, demonstrating the importance of PAH hydrophobicity on elimination, most notably in

organisms which possess only minimal capabilities to metabolize these compounds. The plateau in K_2 values observed for PAH with log K_{ow}>6 is corroborated by PCBs in another freshwater mussel, *D. polymorpha*, and is likely due to the contaminants difficulty re-crossing membranes as a result of larger molecular size and/or lipid interactions. Additionally, effects of various forms of stress on mussel toxicokinetics should be considered in terms of data application in case of over or under-estimation of bioaccumulation and resultant implications for toxicity.

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Table 1. Individual PAH symbol, log K_{ow} and elimination rate constants across studies

Analyte	Symbol	log K_{ow}	K_2 (study B)	K_2 (Gewurtz)
Naphthalene	N0	3.37	0.2217+/-0.059	
2-Methylnapthalene	2MN0	4.00	0.2059+/-0.024	
1-Methylnapthalene	1MN0	3.87	0.2486+/-0.018	
Biphenyl	BP	4.14	0.2128+/-0.017	
2,6-Dimethylnapthylene	26DMN0	4.31	0.2650+/-0.021	
Acenapthylene	AY	4.07	0.1847+/-0.021	0.046+/-0.031
Dibenzofuran	DBF	4.12	0.2316+/-0.028	
Acenapthene	AC	3.92	0.2372+/-0.010	0.095+/-0.046
2,3,5-Trimethylnapthalene	235TMN0	4.90	0.1793+/-0.016	
C1 - Napthalenes	N1	3.87	0.2364+/-0.026	
C2 - Napthalenes	N2	4.37	0.2124+/-0.018	
C3 - Napthalenes	N3	5.00	0.1793+/-0.020	
C4 - Napthalenes	N4	5.55	0.1543+/-0.019	
Fluorene	F0	4.18	0.1902+/-0.038	0.217+/-0.037
1-Methylfluorene	1MF0	4.97	0.1250+/-0.010	
C1 - Fluorenes	F1	4.97	0.1431+/-0.018	
C2 - Fluorenes	F2	5.20	0.1749+/-0.033	
C3 - Fluorenes	F3	5.50	0.1078+/-0.013	
Dibenzothiophene	D0	4.49	0.1611+/-0.020	
C1 - Dibenzothiophenes	D1	4.86	0.1473+/-0.011	
C2 - Dibenzothiophene	D2	5.50	0.0821+/-0.010	
C3 - Dibenzothiophene	D3	5.73	0.0687+/-0.008	
Phenanthrene	P0	4.57	0.1707+/-0.028	0.177+/-0.029
Anthracene	AN	4.54	0.1792+/-0.024	0.163+/-0.043
1-Methylphenanthrene	1MP0	5.14	0.1387+/-0.025	
C1 - Phenanthrenes/Anthracenes	P1	5.14	0.1656+/-0.013	
C2 - Phenanthrenes/Anthracenes	P2	5.51	0.1318+/-0.014	
C3 - Phenanthrenes/Anthracenes	P3	6.00	0.0939+/-0.012	
C4 - Phenanthrenes/Anthracenes	P4	6.51	0.1071+/-0.009	
Fluoranthrene	FL	5.22	0.1257+/-0.013	0.130+/-0.024
Pyrene	PY	5.18	0.1635+/-0.025	0.144+/-0.023
C1 - Fluoranthenes/Pyrenes	FP1	5.72	0.0919+/-0.017	
Benz[a]anthracene	BaA	5.91	0.0924+/-0.014	0.148+/-0.038
Chrysene	C0	5.86	0.0836+/-0.017	0.105+/-0.026
C1 - Chrysenes	C1	6.42	0.0838+/-0.004	
C2-Chrysenes	C2	6.88	0.0697+/-0.011	
C3-Chrysenes	C3	7.44	0.0487+/-0.008	
Benzo[b]fluoranthene	BbF	5.80	0.0827+/-0.010	0.103+/-0.029
Benzo[k]fluoranthene	BkF	6.00	0.0589+/-0.007	0.037+/-0.063
Benzo[e]pyrene	BeP	6.20	0.0727+/-0.012	
Benzo[a]pyrene	BaP	6.04	0.0755+/-0.008	
Perylene	PE	6.30	0.0421+/-0.009	
Indeno[123cd]pyrene	IP	7.00	0.0471+/-0.006	0.162+/-0.046
dibenz[ah]anthracene	DA	6.75	0.0687+/-0.014	0.048+/-0.047
benzo[ghi]perylene	BghiP	6.50	0.0599+/-0.013	0.080+/-0.044
Coronene	C0	7.64	0.0501+/-0.009	

Table 2. $T_{1/2}$ and T_{95} for individual analytes across studies

Symbol	log K_{ow}	$T_{1/2}$ (study B)	$T_{1/2}$ (Gewurtz)	T_{95} (study B)	T_{95} (Gewurtz)
N0	3.37	3.1		13.5	
2MN0	4.00	3.4		14.6	
1MN0	3.87	2.9		12.1	
BP	4.14	3.3		14.1	
26DMN0	4.31	2.6		11.3	
AY	4.07	3.8	15.1	16.2	64.7
DBF	4.12	3.0		13.0	
AC	3.92	2.9	7.3	12.6	31.6
235TMN0	4.90	3.9		16.7	
N1	3.87	2.9		12.7	
N2	4.37	3.3		14.1	
N3	5.00	3.9		16.7	
N4	5.55	4.5		19.4	
F0	4.18	3.6	3.2	15.8	13.8
1MF0	4.97	5.5		24.0	
F1	4.97	4.8		21.0	
F2	5.20	4.0		17.2	
F3	5.50	6.4		27.8	
D0	4.49	4.3		18.6	
D1	4.86	4.7		20.4	
D2	5.50	8.4		36.5	
D3	5.73	10.1		43.7	
P0	4.57	4.1	3.9	17.6	16.9
AN	4.54	3.9	4.3	16.7	18.4
1MP0	5.14	5.0		21.6	
P1	5.14	4.2		18.1	
P2	5.51	5.3		22.8	
P3	6.00	7.4		31.9	
P4	6.51	6.5		28.0	
FL	5.22	5.5	5.3	23.9	23.0
PY	5.18	4.2	4.8	18.3	20.8
FP1	5.72	7.5		32.6	
BaA	5.91	7.5	4.7	32.5	20.2
C0	5.86	8.3	6.6	35.9	28.6
C1	6.42	8.3		35.8	
C2	6.88	9.9		43.0	
C3	7.44	14.2		61.6	
BbF	5.80	8.4	6.7	36.3	29.1
BkF	6.00	11.8	18.7	50.9	81.8
BeP	6.20	9.5		41.3	
BaP	6.04	9.2		39.7	
PE	6.30	16.5		71.3	
IP	7.00	14.7	4.3	63.7	18.5
DA	6.75	10.1	14.4	43.7	63.0
BghiP	6.50	11.6	8.7	50.1	37.5
C0	7.64	13.8		59.9	

Table 3. Regression equations across studies. All data for *E. complanata*

Study	K_2 range	Slope	y-intercept	r^2
Study B	0.04-0.22	-0.0561	0.4356	0.83
Gewurtz*	0.11-0.22	-0.05	0.39	0.71
Russel (HCB, OCS)	0.070-0.178	-0.04	0.34	0.44

* further comparison between regression equations reported in Gewurtz et al (2002)

Chapter 2: Figure Legends

Figure 1: Elimination plots (ln C_m vs time) for individual PAH across studies. Plots a and b are from study A (water only exposure during uptake phase), while plots c and d are from study B (sediment exposure during uptake phase). Plot a and b represent 2MN0, and C0 and follow an apparent second-order elimination. Plot c and d are 1MN0 and BaP. 1MN0 demonstrates apparent second order kinetics (only analyte in this study where this was observed), while BaP elimination appears to be first order.

Figure 2a: Summary plot of K_2 vs log K_{ow} for all PAH analytes (Study B). Behavior of K_2 is well described by PAH hydrophobicity, as represented by the r^2 value of 0.8344. As the log K_{ow} of each PAH increases, the elimination rate constant decreases, until at approximately log K_{ow}=6, there is a leveling off effect observed.

Figure 2b: Summary plot of K_2 vs log K_{ow} for all PAH analytes (Study A). Behavior of K2 is not as well described by PAH hydrophobicity, with an r^2 value of 0.6638. However, the general trend is similar to that in Figure 2a, with an overall decrease in K_2 with increasing log K_{ow}. A similar, though less pronounced leveling off effect is observed at log K_{ow} of approximately 6.

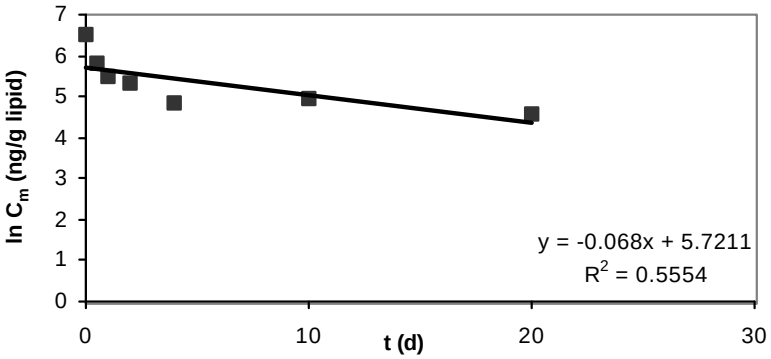

Figure 1a: Data from study A: 2-methyl napthalene

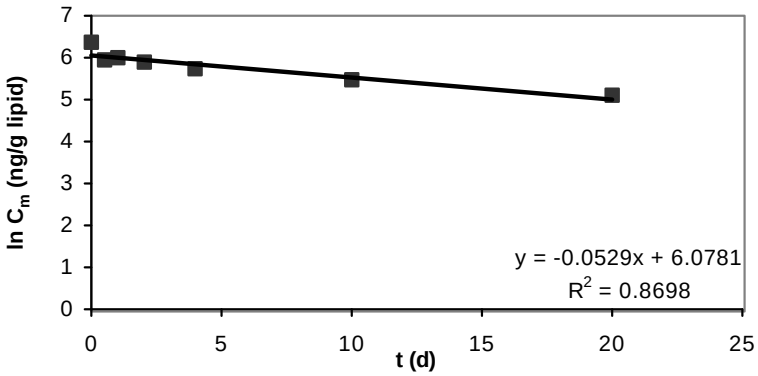

Figure 1b: Data from study A: chrysene

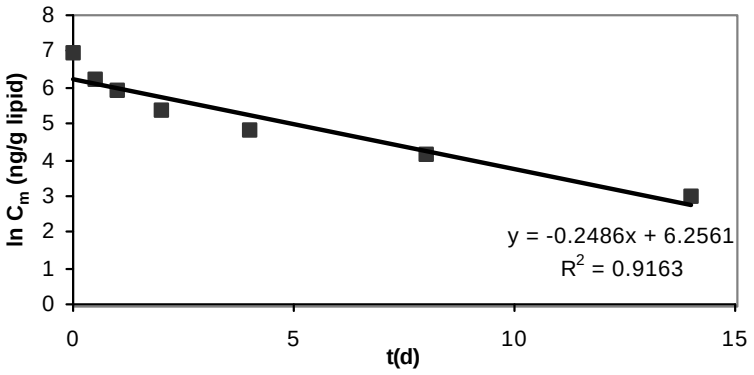

Figure 1c: Data from study B: 1-methylnapthalene

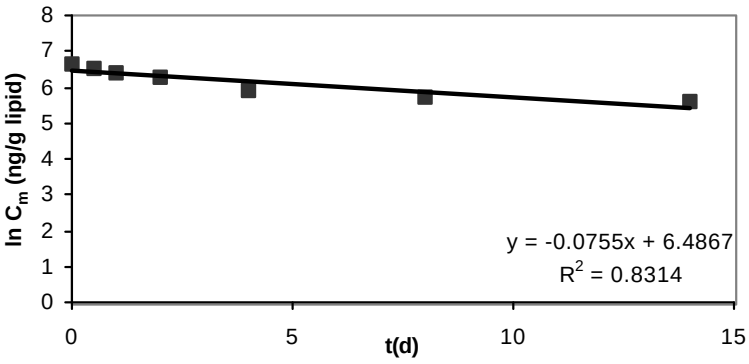

Figure 1d: Data from study B: Benzo(a)pyrene

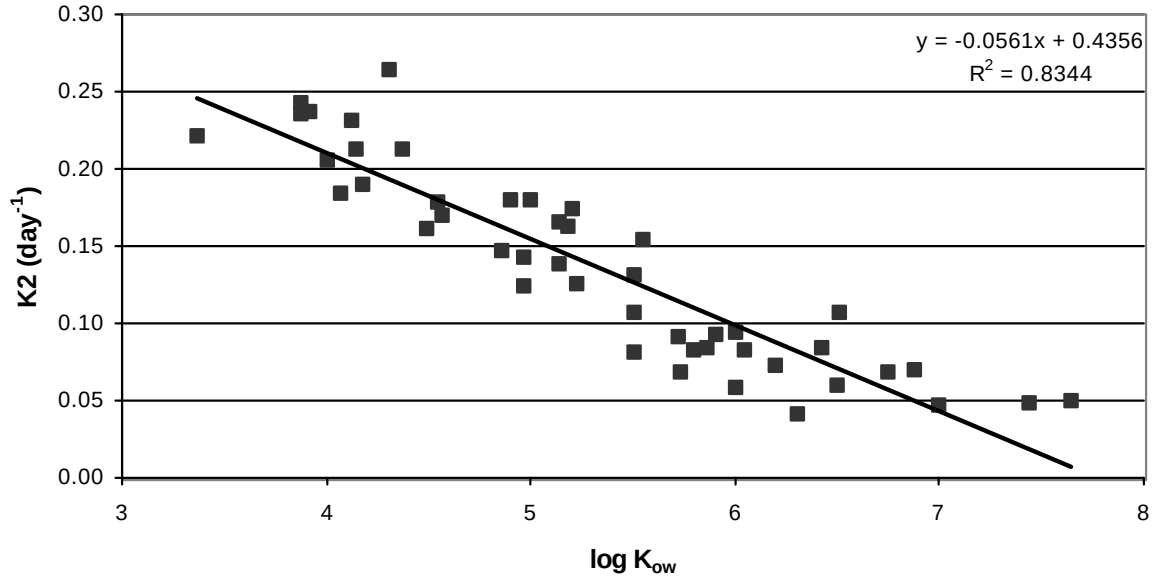

Figure 2a. Linear regression of K_2 vs log K_{ow}-Study B

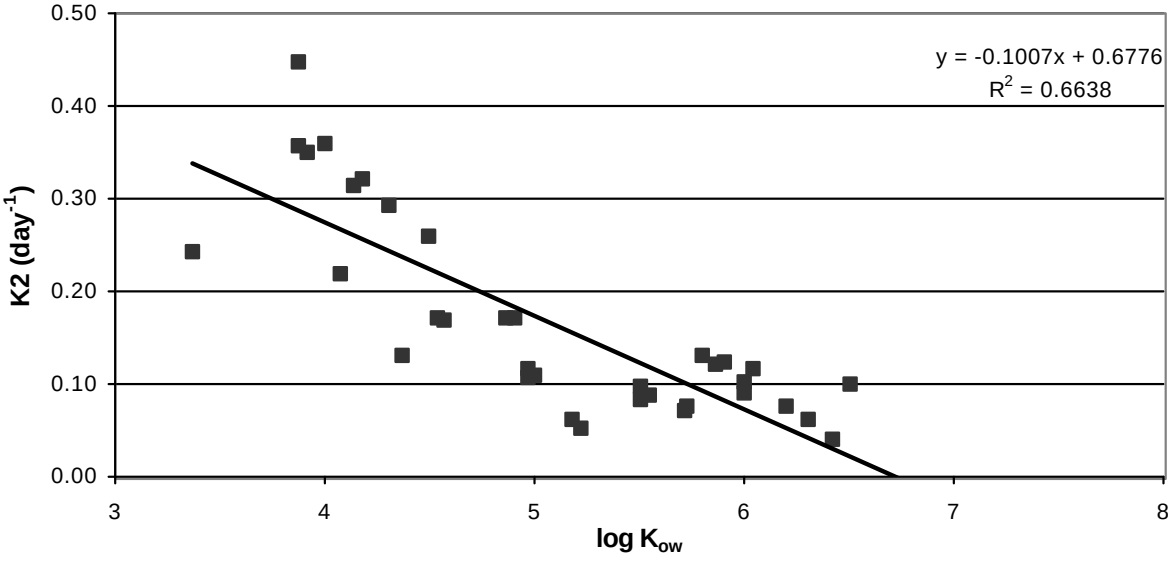

Figure 2b. Linear regression of K_2 vs log K_{ow}- Study A: *data for 10 and 20 day timepoints has been removed*

CHAPTER 3

EFFECTS OF DOC AND POC ON PAH BIOAVAILABILITY: SENSITIVITY OF BCF TO EXPOSURE ENVIRONMENT

ABSTRACT. Three different uptake studies, including one water-only exposure and two sediment exposures, were conducted using a freshwater mussel, *Elliptio complanata*, to determine bioconcentration factors (BCF) for 45 individual polycyclic aromatic hydrocarbons (PAH). Comparison of BCF values across water vs. sediment exposures suggested no difference in PAH bioconcentration patterns. BCF values were used to calculate steady-state bioconcentration regression equations which were compared to other regression equations in the literature and demonstrated no major differences between freshwater and marine bivalve species. However, regression equations did exhibit sensitivity to alterations in dissolved and particulate organic carbon-water partition coefficients (K_{DOC}, K_{POC}) as well as changes in dissolved (DOC) and particulate organic carbon (POC) water concentrations. Thirty-six different regression equations were derived using multiple combinations of K_{DOC} values ranging from 0.02 to $1.86K_{ow}$, K_{POC} values from 0.40 to $1K_{ow}$, and DOC and POC concentrations from 1 to 30 and 1 to 6mg/L, respectively. Ranges of predicted water concentrations from lowest to highest for individual PAH analytes calculated using these different regression equations were as small as less than one order of magnitude to greater than 7 orders of magnitude. These data offer quantitative evidence for the importance of understanding the sensitivity of bioconcentration to environmental parameters, as well as the implications for error associated with the application of predicting aqueous exposure concentrations.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are contaminants that are ubiquitous in the environment, are produced primarily as a result of anthropogenic activity [1,2], and span a wide range of physical and chemical parameters, for example, log octanol-water partition coefficients (K_{ow}) that range from approximately 3-8 (Table 1). PAHs exhibit carcinogenic and mutagenic effects [1,2], and recently have been associated with other non-cancer adverse effects such as immune suppression [3]. As a result of their relatively low water solubilities, PAHs possess the potential to bioconcentrate to high levels in organisms that are only minimally capable of metabolizing them [2,4,5], such as bivalves. Therefore, these organisms can provide important information on PAH concentrations in the environment [5,6,7]. However, little information exists for freshwater bivalve PAH toxicokinetics, such as uptake and elimination rates (K_1, K_2, respectively) [8], bioconcentration factors (BCF), and how these values compare to other mussel/bivalve species. Moreover, current published literature provides inconclusive evidence as to the relative contribution of pedal feeding/gut ingestion (vs. filter feeding) to bioconcentration of PAHs for the freshwater *Elliptio* mussel [9,10,11].

One way to compare differences in bioconcentration patterns for a suite of analytes across different studies, is to calculate steady-state bioconcentration regression equations. Steady-State bioconcentration regression equations are derived from known BCF values, by linearly regressing a plot of log BCF vs log K_{ow} (for example, [4]).These equations account for the extent of bioconcentration in organism (mussel) tissue of PAH analytes of varying physical-chemical characteristics. The importance of a steady-state bioconcentration regression equation is multi-faceted. They can be used to predict tissue

burden, if aqueous contaminant concentrations are known, or they can be used to predict aqueous exposure concentrations, which may be more difficult to measure due to the large volumes of water required for adequate analytical analysis.

Because the prediction of aqueous exposure concentrations can be vital in assessing water quality and ensuring the safety of aquatic organisms, the validity of the regression equation terms hinges on the quality and certainty of the input parameters. For example, different concentrations of dissolved (DOC) and particulate (POC) organic carbon as well as the dissolved or particulate organic carbon-water partition coefficient (K_{DOC}, K_{POC}) used can result in inconsistent regression equations. Uncertainty in the affinity of PAHs for different types of DOC and the difficulty in measurement techniques [12], further complicate predictions of water concentrations. However, BCFs and regression equations are routinely used to estimate aqueous contaminant concentrations without regard for species or environmental conditions: generally regression equations developed under a certain set of conditions are applied unconditionally across many different data sets. It may be unclear whether published BCF values have been corrected for DOC and POC concentrations, and what K_P values have been used.

Underlying the importance of correcting for the fraction of PAH associated with the dissolved or particulate phase is the concept of PAH bioavailability. PAHs are not always fully available to organisms, due to their low water solubility and affinity for lipid/organic carbon phases [2,13,14]. Frequently, PAHs may be associated with a colloidal or particulate phase, making them less bioavailable. Bioavailability depends on many factors including the rate of sorption/desorption of the PAH to/from the particle as well as the timing of the co-occurrence of the organism and the PAH-associated particle.

Therefore, the most accurate way to determine a BCF value is to account for the fraction of PAH truly dissolved ($C_{w,fd}$) in the water column. BCF values calculated using total PAH water concentrations result in the inclusion of particulate and colloidal water concentrations that might cause drastic underestimation of the BCF, by overestimating the bioavailable water values. The fraction of PAH that is freely dissolved in the water column can be calculated using the following equation which accounts for DOC and POC concentrations as well as dissolved and particulate organic carbon-water partition coefficients (K_{DOC}, K_{POC}):

$$F_{fd} = 1/(1+[DOC]K_{DOC}) + ([POC]K_{POC})$$

where F_{fd} is the fraction of PAH freely dissolved. Subsequently, in order to consider F_{fd} in water concentrations, the following calculation can be performed:

$$C_{w,fd} = C_{w,t} \text{ x } F_{fd}$$

where $C_{w,t}$ is the total PAH concentration in the water column (ug/L). To calculate the BCF value for PAHs using water concentrations based on the freely dissolved water concentrations, one can use the following equation:

$$BCF = C_m/C_{w,\,fd}$$

where C_m is the PAH concentration in mussel tissue (ng/g), and $C_{w, fd}$ is the PAH concentration freely dissolved in the water column (ug/L).

Although the effects of DOC and POC on BCFs in both laboratory and environmental systems are intuitive and relatively straightforward, they are generally either ignored or unaccounted for. Understanding the effects of different partition coefficients and changing DOC and POC concentrations is most important when one uses regression equations developed under one set of conditions to apply to data derived under a different set of environmental conditions. This is best exemplified when using regression equations to predict aqueous exposure concentrations based on organism tissue burdens. Multiple regression equations can result in ranges of predicted concentrations for individual PAH analytes. In this paper, we present 36 different regression equations developed using combinations of K_{DOC} and K_{POC} as well as varying DOC and POC concentrations in order to understand the sensitivity of BCF, steady-state bioconcentration regression equations, and ultimately, predicted aqueous exposure concentrations to these environmental parameters.

METHODS

Study designs

Data presented in this paper originate from three studies that were conducted by this laboratory, which will be referred to as Study A, Study B and Study C.

Study A

Study A was a laboratory, flow-through, water-only, uptake study designed to measure the bioconcentration of PAHs by the freshwater unionid mussel, *Elliptio complanata*. Twenty-three, field collected reference mussels, ranging in size from 60 to 90 mm were placed in each of three 70L exposure tanks fed stock water spiked with Alaskan north slope crude oil (Battelle, Duxbury, MA) and creosote (Accustandard Inc, New Haven, CT). To prevent depletion of PAH concentrations in the exposure system, each tank was replenished approximately 5x/day. Three mussels were collected from each tank and composited by tank for analysis during the following timepoints: field background, 0, 12, 24, 48, 96, 240, 480 (10day) hrs. Steady-state was reached between mussel tissue and the water column very rapidly for most analytes (within 24 to 48 hrs), and within the first 10 days for all PAH analytes. One to 3 L. water samples were collected twice daily, composited over two to three days to monitor PAH concentrations. Water samples were filtered through tared, pre-combusted 1.0uM glass-fiber filters (Whatman) for particle removal. Additional water samples (100mL) were taken for dissolved and particulate organic carbon analysis, as well as to monitor water quality parameters including hardness, alkalinity, pH, temperature and dissolved oxygen. The light/dark cycle was 12 hr/12hr.

Study B

Study B was a sediment exposure uptake study conducted in the field to determine PAH bioconcentration factors for the freshwater mussel, *Elliptio complanata*. This field location was a constructed-wetland site that received non-point source PAH

pollution from a nearby urban area. The mussels were collected at a separate field reference site and placed in individual sections in crates directly into the sediment phase. During collection, it was observed that the mussels had burrowed deep into the sediment. Three mussels were collected at the following timepoints: 0, 6, 18, 24, 48, 72, 96, 168, and 264 hrs. Steady state between mussel and environment was reached in the first 48 to 72 hours for most analytes, and for all analytes after the first 7 to 8 days (168 hrs). Three, 1L water samples were collected and composited at the same timepoints as mussel collections. Water samples were filtered similarly to those collected in Study A to determine particulate PAH. Additional water samples (3x100mL) were collected for DOC and POC analysis.

Study C

Study C was a laboratory, sediment exposure, static renewal, PAH uptake study also conducted with the *Elliptio complanata* mussel. To simulate and encourage burrowing behavior, field collected reference mussels were placed directly into the sediment phase, (sediment highly contaminated with creosote (Elizabeth River, VA)), which served as the source of PAH exposure. Mussels were collected once at 10d. Dissolved and particulate organic carbon concentrations in water were measured for partitioning calculations. Water quality parameters were measured including hardness, alkalinity, pH, temperature and dissolved oxygen.

Extraction procedures and instrumentation

Water samples

Water samples were analyzed for PAH concentrations using either liquid-liquid extraction techniques or Empore (0.45 uM, C-18) (3M, St. Paul, MN) extraction disks. Briefly, for liquid-liquid extractions, filtered water was added to a separatory funnel and spiked with surrogate internal standard mix (naphthalene-d8, acenapthene-d10, chrysene-d12 and perylene-d12) and 60mL methylene chloride (DCM) in a ratio of 60mL:1L water. The mixture was shaken for 1 to 2 minutes, allowed to separate and the organic layer allowed to run out through anhydrous sodium sulfate and a 1uM filter into a collection flask. This extraction was repeated three times and the extract was concentrated to 20mL using Kuderna-Danish evaporative techniques with a 70°C water bath. The extract was then further concentrated to 1.5mL using a gentle stream of dry nitrogen and cleaned using a 0.45 Uni-Prep® (Whatman) syringeless filter. Samples were then further concentrated to <1mL, and transferred with addition of recovery internal standard (phenanthrene-d10 and benzo(a)pyrene-d12) for analysis using GC/MS quantification in the SIM mode.

For Empore analysis, following conditioning of the disk with DCM, methanol and deionized water, a 1-3L water sample was extracted. The Empore disk was then extracted using DCM and anhydrous sodium sulfate, concentrated under a gentle stream of nitrogen, and cleaned, further concentrated and analyzed as described above.

Mussel samples

Mussel samples were composited in triplicate in each study. In study A, approximately 15 to 20 g wet homogenized mussel tissue were macerated and extracted with a Polytron tissuemizer (Teckman). Briefly, surrogate internal standard, 60mL of DCM and approximately 40g of anhydrous sodium sulfate were added to the sample jar. Extraction was performed using the tissuemizer to macerate the sample for 2 minutes at high speed. The probe was quantitatively rinsed with DCM, and the sample was centrifuged (<10 min at 3000g), the solvent decanted into a collection flask and the extraction repeated twice more. All extracts were collected and concentrated to 30mL using a rotary-evaporator under reduced pressure (40°C), and further to <1mL under a gentle stream of dry nitrogen. Extracts were fractionated and cleaned using a pre-combusted syringe equipped with a 0.45uM filter (Omni-Disk®, Whatman) and gel-permeation chromatography. Specific samples were run through additional silica clean-up prior to sample analysis.

Mussels in studies B and C were first freeze-dried to remove excess water, and then 5 g of dry tissue was combined with surrogate internal standard and 25mL of DCM into a 150 to 200 mL Teflon bottle. The mixture was allowed to shake vigorously for 24 hours for extraction. Following overnight extraction, the solvent was decanted through a pre-combusted glass fiber filter containing anhydrous sodium sulfate, into a graduated centrifuge tube. Two more extractions of 2 to 3 hours each were performed with 25 to 30 mL DCM, and the decanted extracts combined. The extract was concentrated under a gentle stream of dry nitrogen, filtered and fractionated as described above.

Sample quantification

Extracts were analyzed using an HP 5890 series II GC coupled to an HP 5970 MSD using an RTX-5 MS (Restek, 30mmx0.25mmx0.25um film thickness) with a 5m Integra-Guard column. Analysis was run in the SIM mode using the following temperature program: Injection port: 300 ° C, Transfer line: 280 ° C, Initial temp: 40° C, Initial Hold: 1 min., Ramp rate: 6°C/min, Final temp: 290°C, Final hold: 30 min. One uL of sample was injected. Method detection limits varied for individual PAH analyte, but were approximately 0.2-0.5 ng/g for tissue, and 1 ng/L for aqueous samples. Recoveries were within acceptable ranges (40 to 120%) and were uncorrected for SIS loss.

Quality control and quality assurance protocol

Quality control and quality assurance procedures included the addition of a surrogate internal standard solution (Napthalene-d8, Acenapthene-d10, Chrysene-d12 and Perylene-d12, Accustandard Inc, New Haven, CT) to all samples (excluding POC, DOC measurements and water condition analyses) prior to experimental procedures. A matrix spike and matrix spike duplicate consisting of all individual PAH analytes except the alkylated homologues were run once for each matrix analysis (water and mussel tissue samples). Procedural blanks were run with each sample batch and recovery internal standards (Phenanthrene-d10 and Benzo[a]pyrene-d12, Accustandard Inc, New Haven, CT) were added immediately prior to sample analysis on the GC-MS for analyte peak quantification. Where applicable, dissolved oxygen probes, temperature and pH probes were calibrated before each use.

RESULTS

Calculation and comparison of BCF values

Bioconcentration factors were calculated using steady-state PAH mussel tissue concentrations and two to three day averaged freely dissolved PAH water concentrations (corrected for DOC and POC). Log BCF values increase with increasing PAH hydrophobicity, and are listed for study A, B and C in Table 1. BCF values for study A represent water-only PAH exposure, while BCF values for studies B and C represent field, sediment PAH exposure.

The log BCF values range from as low as 1.54 for N0 to as high as 5.20 for CO, and are fairly consistent across studies. For most analytes, the log BCF values are within an order of magnitude, however, the analytes AN, FP1, PY and BbF are exceptions to this. For these four analytes, the log differences are as high as 1.99, but do not appear to represent any trends in variations across studies. For example, the differences in log BCF values are not observed strictly between water-only (study A) vs. sediment exposure studies (study B and C). The overall differences in BCF values for these analytes ranged from 1.47 (BbF- between study A and B) to 1.99 (AN- between study B and C) and appear to result from lower values for these PAH in study C. For 3 of the 4 compounds, the log BCF values compare well between study A and B. Log BCF values listed in Table 1 were calculated using $K_{DOC}=0.1K_{ow}$ and $K_{POC}=0.41K_{ow}$.

Calculation of steady-state bioconcentration regression equations

The BCF data presented in Table 1 were used to derive steady-state bioconcentration regression equations for all three studies. As would be expected based on the similarity between log BCF values, the linear regression equations also compare well, with slopes close to 1 and y-intercepts close to -1 (Table 2):

$$\text{Study A: } \log BCF = 0.895 \log K_{ow} - 1.21 \quad (1)$$

$$\text{Study B: } \log BCF = 0.786 \log K_{ow} - 0.98 \quad (2)$$

$$\text{Study C: } \log BCF = 0.807 \log K_{ow} - 1.12 \quad (3)$$

These regression equations are comparable to others published in the literature [15], as well as to two additional field studies previously conducted in this laboratory (not yet published) using a marine soft-shell clam (*Mya arenaria*) exposed to PAHs in both a water-only exposure (study D) as well as a sediment exposure (study E) (Table 2):

$$\text{Pruell et al.: } \log BCF = 0.965 \log K_{ow} - 1.4 \quad (6)$$

$$\text{Study D: } \log BCF = 1.097 \log K_{ow} - 1.54 \quad (4)$$

$$\text{Study E: } \log BCF = 1.042 \log K_{ow} - 1.28 \quad (5)$$

The similarity in slope (m) and y-intercept (b) between equations 1 to 6 is interesting because it represents not only similarities among species (freshwater vs freshwater; marine vs marine), but it also represents similarities in PAH bioconcentration patterns across species (freshwater vs marine bivalves) and exposure environment (water only vs sediment exposure; field vs laboratory).

DISCUSSION

Comparison of regression equations across species and exposure environment

Equations 1,2 and 3 are based on data from PAH uptake studies using a freshwater mussel, while equations 4,5 and 6 are based on data from marine species. As previously mentioned, studies D and E were conducted using the marine clam, *Mya arenaria*, while Pruell [15] used the marine mussel, *Mytilus edulis* to measure the bioconcentration of PAHs. Although one might expect similarities in PAH bioconcentration patterns across freshwater and marine bivalves based on feeding mechanisms (i.e: filter feeders), this is not always the case. For example, BCF values can sometimes vary by many orders of magnitude for similar species even when studies are conducted under similar circumstances [16], yet BCFs often are reported and utilized without regard for species differences. The comparison between the mussel species above demonstrates that these bivalves can be used as surrogates for each other, and ostensibly, can be used with caution, for other bivalve species as well.

The similarities of the mix of field vs laboratory data as well as water only vs sediment exposure environment suggest that BCF values, and subsequently steady-state regression equations, may be independent of these variables. Equations 1 to 6 allow one to compare across these different exposure environments, and their similarities demonstrate a few important implications: 1) assumptions of equilibrium partitioning theory are adequate. For example, there appears to be no influential PAH gradient between water at the sediment-water interface and the overlying water phase; 2) if

particle ingestion occurs with sediment exposure, either the elimination (K_2) is rapid enough to re-establish equilibrium, or pedal feeding/gut ingestion is not an important primary or secondary route of exposure for these mussels; and 3) physiological stressors that might vary with environmental and laboratory conditions (i.e: filtration rates) do not appear to have a significant impact on BCF values. It is important to note that these implications may not apply for all mussel species, and certainly, these conclusions may not carry further across species to vertebrates, such as fish. An extensive list of regression equations for fish exists in the literature [17], some of which compare well to those presented in this paper for bivalves. This may simply be coincidence, as vertebrates generally have a greater capacity to metabolize PAHs than invertebrates [2,4], which can result in deflated BCF values for fish. These decreased BCF values translate into lowered slopes and y-intercepts in subsequent regression equations. This may partially explain the regression equation for freshwater fish (and a few marine) species as published by Veith [18] (Table 2), with a much less negative y-intercept than for the bivalve regressions (-0.40 vs. others near -1.00). However, the simple, yet well known regression reported by Makay [19] ($BCF=0.048K_{ow}$; $\log BCF = \log K_{ow} -1.32$), compares very well with equations 1 to 6 presented in this paper. Calculation of BCF values without consideration for steady-state conditions may also change the regression equations.

The addition of BCF values over multiple chemical classes and multiple species into a single regression equation can also alter the slope and y-intercepts. Although this paper is not meant to provide an exhaustive review of regression equations, for comparison, a few regression equations based on multiple chemical classes (i.e: Geyer [20]: hydrophobic organic compounds: organochlorines, polychlorinated biphenyls, and

PAHs), and in multiple species (i.e: Hawker [16]: multiple marine bivalve species) are listed in Table 2.

The discussion of the calculation of BCF values and steady-state regression equations has not yet included accounting for measurable environmental/study parameters such as dissolved and particulate organic carbon. While most of the equations presented above compare well across bivalve species and exposure conditions (in terms of water-only and sediment exposure), BCF values and regression equations can be greatly altered as DOC and POC concentrations change and as dissolved/particulate organic carbon-water partition coefficients are varied. Often, it is unclear or not discussed in the literature whether reported BCF values have been corrected for DOC or POC, but the implications can be very important, and thus need to be considered.

Comparison of regression equations calculated with different K_{DOC}, K_{POC} values

Just as BCF values are frequently utilized without regard for species or exposure phase (water vs sediment), they are also generally reported/used without consideration for DOC and POC. One factor which can complicate the effective and accurate incorporation of DOC and POC concentrations into BCF calculations is the variation in DOC/POC-water partition coefficients depending on the source and character of organic carbon. This nature of dissolved and particulate organic carbon will vary based on composition and location, and the binding capacities of dissolved organic matter have been correlated to polarity, molecular size, and aromaticity [14]. Burkhard [12] compiled a large database (73 references) of DOC source data and published different K_{DOC}-K_{ow} relationships

according to specific DOC sources including Aldrich humic acids, naturally occurring humic and fulvic acids, sediment and soil porewaters, and surface waters. Table 3 lists the DOC source and K_{DOC}-K_{ow} value calculated from Burkhards geometric mean regression equations [12] by assuming a slope of 1 and taking the antilog of the y-intercept. The resultant range of values is extremely large, spanning from a low of $K_{DOC}=0.02K_{ow}$ for PAHs associated with naturally occurring DOC, to $K_{DOC}=1.86K_{ow}$ for Aldrich humic acid. As discussed by Burkhard [12] the large variability in these values arises from a combination of various DOC sources and characteristics, as well as measurement limitations. Hence, the difficulty in determining K_P values and incorporating DOC, POC concentrations into BCF calculations.

The EPA has recommended a value of $K_{DOC}=0.10K_{ow}$ which is similar to Burkhard's overall average of $K_{DOC}=0.08K_{ow}$ (or $K_{DOC}=0.11K_{ow}$, when Aldrich humic acids are included) [12]. But, difficulties arise when utilizing one broad-based, generalized relationship to K_{ow} for multiple environmental sources of DOC, and one can see how it could be expensive and potentially time-consuming to perform DOC analysis in order to determine and confirm the affinity of PAHs (or hydrophobic compound(s) of interest) for the uniqueness of DOC in a specific system. However, an educated guess with the use of Burkhard's values for various sources including sediment porewaters, surface waters and groundwaters seemingly allows for at least a 'ballpark' measurement, and appropriate BCF DOC correction.

Table 4 lists log BCF values for PAHs that have been calculated using no DOC corrections (uncorrected), and $K_{DOC}=0.02$, 0.1, 0.41, 1, and $1.86xK_{ow}$. The correction for DOC, and value of K_{DOC} used does not affect lower hydrophobicity analytes such as N0,

where log BCF is held constant at 1.54, and only mildly influences log BCF for 2MN0 with a slight, but negligible, increase from 2.14 to 2.15 as K_{DOC} increases from 0.41 to $1xK_{ow}$. However, for those PAH analytes with higher K_{ow} values, the effect is much more apparent. For example, for BaA, C0, C1, BbF, BkF, BeP, BaP and PE, the log BCF values increase by an amount equal to or greater than approximately 0.5 log units. The effect is more drastic for CO, where the uncorrected log BCF is 3.81, and the greatest corrected log BCF is 5.84 ($K_{DOC}=1.86K_{ow}$), an increase of over two orders of magnitude. Thus, the effect of changing K_{DOC} is more dramatic for lower solubility PAHs, and does not appear to significantly influence the higher solubility analytes.

The change in BCF values for the higher K_{ow} compounds affects the regression equation derived from the data resulting in a positive shift to the right: an increase in slope (becomes more positive) and y-intercept (becomes more negative). For example, as shown in Table 5, when K_{DOC} is increased from 0.1 to $0.41K_{ow}$, and K_{POC} is held constant ($0.41K_{ow}$ in study B), the slope of the resultant regression line increases from 0.78 to 0.95, while the y-intercept becomes more negative: -0.98 to -1.59. The effect on slope and y-intercept is greater for increases in K_{DOC} than K_{POC}: the change in slope as K_{POC} increases from 0.41 to $1K_{ow}$ and K_{DOC} is held at $0.41K_{ow}$, is an increase of only 0.01, and a slight increase in the negative slope of -0.04. This effect is less noticeable for changes in K_{POC} because the concentrations of POC are much less than DOC, particularly in study B (1.50 vs 21.0 mg/L, respectively). As K_{DOC} and K_{POC} values are increased further, the resulting increase in the slope and y-intercept is minimized, but still occurs.

Table 5 is a comprehensive list of slopes and y-intercepts generated when log BCF (calculated using different K_{DOC} and K_{POC} values) vs log K_{ow} are linearly regressed.

Regression equations 1 through 24 represent those generated with different combinations of K_{DOC} and K_{POC} values. The most apparent change in slope and y-intercept is observed when comparing regression equations from uncorrected vs corrected BCF data. For data uncorrected for DOC and POC, the slope is much lower, and the y-intercept much less negative, and in study B and C, a positive value. Additionally, the scatter is greater and R^2 values farther from 1 for the uncorrected data. The decrease in linearity (as measured by R^2) exhibited by the uncorrected regressions is a result of lowered log BCF values for the higher K_{ow} PAHs.

It should be noted that the regression equations listed in Table 5 are all based on a large suite of PAH analytes ranging in number from 34 to 45 (Table 1 for specific analytes). However, Pruell's regression equation [15] was based on a smaller subset of 6 PAHs. The closeness of Pruell's regression equation [15] to those presented in the beginning of this paper (equation 1 to 6) does suggest that a smaller suite of PAH analytes can be used to predict bioconcentration of a larger PAH suite. The quality of the data can also have an effect on the regression equation: the analytes AY, AC and AN were omitted from the study B data set because their values were at or below the method detection limit. This may also explain the larger log BCF values reported for these analytes relative to the other two studies. Had these three analytes been included in the regression analysis, the slope and y-intercept would have been very different (i.e: for $K_{DOC}=0.1K_{ow}$, $K_{POC}=0.41K_{ow}$, 'm' decreases from 0.78 to 0.66, and 'b' becomes much less negative from -0.98 to -0.22).

Effect of different K_{DOC}, K_{POC} on predicted water concentrations

Based on how sensitive regression equations can be to seemingly minor fluctuations in input data, complications can arise when a published regression equation generated under a certain set of environmental parameters is used to predict aqueous exposure concentrations based on mussel tissue burdens from a completely different environmental site, without concern for the parameters. Steady-state bioconcentration regression equations are frequently used to predict aqueous exposure concentrations from organism tissue residues, or to predict tissue burdens based on known water concentrations.

In order to place the changes in regression equations caused by alterations in K_{DOC}, K_{POC} values in context, predicted water concentrations are presented for three PAH analytes using each regression equation. Table 6 depicts actual water concentrations (ug/L) for N0, BeP and CO calculated using each regression equation (1 to 44) representing changes in both K_{DOC}, K_{POC} and DOC and POC concentrations, as well as other published equations discussed in a previous section. Note the columns summarizing the individual analyte concentrations from lowest to highest as a quick reference for order of magnitude differences: N0 exposure concentrations from lowest to highest range about 2 orders of magnitude, Bep almost 4 orders of magnitude, and CO 6 orders of magnitude.

Equation 39, a simple equilibrium partitioning equation predicts the highest water concentration for N0; equation 7 (uncorrected data) predicts the highest water concentrations for BeP and CO. The lowest water concentrations for all three analytes are

calculated from equation 24, a regression equation derived with $K_{DOC}=1.86K_{ow}$, and $K_{POC}=1.0K_{ow}$, which makes sense, as these are the highest partition coefficients used. The range of predicted water concentrations is quite large for the two lower water solubility PAHs, when $K_{DOC/POC}$, DOC, POC concentration changes and other published regression equations are all compiled. The resulting ranges in predicted water concentrations separated into single variables (only $K_{DOC/POC}$ changes, only DOC, POC concentration changes, and other published regression equations only) are found in tables 7,8 and 9.

Table 7 shows the range, lowest to highest, of water concentrations calculated using regression equations developed with different K_{DOC} and K_{POC} values only. If one assumes a range of K_{DOC} values from 0.02 to $1.86K_{ow}$, as presented in Burkhard [12], the predicted water concentrations have not changed when all three variables are combined into one table. Again, for N0 the water concentrations span two orders of magnitude, for BeP nearly 4 orders of magnitude, and for CO almost 7 orders of magnitude. While the toxicological relevance of 2 orders of magnitude is minimal and could be partially explained by the uncertainty associated with individual K_{DOC} measurements and compilation of data, 4 to 7 orders of magnitude are more likely of toxicological significance, and require consideration of the 'error' associated with one's data. Moreover, if different combinations of DOC and POC concentrations are used, even greater ranges would result. This is a particularly important point, as the ranges used in this paper are meant to serve only as examples. Real field values vary significantly from site to site. If a regression equation is used to predict aqueous exposure concentrations based on tissue burdens, for comparison to water quality criteria to protect aquatic species, without proper consideration of both the parameters used in developing the

equation and the parameters in the study of interest, two scenarios could occur: 1) Exposure concentrations are underpredicted, potentially putting aquatic organisms at risk, and 2) Exposure concentrations are overpredicted, possibly resulting in costly or excessive remediations.

When Table 7 is further analyzed, it is apparent that the lowest predicted water concentrations result from the regression equations (24, 23, 6, 7 etc.) that are based on either high K_{DOC} (1.86, $1.0K_{ow}$) and K_{POC} ($1.0K_{ow}$) values, or on uncorrected data (U). To assess the appropriateness of these water concentration ranges, one must consider how reasonable a K_{DOC} value of $1.86K_{ow}$ (for Aldrich humic acids) is in a true environmental setting. It is most likely very uncommon to have a value this high for K_{DOC} in the majority of field locations. The reported K_{DOC} for naturally occurring humic acids as compiled by Burkhard [12] is much lower, $0.77K_{ow}$, which results in a smaller range of individual analyte water concentrations. For example, if the water concentrations predicted from regression equations from uncorrected data and regression equations based on K_{DOC} of >1.0 ($1.86K_{ow}$) are removed from Table 7, the predicted water concentrations for N0, BeP and CO now are different by a less than one order of magnitude (a factor of 2), an order of magnitude (x7.4), and almost two orders of magnitude (x86.8), respectively. The ranges are much less than when the regressions from larger K_{DOC} values are included, but still are important to consider, depending on the application of these values to a real world context.

An analysis of Tables 8 and 9 demonstrates smaller ranges in lowest to highest predicted water concentrations for the three PAH analytes comparable to Table 7 when no data are omitted (i.e: those data including $K_{DOC}>1K_{ow}$): for changing DOC, POC

concentrations (equations 25 to 36), concentrations of N0 increase by a factor of 2.6, BeP concentrations by a factor of 42, and CO concentrations by a factor of 454; for differences between other published regression equations (37 to 44), N0 concentrations increase by x20, BeP by x29 and CO by x46. It would be difficult to tease out the variable or combination of variables contributing to these concentration ranges. The ranges are no doubt a combination of variability in the measurements and methods used to determine K_{DOC} values, error inherent in compiling data sets, as well as the changes in K's, and DOC, POC concentrations introduced in this paper. In a perfect scenario, every study would include DOC and POC measurements as well as DOC source characterization to determine the best K_{DOC}/K_{ow} relationship to use in BCF calculations, which would likely reduce at least some of the 'error' associated with these values.

Effect of different DOC and POC concentrations on predicted water values

Just as predicted aqueous concentrations vary with dissolved and particulate organic carbon-water partition coefficients, they are also altered by changes in DOC and POC concentrations. As would be expected, as DOC concentrations increase from 1 to 30mg/L, and POC concentrations are held constant at 1 mg/L, the log BCF values increase (Table 10). The increases are again minimal for lower K_{ow} PAHs, and greater for higher Kow PAHs. When K_{DOC} and K_{POC} values are held constant at 0.10 and $0.41K_{ow}$, respectively, log BCF values for N0 remain unchanged (1.54), while the log BCF values for BeP increase from 4.72 to 5.27, and to 5.45 (with DOC held at 30mg/L) when POC is

increased from 1 to 6mg/L. The greater effect of increasing DOC and POC on lower solubility PAHs again results in a shift in the regression equations.

Table 5 lists the slopes and y-intercepts for 12 regression equations calculated using different DOC, POC values. As with changes in K_{DOC} and K_{POC}, an increase in slope (becomes more positive) and y-intercept (becomes more negative) is observed as DOC concentrations are increased. For example, the slope in study C increases from 0.67 to 0.92 as DOC increases from 1-30mg/L. Moreover, the y-intercept becomes more negative; -0.52 to -1.57. These regression equations result in ranges of predicted water concentrations that are not as dramatic as those for increasing K_{DOC}, K_{POC}; however, these ranges would increase (greater gap between low and high predicted water concentrations) with increases in DOC to greater than 30mg/L, POC values to greater than 6 mg/L, as well as increases in K_{DOC} and K_{POC}, which were held at 0.1 and $0.41K_{ow}$, respectively for this exercise. DOC values in the environment have been reported with much greater concentrations, for example 50mg/L [14].

Table 8 lists the predicted water concentrations for N0, BeP, and CO as calculated by regression equations with variations in DOC and POC concentrations only (equations 25 to 32). The lowest N0 concentration is 0.060ug/L, while the highest N0 concentration is 1.6ug/L, a factor of 2.7 times greater. The difference between lowest and highest BeP and CO water concentrations is a factor of 42, and 454, respectively, as previously mentioned. Because the source and concentrations of DOC and POC can vary to a large extent in the environment, it is important to take DOC/POC measurements, but perhaps more important to further characterize DOC in order to accurately determine BCF values and steady-state bioconcentration regression equations.

CONCLUSIONS

One needs to consider the application of the regression equation in terms of evaluating the importance of assessing organic carbon source, concentration and partition coefficients. How much error is acceptable based on the purpose and application of one's work? In some cases, more than one log unit difference may not pose a concern. However, in other circumstances, it may be of utmost importance to predict exposure concentrations as accurately as possible.

In summary, a combination of factors can influence the accuracy of using steady-state regression equations to predict exposure PAH concentrations. The factors that must be considered include: A) parameters used to derive the regression equation, including nature and source of DOC, POC- as described mathematically by K_{DOC}, K_{POC} values, and the concentrations of DOC and POC; B) consideration for the species and chemical class used to determine BCF values utilized in regression equations; and C) the application of the resulting water values and appropriate understanding of the potential error associated with those values.

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Table 1. List of PAHs, Symbols, log K_{ow}, log BCF (K_{DOC}=0.10K_{ow},K_{POC}=0.41K_{ow})

Analyte	Symbol	log K_{ow}	log BCF [A]	log BCF[B]	log BCF[C]
Napthalene	N0	3.37	1.54	1.90	1.69
2-Methylnapthalene	2MN0	4.00	2.14		1.71
1-Methylnapthalene	1MN0	3.87	2.01		2.24
Biphenyl	BP	4.14	2.23		3.00
2,6-Dimethylnapthylene	2,6DMN0	4.31	2.50		1.92
Acenapthylene	AY	4.07	2.84	3.40	2.23
Acenapthene	AC	3.92	2.42	3.30	2.61
2,3,5-Trimethylnapthalene	2,3,5TMN0	4.90	2.71		2.75
C1 - Napthalenes	N1	3.87	2.10	2.36	2.46
C2 - Napthalenes	N2	4.37	2.51	2.39	2.24
C3 - Napthalenes	N3	5.00	2.83	2.04	3.00
C4 - Napthalenes	N4	5.55	2.92	2.65	3.21
Fluorene	F0	4.18	2.58	2.41	1.84
1-Methylfluorene	1MF0	4.97	2.89		2.68
C1 - Fluorenes	F1	4.97	2.94	3.08	3.16
C2 - Fluorenes	F2	5.20	3.50	2.35	2.84
C3 - Fluorenes	F3	5.50		3.40	2.31
Dibenzothiophene	D0	4.49	2.86	2.93	2.69
C1 - Dibenzothiophenes	D1	4.86	3.43	3.07	3.38
C2 - Dibenzothiophene	D2	5.50	4.08	3.64	3.58
C3 - Dibenzothiophene	D3	5.73		2.65	3.63
Phenanthrene	P0	4.57	3.02	3.06	3.44
Anthracene	AN	4.54	3.35	3.59	1.60
1-Methylphenanthrene	1MP0	5.14			2.92
C1 - Phenanthrenes/Anthracenes	P1	5.14		2.87	3.50
C2 - Phenanthrenes/Anthracenes	P2	5.51	3.75	2.71	3.34
C3 - Phenanthrenes/Anthracenes	P3	6.00		4.39	4.03
C4 - Phenanthrenes/Anthracenes	P4	6.51		4.46	3.30
Fluoranthrene	FL	5.22	3.70	3.19	2.75
C1 - Fluoranthenes/Pyrenes	FP1	5.72	3.99	3.70	2.51
Pyrene	PY	5.18	3.79	3.45	2.29
Benz[a]anthracene	BaA	5.91	4.21	3.34	3.19
Chrysene	C0	5.86	4.18	3.67	3.23
C1 - Chrysenes	C1	6.42	4.38	4.27	4.50
C2 - Chrysenes	C2	6.88		4.33	5.16
C3 - Chrysenes	C3	7.44		5.21	5.51
Benzo[b]fluoranthene	BbF	5.80	4.73	3.78	3.26
Benzo[k]fluoranthene	BkF	6.00	4.70	3.59	3.84
Benzo[e]pyrene	BeP	6.20	4.54	4.23	3.48
Benzo[a]pyrene	BaP	6.04	4.66	3.50	3.94
Perylene	PE	6.30	4.49	3.57	3.80
Indeno[1,2,3-c,d]perylene	IP	7.00		4.39	4.55
Dibenz[a,h]anthracene	DA	6.75		4.77	5.22
benzo[g,h,i]perylene	BghiP	6.50		4.06	4.85
Coronene	CO	7.64	4.64	5.20	4.56

Table 2. Comparison of log BCF vs log Kow regression equations for different chemical classes versus exposure environment and location for marine and freshwater mussels

Study	Species	Exposure	Chemical Class	Location	m	b	r^2
Study A	freshwater, E. complanata	water only	PAH	lab	0.895	-1.21	0.8325
Study B	freshwater, E. complanata	sediment	PAH	field	0.786	-0.98	0.7847
Study C	freshwater, E. complanata	sediment	PAH	lab	0.807	-1.12	0.7268
Pruell et al	marine, M. edulis	sediment slurry	PAH	lab	0.965	-1.40	
Study D	marine, M. arenaria	water only	PAH	field	1.097	-1.54	0.8472
Study E	marine, M arenaria	sediment	PAH	field	1.042	-1.28	0.8472
Geyer et al	marine, M. edulis		multiple	lab	0.858	-0.81	0.9550
Veith	freshwater, fish		multiple		0.790	-0.40	0.9300
Hawker	marine, bivalves		multiple		0.844	-1.23	0.8320
Makay	fish, simple regression				1.000	-1.32	

Table 3. List of different K_{DOC};K_{ow} relationships based on DOC source[1]

Source of DOC	$K_{DOC}=xK_{ow}$
Aldrich Humic Acid	1.86
all DOC including Aldrich H.A	0.77
humic, fulvic acid w/o Aldrich H.A	0.77
soil porewaters and groundwaters	0.60
naturally occuring DOC	0.56
overall PAHs	0.26
sediment porewaters	0.13
surface waters	0.05
PAH's, naturally occuring DOC	0.02

1. Burkhard LP. 2000. Environ Sci Technol 34:4663-4668 [12].

Table 4. Data from Study A: Sensitivity of BCF values to Changes in K_{DOC}

Analyte	Uncorrected log BCF	$K_{DOC}=0.02K_{ow}$ log BCF	$K_{DOC}=0.1K_{ow}$ log BCF	$K_{DOC}=0.41K_{ow}$ log BCF	$K_{DOC}=K_{ow}$ log BCF	$K_{DOC}=1.86K_{ow}$ log BCF
N0	1.54	1.54	1.54	1.54	1.54	1.54
2MN0	2.14	2.14	2.14	2.14	2.15	2.15
1MN0	2.01	2.01	2.01	2.01	2.02	2.02
BP	2.23	2.23	2.23	2.23	2.24	2.25
2,6DMN0	2.50	2.50	2.50	2.50	2.51	2.52
AY	2.84	2.84	2.84	2.84	2.84	2.85
AC	2.42	2.42	2.42	2.42	2.43	2.43
2,3,5TMN0	2.71	2.71	2.71	2.73	2.75	2.79
N1	2.10	2.10	2.10	2.10	2.10	2.11
N2	2.50	2.50	2.51	2.51	2.52	2.53
N3	2.82	2.82	2.83	2.85	2.88	2.92
N4	2.90	2.91	2.92	2.98	3.07	3.18
F0	2.58	2.58	2.58	2.59	2.59	2.60
1MF0	2.89	2.89	2.89	2.91	2.94	2.98
F1	2.93	2.93	2.94	2.95	2.98	3.02
F2	3.49	3.50	3.50	3.53	3.58	3.64
D0	2.86	2.86	2.86	2.86	2.87	2.89
D1	3.43	3.43	3.43	3.45	3.47	3.50
D2	4.06	4.06	4.08	4.13	4.21	4.31
P0	3.02	3.02	3.02	3.02	3.04	3.05
AN	3.34	3.34	3.35	3.35	3.36	3.38
P2	3.73	3.73	3.75	3.80	3.88	3.98
FL	3.69	3.69	3.70	3.73	3.77	3.84
PY	3.78	3.78	3.79	3.81	3.86	3.91
FP1	3.96	3.97	3.99	4.07	4.19	4.32
BaA	4.17	4.18	4.21	4.32	4.48	4.64
C0	4.14	4.15	4.18	4.28	4.43	4.58
C1	4.25	4.27	4.38	4.63	4.90	5.12
BbF	4.69	4.70	4.73	4.82	4.96	5.10
BkF	4.65	4.66	4.70	4.84	5.02	5.19
BeP	4.46	4.48	4.54	4.73	4.95	5.15
BaP	4.60	4.61	4.66	4.80	4.99	5.16
PE	4.39	4.41	4.49	4.71	4.95	5.16
CO	3.81	4.14	4.64	5.20	5.58	5.84

Table 5. Comparison of regression equations across studies with variations in DOC, POC and K parameters

STUDY	m	b	[DOC]	[POC]	$K_{DOC(xKow)}$	$K_{POC(xKow)}$	r^2	REG EQ	Analyte #	REG CODE
Study A	0.79	-0.77	1.3	nd			0.74	y=0.79x-0.77	34	1
Study A	0.83	-0.93	1.3	nd	0.02		0.79	y=0.83x-0.93	34	2
Study A	0.89	-1.21	1.3	nd	0.10		0.83	y=0.89x-1.21	34	3
Study A	0.99	-1.65	1.3	nd	0.41		0.87	y=0.99x-1.65	34	4
Study A	1.09	-2.04	1.3	nd	1.00		0.89	y=1.09x-2.04	34	5
Study A	1.17	-2.37	1.3	nd	1.86		0.90	y=1.17x-2.37	34	6
Study B	0.28	1.34	21.0	1.50			0.33	y=0.28x+1.34	35	7
Study B	0.65	-0.41	21.0	1.50	0.02	0.41	0.72	y=0.65x-0.41	35	8
Study B	0.78	-0.98	21.0	1.50	0.10	0.41	0.79	y=0.78x-0.98	35	9
Study B	0.95	-1.59	21.0	1.50	0.41	0.41	0.85	y=0.95x-1.59	35	10
Study B	0.74	-0.78	21.0	1.50	0.02	1.00	0.76	y=0.74x-0.78	35	11
Study B	0.82	-1.14	21.0	1.50	0.10	1.00	0.80	y=0.82x-1.14	35	12
Study B	0.96	-1.63	21.0	1.50	0.41	1.00	0.85	y=0.96x-1.63	35	13
Study B	1.05	-1.89	21.0	1.50	1.00	1.00	0.88	y=1.05x-1.89	35	14
Study B	1.10	-1.98	21.0	1.50	1.86	1.00	0.88	y=1.10x-1.98	35	15
Study C	0.43	0.58	11.2	1.05			0.43	y=0.43x+0.58	45	16
Study C	0.70	-0.66	11.2	1.05	0.02	0.41	0.67	y=0.70x-0.66	45	17
Study C	0.81	-1.12	11.2	1.05	0.10	0.41	0.73	y=0.81x-1.12	45	18
Study C	0.97	-1.79	11.2	1.05	0.41	0.41	0.79	y=0.97x-1.79	45	19
Study C	0.78	-1.02	11.2	1.05	0.02	1.00	0.72	y=0.78x-1.02	45	20
Study C	0.85	-1.31	11.2	1.05	0.10	1.00	0.75	y=0.85x-1.31	45	21
Study C	0.98	-1.85	11.2	1.05	0.41	1.00	0.79	y=0.98x-1.85	45	22
Study C	1.09	-2.23	11.2	1.05	1.00	1.00	0.83	y=1.09x-2.23	45	23
Study C	1.17	-2.46	11.2	1.05	1.86	1.00	0.85	y=1.17x-2.46	45	24
Study A	0.99	-1.63	1.0	1.00	0.10	0.41	0.87	y=0.99x-1.63	34	25
Study A	1.21	-2.56	30.0	1.00	0.10	0.41	0.90	y=1.21x-2.56	34	26
Study A	1.17	-2.39	1.0	6.00	0.10	0.41	0.90	y=1.17x-2.39	34	27
Study A	1.28	-2.83	30.0	6.00	0.10	0.41	0.91	y=1.28x-2.83	34	28
Study B	0.56	0.00	1.0	1.00	0.10	0.41	0.66	y=0.56x-0.001	35	29
Study B	0.81	-1.10	30.0	1.00	0.10	0.41	0.79	y=0.81x-1.12	35	30
Study B	0.78	-0.94	1.0	6.00	0.10	0.41	0.78	y=0.78x-0.94	35	31
Study B	0.88	-1.34	30.0	6.00	0.10	0.41	0.82	y=0.88x-1.34	35	32
Study C	0.67	-0.52	1.0	1.00	0.10	0.41	0.66	y=0.67x-0.52	45	33
Study C	0.92	-1.57	30.0	1.00	0.10	0.41	0.77	y=0.92x-1.57	45	34
Study C	0.88	-1.41	1.0	6.00	0.10	0.41	0.76	y=0.87x-1.41	45	35
Study C	0.98	1.84	30.0	6.00	0.10	0.41	0.80	y=0.98x-1.83	45	36
Pruell	0.97	-1.40						y=0.965x-1.4	5	37
Smpl Reg	1.00	-1.32						y=1.00x-1.32		38
EQP		Cw,m = Cm/(fL*Kow)								39
Study D	1.10	-1.54						y=1.10x-1.54		40
Study E	1.04	-1.28						y=1.04x-1.28		41
Geyer	0.86	-0.81						y=0.85x-0.81		42
Hawker	0.84	-1.23						y=0.84x-1.23		43
Veith	0.79	-0.40						y=0.79x-0.40		44

Table 6. Predicted water concentrations using each regression equation

REG EQ	R.Code	N0	BeP	CO	L-->H N0	L-->H BeP	L-->H CO
y=0.79x-0.77	1	6.0E-01	7.8E-02	1.0E-04	1.4E-02	9.5E-06	8.7E-10
y=0.83x-0.93	2	6.3E-01	6.4E-02	7.5E-05	1.2E-01	1.8E-04	2.2E-06
y=0.89x-1.21	3	7.6E-01	5.1E-02	5.0E-05	2.4E-01	5.5E-03	2.6E-06
y=0.99x-1.65	4	9.6E-01	3.4E-02	2.3E-05	2.5E-01	7.1E-03	4.0E-06
y=1.09x-2.04	5	1.1E+00	2.0E-02	9.9E-06	2.8E-01	8.2E-03	4.2E-06
y=1.17x-2.37	6	1.2E+00	1.4E-02	5.2E-06	3.2E-01	1.2E-02	5.2E-06
y=0.28x+1.34	7	2.4E-01	8.8E-01	6.4E-03	4.1E-01	1.4E-02	5.4E-06
y=0.65x-0.41	8	7.7E-01	2.5E-01	5.3E-04	4.1E-01	1.4E-02	6.1E-06
y=0.78x-0.98	9	1.0E+00	1.5E-01	2.0E-04	4.4E-01	1.4E-02	7.2E-06
y=0.95x-1.59	10	1.1E+00	5.2E-02	4.1E-05	6.0E-01	1.5E-02	9.2E-06
y=0.74x-0.78	11	9.0E-01	1.6E-01	2.6E-04	6.0E-01	2.0E-02	9.9E-06
y=0.82x-1.14	12	1.1E+00	1.2E-01	1.4E-04	6.3E-01	2.5E-02	1.4E-05
y=0.96x-1.63	13	1.2E+00	5.0E-02	3.8E-05	6.5E-01	2.7E-02	2.0E-05
y=1.05x-1.89	14	1.0E+00	2.5E-02	1.4E-05	7.6E-01	3.2E-02	2.2E-05
y=1.10x-1.98	15	8.7E-01	1.5E-02	7.2E-06	7.7E-01	3.3E-02	2.3E-05
y=0.43x+0.58	16	4.4E-01	5.9E-01	2.6E-03	8.6E-01	3.4E-02	3.8E-05
y=0.70x-0.66	17	9.3E-01	2.2E-01	3.9E-04	8.7E-01	3.6E-02	4.0E-05
y=0.81x-1.12	18	1.1E+00	1.3E-01	1.6E-04	9.0E-01	5.0E-02	4.1E-05
y=0.97x-1.79	19	1.5E+00	6.2E-02	4.6E-05	9.1E-01	5.1E-02	4.2E-05
y=0.78x-1.02	20	1.1E+00	1.6E-01	2.2E-04	9.3E-01	5.2E-02	4.4E-05
y=0.85x-1.31	21	1.3E+00	1.1E-01	1.3E-04	9.5E-01	5.9E-02	4.5E-05
y=0.98x-1.85	22	1.6E+00	6.2E-02	4.4E-05	9.6E-01	6.2E-02	4.6E-05
y=1.09x-2.23	23	1.2E-01	1.8E-04	6.1E-06	1.0E+00	6.2E-02	5.0E-05
y=1.17x-2.46	24	1.4E-02	9.5E-06	8.7E-10	1.0E+00	6.4E-02	6.7E-05
y=0.99x-1.63	25	9.1E-01	3.2E-02	2.2E-05	1.1E+00	7.7E-02	7.5E-05
y=1.21x-2.56	26	1.4E+00	1.2E-02	4.0E-06	1.1E+00	7.8E-02	8.0E-05
y=1.17x-2.39	27	1.3E+00	1.4E-02	5.4E-06	1.1E+00	8.0E-02	1.0E-04
y=1.28x-2.83	28	1.5E+00	8.2E-03	2.2E-06	1.1E+00	1.1E-01	1.1E-04
y=0.56x-0.001	29	6.0E-01	3.5E-01	1.0E-03	1.1E+00	1.1E-01	1.1E-04
y=0.81x-1.12	30	1.1E+00	1.3E-01	1.6E-04	1.1E+00	1.1E-01	1.2E-04
y=0.78x-0.94	31	9.5E-01	1.3E-01	1.8E-04	1.1E+00	1.2E-01	1.3E-04
y=0.88x-1.34	32	1.1E+00	8.0E-02	8.0E-05	1.2E+00	1.3E-01	1.4E-04
y=0.67x-0.523	33	8.6E-01	2.4E-01	4.9E-04	1.2E+00	1.3E-01	1.6E-04
y=0.92x-1.57	34	1.4E+00	7.7E-02	6.7E-05	1.2E+00	1.3E-01	1.6E-04
y=0.87x-1.41	35	1.4E+00	1.1E-01	1.1E-04	1.3E+00	1.5E-01	1.8E-04
y=0.98x-1.83	36	1.6E+00	5.9E-02	4.2E-05	1.3E+00	1.6E-01	2.0E-04
y=0.965x-1.4	37	6.5E-01	2.7E-02	2.0E-05	1.4E+00	1.6E-01	2.2E-04
y=1.00x-1.32	38	4.1E-01	1.4E-02	9.2E-06	1.4E+00	1.6E-01	2.6E-04
$C_{w,m} = C_m/(f_L*K_{ow})$	39	5.0E+00	1.6E-01	1.1E-04	1.4E+00	2.2E-01	3.9E-04
y=1.10x-1.54	40	3.2E-01	5.5E-03	2.6E-06	1.5E+00	2.4E-01	4.9E-04
y=1.04x-1.28	41	2.8E-01	7.1E-03	4.2E-06	1.5E+00	2.5E-01	5.3E-04
y=0.85x-0.81	42	4.1E-01	3.6E-02	4.0E-05	1.6E+00	3.5E-01	1.0E-03
y=0.84x-1.23	43	1.2E+00	1.1E-01	1.2E-04	1.6E+00	5.9E-01	2.6E-03
y=0.79x-0.40	44	2.5E-01	3.3E-02	4.5E-05	5.0E+00	8.8E-01	6.4E-03

Table 7. $C_{w,m}$ from lowest to highest calculated from each regression: changing K_{DOC}, K_{POC} only

REG CODE	L-->H N0	REG CODE	L-->H BeP	REG CODE	L-->H CO
18	1.4E-02	18	9.5E-06	18	8.7E-10
17	1.2E-01	17	1.8E-04	24	5.2E-06
1	2.4E-01	24	1.4E-02	17	6.1E-06
10	4.4E-01	9	1.5E-02	9	7.2E-06
19	6.0E-01	23	2.0E-02	23	9.9E-06
20	6.3E-01	8	2.5E-02	8	1.4E-05
21	7.6E-01	22	3.4E-02	22	2.3E-05
2	7.7E-01	7	5.0E-02	7	3.8E-05
9	8.7E-01	21	5.1E-02	4	4.1E-05
5	9.0E-01	4	5.2E-02	16	4.4E-05
11	9.3E-01	16	6.2E-02	13	4.6E-05
22	9.6E-01	13	6.2E-02	21	5.0E-05
3	1.0E+00	20	6.4E-02	20	7.5E-05
8	1.0E+00	19	7.8E-02	19	1.0E-04
23	1.1E+00	15	1.1E-01	15	1.3E-04
6	1.1E+00	6	1.2E-01	6	1.4E-04
4	1.1E+00	12	1.3E-01	12	1.6E-04
12	1.1E+00	3	1.5E-01	3	2.0E-04
14	1.1E+00	14	1.6E-01	14	2.2E-04
7	1.2E+00	5	1.6E-01	5	2.6E-04
24	1.2E+00	11	2.2E-01	11	3.9E-04
15	1.3E+00	2	2.5E-01	2	5.3E-04
13	1.5E+00	10	5.9E-01	10	2.6E-03
16	1.6E+00	1	8.8E-01	1	6.4E-03

Table 8.$C_{w,m}$ from lowest to highest calculated from each regression:changing [DOC,POC] only

REG CODE	L-->H N0	REG CODE	L-->H BeP	REG CODE	L-->H CO
29	6.0E-01	28	8.2E-03	28	2.2E-06
33	8.6E-01	26	1.2E-02	26	4.0E-06
25	9.1E-01	27	1.4E-02	27	5.4E-06
31	9.5E-01	25	3.2E-02	25	2.2E-05
30	1.1E+00	36	5.9E-02	36	4.2E-05
32	1.1E+00	34	7.7E-02	34	6.7E-05
27	1.3E+00	32	8.0E-02	32	8.0E-05
34	1.4E+00	35	1.1E-01	35	1.1E-04
35	1.4E+00	30	1.3E-01	30	1.6E-04
26	1.4E+00	31	1.3E-01	31	1.8E-04
28	1.5E+00	33	2.4E-01	33	4.9E-04
36	1.6E+00	29	3.5E-01	29	1.0E-03

Table 9. $C_{w,m}$ lowest to highest calculated from miscellaneous equations

REG CODE	L-->H N0	REG CODE	L-->H BeP	REG CODE	L-->H CO
44	2.5E-01	40	5.5E-03	40	2.6E-06
41	2.8E-01	41	7.1E-03	41	4.2E-06
40	3.2E-01	38	1.4E-02	38	9.2E-06
42	4.1E-01	37	2.7E-02	37	2.0E-05
38	4.1E-01	44	3.3E-02	42	4.0E-05
37	6.5E-01	42	3.6E-02	44	4.5E-05
43	1.2E+00	43	1.1E-01	39	1.1E-04
39	5.0E+00	39	1.6E-01	43	1.2E-04

Table 10. Data from Study A: Changes in BCF values with increasing [DOC,POC]

Analyte	Uncorrected log BCF	[DOC=POC=1mg/L] log BCF	[DOC=1,POC=6] log BCF	[DOC=30, POC=1] log BCF	[DOC=30, POC=6] log BCF
N0	1.54	1.54	1.54	1.54	1.54
2MN0	2.14	2.14	2.15	2.16	2.16
1MN0	2.01	2.01	2.02	2.02	2.03
BP	2.23	2.23	2.25	2.25	2.26
2,6DMN0	2.50	2.50	2.52	2.53	2.54
AY	2.84	2.84	2.85	2.85	2.86
AC	2.42	2.42	2.43	2.43	2.44
2,3,5TMN0	2.71	2.73	2.79	2.81	2.86
N1	2.10	2.10	2.11	2.11	2.12
N2	2.50	2.51	2.53	2.54	2.56
N3	2.82	2.85	2.92	2.95	3.01
N4	2.90	2.98	3.19	3.25	3.37
F0	2.58	2.59	2.60	2.61	2.62
1MF0	2.89	2.91	2.98	3.01	3.06
F1	2.93	2.95	3.03	3.05	3.11
F2	3.49	3.53	3.64	3.68	3.77
D0	2.86	2.86	2.89	2.90	2.92
D1	3.43	3.45	3.50	3.53	3.58
D2	4.06	4.12	4.32	4.38	4.49
P0	3.02	3.02	3.06	3.07	3.10
AN	3.34	3.35	3.38	3.39	3.42
P2	3.73	3.80	3.99	4.05	4.17
FL	3.69	3.72	3.84	3.88	3.97
PY	3.78	3.81	3.92	3.96	4.04
FP1	3.96	4.07	4.33	4.41	4.55
BaA	4.17	4.32	4.65	4.74	4.90
C0	4.14	4.28	4.60	4.68	4.84
C1	4.25	4.62	5.13	5.24	5.43
BbF	4.69	4.82	5.11	5.19	5.34
BkF	4.65	4.83	5.20	5.29	5.46
BeP	4.46	4.72	5.17	5.27	5.45
BaP	4.60	4.79	5.18	5.27	5.44
PE	4.39	4.70	5.18	5.28	5.47
CO	3.81	5.18	5.86	5.98	6.19

CHAPTER 4

BIOAVAILABILITY OF PAHS: EFFECTS OF SOOT CARBON AND PAH SOURCE

ABSTRACT. The bioavailability of 39 individual polycyclic aromatic hydrocarbon (PAHs) compounds was determined through calculation of Biota-Sediment-Accumulation Factors (BSAF). BSAF values were calculated from individual PAH concentrations in mussel, clam, and sediment obtained from field and laboratory bioaccumulation studies. Sediment that was amended with different types of soot carbon (SC) was also used in the bioaccumulation experiments. BSAF values for petrogenic PAH were on average, greater than one (i.e.: 1.57+/-0.53), while those for the pyrogenic PAH were nearly all less than one (i.e.: 0.25+/-0.23) indicating a greater bioavailability of petrogenic PAH over pyrogenic PAH ($P<0.05$). This trend was conserved across both marine and freshwater sites. Increases in SC content resulted in decreases in bioavailability of pyrogenic PAHs; however, the effect was moderate and not completely linear ($r^2=0.85$). The effect of increasing SC content on petrogenic PAH was negligible. SC was considered as an additional sorptive phase when calculating BSAF values and resulted in unreasonably large BSAF values for all petrogenic PAH, and some pyrogenic PAH which led us to suggest that a quantitative model to assess bioavailability through a combination of organic carbon and soot carbon sorption is not applicable across field sites with a wide range of soot carbon fractions and PAH sources. Our data offer evidence that many factors, including analysis of a full suite of PAH analytes, PAH hydrophobicity, sediment organic carbon content, sediment soot carbon content and PAH source, are extremely important in adequately assessing PAH bioavailability in the environment.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of contaminants that are ubiquitous in the environment and are produced primarily as a result of anthropogenic activities including the production, transport and use of petroleum (1, 2). It is important to study the fate of these contaminants because of their carcinogenic, mutagenic and teratogenic properties (1, 3). For example, benzo(a)pyrene, benzo(b)fluoranthene, and benzo(k)fluoranthene have been reported to be carcinogenic (3). Additionally, PAHs have been shown to cause other, non-cancer adverse effects, such as immune suppression (4,5,6). PAHs can be broadly separated into three categories based on their source (2). This includes biogenic, petrogenic and pyrogenic PAHs. Biogenic PAH are formed from natural biological processes including diagenesis; petrogenic PAH are derived from petroleums and may enter the environment primarily associated with a dissolved phase (aqueous or air), for example as crude oil; and pyrogenic PAH are formed as a result of incomplete combustion of fuels, and largely enter the environment strongly sorbed to particulate matrices. Pyrogenic PAH are also produced in tandem with combustion products such as soot (1, 7, 8). This classification is presented in a simplified form, as different PAH sources can produce varying relative abundances of individual PAH analytes.

In the aquatic environment, PAHs are taken up by aquatic invertebrates through bioconcentration (water-only) and bioaccumulation (food and environment) (1). The extent to which PAHs are accumulated in an organism depends primarily on the ratio of the PAH uptake rate to the depuration rate, the metabolic capacity of the organism for

PAHs (i.e.: Cytochrome P450 activity), the mobility of the organism, and on various physical-chemical parameters of the individual compounds. For example, bivalves are frequently used as sentinel organisms because of their low metabolic capability for PAHs (9), and their relatively sessile character, therefore providing a time-integrated measurement of PAH contamination. Additionally, aquatic invertebrates, such as mussels, generally possess lower capacity for metabolizing PAHs (as well as other hydrophobic organic contaminants, HOCs) than aquatic vertebrates, for example, fish (9). Bioaccumulation potential will also depend on the desorption rate of the PAH from the sediment or particle matrix (10, 11, 12, 13, 14). If desorption kinetics are fast relative to the co-occurrence of the PAH and the organism, the PAH will be available for uptake. However, if desorption rates are slow relative to the co-occurrence of the PAH and the organism, the contaminant may not be available for uptake. Therefore, it is important to consider the bioavailablity of PAHs when assessing their bioaccumulation potential.

Others have previously reported that PAHs may exhibit low chemical and biological availability (1, 14, 15, 16, 17, 18). This has been described in terms of field-derived solid-water partition coefficients (K_D) values being greater than predicted (19, 20, 21), as fractions available for equilibrium partitioning (AEP) being less than predicted (18), and culminates in toxicity values that are less than predicted based on equilibrium partitioning theory. When identifying the potential for sediment toxicity to aquatic organisms, determining sediment PAH concentration is only one aspect of evaluation. Particularly when investigating PAHs, it is important to adequately assess this availability to organisms of individual PAH analytes. High total sediment PAH concentrations may not confer toxic levels to organisms, if individual PAH analytes are sequestered, or

tightly sorbed, and are unavailable for rapid desorption, as mentioned above (16, 17). One way to assess the bioavailability of PAHs in the environment is to compare individual PAH concentrations in benthic organisms to individual PAH concentrations in sediment. This is described as the Biota-Sediment Accumulation Factor Model (BSAF):

$$BSAF = (C_m/f_L)/(C_s/f_{oc}) \qquad \text{(eq.1)}$$

where C_m is the individual PAH concentration in mussel tissue (ng PAH/g mussel dry wt.), f_L is the organism lipid fraction (g lipid /g mussel dry wt), C_s is the individual PAH concentration in sediment (ng PAH/g sediment dry wt.), and f_{oc} is the mass fraction of organic carbon (g organic C/g sediment dry wt). The BSAF theory models the partitioning of PAHs between the hydrophobic (sorptive) phases present in a benthic organism and sediment. These sorptive phases are traditionally the lipid fraction in the organism and the organic carbon fraction in sediment. One must consider specific factors and assumptions when using the BSAF model:

1) The organism possesses minimal metabolic capability (BSAF values of <1 may suggest metabolism has occurred)
2) PAHs are 100% available (BSAF values may be <1 if bioavailability is decreased)
3) The affinity of PAHs for lipid and organic carbon are equivalent (i.e.: Octanol-water partition coefficient (K_{ow}) = Organic carbon normalized partition coefficient (K_{oc}))
4) Organic carbon is the only sorptive phase present in sediment

While the traditional form of the BSAF model considers only organic carbon as the sorptive phase, the presence of an additional sorptive phase in sediment has been discussed recently in the literature (19, 20, 21). Others have suggested that soot carbon (SC) present in the sediment may provide an additional sorptive phase for which PAHs have greater affinity than for organic carbon (7, 20, 21). We will present data calculated using both the traditional and 'modified' BSAF, which includes a term for the mass fraction of soot carbon in sediment (f_{sc}), as well as a quantity that accounts for the greater affinity of PAHs for soot carbon than for organic carbon (K_{sc}/K_{oc}, where K_{sc} is the soot carbon-water distribution coefficient):

$$BSAF = (C_m/f_L)/(C_s/(f_{oc} + f_{sc} (K_{sc}/K_{oc}))) \qquad (eq.2)$$

In this study, K_{sc} values were estimated from a quantitative-structure-activity relationship (QSAR) calculated from existing laboratory derived K_{ac} values (20, 23), extrapolated for 39 PAH analytes. The use of the additional assumptions mentioned above (1 to 3) will be discussed. It is also important to mention that if the system is in equilibrium, and the above listed assumptions are considered, BSAF values should be close to or greater than one (a BSAF value of 1 to 1.5, and sometimes greater values are acceptable because $K_{ow} > K_{oc}$). A BSAF value of one or greater indicates an increased concentration of PAH in an organism (mussel) relative to sediment, while a BSAF value of less than one indicates enrichment of PAH in the sediment source. Additionally, values of less than one can suggest a decreased bioavailability of certain contaminants. The objectives of this paper are to present BSAF values for 39 individual PAH analytes using both marine and

freshwater bivalves, assess potential differences in accumulation related to PAH source (petrogenic vs pyrogenic), and discuss the applicability across field sites, of a modified BSAF model considering soot carbon as an additional sorptive phase present in sediment.

MATERIALS AND METHODS

Sample Collection-Freshwater Sites

Transplanted mussels (*Elliptio spp*.) taken from a control site on an un-named tributary of the Steele River in western NC were deployed in polyethylene crates bottom-lined with fine-mesh wire at two sites (Site 1a, and Site 2) located in Gaston County, NC. Site 1a is a relatively remote site, while site 2 is a constructed wetland that receives direct urban run-off. Field survival of the mussels was good, with 68% recovery (due to vandalism), and a 94% survival rate of those recovered, at site 1a. At site 2, 100% of mussels deployed were recovered, with 100% survival. At site 1a, mussels were collected individually in two replicates of 5 individuals each following a 6 month exposure period. At site 2, individual mussels were collected in triplicate and composited after 48 and 80 hour exposures. Triplicate sediment samples were collected from the top 0 to 2 cm surface layer.

Sample Collection- Marine Sites

Native marine clams (*Mya spp*.) were collected from multiple marine sites and laboratory and field bioaccumulation studies were performed using unamended field sediment, and field sediment amended with different types of soot carbon sources (diesel

soot, urban dust, and oil fired power plant soot). Amended sediments were mixed for 14 days prior to conducting the bioaccumulation studies.

Sample Extraction

Mussel, clam, and sediment samples were extracted as described by Short et al (24) with the following modifications: samples were lyophilized and shaker-extracted (200 rpm) for 24 hrs, sediment samples were extracted with methylene chloride:acetone (1:1) (v:v). Mass fraction of organic carbon in sediment was determined by CHN analysis using an elemental analyzer. Soot carbon content was also determined using CHN analysis, following combustion of lyophilized sediment at 375° C, to remove the thermally labile fraction (20). Mussel lipid content was determined through gel permeation chromatography (GPC) fractionation and subsequent evaporation. Clam lipid content was determined by similar means.

Sample Analytical PAH Analysis

Sediment and bivalve samples were analyzed for 39 PAH analytes (Table 1) using an HP 5890 series II GC coupled to an HP5970 MSD using an RTX-5 MS (Restek, 30mmx0.25mmx0.25µm film thickness) with a 5m Integra-Guard column. Analysis was run in the SIM mode using the following temperature program: Injection port: 300° C, Transfer Line: 280° C, Initial Temp: 40° C, Initial Hold: 1 min., Ramp Rate: 6 ° C/min, Final Temp: 290° C, Final Hold: 30 min. One µL of sample was injected. Quality assurance was monitored using procedural blanks, and recovery (phenanthrene-d10, benzo(a)pyrene-d12) and surrogate internal standards (SIS) (naphthalene-d8,

acenapthene-d10, perylene-d12, chrysene-d12). Percent relative standard deviation (RSD) ranged between 8 to 25%, SIS and matrix spike recoveries were 55 to 105%, lab replicate RSDs were <15%, and method blanks were either not dectected or <10% of the measured value. Method detection limits were 0.2 to 0.5 ng/g.

RESULTS AND DISCUSSION

Calculation of BSAF values and Assessment of Bioavailability

BSAF values were calculated for 39 individual PAH analytes (Table 1), using equation 1. This will be referred to as the 'traditional' (T) BSAF equation, where organic carbon is considered the only sorptive phase present in sediment. Figure 1a and 1b represent the BSAF values plotted against individual PAH calculated for freshwater (1a) and marine (1b) sites. In general, PAH are increasing in molecular weight and ring number along the x-axis. Petrogenic PAH have BSAF values that are equal to or greater than one, in most cases, whereas pyrogenic PAH generally have BSAF values that are less than one. For example, the petrogenic PAHs parent naphthalene (N0), and its alkylated homologues (C1-C4- napthalenes, N1-N4) have BSAF values that range from 1.31 to greater than 2. These analytes are fully available to mussels and clams in both systems. However, AN, FL, BaA, IP and CO, among other pyrogenic PAH, have BSAF values that are less than one, in some cases as low as only a few percent (IP BSAF = 4%). There is a visible differential accumulation between petrogenic and pyrogenic PAH that is conserved across both the freshwater and the marine sites. The observation that

petrogenic PAH are readily available and pyrogenic PAH are not is further substantiated by the fact that the two sites are characteristically very different. The freshwater site is not heavily polluted (Total PAH concentration = 325.26ng/g) and has a very low organic carbon content (f_{oc}=0.64%), while the marine site is heavily polluted (Total PAH concentration = >2000ng/g), and has a much higher organic carbon content (f_{oc}=3.74%). There are however, certain differences in the relative abundances of individual analytes at each site. At the freshwater site, AY and AC are fully available, but the BSAF values are questionable due to concentrations for these analytes that are lower than our detection limit. At the marine site, AY and AC are less available, suggestive of a pyrogenic source. Regardless of location, it appears that petrogenic PAH are nearly all more bioavailable than pyrogenic PAH. This is also depicted in Table 2, where the average BSAF values for both the petrogenic and pyrogenic PAH are presented. The petrogenic PAH average BSAF values are greater than one (2.44+/-1.15, and 1.57+/-0.53), while the average pyrogenic BSAF values are less than one (0.63+/-0.60 and 0.25+/-0.23). It should be noted that the average petrogenic PAH BSAF values for the freshwater and marine sites do not include the alkylated homologues of C0, because the origin (petroleum vs coal source) of these analytes is questionable. Additionally, the biogenic PAH PE has been excluded from all BSAF averages, P0 and C0, have been excluded from the pyrogenic BSAF averages at both sites, AC and AY at only the freshwater site, and PY at only the marine site. The reasoning behind this will be discussed below, and takes into account different sources of PAH to both sites. In summary, bioavailability appears to depend on the source of the PAHs: where petrogenic PAH are readily available, and pyrogenic PAH are less available.

PAH source can yield a prediction of PAH bioavailability, but PAH bioavailability can also offer information *a priori* on PAH source. Although nearly all petrogenic PAH are bioavailable, and nearly all pyrogenic PAH exhibit lower availability, there are some exceptions to this classification. From this arises our logic in determining which PAHs to consider when calculating average petrogenic and pyrogenic PAH BSAF values. For example, PAH that enter the environment associated with neat, dissolved petroleum would be expected to exhibit greater availability than PAH that enter the environment tightly sorbed to a particulate phase, such as soot. In figure 1a, P0 is fully available (BSAF = 1.77), suggesting it may be in a more readily exchangeable state, perhaps associated with a coal tar, or a refined or diesel oil source, rather than a high temperature combustion source. However, the alkylated homologues of chrysene (C1-C3 Chrysenes) have BSAF values that are less than one, and exhibit bioavailability more similar to that of pyrogenic PAH. These alkylated chrysenes may be strongly bound in a coal or tar matrix and not as rapidly available for equilibrium partitioning. In contrast, in figure 1b, P0 is not fully available (BSAF = 0.16), suggesting a different source of P0, perhaps of pyrogenic origin, to this system as compared to the freshwater system. Moreover, PY and parent C0 are more available at the marine site, which may suggest the presence of creosote, providing an available source of PY and C0. This suggests that the type of PAH entering the system will influence bioavailability in different ways, and that source may be a very important factor to consider when examining PAH bioavailability.

Consideration of PAH Hydrophobicity

The sharp cut-off in bioavailability that occurs in figures 1a and 1b suggests that bioavailability is independent of K_{ow}. We observe a distinct decrease in BSAF values approximately corresponding to log K_{ow} 4 (AC, 3.92) at the marine site, and to log K_{ow} 5 (AN, 5.54) at the freshwater site, suggesting that BSAF values are only weakly dependent on log K_{ow} values. The two parameters are not significantly correlated in regression analysis (r^2 = 0.42, negative slope) and hydrophobicity explains only 42% of the variation in the BSAF values (Figure 2). For example, C4-phenanthrene (P4) has a log K_{ow} value of 6.51, and is readily bioavailable (BSAF approximately 1.6); however, BbF has a lower log K_{ow} value (5.80), and is not readily bioavailable (BSAF = 0.18-0.35). Other literature studies provide conflicting information in terms of log BSAF vs log K_{ow} relationships in that some demonstrate a maximal BSAF at log K_{ow} 5.5-6 (12, using an amphipod) while others have positive slopes (25, using a deposit feeding clam) that are in contrast to our negative slope. The discrepancy appears to arise when only a small subset of PAHs are used, and sometimes when different log K_{ow} values are used (i.e. Landrum (12) uses a higher log K_{ow} value for AN (4.5) than for P0 (4.3), while our log K_{ow} value is larger for P0 (AN=4.53, P0=4.57).

When a full suite of analytes, including alkylated homologues, is used in the analysis, a more robust data set is obtained for which the true relationship between log BSAF and log K_{ow} is determined. Ferraro et al (26), and Krauss et al (28) also report an independent relationship between log BSAF and log K_{ow} for PAH in earthworms and clams, respectively, and their corresponding BSAF values for individual PAHs agree well

with ours (Table 3). Moreover, Maruya (27) reports a negative slope for log BSAF vs log K_{ow}, and the BSAF values also compare well (Table 3).This suggests that hydrophobicity is not always a good predictor of bioavailability, and that additional factors, such as the source of individual PAHs may be a better predictor of bioavailability.

Other Considerations of Decreased BSAF values

Other possibilities in addition to decreased bioavailability should be considered to potentially explain the decreased BSAF values of pyrogenic PAH. These include: a) differences in hydrophobic character between mussel lipid and sediment organic carbon, b) a lack of steady-state condition for mussels, sediment phase, and the water column, and c) a preferential metabolic capacity of mussels for 3+ ring PAH analytes. While there is evidence that organism lipid quality may be more 'lipid-like' than sediment organic carbon (i.e.: $K_{ow}=0.41K_{oc}$ (28), because organic carbon can contain hydrophilic components), any difference would presumably affect our entire suite of analytes equally and would not result in a decrease in availability unassociated with PAH hydrophobicity. For example, if all PAH analytes are 100% available for equilibrium partitioning, they should preferentially partition into mussel lipid based on their individual affinity for adipose tissue, unless steric problems exist, or particular analytes remain unavailable for equilibrium partitioning due to surface association with particulates, micropore sediment association or occlusion. While lipid pool inequality could explain part of our data, it is potentially masked by a larger effect, notably, decreased bioavailability of the pyrogenic

compounds. Therefore, lipid pool inequalities do not appear to explain the dichotomy between petrogenic and pyrogenic PAH BSAFs we observe in figures 1a+b.

Bioaccumulation experiments with Mytids show rapid uptake of organic compounds, and that steady state is reached on average within eight days (29). Additionally, Obana (30) reported that clams (Tapes japonica) reached steady state levels with the water column in two days, and with the sediment layer in seven days. This included high K_{ow} compounds B(b)F, B(k)F, IP, and B(g)P. While Pruell (31) reports steady state is reached in *M. edulis* at 20 days, an 'apparent' steady state appears to be reached at 10 days for the 4 ring PAH. Based on preliminary results from a separate bioaccumulation study performed by this laboratory, Unionid mussels appear to reach steady state conditions within 4 days of exposure (32). Therefore, we believe that our 4 day (Unionids), 14 day (Mya), and 180 day (Unionids) exposures for data presented in this paper, are more than sufficient to reach steady state between mussels and the rapidly reversible sediment PAH pool. It is important to make this distinction between steady state levels for the sediment-sorbed PAH available for rapid exchange with the water column, and those sediment associated PAHs that are only slowly reversible and not as readily available for equilibrium partitioning (12). The depletion of pyrogenic PAH in our bivalves may be a result of a lack of steady state between mussels and the slowly reversible PAH pool contained within the sediment, or the pool that is associated with an additional sorptive phase, such as soot carbon (SC). The apparent steady state that has been reached for PAH analytes with high log K_{ow} values (P4, 6.51) suggests that the lower BSAF values are not related to a lack of equilibrium but rather to a lack of bioavailability.

It is unlikely that bivalves possess preferential metabolic capacity for 3+ ring PAHs. Narbonne (33) reported that the number of rings in a PAH compound influences their metabolic velocities. However, the relationship between the number of rings and metabolic rate is reported as a shorter half-life for fewer ringed PAHs (5h for N0, and 8h for BaP), which is the opposite of what our data portray. If metabolism is occurring, one might expect to see depleted BSAF values for the lower ring numbered (<3) PAH analytes. Additionally, because bivalves exhibit minimal enzymatic capacity for metabolizing HOCs, including PAHs (1,9), it is extremely unlikely that the mussels and clams in our study are selectively metabolizing the 3+ ring analytes.

Consideration of Soot Carbon (SC) as an Additional Sediment Sorptive Phase

In an attempt to evaluate and further understand our data, we hypothesize that SC present in our sediment may help to explain the decreased bioavailability of pyrogenic PAH. While PAHs partition (absorb) into organic carbon, they also adsorb to SC (20), and this may help explain where the unavailable pool of PAHs reside. When we apply equation 2 to our data, we find that the modified BSAF values unreasonably overestimate bioavailability for petrogenic PAH (Table 1) but increase the BSAF values for the pyrogenic PAH (i.e: they approach and sometimes exceed one, only overpredicting bioavailability in some cases). This could, in part, be a function of using activated carbon-water partition coefficients (K_{ac}) to estimate soot carbon-water partition coefficients (K_{sc}). Correcting for SC content may only be necessary for pyrogenic rather than petrogenic PAH. For example, the uncorrected BSAF value for N0 at the freshwater

site is 2.51, and for the marine site is 1.31, but once these values are corrected for SC, the BSAF values increase to 36.24 and 56.86, respectively. This is an increase of 14 to 50 fold. However, when pyrogenic analytes are corrected for SC, BSAF values increase from 0.48 to 1.49 for BaA at the freshwater site, and from 0.05 to 0.72 at the marine site. This suggests that petrogenic PAH may be much less affected, if affected at all, by SC present in sediment, and that K_{ac} values may be overestimating K_{sc} values. K_{ac} values may not reflect K_{sc} values in the field if competitive sorption interactions are involved. If other HOCs such as pesticides and polychlorinated biphenyls (PCBs) as well as natural organic matter (NOM) compete for active surface sorption sites on the exterior of the SC particles, laboratory derived K_{sc} values may not adequately estimate field interactions. Additionally, different types of SC used in the laboratory, such as NIST SRM standards and SC present in various field sites, which can be as high as 30% of total organic carbon (19) may possess different affinities for PAHs. Thus, it is difficult to form a solid conclusion on this matter, until competitive sorption studies in both laboratory and field settings are conducted.

The SC fractions at the freshwater and marine sites are quite different: f_{sc} at the freshwater site is 0.03, and at the marine site is 0.19 (f_{sc} values are reported as g SC/g TOC). In figures 1a+b, we see that overall the relative petrogenic PAH BSAF values do not differ between the two sites (BSAF values are equal to or greater than 1), even though SC fraction at the marine site is nearly three times that observed at the freshwater site. However, there is a visible difference in the availability of the pyrogenic PAH at the two sites, particularly P0, AN, FL, BaA, BbF, BkF, BaP, IP, and CO. At the freshwater site, with lower SC, the BSAF values for the pyrogenic PAH are, for the most part, greater

than for those at the marine site. For example, AN, with a BSAF value of 0.75 at the freshwater site, is about 75% bioavailable to mussels; however, when the soot carbon fraction is increased at the marine site, AN has a BSAF value of 0.10, or only 10% availability to clams. Therefore, these data suggest that as f_{sc} value increases, the bioavailability of pyrogenic PAH decreases, but the bioavailability of the petrogenic PAH remains essentially unaffected.

This dichotomy between petrogenic and pyrogenic PAH and their interaction with SC may offer indirect evidence on the mechanism(s) of action of sorption of PAH onto SC active sites. For example, petrogenic PAH may not be affected by SC because pyrogenic PAH are formed in tandem with SC, and enter the environment already associated with SC, whereas petrogenic PAH do not. Others have reported that PAHs are associated primarily with the exterior of the SC particle (35, 36). This arises from the similarity in 2D and 3D surface areas of soot (37, 38), as well as the use of cryomicrotome sectioning that found most PAHs are present on the external surfaces of coal particles (36). Additionally, some contrasting evidence exists for external vs internal sorption: Gustafsson et al (38) reported that slow gas sorption kinetics suggest internal interactions, whereas Ghosh et al (35) suggest desorption models with a ‘rind’ type outer layer of PAHs on coal-derived particles best explains PAH interaction. In order to understand why SC appears to only influence pyrogenic PAH, the microscale mechanism behind the interaction must be resolved.

Ideally, accounting for the SC fraction and affinity of PAHs for soot carbon, as modeled in equation 2, would result in adequate predictions of bioavailability across different field sites with varying organic carbon and SC fractions, and across different

PAH sources. However, when we look at a broader picture incorporating many sites (additional freshwater and marine) with different soot carbon fractions, including data from sediment amended with various sources of soot carbon (Figure 3), the effect of increasing soot carbon and decreasing bioavailability persists, but the effect is only moderately linear (r^2=0.85). For example, at the site where f_{sc} is 0.02, the range of BSAF values (BSAF range from 0.25 to 1.25) is similar to those for sites with f_{sc} values of 0.29-0.30 (BSAF range from 0.08 to 0.98). Even with very large SC contents, individual analytes still exhibit BSAF values that are at or near one. If the effect of SC is as dramatic as might be expected, one might observe significantly lower BSAF values at sites with very high SC content. Therefore, there appears to be a more important contributing factor for differing bioavailability across sites.

Variation of PAH Source and Subsequent Effect on Bioavailability

Because the effect of increasing soot carbon concentration and decreasing bioavailability is not drastic (Figure 3), we attempt to further elucidate dominating factors by evaluating the effect of PAH source on BSAF values. Using sediment that was amended for 14 days with soot carbon from various sources including crude oil, diesel soot, urban dust and oil fired power plant soot, we hold the soot carbon fraction constant, and examine the effect of source on bioavailability. It is apparent from table 4 that the effect of soot carbon content on PAH bioavailability is overwhelmed by the change in PAH source, although the effect is still only observed with pyrogenic PAH, rather than

for petrogenic PAH. While there is some decrease in petrogenic BSAF values as f_{sc} increases, the effect is not as marked as for pyrogenic PAH. For example, in the unamended sediment collected from a different marine site than that presented in figure 1b, the dichotomy between the availability of petrogenic and pyrogenic PAH is conserved; however, the pyrogenic PAH are more bioavailable than in the sediment amended with SC. As soot carbon fraction is increased from the unamended sediment (0.02) to each of the three amended sediments (0.29-0.30), the bioavailability of the pyrogenic PAH decreases, but these lower BSAF values do not remain consistent as PAH source changes, and f_{sc} is held constant. This is described best by the individual pyrogenic PAH analyte BeP, which varies from 57% bioavailable in low SC, unamended sediment, to 28% in high SC sediment amended with diesel soot, but when PAH source changes with no change in f_{sc}, the bioavailability drops to 9% for sediment amended with urban dust and sediment amended with oil-fired power plant soot. This is particularly interesting due to the fact that urban dust is partially comprised of diesel soot, sometimes in quantities as high as 30%. The variation in BSAF values that occurs when the source of the amended quantity changes, as SC content remains the same, suggests that PAH source is a more important determining factor of bioavailability than both f_{sc} and PAH hydrophobicity. The differences in BSAF values that are observed with changes in PAH source are most likely related to different compositions of starting material, combustion temperature, and weathering. For example, diesel soot is formed at a lower burning temperature than urban dust, and oil-fired power plant soot. Moreover, diesel soot PAH signatures will exhibit less weathering than urban dust PAH patterns. This implies that establishing a quantitative BSAF model as previously presented in equation 2, that

accounts for f_{sc} may not be applicable across environmental sites. In terms of bioavailability, assessing SC content at field sites may be helpful only in a qualitative sense, because SC appears to have little effect on the bioavailability of petrogenic PAH and its overall effect on pyrogenic PAH is overpowered by the specific source of the PAH.

Applicability of Modified BSAF Model Across Different Field Systems

Due to the greater importance of PAH source over SC content or PAH hydrophobicity in bioavailability assessments, the use of the modified BSAF model as presented in this paper, may not be applicable across a wide range of locations, including varying f_{sc} values and different PAH sources. The data presented offer evidence that the use of a qualitative model may not be appropriate when assessing the bioavailability of PAHs. The usefulness of a model requires that variables that exist between different locations be accounted for in a reasonable and ideally, simplistic way. For example, the traditional method of normalizing sediment HOC concentrations to sediment organic carbon allows comparison of HOC concentrations across different sites (though PAHs are frequently an exception to this). Based on the data presented in this paper, it is questionable whether a PAH-soot carbon bioavailability model can be developed that can be applied universally across sites with varying SC concentrations, and with different sources of PAH. However, further research on determining actual K_{sc} values and the applicability of laboratory K_{sc} determinations to field settings must be considered. Competitive interactions in the environment, such as those between other HOCs, and

NOM for SC active sites should be studied to further our overall understanding on the mechanisms involved between contaminants and SC in the environment. The data presented exhibit only moderate decreases in bioavailability with subsequent increases in SC content in sediment, typically with BSAF values declining by factors of 2 to 3 with substantial increases in SC. This questions the toxicological relevance of SC as a major player in site hazard assessment and remediation technologies.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the following funding sources: The North Carolina Cooperative Extension Group, Gaston County, NC; National Institute of Environmental Health Sciences Training Grant; and the U.S. Environmental Protection Agency. Additionally, we would like to thank Peter Lazaro for field and technical assistance, and Battelle for collaborative soot carbon research.

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Table 1. List of traditional (T) and modified (M) BSAF values at sites 1a and 2

Analyte	Symbol	log Kow[1]	1a T BSAF	2 T BSAF	1a M BSAF	2 M BSAF
Napthalene	N0	3.37	2.11	1.31	13.97	56.86
C1-Napthalenes	N1	3.87	2.19	1.87	12.01	65.12
C2-Napthalenes	N2	4.37	2.44	2.14	11.20	59.88
C3-Napthalenes	N3	5.00	2.96	1.95	10.94	41.53
C4-Napthalenes	N4	5.55	2.49	2.07	7.72	34.84
Acenapthylene	AY	4.07	2.15	0.31	10.97	9.89
Acenapthene	AC	3.92	1.96	0.17	10.56	5.79
Fluorene	F0	4.18	4.07	0.40	19.95	12.16
C1-Fluorenes	F1	4.97	5.53	1.65	20.64	35.60
C2-Fluorenes	F2	5.20	2.71	2.30	9.38	44.95
C3-Fluorenes	F3	5.50	2.26	2.11	7.11	36.27
Dibenzothiophene	D0	4.49	2.85	1.74	12.51	46.21
C1-Dibenzothiophenes	D1	4.86	3.52	1.39	13.64	31.44
C2-Dibenzothiophenes	D2	5.50	2.18	1.70	6.86	29.22
C3-Dibenzothiophenes	D3	5.73	1.53	1.21	4.50	18.87
Phenanthrene	P0	4.57	1.77	0.16	7.54	4.10
C1-Phenanthrenes/Anthracenes	P1	5.14	1.20	1.48	4.22	29.68
C2-Phenanthrenes/Anthracenes	P2	5.51	1.45	1.52	4.55	26.02
C3-Phenanthrenes/Anthracenes	P3	6.00	2.46	1.49	6.67	20.73
C4-Phenanthrenes/Anthracenes	P4	6.51	1.56	1.63	3.70	18.35
Anthracene	AN	4.54	0.75	0.06	3.23	1.56
Fluoranthene	FL	5.22	0.47	0.28	1.61	5.42
C1-Fluoranthenes/Pyrenes	FP1	5.72	0.43	0.34	1.27	5.32
Pyrene	PY	5.18	0.76	0.92	2.66	18.13
Benzo[a]Anthracene	BaA	5.91	0.27	0.05	0.77	0.72
Chrysene	C0	5.86	0.50	0.55	1.40	8.12
C1-Chrysenes	C1	6.42	0.77	0.72	1.85	8.41
C2-Chrysenes	C2	6.88	0.41	0.84	0.88	8.13
C3-Chrysenes	C3	7.44	0.74	0.89	1.40	6.89
Benzo[b]Fluoranthene	BbF	5.80	0.35	0.18	1.01	2.72
Benzo[k]Fluoranthene	BkF	6.00	0.30	0.21	0.83	2.92
Benzo[e]Pyrene	BeP	6.20	0.58	0.30	1.48	3.84
Banzo[a]Pyrene	BaP	6.04	0.18	0.12	0.49	1.64
Perylene	PE	6.30	0.18	0.11	0.44	1.35
Indeno[1,2,3-c,d]Pyrene	IP	7.00	0.27	0.04	0.56	0.37
Dibenzo[a,h]Anthracene	DA	6.75	0.41	0.07	0.91	0.71
Benzo[g,h,i]Perylene	BgP	6.50	0.29	0.34	0.70	3.84
Coronene	CO	7.64	0.26	0.15	0.46	1.07
foc			0.64	3.74	0.64	3.74
fsc			0.02	0.88	0.02	0.88

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Table 2. Average BSAF values for petrogenic and pyrogenic PAH across sites (freshwater, marine).

Site	BSAF	Sd. Dev
1a-Petro	2.44	1.15
1b-Petro	1.57	0.53
1a-Pyro	0.63	0.60
1b-Pyro	0.25	0.23

Table 3. Comparison of BSAF values (This study) to various literature values

Study	Species	PY	BaA	C0	Bb,k,jF	BaP
This study	*E. complanata*	0.76	0.27	0.50	0.30-0.35	0.18
This study	*M. arenaria*	0.92	0.05	0.55	0.18-0.21	0.12
Ferraro et al.[14]	*M. nasuta*	0.37-0.53	0.15-0.62	0.21-0.61	0.21-1.02	0.05-0.85
Maruya et al.[26]	*P. amurensis*	0.12-1.50	0.09-2.48	0.13-0.97	0.08-1.20	0.06-0.19
Krauss et al. [27]*	*L. terrestris*	0.16	0.2	0.18	0.16	0.16

* values approximated from figure 4 in Krauss [27]. C0 represents both chrysene and triphenylene

Table 4. BSAF values with changing soot carbon content and PAH source

	Field Wlft	w/Diesel Soot	w/Urban Dust	OilFiredPPlt Soot
fsc	0.02	0.29	0.29	0.30
N0	1.51	1.28	1.29	1.03
N1	1.24	1.27	1.32	0.90
N2	1.64	1.23	1.09	0.82
N3	1.77	1.55	1.21	0.92
N4	1.62	1.44	1.14	0.88
AY	0.42	0.31	0.22	0.16
AC	0.51	0.26	0.09	0.14
F0	1.36	1.51	1.05	0.84
F1	1.75	1.53	1.46	1.22
F2	1.48	1.18	1.29	1.07
F3	1.30	1.42	1.38	1.18
D0	1.57	1.15	1.05	0.91
D1	1.49	1.21	1.36	0.82
D2	1.33	1.07	1.27	0.93
D3	1.20	1.19	0.94	1.04
P0	0.42	0.47	0.11	0.08
P1	1.36	1.41	1.36	0.99
P2	1.44	1.20	1.06	1.08
P3	1.59	1.65	0.96	0.88
P4	1.57	0.95	1.14	1.16
AN	0.60	0.12	0.08	0.08
FL	0.73	0.27	0.13	0.13
FP1	0.89	0.39	0.20	0.15
PY	1.25	1.15	1.01	0.97
BaA	0.72	0.28	0.17	0.17
C0	1.03	0.84	0.33	0.38
C1	1.18	0.88	0.72	0.49
C2	1.27	1.07	0.82	0.58
C3	1.34	1.12	1.15	0.72
BbF	0.62	0.26	0.13	0.14
BkF	0.57	0.37	0.24	0.17
BeP	0.57	0.28	0.09	0.09
BaP	0.45	0.17	0.08	0.07
PE	0.46	0.21	0.17	0.14
IP	0.29	0.14	0.08	0.06
DA	0.49	0.18	0.12	0.12
BgP	0.78	0.24	0.16	0.17
CO	0.90	0.14	0.11	0.10

Chapter 4: Figure Legends

Figure 1: Traditional BSAF values (no soot carbon corrections) for individual PAH at a freshwater (a) and marine (b) site.

Figure 2: Log BSAF vs log K_{ow}: plot of data from this study (squares), data from Landrum [12] (triangles), and data from Lamoureux [25](diamonds). Note the differences in relationships between BSAF and PAH hydrophobicity: our data exhibits a negative slope, while Landrum and Lamoureux's data show a maximum at log K_{ow}=5, and a positive slope, respectively.

Figure 3: Average BSAF values for pyrogenic PAH vs soot carbon content over all sites, freshwater and marine. The relationship between BSAF value and increasing soot carbon content is less drastic than one might expect, suggesting that PAH source may be contributing to the decreased bioavailability of the pyrogenic PAH as well.

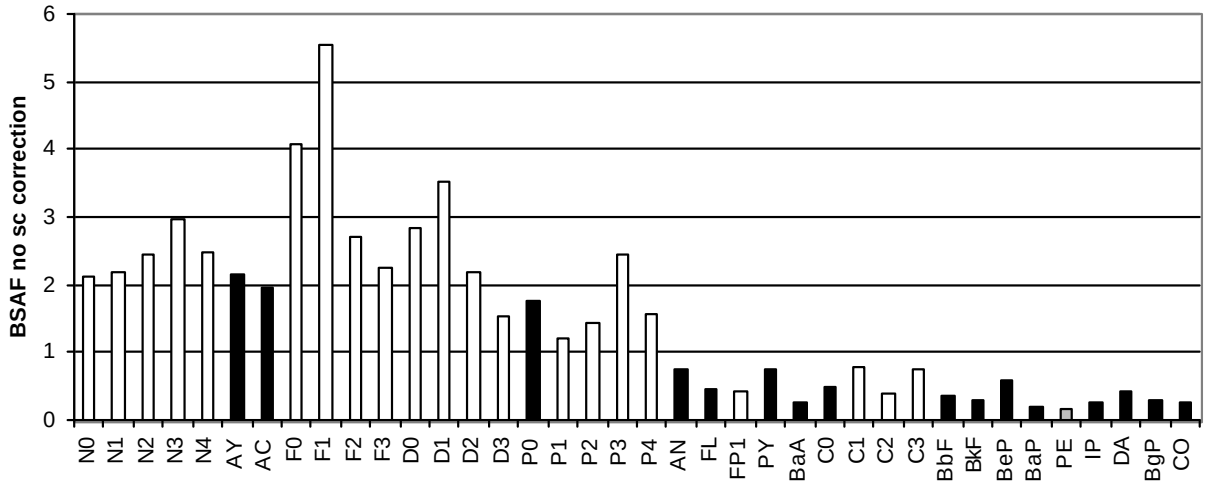

Figure 1a: BSAF values at freshwater site

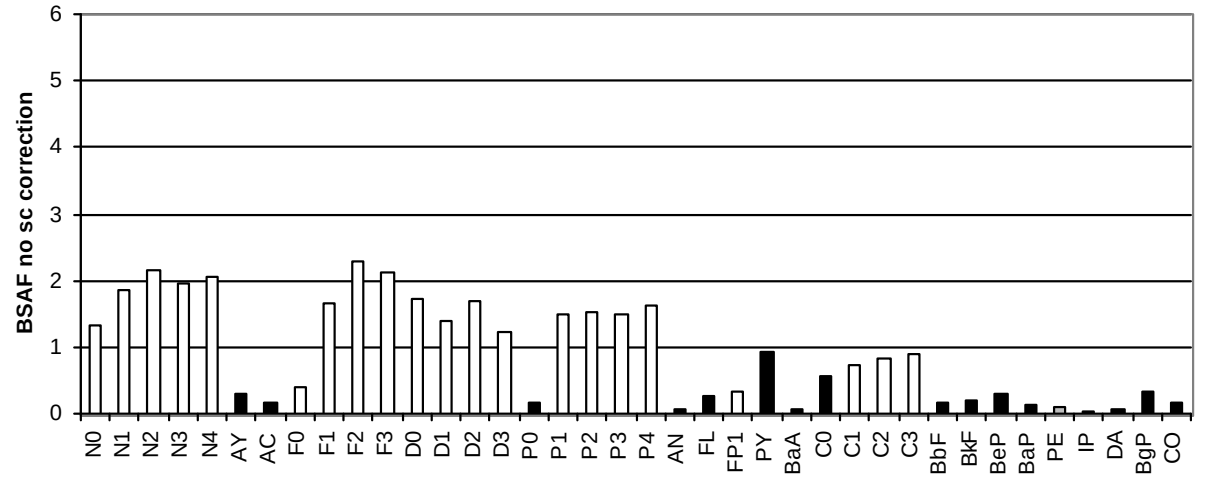

Figure 1b: BSAF values at marine site

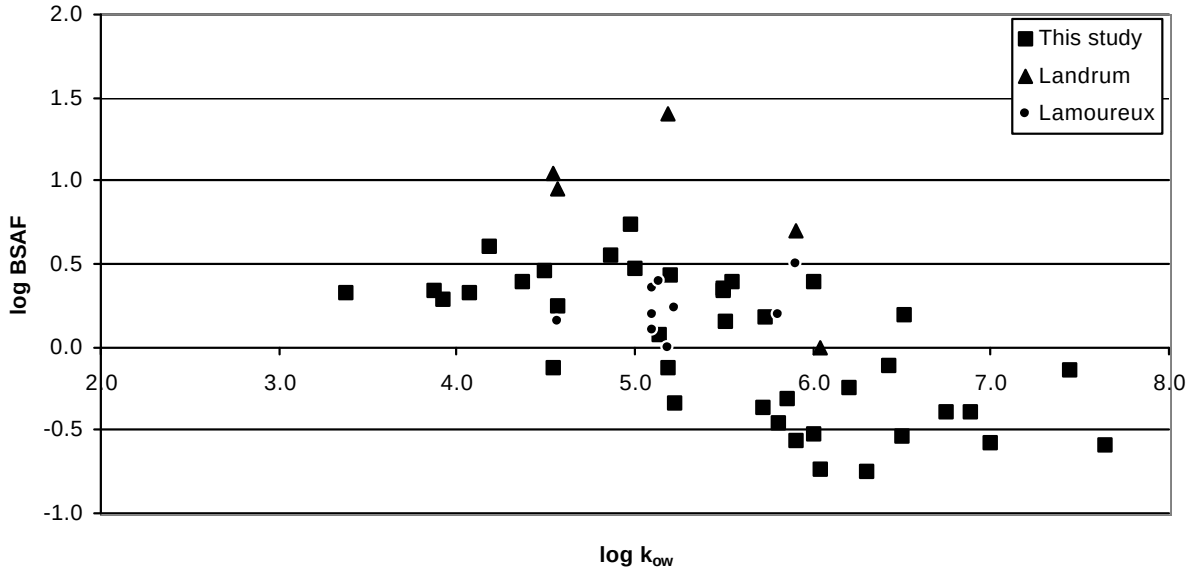

Figure 2: log BSAF vs log K_{ow} for this study and others. Note negative slope for this study, peak at log K_{ow}=5 for Landrum, and positive slope for Lamoureux

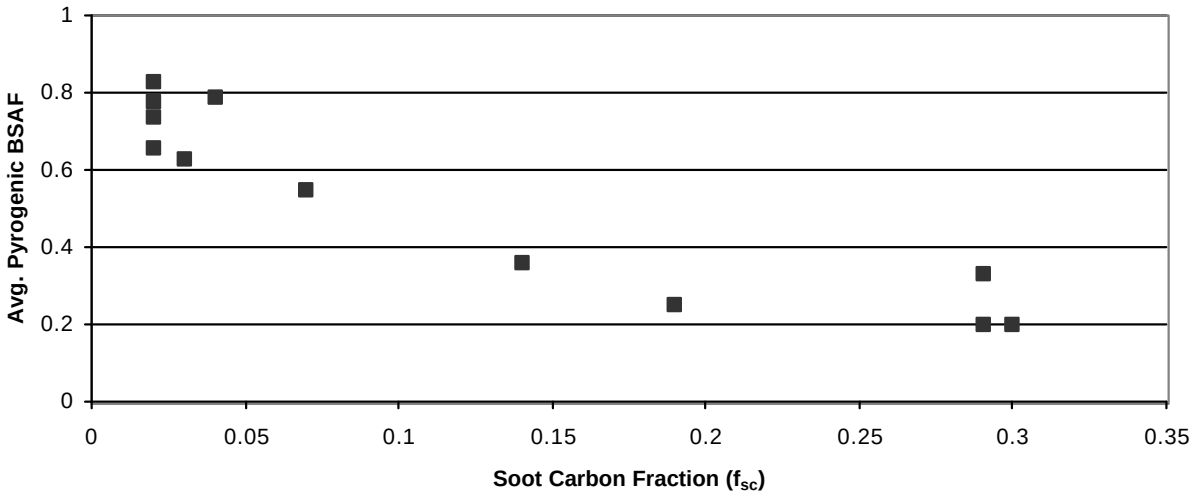

Figure 3: Average pyrogenic BSAF values vs f_{sc} over all sites