## AN ABSTRACT OF THE THESIS OF

Takashi Fuji for the degree of Doctor of Philosophy in Toxicology presented on May 30, 1997. Title: Biotic and Abiotic Factors Influencing the Bioavailability of Sediment-Associated Phenanthrene to Marine Amphipods.

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The "equilibrium partitioning theory" is one of the most widely used models to evaluate the bioavailability of sediment-associated, nonpolar, organic contaminants and it makes specific assumptions regarding the factors that influence this bioavailability. The objective of this research was to test two assumptions of this theory: (1) that benthic organisms are exposed to a constant, equilibrium-predicted concentration of a contaminant in interstitial water, regardless of the behavior of the organism; and (2) that exposure to interstitial water in a sediment exposure system is equivalent to the exposure in a water-only exposure system. The effect of behavior on the exposure to sediment-associated phenanthrene was tested by exposing three marine amphipod species (with different burrowing behaviors) to the polycyclic aromatic hydrocarbon (PAH) phenanthrene under two exposure conditions, one with spiked sediment and clean overlying water and the other with spiked sediment and contaminated overlying water. This was done to evaluate the extent to which the burrow irrigating behavior and the different tube or burrow building behavior exhibited by the amphipod species could effect the accumulation of sediment-associated phenanthrene. The assumption of equivalent exposure between sediment and water systems was tested by exposing the amphipods to the same concentration of phenanthrene in a water-only versus sediment exposure system. In both series of experiments, the bioaccumulation of phenanthrene by the amphipods was followed over 72 hours and bioaccumulation kinetics calculated for each species and exposure treatment. The results indicated that the burrow irrigating behavior of benthic marine amphipods can significantly affect the exposure of these amphipods to sediment-associated contaminants by diluting the concentration of contaminant in the interstitial water surrounding the organisms with overlying water. Additionally, there was a species dependent decrease in exposure based upon the tube or

burrow building strategy used by the amphipod species. The results also indicated that exposure in a sediment system was not equivalent to exposure in a water-only system. The bioaccumulation of phenanthrene was significantly higher for all three species in water versus sediment. However, the interpretation of the results from this second series of experiments was complicated by the degradation of phenanthrene in the sediment-only exposure.

# Biotic and Abiotic Factors Influencing the Bioavailability of Sediment-Associated Phenanthrene to Marine Amphipods

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Takashi Fuji, Author

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## CHAPTER 1

## INTRODUCTION

### 1.1 STATEMENT OF PURPOSE

The purpose of the research presented in this thesis is to test two assumptions of the "equilibrium partitioning theory". The "equilibrium partitioning theory" is one of the most widely used models to evaluate the bioavailability of sediment-associated, nonpolar, organic contaminants. The two specific assumptions of the "equilibrium partitioning theory" that will be tested are: (1) that benthic organisms are exposed to a constant, equilibrium predicted concentration of a contaminant in interstitial water, regardless of the behavior of the organism and differences in behavior among different benthic species; and (2) that exposure to interstitial water in a sediment exposure system is equivalent to the exposure in a water-only exposure system. Additionally, the results of this research will provide insight into the influence of biological, physical, and chemical factors in determining the bioavailability of sediment-associated phenanthrene. Further details of the "equilibrium partitioning theory", its assumptions and uses, are described in subsequent sections of this introduction.

The objective of this introduction is to place the research topic in perspective with current methods used to assess contaminated sediments. Understanding the bioavailability of sediment-associated contaminants is fundamental in assessing the risk posed by a particular level of contamination to the aquatic environment. The bioavailability of a contaminant in sediment is defined as the total pool of material that is potentially available to organisms and includes the biological, physical, and chemical processes that can modify this availability. There is ample evidence that sediment-associated contaminants are entering and affecting aquatic systems. However, the processes responsible for the transfer of a contaminant from sediment to biota and the physiochemical and biological factors modifying these processes remain relatively ill-defined. The public attention and

regulatory scrutinity given to sediment contamination issues have increased over the past few years. The use of sediment toxicity tests utilizing benthic invertebrates for regulatory purposes have also increased. Therefore an understanding of the factors influencing the bioavailability of contaminants in sediments has become critical in developing appropriate management strategies for addressing contaminated sediments.

## 1.2 SEDIMENT CONTAMINATION ISSUES

As the ultimate sink for many pollutants entering the aquatic environment, sediments integrate contaminants from a variety of sources. Contaminants can enter the aquatic environment via point, non-point, and aerial sources and deposit and accumulate in sediments. As sediments retain persistent compounds, chemical contamination currently present in sediments may be the result of either historical or recent activities.

In the past, sediment contamination issues came to the forefront during dredging operations required by ports and harbors in order to maintain navigational and operational channel depths. Issues related to dredge material disposal required the establishment of assessment techniques (EPA/USACE, 1977, EPA/USACE, 1991) to ensure the adequate classification of material to determine appropriate disposal options. A tiered dredge material evaluation procedure was developed to assess; toxicity during dredging and disposal, toxicity of inplace sediment after disposal, and bioaccumulation potential of contaminants in the dredge material.

The extent of sediment contamination, as a whole, has only begun to be recognized within the past decade. The National Research Council in 1989 (NRC, 1989) reported that "The problem of contaminated marine sediments has emerged as an environmental issue of national importance". In 1994, the EPA determined that "the contamination of sediments in waterbodies of the United States has emerged in recent years as an ecological and human health issue of national proportions" (EPA, 1994a).

In recent years, attention has been focused on evaluating sediment quality regionally (Overton et al., 1991, Lauenstein and Cantillo, 1993, and Daskalakis and O'Connor, 1995) and at specific sites in hazardous waste assessment and remediation programs and projects (Chapman et al., 1987, Becker et al., 1990, Ankley et al., 1992, and Swartz et al., 1994). A recent evaluation of sediment toxicity in major U.S. estuaries (Long et al., 1996)

revealed that sediments contamination and toxicity problems may be small and isolated in certain areas or represent larger, pervasive toxicity problems encompassing entire sections of industrial bays and estuaries.

A wide variety of adverse biological effects have been associated with sediment contaminants. These include both acute and chronic effects documented at the individual organism level as well as population and community level effects in the benthos. Additionally, geochemical processes have been implicated in the release (mobilization) and transport of pollutants from contaminated sediments to the water column (Burgess and Scott, 1992). Such processes have resulted in the bioaccumulation of contaminants via exposure to dissolved and particulate phases present in the water column that originate in the sediments (Mac and Schmitt, 1992, Dabrowska and Fisher, 1993). Additionally, sediment-associated contaminants have shown the ability to transfer through aquatic food webs (Clements et al., 1994).

A notable issue is that the relative importance of sediment as a pollution source increases as other industrial and point sources decrease because of increased regulatory vigilance. Environmental regulations, both in the U.S. and abroad, have resulted in the reduction of loadings of pollutants from atmospheric and terrestrial sources (Burgess and Scott, 1992). This is particularly true of banned persistent compounds, such as the pesticides DDT and tributyltin, and industrial chemicals such as PCBs (Fent, 1996). Therefore, while pollutant loading will have diminished using current water column based measurements, the sediments will continue as a source of these compounds to the aquatic environment.

## 1.3 EXPOSURE PATHWAYS AND BIOAVAILABILITY OF SEDIMENT-ASSOCIATED CONTAMINANTS

Sediment-associated contaminants can exert adverse biological impacts via direct and indirect exposure pathways. Benthic and epibenthic organisms are exposed via interstitial and overlying water which may contain dissolved contaminants solubilizing off of sediment particles and by exposure to the sediment itself, via ingestion and direct contact to respiratory or body surfaces (Barron, 1995, Mayer et al., 1996).

Another exposure pathway is the trophic transfer of sediment-associated contaminants to predatory fish and epibenthic invertebrates that feed upon contaminated benthos. The

bioaccumulation and in some cases, biomagnification, of sediment contaminants provides an important mechanism of transport and exposure to higher trophic levels (Nalepa and Landrum, 1988, Bierman, 1990, and Clements et al., 1994). This is particularly true of persistent chemicals such as PCBs and mercury that enter food chains and concentrate to levels that may induce chronic toxicity, especially in predators at higher trophic levels, or accumulate to concentrations that render fish and wildlife unsuitable for human consumption (Mac and Schmitt, 1992).

To evaluate the ecological and public health risks posed by sediment-associated contaminants, the factors governing the bioavailability of sediment-associated contaminants must be understood. Bioavailability of sediment-associated contaminants can be defined as "the fraction of the total contaminant in the interstitial water and on the sediment particles that is available for bioaccumulation" (Landrum and Robbins, 1990). The main factors that affect bioavailability can be divided into two major areas: factors that alter the partitioning of contaminant between sediment and interstitial water and biological factors that alter the exposure and accumulation of the contaminant by benthic organisms.

The potential toxicity of sediment-associated chemicals; in particular, nonionic organic chemicals, organotin compounds, and divalent metals, is related to the amount of chemical that is freely available in the interstitial water of the sediment (Kemp and Swartz, 1988, DiToro et al., 1991, and Fent, 1996). The amount of uncomplexed, bioavailable chemical can be affected by several physical, chemical, and biological factors. The most important factor determining bioavailability of nonionic organic chemicals is sorption to the organic carbon dissolved in the pore water and bound to sediment particles; for divalent metals, sulfide, especially the reactive sulfide fraction known as acid-volatile sulfide (AVS), controls bioavailability (Ankley et al., 1996). The partitioning of a nonionic organic chemical between organic carbon and pore water and the partitioning of a divalent metal between solid phase and solution phase are assumed to be at equilibrium (DiToro et al., 1991, Ankley et al., 1996).

The sediment properties that can regulate this partitioning include pH, redox potential, organic carbon content, particle size distribution, and cation exchange capacity. The influence of organic carbon is greater for compounds of higher octonal-water coefficients that sorb primarily to the organic fraction of sediments (Landrum and Robbins, 1990). Additionally, the chemical characteristics of the contaminant itself is important. Solubility, octonal-water partition coefficient, and speciation all can play an important role in

influencing the adsorption and desorption of material into and out of the sediment matrix. Of these chemical properties, the octonal-water coefficient is the major characteristic which controls the partitioning between the soluble phase and the sediment organic carbon.

Biological and behavioral characteristics of the benthos themselves can influence exposure. As reported by Lee (1991), factors that can influence the microhabitat and the interstitial water surrounding the organisms will in turn affect its exposure to sediment-associated contaminants. Such processes include the advection of overlying water into sediments, which can act to dilute the interstitial water concentration of a chemical or ion present (Wasenchuck et al., 1983). For example, free burrowing benthic organisms meet their oxygen requirements by ventilating overlying water into the sediment prism (Lee, 1991). Therefore, assumption of equilibrium pollutant concentration in the ventilated interstitial water would overestimate exposure if pollutant concentration in the microhabitat surrounding the free-burrowing species are reduced by organism uptake and advection of overlying water.

This situation can be exacerbated in situation where the organisms build tubes or burrows in the sediment. Tubes and well-defined burrows are common among polychaetes, amphipods and other infaunal taxa. Matisoff et al. (1985) found that dwelling and feeding burrows act as conduits of matter exchange between interstitial water and overlying water via fluid advection associated with feeding and respiratory behavior. It was found that the pore water concentration were low within irrigated burrow zones for chemicals species normally high in sediments. There is no definitive difference between a tube and a burrow, but in general a tube has a more defined structure whereas a burrow is the modification of the surrounding sediment (Lee, 1991). In theory, the tubes or burrows facilitates the movement of overlying water into the sediment prism by providing a direct access to the sediment surface. The dilution of interstitial water should be greater for those species utilizing such structures.

An additional factor that can serve to decrease the concentration of contaminants the benthos is exposed to is the situation in which the uptake and dilution of contaminant in interstitial water is greater than the desorption rate of the contaminant from the sediment organic carbon (Lee, 1991, Landrum and Robbins, 1990). It has been reported that the desorption rate from sediments can be much longer than expected for PAHs and other hydrophobic contaminants (Landrum and Robbins, 1990, Borglin, 1996). In such situations, the desorption rate can become controlling factor determining the ultimate

concentration of contaminant in the micro-habitat surrounding the organisms. This may be particularly true for lipophilic compounds that tend to have the highest uptake rates from water and in situations in which biological processes such as respiration and filter feeding are contributing to the loss if the compound from interstitial water.

All of these factors must be considered in evaluating the bioavailability of pollutants associated with sediments and in determining the ultimate risk or threat posed by a particular contaminated sediment to the environment.

## 1.4 SEDIMENT ASSESSMENT TECHNIQUES

At the present time, sediment assessment strategies can be divided in two basic categories: one that relies strictly on measurements of chemical concentrations in sediment and subsequent comparisons with numeric criteria and a second of biological investigations which involve laboratory toxicity testing or *in-situ* evaluation of biological communities. Comparisons of bulk chemistry data with regulatory benchmarks or criteria have regulatory history being the preferred method for evaluating contamination issues in ground water, surface water, and surface soils under a variety of regulatory programs (i.e. Clean Water Act, CERCLA). However, a wealth of data exists that suggest that bulk chemistry information, by itself, is insufficient to accurately predict the toxicity or bioavailability of contaminated sediments (DiToro et al., 1991, Ankley et al., 1996).

Biological assessment techniques have included the use of laboratory sediment toxicity tests that involve the exposure of standardized benthic species to field collected or laboratory spiked sediment. Various toxicological endpoints have been developed to evaluate both acute (mortality) and chronic (growth and reproduction) endpoints (Lamberson et al., 1992, Burton et al., 1992, and DeWitt et al., 1992). These biological methods can provide the most direct evidence of sediment toxicity but care must be taken to distinguish adverse biological effects due to contaminants effects versus those resulting from physical characteristics of the sediment (i.e. grain size) or the environment temperature, salinity, or habitat changes. Bioassays for marine/estuarine sediments have been developed for amphipods, cumaceans, copepods, shrimp, isopods, bivalves, polychaetes, echinoderms, and fish (Lamberson et al., 1992, ASTM, 1995).

In situ evaluations of biological communities have also been utilized in evaluating the effects of contaminated sediments. The accuracy of these techniques have been dependent on the ability of the method to discriminate contaminant specific changes in community parameters versus natural variability, both spatially (in cases where community data at a contaminated site is compared against a uncontaminated reference location) and temporally (in cases where data are collected at only one time and therefore can only give a "snapshot" of the community) (Ferraro et al., 1991).

Integrative methodologies that combine biological and chemical measurements to assess risk posed by contaminated sediments have also been developed to assess site specific toxicity of sediments and are also being utilized to derive sediment quality benchmarks for use in regulatory screening of sediment quality (MacDonald et al., 1992, Smith et al., 1996). Additionally, as is the case in Washington State, the sediment regulations have adopted a integrated strategy that combines numeric criteria with confirmatory sediment bioassays if there is a question on the site specific application of the numeric criteria (Ecology, 1991).

In the United States, the EPA embarked on a substantive effort in the late 1980's and early 1990's to develop chemical-specific numeric criteria for the evaluation of sediment contamination issues. The Clean Water Act (CWA) and its 1987 amendments are the primary legislation governing the protection of the nation's waterways. It's two principle goals are to maintain the physical, chemical, and biological integrity of surface waters and to achieve water quality that provides for the protection and propagation of fish. As part of the CWA, the EPA was issued the mandate by Congress to derive Water Quality Criteria for a defined set of pollutants. These Federal criteria provided the basis for each state to develop its own water quality standards and were intended to provide comprehensive environmental protection. However, there is evidence of environmental degradation in areas where Water Quality Criteria are not being exceeded (EPA, 1987). Specifically, organisms living in or near sediments are being adversely affected, apparently by chemical contamination (EPA, 1987). Thus, although Water Quality Criteria are intended to protect water column organisms, they are not protecting communities associated with sediments. Hence, there is a primary need to determine what levels of contaminants in sediment could be correlated with detrimental biological and ecological responses. As the need to evaluate contaminated sediments grew in the 1980's, EPA increased efforts to develop a methodology that would generate a chemical-by-chemical criteria that if not exceeded, would protect the biological integrity of aquatic ecosystems. EPA evaluated a variety of

methods and decided to pursue the "equilibrium partitioning approach" (EQP) to generate sediment quality criteria.

Setting numerical criteria for sediment is a complicated task. Numerous investigations have noted that sediments containing the same or very similar total chemical concentrations of a contaminant or suite of contaminants have exhibited vastly different levels of toxicity (NOAA, 1990). This discrepancy can be explained because sediments are a very heterogeneous environment, varying in such parameters as grain size, organic content, and redox potential. These parameters can act alone or together to modify the availability of a particular toxicant or contaminant to an aquatic organisms (Shea, 1988, Lee, 1991).

The EPA evaluated many different approaches to estimate the toxicity of sediment-associated contaminants to determine underlying factors that could be used to normalize the observed toxicity of contaminated sediments. Two key observations provided insights on quantifying the bioavailability of chemicals in sediments. First, the concentration-response curve for biological effects could be correlated, not to total chemical concentrations, but to the interstitial (pore-water) concentration. Organism mortality, growth rate, and bioaccumulation data appear to support and demonstrate this correlation (DiToro et al., 1991). Second, for a certain class of compounds, the non-polar organics which include a variety of pesticides and polycyclic aromatic hydrocarbons, the movement or partitioning of the chemical from the sediment to the pore-water is controlled by the organic carbon content of the sediment. Therefore, the toxicity or bioavailability of these compounds is controlled by the "equilibrium partitioning" of the compound between sediment particles and the biologically active phase, the pore water. Thus, if the partitioning coefficients are known for a given contaminant, then measuring its concentration in one environmental phase (e.g. sediment) allows one to predict its concentration in the other (e.g. pore water) (Shea, 1988, DiToro et al., 1991).

The next step in developing the model was to select the toxicity endpoints to be used in determining unacceptable adverse biological effects. The EPA has decided to use existing Water Quality Criteria (WQC) as the toxicological benchmark to compare the predicted pore-water concentrations. Briefly, the WQC are based upon a wide range of aquatic water column toxicity data and is currently considered the best available estimate of cause-effect relationships in the water column (EPA, 1989b). The use of the WQC as the toxicological benchmark for pore-water assumes that (a) the sensitivity of benthic species and species

tested to derive WQC are similar, and (b) that exposure to the same concentration of a contaminant in interstitial water is equivalent to exposure in the water column.

## 1.5 JUSTIFICATION FOR SPECIES AND CONTAMINANT SELECTION

The following section provides the rationale for the selection of the test organisms and the contaminant used in this research.

### Amphipods

Amphipods were selected for this research project because they are an important component of tidal and intertidal ecosystems. Amphipods are an important prey item for many fish, birds, and marine mammal species. Additionally, amphipods are commonly used sediment toxicity research and in regulatory sediment toxicity testing. Because of these uses, considerable amount of information exists on the physical/chemical tolerances of these organisms, the response that these organisms exhibit when challenged with physical and chemical stress, and the life history for individual species (Lamberson et al., 1992, Burton et al., 1992, and ASTM, 1995). The species that have been approved for regulatory and scientific sediment toxicity testing exhibit characteristics recognized as desirable in test organisms.

Desirable properties for a sediment toxicity test species are: (1) broad salinity tolerances; (2) high sensitivity to common sediment contaminants; (3) high survival under control conditions; (4) occupation of microhabitat(s) at, or preferably, below the sediment water interface to ensure maximum and consistent exposure to sediment contaminants; (5) low sensitivity to natural sediment variables; (6) broad geographical range to enhance the breadth of its application as a test species; (7) ease of collection, handling, and maintenance in the laboratory; (8) ecological importance in estuarine systems; and (9) the ability to be cultured or year round availability from the field (DeWitt et al., 1989, Shuba et al., 1978).

For these particular experiments, the following species were selected; *Eohaustorius estuaries, Grandidierella japonica, and Leptocheirus estaurius.* These species were selected because they cover the range of different burrowing strategies employed by benthic amphipods and each of these species have been recognized by ASTM and other regulatory

agencies (USF&W, U.S. Army Corps of Engineers, PSDDA, Washington State Department of Ecology) as appropriate test species for sediment toxicity testing.

Eohaustorius estuarius:

*E. estaurius* lives in intertidal sands along the North American west coast from British Columbia south to at least central California. It is a non-structure forming (free burrowing) species that normally resides in fine, intertidal sands. The feeding habits of *E. estuarius* are unknown but suspected to be deposit-feeding because its offshore congener, *Eohaustorius sencillus*, and other haustoriid amphipods are infaunal deposit feeders (DeWitt et al., 1989). E. estuarius is a desirable test species for sediments which have interstitial salinities ranging between 2 and 28 °/oo and is tolerant of a wide variety of sediment types and grain sizes. *E. estuarius* appears to have an annual life cycle with reproduction occurring between February through July (Bosworth, 1976). It is recognized by ASTM (1995) as a marine sediment bioassay species and has been used in research, sediment toxicity tests and for regulatory purposes (DeWitt et al., 1989, PSEP, 1995).

Grandidierella japonica:

*G. japonica* is an introduced species to central and southern California. This species was first described in Japan in 1938. *G. japonica* was first reported from California in 1966 (from San Francisco Bay). This species was probably introduced to the area with transplanted oyster spats, which began in 1928. The first record of *G. japonica* in southern California was in 1979, when it was reported in Newport Bay, CA (Nipper et al., 1989). The species survives well in laboratory cultures at temperatures of 15 to 23°C and has a short reproductive cycle in laboratory cultures (30 days at 19°C). *G. japonica* is builds porous U-shaped tubes in the sediment and lives in a variety of sediment types (ASTM, 1995). It has been used for toxicity testing (Hong and Reish, 1987, Nipper et al., 1989) and is a ASTM recognized species for the toxicity testing of marine sediments (ASTM, 1995).

Leptocheirus plumulosus:

*L. plumulosus* ranges from Massachusetts to Florida, from the intertidal zone to water approximately 5 meters in depth. It builds an unlined U-shaped burrow of sand grains and debris in the upper 5-7 cm of sediment, and is typically found in mud to sandy mud and detritus, especially in areas with a current. *L. plumulosus* is reported to be a surface deposit feeder and it has been observed to leave its burrow to forage on the sediment surface. It has the ability to tolerate a wide range of salinities (3 - 31°/oo) and sediment types (DeWitt et al., 1992). It has been used for both the acute and chronic toxicity testing of marine and estuarine sediments and is recognized by ASTM as a marine sediment bioassay species (Schlekat et al., 1991, DeWitt et al., 1992, and ASTM, 1995)

Phenanthrene

Phenanthrene is a constituent of a class of petroleum compounds identified as polycyclic aromatic hydrocarbons (PAH), which are naturally occurring, fused-ring, aromatic compounds whose primary pre-industrial route of entry into the environment was via oil seeps and forest fires. As a component of fossil fuels and a product of its combustion, modern day sources of PAH are numerous and plentiful (Neff, 1979). Generally, PAHs are considered a signature of human activity as a result of industrial processes, however, localized accumulation may results from natural events such as forest fires, volcanism, and petroleum seeps (Meador et al., 1995b). PAHs are ubiquitous in the marine environment and occur at their highest concentration near urban centers of human population (LaFlamme and Hites, 1978). The composition and typical depth profiles of PAHs in sediment correspond to anthropogenic fossil fuel utilization. The primary sources of PAHs for these aquatic ecosystems is thought to be atmospheric deposition of PAHs produced from pyrolysis of fossil fuels and the accidental discharge of petroleum products directly into the aquatic systems (McElroy et al., 1990).

Because of their hydrophobic nature, PAHs released into the aquatic environment rapidly become associated with particles, and are deposited in sediments (Gearing et al., 1980). The importance of sediments as reservoirs for PAHs has been well documented (McElroy et al., 1990). Small-scale physical and biological processes such as local turbulence and sediment-mixing by burrowing organisms, and large-scale physical forces

such as tides and currents which redistribute particulate material will also affect the distribution of PAHs.

In a recent assessment of the distribution of chemical contaminants in US coastal and estuarine sediments (Daskalakis and O'Connor, 1995), a dataset containing nearly 13,500 coastal sediment samples was evaluated to determine the relative extent of elevated levels of various contaminants. For PAHs, a total of 5.5% of all sites contained concentrations of PAHs above a operationally defined "5 x high" level, a level thought to be associated with adverse biological effects. These sites were not evenly distributed along coastal waters, but were rather clustered in relatively small, industrial and urban areas. The greatest concentrations of these "5 x high" sites were in locations with high ship traffic, industrial activity, and relatively poor water flushing, as is the case of harbors, canals, and intracoastal waterways (Daskalakis and O'Connor, 1995).

Phenanthrene is a major component of the PAH assemblage in sediments (Barrick, 1982). Because of its intermediate solubility (1.0 ug/ml) and volatility (vapor pressure 6.8 x $10^{-4}$ mm Hg), phenanthrene is an excellent model compound for studying PAHs (Guerin and Jones, 1989). Although itself noncarcinogenic, phenanthrene is the smallest of the angular PAHs possessing the so-called bay region which is characteristic of the carcinogenic members of this class of hydrocarbons (Williams and Weisburger, 1991). Phenanthrene has a three ring structure and exists as colorless leaflets (EPA, 1991).

The PAH phenanthrene was selected for use in this research because it is an important component of petroleum mixtures and has physical/chemical characteristics similar to other hydrocarbon contaminants found in aquatic systems. Phenanthrene has been shown to be acutely toxic to the amphipods *Eohaustorius estuarius* and *Leptocheirus plumulosus* (EPA, 1991) and *Diporeia* spp. (Landrum et al., 1994). Both acute and sublethal effects have been reported for the oligochaete *Limnodrilus hoffmeisteri* (Lotufo and Fleeger, 1996) and growth and reproductive effects observed for the polychaete *Neanthes arenaceodentata* (Emery and Dillon, 1996) following exposure to phenanthrene spiked sediments. Additionally, phenanthrene has been widely used as a model chemical to represent PAHs as a class and other hydrophilic hydrocarbon pollutants. Studies have been conducted on the kinetics and mechanism of phenanthrene's sorption to dissolved and particulate organic matter (Piatt et al., 1996, Brunk et al., 1997), desorption from sediments (Helmstetter and Alden, 1994, McGroddy et al., 1996), and biodegradation in estuarine sediments (Guerin

and Jones, 1989) to evaluate hydrocarbon fate, transport, and persistence in aquatic systems.

The direct mechanism of phenanthrene's toxicity to invertebrates is unknown. However it is hypothesized that it exerts it's toxic effects through a reversible dysfunction called narcosis. The chemicals exhibiting this form of toxicity, termed nonpolar narcotics, are a group of organic compounds that are widely used in industry and are common environmental pollutants (Pawlisz and Peters, 1993). These chemicals do not affect any specific organ, organ system, or biochemical pathway. The exact mechanism of narcosis is not known, but the leading theories propose that narcotic molecules disrupt cell membranes, enzymes, or other organic structures to which they adsorb (Suter, 1993). This induces paralysis and subsequent death in exposed organisms.

## 1.6 SUMMARY

Contaminated sediments have become a demonstrated environmental concern and petroleum products have been identified as a major source of sediment pollution. The PAH phenanthrene is a suitable model for these types of chemical contaminants. To assess the threat posed by contaminated sediments, it is necessary to have an understanding of the biological, physical, and chemical factors that influence the bioavailability of the contaminant in question. Under regulatory and research programs, hazard assessments that use benthic amphipods for sediment toxicity testing have increased. This research was conducted to evaluate the biological, and physio/chemical processes at work in determining the bioavailability of the PAH phenanthrene to assist the refinement of sediment assessment techniques and theories at both operational and theoretical levels.

# CHAPTER 2

# EFFECT OF BURROWING BEHAVIOR ON THE BIOACCUMULATION OF SEDIMENT-ASSOCIATED PHENANTHRENE BY THREE SPECIES OF MARINE AMPHIPODS

## 2.1 INTRODUCTION:

Sediments are the ultimate sink for a variety of pollutants. Chlorinated hydrocarbons, petroleum hydrocarbons, nutrients, metals, and oxygen demanding substances can be found in freshwater and marine sediments throughout the world (International Joint Commission, 1988, EPA, 1987). While elevated levels of contaminants can, in some cases, be tied to natural processes, most often they are due to anthropogenic activities. Contaminated sediments pose risks to aquatic ecosystems through both direct and indirect means. Benthic and epibenthic organisms may be directly exposed to contaminated sediment particles or contaminated interstitial water. The contaminant may cause mortality in resident biota, rendering an area uninhabitable, or may selectively affect sensitive populations in a community, changing the community's structure and function. Aquatic organisms may also be indirectly exposed to sediment contaminants through trophic transfer and the impact of such contamination can be felt at upper levels of aquatic food webs. Public health is also endangered through the processes of bioaccumulation and trophic transfer to commercially and recreationally harvested species. There are numerous examples of cases where fish/shellfish consumption warnings have been issued for pollutants such as PCBs, mercury, dioxin, kepone, and others due to contaminated sediments (EPA, 1987, EPA, 1989a).

The risks posed by this reservoir of contaminants is dependent upon the factors that govern the bioavailability of the contaminants (Swartz and Lee, 1980). It is the bioavailability of a contaminant that determines the concentration of a particular chemical to which biota is exposed to. Increasing our understanding of the factors affecting the bioavailability of sediment-associated contaminants, including evaluating the influence of physical-chemical characteristics of the sediment and the biological behavior of the resident biota, is necessary to more accurately assess the risks posed by contaminated sediments. Biological processes that influence the transfer of contaminants from sediment include organism behavior, modes and rates of feeding, and sources of water for respiration and

feeding. These processes can work to increase and/or decrease exposure to sediment contaminants. For example, preferential feeding upon fine, organic rich sediment particles rather than bulk sediment can increase exposure because of the higher contaminant concentrations associated with these sediment fractions. In contrast, active irrigation of living tubes and burrows for feeding and respiration may work to decrease the concentration of contaminants in the microhabitat surrounding the organism. This research was conducted to investigate the degree to which the respiratory behavior (via irrigating overlying water into sediment) and the tube or burrow building strategy employed by benthic infaunal invertebrates could influence the exposure and bioaccumulation of sediments-associated petroleum hydrocarbons.

Background:

Sediments are the organic and particulate matter that settle out of all bodies of water. The physio-chemical characteristics of sediments allow for the concentration of persistent aquatic pollutants to much higher levels than seen in the water column. Pollutants will adsorb onto particles and/or organic components of the sediment and the sediment may act as a temporary or long term sink for these pollutants (Wakeham and Farrington, 1980). This contaminated sediment can become a long term reservoir for pollutants and serve as a source of contamination long after the original source of pollution has been eliminated (Swartz and Lee, 1980).

While contamination of sediments has been recognized as a potential environmental problem for many years, it has only been in the last decade or so that systematic surveys have been performed to investigate the extent of the contamination (EPA, 1987, International Joint Commission, 1988, and NOAA, 1990). One factor which has hampered such efforts was the lack of national guidelines and a well developed scientific basis for determining what levels of various pollutants in sediments constitute a problem (EPA, 1987). Recent examinations of the bioaccumulation and toxicity of sediment contaminants to benthic invertebrates have shown, in general, that they are better correlated with dissolved contaminant concentrations in interstitial water than with bulk sediment concentrations (EPA, 1989b, Di Toro et al., 1991) In addition, experiments quantifying the concentration-response curve for a biological effect of concern found that the response could not be correlated with the bulk sediment concentration, but correlated well with the concentration of contaminant in interstitial water and in sediment on a sediment-organic

carbon basis. Furthermore, the interstitial water concentration-response curves resembled the concentration-response curves from water-only exposure.

This analysis led to the development of the "equilibrium partitioning" (EqP) theory for the development of national sediment quality criteria for nonionic chemicals. EqP is used to predict interstitial concentrations of organic chemicals based on distribution coefficients between particle bound and dissolved phases (Swartz et al., 1990, Di Toro et al., 1991). This theoretical model uses organic-carbon normalized partition coefficients for individual nonpolar organic compounds to establish chemical concentrations in sediments that will yield concentrations in the interstitial water that are equivalent to established water quality criteria. Assuming that such a system is in equilibrium, existing federal ambient water quality criteria can be used as a threshold in interstitial water above which biological effects would be expected in benthic populations. Therefore, based upon sediment organic carbon content, bulk sediment concentrations of specific chemicals can then be calculated to determine sediment specific criteria (Shea, 1988, EPA, 1989b). This approach makes several other important assumptions: it is assumed that the partitioning of nonpolar organic chemicals can be explained on the basis of organic carbon content of the sediment; it assumes that water column organisms, used to develop the ambient water quality criteria, have the same sensitivities to toxicants as benthic organisms; and that the ingestion of sediment particles by the organisms will not significantly effect the distribution of a chemical within the organic carbon - interstitial water - benthic organisms matrix (Di Toro et al., 1991).

A key principle of EqP is that benthic organisms are exposed to a constant concentration of chemical in the interstitial water. However, certain benthic species have the ability to dilute interstitial water by ventilating its microhabitat with overlying water (Lee, 1991). Several studies in the field of geochemistry have demonstrated the ability of certain benthic organisms to decrease the concentration of dissolved chemicals (e.g. $NH_4^+$, $HS^-$, Fe) in either the water within burrows, or in the interstitial water of bulk sediments. (Aller, 1978, Aller et al., 1983, Wasenchuk et al., 1983, and Matisoff et al., 1985). This process could have a dramatic effect on animal exposure to any contaminant for which interstitial water is the dominant uptake route e.g. relatively soluble polycyclic aromatic hydrocarbons (PAHs) and cadmium (Kemp and Swartz, 1988, Landrum and Robbins, 1990).

The ventilation of overlying water through the portion of the sediment inhabited by the individual amphipod is done for respiration. Additionally, some burrow or tube building

species can use this same ventilatory behavior to assist in filter feeding. Differences in burrowing behavior have been implicated as a potential cause for observed differences in sensitivities among species of amphipods exposed to contaminated sediments (Swartz et al., 1990) and ASTM (1995) has raised this concern in its guidance on selecting appropriate amphipod species to be used in sediment bioassays. However, there has not been any quantitative investigation linking the ventilatory behavior of individual amphipods and differences in burrowing behavior among different amphipod species with changes in contaminant exposure. For this reason, I propose to compare and contrast exposure among three species of amphipods by relating differences in tissue residue and uptake kinetics to known differences in burrowing behavior. The three species of amphipods chosen for this research were selected based upon known differences in burrowing behavior and are: *Eohaustorius estuarius*, a mobile free-burrowing amphipod, *Leptocheirus plumulosus*, a sedentary, soft-walled burrow building amphipod, and *Grandidierella japonica*, a sedentary tube-building amphipod

Research objectives:

The primary objective of my research is to determine to what extent the burrow-irrigating behavior exhibited by benthic marine amphipods can affect exposure to interstitial water borne contaminants. The secondary objective is to determine whether differences among the burrowing behaviors exhibited by the selected species of amphipods could be correlated with differences in the bioaccumulation of the contaminant.

The ability of the irrigating and burrowing behavior of benthic invertebrates to cause differences in exposure to sediment-associated contaminants was investigated by exposing the three amphipod species to the PAH phenanthrene under two exposure conditions. The first involved exposing the three amphipod species to sediment spiked with $^{14}$C-phenanthrene and uncontaminated overlying water. The second exposure treatment involved exposing the three amphipod species to sediment spiked with $^{14}$C-phenanthrene with the overlying water concentration of $^{14}$C-phenanthrene maintained at equilibrium with interstitial water. Therefore, in the second exposure treatment, the concentration of phenanthrene in the interstitial water surrounding the organisms (the exposure concentration for the amphipod) would not be affected by the introduction of overlying water into the sediment.

It was expected that if the irrigation of clean overlying water into the sediment for respiration was decreasing the concentration of phenanthrene in the interstitial water surrounding the amphipod (via dilution), the tissue residue of phenanthrene in the amphipods exposed in the contaminated overlying water treatment would be greater than those exposed in the clean overlying water treatment. To evaluate whether the tube or burrow building behavior exhibited by individual amphipod species could effect the exposure to and accumulation of sediment-associated phenanthrene, the magnitude of the difference in tissue residues between the treatments (expressed as a ratio of tissue concentrations between exposure treatments) was investigated. It was expected that the species able to cause the greatest difference in exposure concentrations between treatments would consequently exhibit the greatest magnitude of difference in the tissue residues found in the amphipods between exposure treatments. The tube or burrow that is built in the sediment would facilitate the movement of overlying water into the sediment by providing a direct connection to the overlying water. It is expected that an organism residing in such a tube or burrow would be able to pump a relatively greater amount of overlying water into the sediment than its free-burrowing counterpart. In one of the exposure treatments, uncontaminated overlying water is available to dilute the concentration of $^{14}$C-phenanthrene in interstitial water and in the other, the overlying water is maintained at the equilibrium predicted concentration of interstitial water, therefore, the pumping of overlying water into the sediment should have no effect on the interstitial water concentration of $^{14}$C-phenanthrene. The ratio of tissue concentrations between exposure treatments can then be used to compare the relative effect of tube building, burrow building, and non-structure forming behaviors among the three amphipod species selected for use in this research.

## 2.2 MATERIALS AND METHODS

Experimental Animals:

*Leptocheirus plumulosus* and *Grandidierella japonica* were obtained from ongoing cultures maintained at the USEPA laboratory facility at Newport, OR. *Leptocheirus plumulosus* and *Grandidierella japonica* were obtained prior to the initiation of the bioaccumulation experiments by sieving culture bins with a 0.5-mm mesh-diameter screen to retain suitable adult amphipods. *Eohaustorius estuarius* was manually collected from the upper 10 cm of intertidal, estuarine sands of Yaquina Bay, Newport, OR. The amphipods

were collected by trowel at low tide and sieved from their native sediments with a 1.0-mm mesh-diameter screen. All amphipods were presorted prior to use. Only non-gravid adult amphipods were used to avoid gender specific total lipid content differences due to the presence of eggs or brood pouches in gravid females.

Sediment:

Clean estuarine sediment was collected from McKinney Slough located in Alsea Bay, Waldport, Oregon. The field sediment was washed sequentially through a 1.0-mm mesh-diameter screen and a 0.25-mm mesh diameter screen to remove indigenous macroinvertebrates. The organic carbon content of the sediment was determined by acidifying and drying the sediment and analyzing on a Perkin-Elmer CHON analyzer. Percent total solids was determination was made by measuring weight loss of samples dried at 90°C for 12 hours and 110°C for one additional hour. Based on this data, the sediment was spiked to reach a target whole sediment concentration of 37 ug/g organic carbon (OC) dw phenanthrene. The sediment was spiked by lining 1 gallon glass jars with $^{14}$C-phenanthrene in an acetone carrier and allowing the acetone to volatilize completely out of the glass jar. This was done to avoid any carrier affect caused by residual levels of acetone in the sediment subsequent to the introduction of the $^{14}$C-phenanthrene. The jars were then filled with the appropriate mass of sediment and placed onto a rolling mill. Sediments were rolled for 2 hours in the dark at room temperature and allowed to settle overnight at 4°C and then rolled an additional 2 hours. The sediments were then allowed to equilibrate for 28 days at 4°C in the dark before use (Ditsworth et al, 1990, Swartz et al., 1990).

Chemicals:

$^{14}$C-Phenanthrene (PHE), labeled on carbon-9, was obtained from Sigma Chemical Company (St. Louis, MO). The specific activity of the $^{14}$C-PHE was 8.3 mCi/mmol and was reported by the manufacturer to be at least 98% radiopure.

Experimental Design - Bioaccumulation Experiments:

For these series of experiments, the exposures were conducted in two separate treatments. In the first, the amphipods were exposed to contaminated sediment with clean overlying water. In the second, the amphipods were exposed to contaminated sediment with the overlying water containing the same concentration of phenanthrene as the sediment interstitial water.

*Exposure Chambers*:

The exposure were carried out in precleaned 400 ml glass beakers containing approximately 85 g of sediment and 300 ml of overlying water at a salinity of 28 °/oo. The test chambers were all covered with a pyrex watch glass to reduce contamination of the contents and evaporation of the water and test material over the course of the experiment. The overlying water was replaced every eight hours in each beaker on an static renewal basis. The old water was carefully siphoned out with a screened siphon to avoid removing any amphipods from the exposure beakers and the new water gently added to avoid resuspension of the sediment in the exposure vessel. For the clean overlying water exposure treatment, fresh filtered sea water at 28 °/oo salinity was used in the overlying water exchanges. For the contaminated overlying water exposure treatment, a fresh batch of water was made up for each water change following this protocol. A stock $^{14}$C-phenanthrene solution was prepared by adding an appropriate volume of $^{14}$C-phenanthrene in an acetone carrier to a pre-cleaned 2-liter volumetric flask. The acetone was then completely evaporated off, again to avoid any carrier effects in the treatment, and 2-liters of fresh filtered sea water at 28 °/oo added. The flask was then placed on a stir plate with a Teflon stir bar and gently agitated for two hours prior to use. This stock was be diluted down to the target $^{14}$C-phenanthrene concentration in sea water immediately before addition to the exposure chambers.

*Sampling Protocol*:

Three days prior to the initiation of the exposure treatments, approximately 400 amphipods of each test species were collected and readied for acclimation to experimental conditions. Field collected *Eohaustorius estuarius* were transferred to an aerated plastic tub containing native Yaquina Bay sand, fresh filtered sea water at 28 °/oo salinity, and

maintained at 20°C. *Leptocheirus plumulosus* and *Grandidierella japonica* were obtained from ongoing cultures and were transferred to an aerated plastic tub containing clean McKinney Slough sediment (same sediment as the test). For *Grandidierella japonica* the salinity in the acclimation tub was maintained at 28 °/oo and the temperature at 20 °C. For *Leptocheirus plumulosus* which is maintained at a salinity of 20 °/oo in culture, the acclimation involved sequentially increasing the salinity in the acclimation tub by a few ppt per day until it reached the test salinity of 28 °/oo. All acclimation tubs were feed daily with a mixture of algae and powdered dry food (DeWitt et al., 1992).

One day before the initiation of the exposure, the test sediment and clean overlying sea water were added to all exposure chambers. This was done to allow suspended sediment particles to settle and to allow time for equilibration of temperature and the sediment-water interface. After the overnight equilibration, the overlying water in each chamber was carefully replaced with either clean sea water or sea water containing 2.5 ug/L $^{14}C$ - phenanthrene two hours prior to the initiation of the exposure treatment. At the initiation of exposure, 20 amphipods were distributed to each of the test chambers and additional clean or spiked sea water added to bring the water level up to the 350-ml level.
There were three replicates beakers per species at each sampling time, and three clean sediment control chambers per species were set up to evaluate mortality and the lipid content at the end of the exposure treatment. There were 5 sampling times for each exposure treatment. The amphipods were sampled at 4, 8, 24, 48, and 72 hours after initiation of exposure. At the time of sampling, the amphipods from each exposure chamber were sieved out of the contaminated sediment using a 500 um mesh-diameter screen and placed in clean sea water for 15 minutes to remove any adhering particles. The amphipods were then blotted dry and placed in polyethylene tubes and frozen at -80 °C until analysis. Table 2.1 presents a summary of the experimental conditions for the bioaccumulation experiments.

Table 2.1: Experimental Parameters for Bioaccumulation Experiments

| | |
|---|---|
| Salinity | 28 °/oo |
| Temperature | 20 °C |
| Exposure Chamber | Covered 400-ml Glass Beakers |
| Overlying Water Renewal Period | 8-Hours |
| Light Cycle | 24-Hour Dark |
| Exposure Time | 72 Hours Total; 5 Sampling Time Points |

Experimental Design - Elimination Experiments:

An additional study was conducted with each species to derive elimination constants ($k_2$'s) for use in the first order uptake model for each species. The elimination rate constants are determined from the elimination rate of the pollutant after the organism has been placed in a clean environment. This study was performed in two phases, first an accumulation phase and second an elimination phase which included the sampling and analysis of amphipods.

In the accumulation phase of the study, each species of amphipods was exposed to 2.5 ug/L $^{14}$C-phenanthrene over 72-hours in an water only exposure system. The water in the exposure vessels (400-ml pre-cleaned glass beakers) were maintained at a relatively constant concentration by a static renewal system with 90% water changes every eight hours. For each species, 14 beakers containing 20 amphipods per beaker were used in the accumulation portion of the study. The exposure vessels contained 15 pieces of 1/3" - 1/2" diameter glass tubing cut to 2" lengths. This glass tubing was added to the beakers to provide refugia for the normally sediment dwelling amphipods. The intent was to decrease the biological activity of the amphipods by reducing swimming in the water column and to decrease cannibalism in the *Grandidierella japonica* . The experimental conditions (e.g. temperature, salinity, light regime) were the same as those presented for the bioaccumulation experiments (Table 2.1).

The elimination of $^{14}$C-phenanthrene was determined by following the decrease of tissue residues of $^{14}$C-phenanthrene in the amphipods after being placed in clean sediment. After exposure the amphipods were placed into beakers containing approximately 85 grams of

clean McKinney Slough sediment with 300 ml of clean overlying sea water. Amphipods from each species were placed in triplicate beakers for each sampling time. The sampling times for this portion of the study were 1, 2, 4, 8, 24, and 48 hours after being removed from the exposure water. In the elimination portion of the study, there were triplicate beakers for each time point containing 15 amphipods each. Similar to the bioaccumulation experiments, the amphipods from each exposure chamber were sieved out of the contaminated sediment using a 500 um mesh-diameter screen and placed in clean sea water for 15 minutes to remove any adhering particles at each sampling time. The amphipods were then blotted dry and placed in polyethylene tubes and frozen at -80 °C until analysis. As in the bioaccumulation study, separate control beakers were set up to evaluate lipid content changes and survival over the course of the experiment. At the beginning of the exposure, four beakers containing 20 amphipods each were set up for each species. During the accumulation phase, these beakers were renewed with fresh clean sea water every eight hours. At the conclusion of the accumulation portion of the study, two beakers were sampled for mortality and lipid content to serve as baseline data for the elimination phase. Two beakers with clean sediment were set up for each species and these were sampled at the end of 48 hours for determining overall survival and total lipid content of the amphipods.

Chemical Analysis:

Overlying water samples were collected from the exposure beakers by taking 2 ml samples and placing in LSC vials with 18 ml of Optiflour scintillation cocktail for aqueous samples (Packard Instrument Co.). Overlying water samples were taken from duplicate beakers for each species at the start of the exposure and before and after each water change. The activity (dpm) of the water samples were counted on Packard Tri-Carb 2000CA Liquid Scintillation Analyzer with external quench control and the actual concentration or mass of $^{14}$C-phenanthrene was quantified according to sample volume and specific activity of the $^{14}$C-phenanthrene.

Total $^{14}$C in non-aqueous samples were determined using a Packard Tri-Carb sample oxidizer (Model 307A, Packard Instrument, Co.). The $^{14}CO_2$ was trapped in Carbo-Sorb (Packard Instrument, Co.) using a volume of 9 ml for sediment and 6 ml for amphipods. The Carbo-Sorb was mixed with scintillation cocktail (12 ml Permofluor E, Packard Instrument, Co.) and quantified by scintillation counting. The sediment was collected in 3-

4 gram aliquots collected from the dedicated chemistry beaker at the beginning and end of exposure. Triplicate samples of approximately 0.2 grams of sediment were analyzed from each of the sediment samples by thawing and placing in paper combustion cones. Cellulose powder and combust-aide (Packard Instrument, Co.) were added to each sample to assist in sample combustion and complete oxidation. Recovery and sample memory were quantified prior to each analysis run by conducting performance verification test with Packard $^{14}C$ Spec-Chec (Packard 1990). Recovery of $^{14}C$ exceeded 95% and memory was less than 0.15%. A $^{14}C$ standard ($^{14}C$-methyl mathacrylate chip, E.I. du Pont de Nemours and Co.) was combusted at the end of each analysis run to confirm $^{14}C$ recovery.

Chemical analysis of amphipod samples was conducted by taking 0.05 gram (wet weight) samples, approximately 4 individual amphipods, and placing in paper combustion cones. Cellulose powder and combust-aide (Packard Instrument, Co.) were added to each sample to assist in sample combustion and complete oxidation.

The interstitial water was collected by placing 35 g of sediment into precleaned 50 ml pyrex centrifuge tubes and centrifuging at 10,000 rpm for 60 minutes. Then, duplicate 2 ml samples were taken of the overlying water in each tube for analysis by LSC. The samples for interstitial water and bulk sediment analysis were collected from triplicate chemistry control beakers (containing no amphipods) that were set up at the beginning of the experiment. For the T=0 hour data, the chemistry control beakers were loaded with sediment as with the experimental beakers and placed with clean overlying water overnight. These beakers were then taken down for sampling sediment and interstitial water prior to the start of exposure. For the T=72 data, the chemistry control beakers were maintained exactly the same as the experimental beakers and were taken down at the conclusion of the experiment.

Lipid Analysis:

The percent total lipids were determined for each species of amphipods extracting with chloroform:methanol previously freeze dried amphipod samples. The percent lipids were then determined by a microgravimetric technique (Gardner et al., 1985). The total percent lipid (dry weight) were measured for each species and exposure treatment at the beginning and end of exposure. The amphipods collected at the end of the exposure were subject to

the same experimental conditions as the test organisms, however they were not exposed to $^{14}C$-phenanthrene.

Three composite samples of 20 amphipods each were selected for each species and frozen at -80°C until analysis. For each species, three separate beakers were set up with uncontaminated McKinney Slough sediment. 20 amphipods were added to each of these beakers and these beakers served the dual purpose of a negative control (to evaluate non-contaminant related responses to test sediment) and to determine the loss of lipid (if any) in the experimental animals over the course of the 72-hour exposure. The lipid/negative control beakers were maintained exactly the same manner as the experimental beakers i.e. 8-hour water changes, same temperature and salinity and light regime. In determining the percent lipid content for use in lipid normalizing the tissue concentration of phenanthrene for each exposure treatment, the mean lipid content was calculated from the results obtained from the beginning and end of the of the exposure period.

Wet to Dry Weight Normalization:

Wet weight to dry weight ratios regression models were developed for each amphipod species from a data set including wet weight and corresponding dry weights from individual organisms and from composites of five to ten animals. To develop these regression models, the dry weights and wet weights were plotted and a simple linear regression line drawn through all of the data points. The following regression were used for each amphipod species to convert wet weight data to dry weight.

| | | |
|---|---|---|
| *Eohaustorius estuarius* (n=16) | $y = 0.221x + 0.547$ | $r^2 = 0.987$ |
| *Leptocheirus plumulosus* (n=14) | $y = 0.243x + 0.056$ | $r^2 = 0.995$ |
| *Grandidierella japonica* (n=19) | $y = 0.256x - 0.037$ | $r^2 = 0.978$ |

Where y = wet weight and x = dry weight.

Data Analysis:

*First Order Accumulation Models:*

A first order bioaccumulation model was used to describe the uptake of $^{14}C$-phenanthrene by amphipods. The first order model has been used to estimate tissue residue resulting from exposure to dissolved (water-borne) contaminants. The objectives of the modeling effort was to compare uptake coefficients between exposure treatments and to compare the linear versus non-linear methods for calculating uptake coefficients. In this model, the change in tissue concentration with time is calculated by:

$$dC_t/dt = k_1C_w - k_2C_t \qquad (1)$$

Where:

$C_t$ = tissue residue (ug/g tissue);
$C_w$ = pollutant concentration in water (ug/g water);
$k_1$ = uptake rate coefficient (mL water/g tissue-time);
$k_2$ = elimination rate constant ($time^{-1}$);
t = time.

Initial uptake and elimination rates determined in short-term experiments can be used to predict steady-state bioconcentration factors (BCFs) and the exposure time necessary to reach steady-state.

Elimination of pollutants from tissues was modeled by:

$$C_tt = C_{t0}(e^{-k2t}) \qquad (2)$$

Where:

$C_{t0}$ = tissue residue at the start of the elimination phase ($t_0$).

The $k_1$ and $k_2$ values used in these equations can be estimated by fitting equation (1) to measured tissue residue values using an iterative least-squares nonlinear regression method. Alternatively, $k_1$ can be estimated from the rate of increase in tissue residue measured in short-term uptake experiments during the initial (linear) phase of contaminant uptake when tissue residue concentrations are low and elimination minimal. The elimination rate constant is often determined by depurating contaminated organisms in

uncontaminated sediment and determining $k_2$ directly. As the model assumes that $k_2$ is not a function of tissue residue, it is possible to determine $k_2$ values for test organisms that have been exposed to contaminants in short-term uptake experiments.

Both the linear and non-linear method for deriving uptake rate constant estimates have been reported in the literature (Boese et al., 1997). While both of these methods should give similar results, the non-linear regression method has the advantage of using a greater proportion of the experimental data. It is not limited to strictly the linear portion of the uptake curve. The accuracy of the $k_1$ estimates will be assesses by calculating the predicted steady state concentration from the uptake constant estimates. The type of data collected in this series of experiments can be used to assess the accuracy of the regression methods.

The predicted steady state concentration can be derived by re-arranging equation (1). When steady-state tissue residues are attained, $dC_t/dt = 0$ and equation (1) can be written as:

$$k_1 C_w = k_2 C_t \qquad (3)$$

By re-arranging (3) to solve for $C_t$, we get the following expression:

$$C_t = C_w k_1 / k_2 \qquad (4)$$

Where:

$C_t$ = tissue residue (ug/g tissue);
$C_w$ = pollutant concentration in water (ug/g water);
$k_1$ = uptake rate coefficient (mL water/g tissue-time);
$k_2$ = elimination rate constant ($time^{-1}$).

Additionally, Boese et al. (1997) reports that the time ($t_f$) required to reach a given Fraction (F) of $C_t$ is :

$$t_f = -\ln(1-F)/k_2 \qquad (5)$$

The time required to attain 95% of steady state (F=0.95) has been used as a practical estimate of the steady-state exposure time (Boese, 1990, Lee and Boese, 1993). Therefore, with F=0.95, equation (5) becomes:

$$t_f = 2.99/k_2 \qquad (6)$$

*Linear Regression Accumulation rates:*

Uptake rate constants ($k_1$) were estimated from short-term uptake experiments with the linear regression of tissue residue (lipid normalized mg/g lipid dry weight) on exposure time (hours). As this method is used only on the linear portion of the uptake curve, the time points to be included in the regression were selected by a visual analysis of the uptake data and an analysis of the $R^2$ values of the regression lines. Generally, only those data in the linear uptake portion of the uptake curve were utilized and the data set with the highest $R^2$ value among those calculated was selected for use.

*Non-linear Regression Accumulation Rates:*

Uptake rate constants ($k_1$) were also estimated by fitting equation to tissue residue data from short-term uptake experiments with the experimentally derived $k_2$ constraining the non-linear regression model. Generally all of the timepoints were included in this method, however in situations where there was a significant decrease in tissue residue at the end of the exposure period, the latter time point data were not included in the $k_1$ estimation. A iterative, nonlinear, least-squares curve fitting technique (SigmaStat, Jandel Scientific Software, San Rafael, CA) was used.

*Elimination Rates Constants ($k_2$):*

The elimination data is modeled as an exponential function. The elimination rate coefficient ($k_2$) is calculated by fitting a linear regression to the natural log of the tissue residue versus time. The elimination coefficient is determined as the absolute value of the slope of this regression line and has the units time$^{-1}$. The elimination rate is determined from a linear regression of the natural log transformed tissue residue data from the elimination study. Generally, all of the data were used in deriving $k_2$ estimates, however, in situation where the natural log transformed data deviated from linearity, a subset of the data was used to generate $k_2$ estimates by trying to maximize $R^2$ values of the regression lines.

*Statistics:*

The mean body burdens of $^{14}$C-phenanthrene between the two exposure treatments for each species were compared by performing a paired student's *t*-test at each time point. Differences between means and slopes were considered significant when $p < 0.05$. Standard deviations (SD) are reported to show the range in the data and the standard error of the mean (SEM) is reported when comparisons of the means are intended.

The regression coefficients generated by the linear and non-linear regression models were compared to determine whether a significant difference exists by Spjotvoll-Stoline T'-method (Sokal and Rolfe, 1981). Differences between regression coefficients were considered significant when $p < 0.05$.

## 2.3 RESULTS:

Interstitial Water, Overlying Water and Sediment Concentrations of $^{14}$C-phenanthrene:

The target interstitial water concentration of phenanthrene for the two exposure treatments was 2.50 ug/l. In both exposure treatments, the bulk sediment was spiked with the appropriate mass of $^{14}$C-phenanthrene and the measured interstitial water concentration of $^{14}$C-phenanthrene after 28-days of equilibration was very close to the target. The amount of radiolabeled compound measured in the interstitial water, compared to the nominal or target concentration, was 94% (2.35 ug/l T=0) for the spiked sediment only exposure and 106.4% (2.66 ug/l T=0) for the spiked sediment and overlying water exposure. The measured interstitial water concentrations of $^{14}$C-phenanthrene at the beginning and end of the accumulation experiments are presented in Table 2.2. The target interstitial water concentration of 2.5 ug/L was selected as a exposure concentration for these amphipods because this concentration was 1/20-1/50 of the reported $LC_{50}$ for phenanthrene for these amphipods (EPA, 1991). As the data in Table 2.2 indicates, there was no significant change in the interstitial water concentration of $^{14}$C-phenanthrene regardless of whether the overlying water was maintained clean or spiked at the equilibrium predicted interstitial water concentration of $^{14}$C-phenanthrene. There appeared to be a slight increase in the interstitial water concentration from 2.35 ug/L to 2.67 ug/L $^{14}$C-phenanthrene when the overlying water was renewed with clean sea water and a slight decrease in the measured interstitial water concentration from 2.66 ug/L to 2.50 ug/L $^{14}$C-

phenanthrene when the overlying water was renewed with sea water containing $^{14}$C-phenanthrene.

The target bulk sediment concentration of $^{14}$C-phenanthrene was 37 ug/g OC dw. The sediment concentration of phenanthrene was measured to be 35.09 ug/g OC dw at T=0 and 35.79 ug/g OC dw at T=72 for the sediment only exposure and be 41.87 ug/g OC dw at T=0 and 43.00 ug/g OC dw at T=72 for the sediment and overlying water exposure. While the bulk sediment concentration was slightly above the target in the sediment and spiked overlying water treatment, the interstitial water concentration in this treatment was still very close to the target.

Table 2.2. Interstitial Water (IW) and Sediment Concentrations of $^{14}$C-phenanthrene.

| Treatment | T=0 (SD) | T=72 (SD) |
|---|---|---|
| Interstitial Water - (IW) | 2.35 ug/L (0.17) | 2.67 ug/L (0.20) |
| Interstitial Water - (IW+OW) | 2.66 ug/L (0.27) | 2.50 ug/L (0.10) |
| Sediment Conc. - (IW) | 35.09 ug/g OC dw (1.99) | 35.79 ug/g OC dw (2.29) |
| Sediment Conc. - (IW+OW) | 41.87 ug/g OC dw (1.94) | 43.00 ug/g OC dw (1.01) |

IW = Contaminated Sediment and Clean Overlying Water Exposure Treatment
IW + OW = Contaminated Sediment and Spiked Overlying Water Exposure Treatment

The measured concentration of $^{14}$C-phenanthrene in overlying water before and after the scheduled water changes, for both exposure treatments, are presented in Table 2.3. In the clean overlying water treatment, the concentration of $^{14}$C-phenanthrene measured after the addition of clean fresh sea water was very low ranging from 0.12 ug/L to 0.19 ug/L for all three species. The small amount of $^{14}$C-phenanthrene measured probably reflects the resuspension of sediment into the water column occurring during the course of the overlying water exchange. However, there was a species specific difference in the measured concentration of $^{14}$C-phenanthrene in the overlying water prior to the scheduled water changes (i.e. after the exposure chambers had been allowed to sit for seven-hours). In this case, there was almost no difference in the measured overlying water concentration for *Eohaustorius estuarius* before and after the water exchange (0.15 ug/L vs. 0.14 ug/L $^{14}$C-phenanthrene). *Grandidierella japonica* exhibited a slight increase in $^{14}$C-phenanthrene prior to the water exchange and *Leptocheirus plumulosus* had an even greater increase in

$^{14}$C-phenanthrene concentration in overlying water prior to the water exchange. This was most likely because of the amount of sediment resuspended by these amphipods in the exposure beakers. *Eohaustorius estuarius* being a free burrower, bioturbated the sediment least and *Leptocheirus plumulosus* being a generally larger, more active species, at least on the sediment surface, noticeably resuspended a greater amount of sediment into the water column than the other two species. In any case, the concentrations of $^{14}$C-phenanthrene in the overlying water was still much less than the interstitial water concentration.

There were species specific differences in the spiked overlying water concentration of $^{14}$C-phenanthrene prior to water renewal. During the time between water renewals, the $^{14}$C-phenanthrene was depleted from the overlying water at a greater rate for *Leptocheirus plumulosus* which exhibited a 36% decrease in the $^{14}$C-phenanthrene while on average the decrease was 15% for *Eohaustorius estuarius* and 21% for *Grandidierella japonica.* For all three species, the $^{14}$C-phenanthrene concentration in the overlying water after the water exchange was very close to the target of 2.50 ug/L.

Table 2.3. Overlying Water Concentrations of $^{14}$C-phenanthrene Before and After Renewal in ug/L.

| Treatment | Species | Before Renewal (SD) | After Renewal (SD) |
|---|---|---|---|
| Overlying Water - (IW) | *E. estuarius* | 0.15 (0.08) | 0.14 (0.07) |
| Overlying Water - (IW+OW) | *E. estuarius* | 2.22 (0.23) | 2.60 (0.14) |
| Overlying Water - (IW) | *G. japonica* | 0.29 (0.09) | 0.12 (0.03) |
| Overlying Water - (IW+OW) | *G. japonica* | 2.02 (0.25) | 2.57 (0.20) |
| Overlying Water - (IW) | *L. plumulosus* | 0.53 (0.19) | 0.19 (0.09) |
| Overlying Water - (IW+OW) | *L. plumulosus* | 1.59 (0.20) | 2.49 (0.18) |

IW = Contaminated Sediment and Clean Overlying Water Exposure Treatment
IW + OW = Contaminated Sediment and Spiked Overlying Water Exposure Treatment

Lipids:

Total lipids (presented on a percent dry weight basis) was measured for each species at the start and end of the exposure period and are presented in Table 2.4. *Eohaustorius*

*estuarius* contained the highest lipid content among the three species tested, ranged from 9.43 % - 11.77 % dw. The lipid contents for *Grandidierella japonica* (range 3.93 % - 4.67 % dw) and *Leptocheirus plumulosus* (range 3.53 % - 4.97 % dw) were similar. Though there was a slight decrease in lipid content in over the course of the exposure period (with the exception of *Leptocheirus plumulosus* in the interstitial water only exposure) there were no statistically significant difference between the two lipid levels for any of the three amphipod species. The mean percent lipid content was calculated for each species and this value was used to lipid normalize the tissue residue data.

Table 2.4. Total Lipid Concentrations (percent dry weight) Measured in the Amphipods.

| | T=0 (SD) | T=72 (SD) | Mean Lipid Value |
|---|---|---|---|
| Lipid (*E. estuarius*) - (IW) | 11.77 % (1.53) | 9.43 % (1.25) | 10.60 % |
| Lipid (*E. estuarius*) - (IW+OW) | 10.03 % (0.51) | 9.50 % (0.26) | 9.77 % |
| Lipid (*G. japonica*) - (IW) | 4.67 % (0.25) | 3.93 % (0.59) | 4.30 % |
| Lipid (*G. japonica*) - (IW+OW) | 4.37 % (0.75) | 4.13 % (0.93) | 4.25 % |
| Lipid (*L. plumulosus*) - (IW) | 4.70 % (0.66) | 4.97 % (0.90) | 4.84 % |
| Lipid (*L. plumulosus*) - (IW+OW) | 4.23 % (0.38) | 3.53 % (0.51) | 3.88 % |

IW = Contaminated Sediment and Clean Overlying Water Exposure Treatment
IW + OW = Contaminated Sediment and Spiked Overlying Water Exposure Treatment

Bioaccumulation Data:

The tissue residue data from the bioaccumulation experiments were converted from wet weight to dry weight using the species specific regression algorithm presented earlier in this document. This data was then lipid normalized to give the final concentration of $^{14}C$-phenanthrene in ug phenanthrene/g lipid dw. The lipid normalized bioaccumulation data (ug/g lipid) for *Eohaustorius estuarius*, *Grandidierella japonica*, and *Leptocheirus plumulosus* are presented in Figures 2.1, 2.3, and 2.5 respectively.

*Eohaustorius estuarius*:

Figure 2.1 presents the tissue residue data for *Eohaustorius estuarius* for both the contaminated sediment and clean overlying water exposure and the contaminated sediment

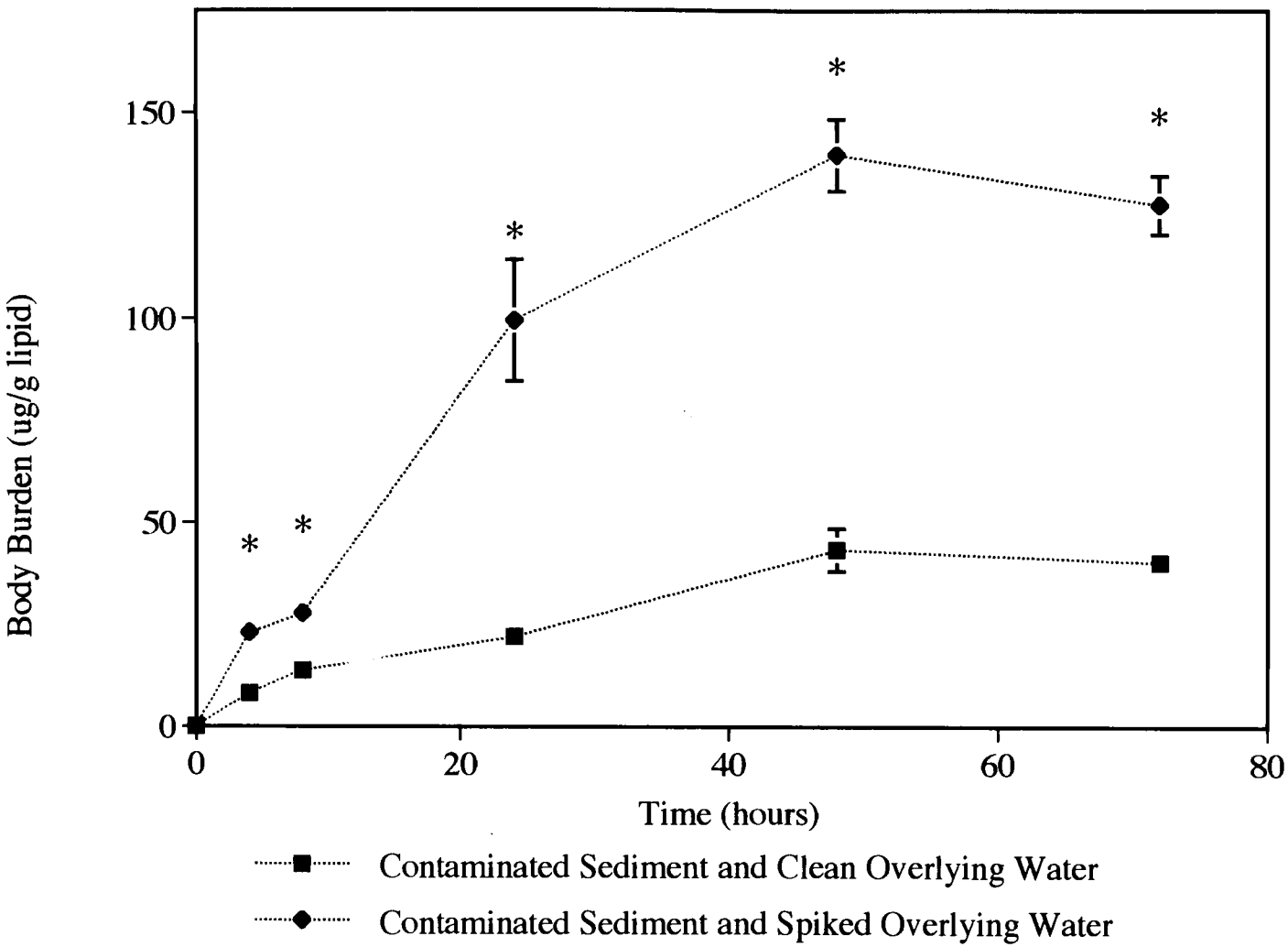


Figure 2.1. Bioaccumulation of $^{14}$C-phenanthrene by Eohaustorius estuarius from sediment with clean overlying water versus sediment with spiked overlying water. All points ± Standard Error of the mean. n = three replicates. * significantly different means.

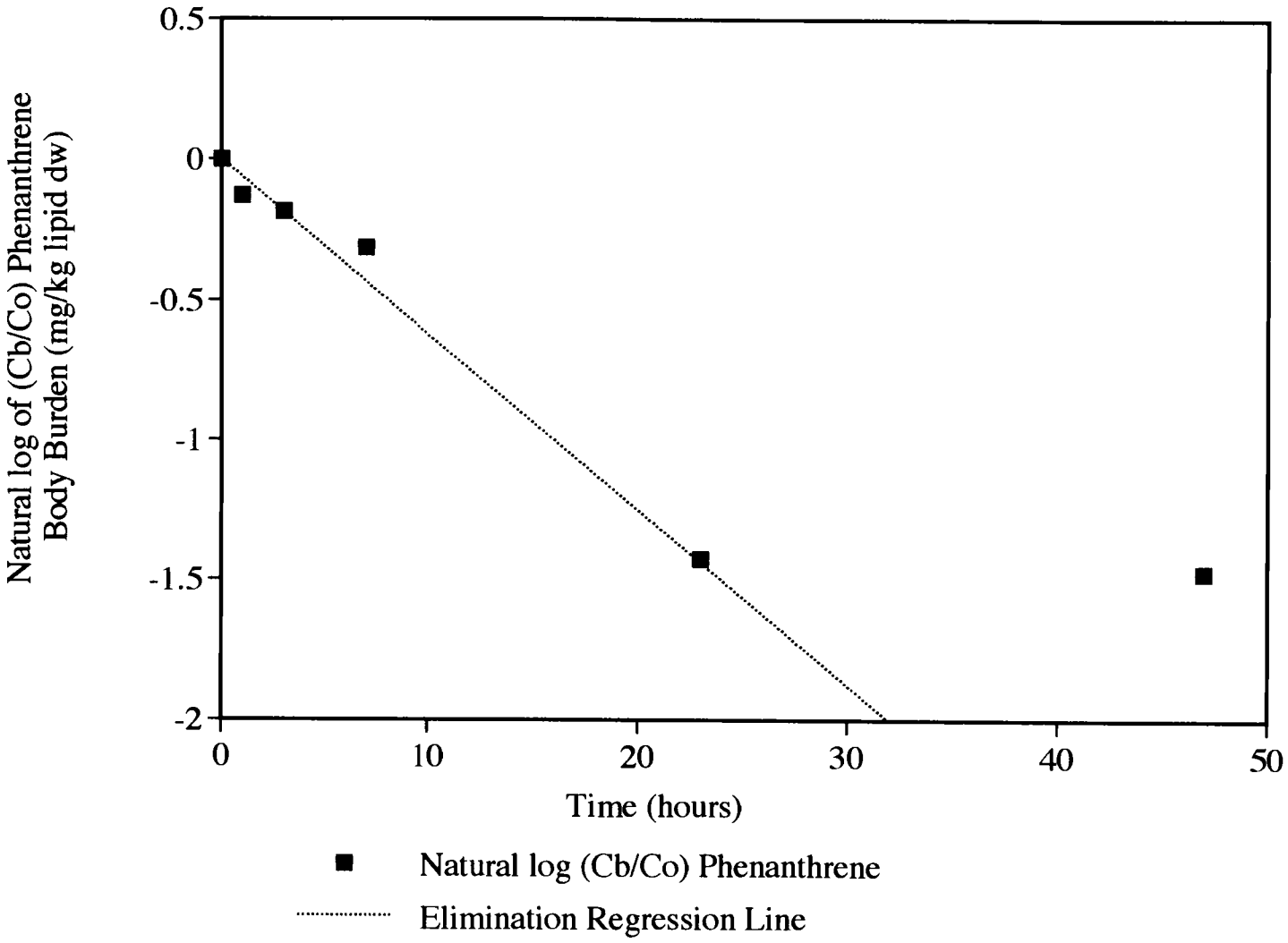


Figure 2.2. Clearance of $^{14}C$-phenanthrene by Eohaustorius estuarius in Sediment

and spiked overlying water exposure. The tissue residue of $^{14}$C-phenanthrene in the contaminated sediment and clean overlying water exposure increased over the first 48 hours and reached an apparent steady state concentration of approximately 41.5 ug/g lipid exhibited during the 48 and 72 hour time points. In the contaminated sediment and contaminated overlying water exposure, again the concentration of $^{14}$C-phenanthrene increased in the tissue over the first 48 hours of exposure, reaching an apparent steady state concentration of approximately 133.5 ug/g lipid exhibited during the 48 and 72 hour time points.

The tissue residue levels of $^{14}$C-phenanthrene were compared between the two exposure treatments by comparing the mean body burdens (in ug/g lipid) at each time point between the two treatments using a student's t-test with a p-value of 0.05. For *Eohaustorius estuarius* , there was a statistically significant difference between the mean body burdens between the two treatments at each time point, with the tissue residues being statistically higher in the contaminated sediment and spiked overlying water exposure.

Uptake coefficients for *Eohaustorius estuarius* ($k_1$) were calculated using a linear regression model and a non-linear regression model. The linear model used data from time points T = 0 through T = 48 hours, which represented the linear portion of the uptake curve. The non-linear regression model utilized all the data points and the model was run with the $k_2$ value being constrained to the experimentally derived value of $0.0626^{-time}$.

Figure 2.2 shows the elimination data for *Eohaustorius estuarius* placed in clean sediment. There is an initial rapid loss of $^{14}$C-phenanthrene over the first 24 hours and then the rate of elimination decreases, represented by a plateau in the elimination curve to the 48 hour time point. A linear regression of the data was conducted to calculate a clearance rate ($k_2$) for *Eohaustorius estuarius*. As this model is best represented by the linear portion of the clearance curve, only the data from T = 0 hours to T = 24 hours was used to calculate the $k_2$ value of $0.0626^{-time}$. Table 5 presents the uptake and elimination coefficients calculated for each species and exposure treatment.

*Grandidierella japonica:*

Figure 2.3 presents the tissue residue data for *Grandidierella japonica* for both the contaminated sediment and clean overlying water exposure and the contaminated sediment and spiked overlying water exposure. The tissue residue for $^{14}$C-phenanthrene in the

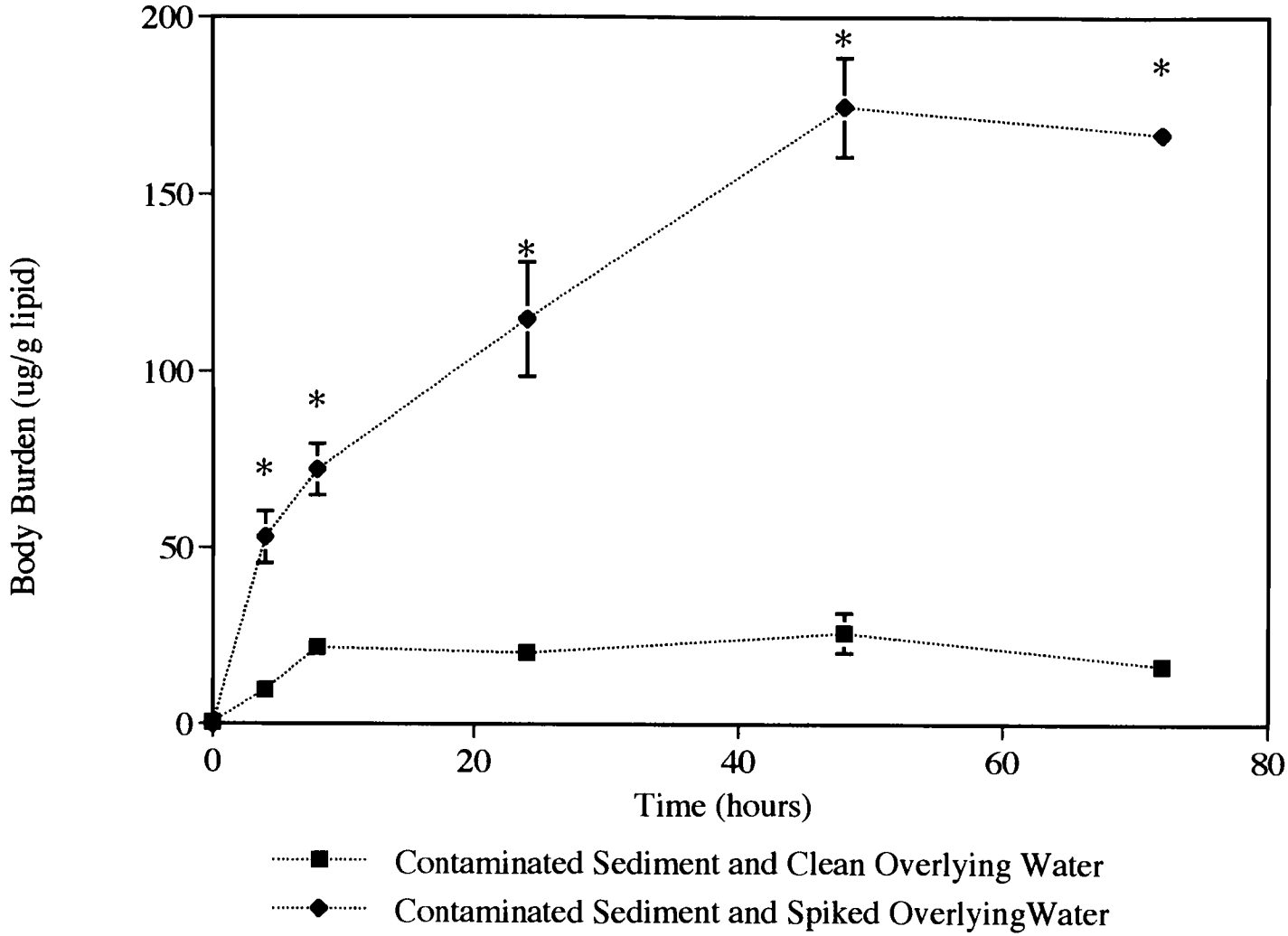


Figure 2.3. Bioaccumulation of $^{14}C$-phenanthrene by Grandidierella japonica from sediment with clean overlying water versus sediment with spiked overlying water. All points ± Standard Error of the mean. n = three replicates. * significantly different means.

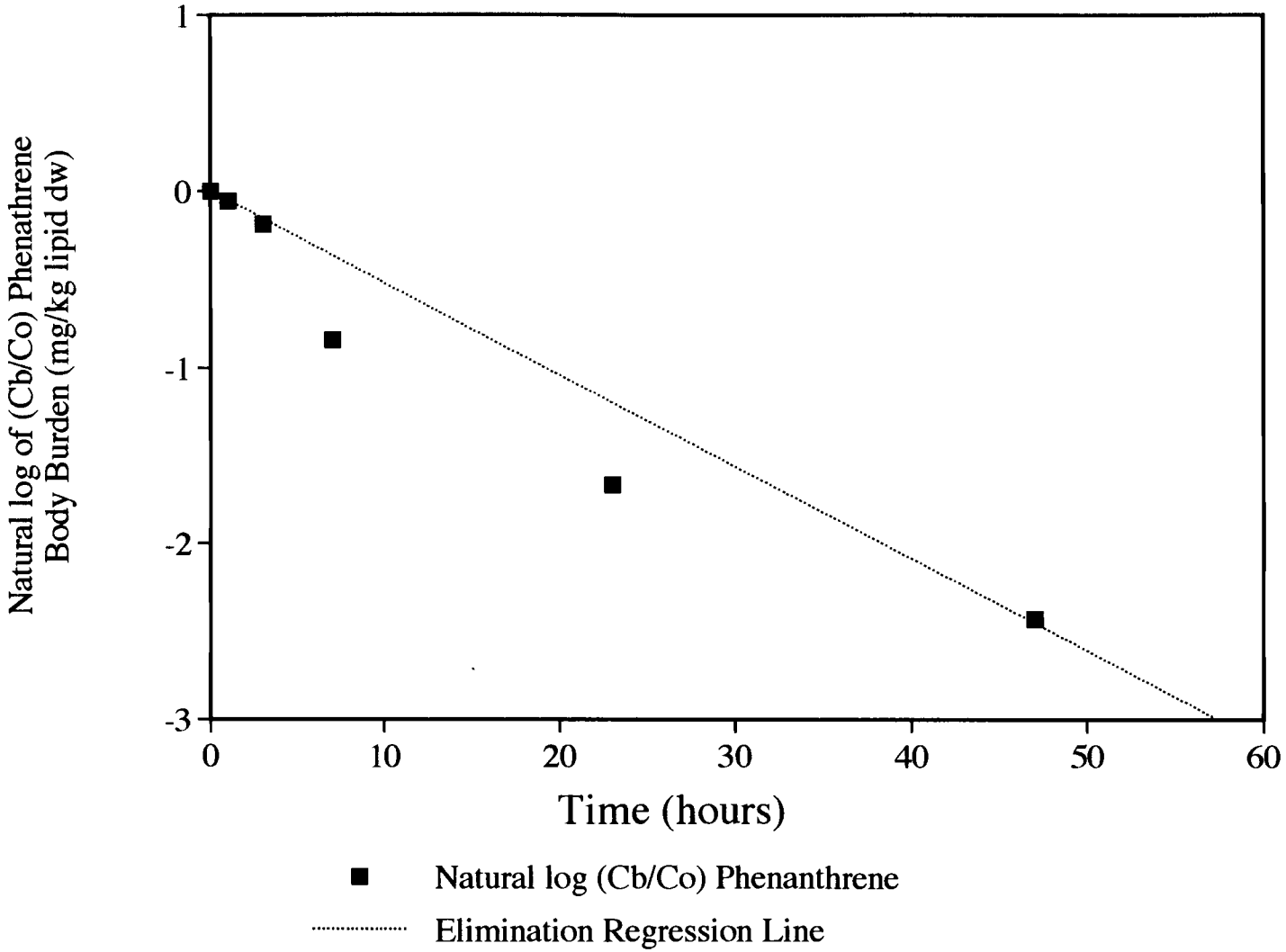


Figure 2.4. Clearance of $^{14}C$-phenanthrene by Grandidierella japonica in sediment

contaminated sediment and clean overlying water exposure increased over the first 8 hours of exposure and reached an apparent steady state concentration of 20 ug/g lipid exhibited during the 8 to 72 hour time points. In the contaminated sediment and contaminated overlying water exposure, the concentration of $^{14}$C-phenanthrene in the amphipods increased over the first 48 hours to reach an apparent steady state concentration of approximately 170 ug/g lipid.

The tissue residue levels of $^{14}$C-phenanthrene were compared between the two exposure treatments by comparing the mean body burdens (in ug/kg lipid) at each time point between the two treatments using a student's t-test with a p-value of 0.05. For *Grandidierella japonica* there was a statistically significant difference between the mean body burdens between the two treatments at each time point, with the tissue residues being statistically higher in the sediment and overlying water exposure

Uptake coefficients for *Grandidierella japonica* ($k_1$) were calculated using a linear regression model and a non-linear regression model. The linear model used data from time points T = 0 through T = 24 hours for the clean overlying water exposure and time points T=0 through T=48 hours for the spiked overlying water exposure. These represented the linear portions of the uptake curve for each treatment. The non-linear regression model utilized all the data points for both exposure treatment and the model was run with the $k_2$ value being constrained to the experimentally derived value of 0.0523 $^{-time}$.

Figure 2.4 shows the elimination data for *Grandidierella japonica* placed in clean sediment. There was a steady decrease in the tissue residue of $^{14}$C-phenanthrene over time and the data from T = 0 hours to T = 48 hours was used to calculate the $k_2$ value of 0.0523 $^{-time}$.

*Leptocheirus plumulosus* :

Figure 2.5 presents the tissue residue data for *Leptocheirus plumulosus* for both the contaminated sediment and clean overlying water exposure and the contaminated sediment and spiked overlying water exposure. The tissue residue for $^{14}$C-phenanthrene in the contaminated sediment and clean overlying water exposure increased over the first 8 hours and reached an apparent steady state concentration of 25 ug/g lipid exhibited during the 8 to 24 hour time points. In the contaminated sediment and contaminated overlying water exposure, the concentration of $^{14}$C-phenanthrene in the tissue increased over the first 48

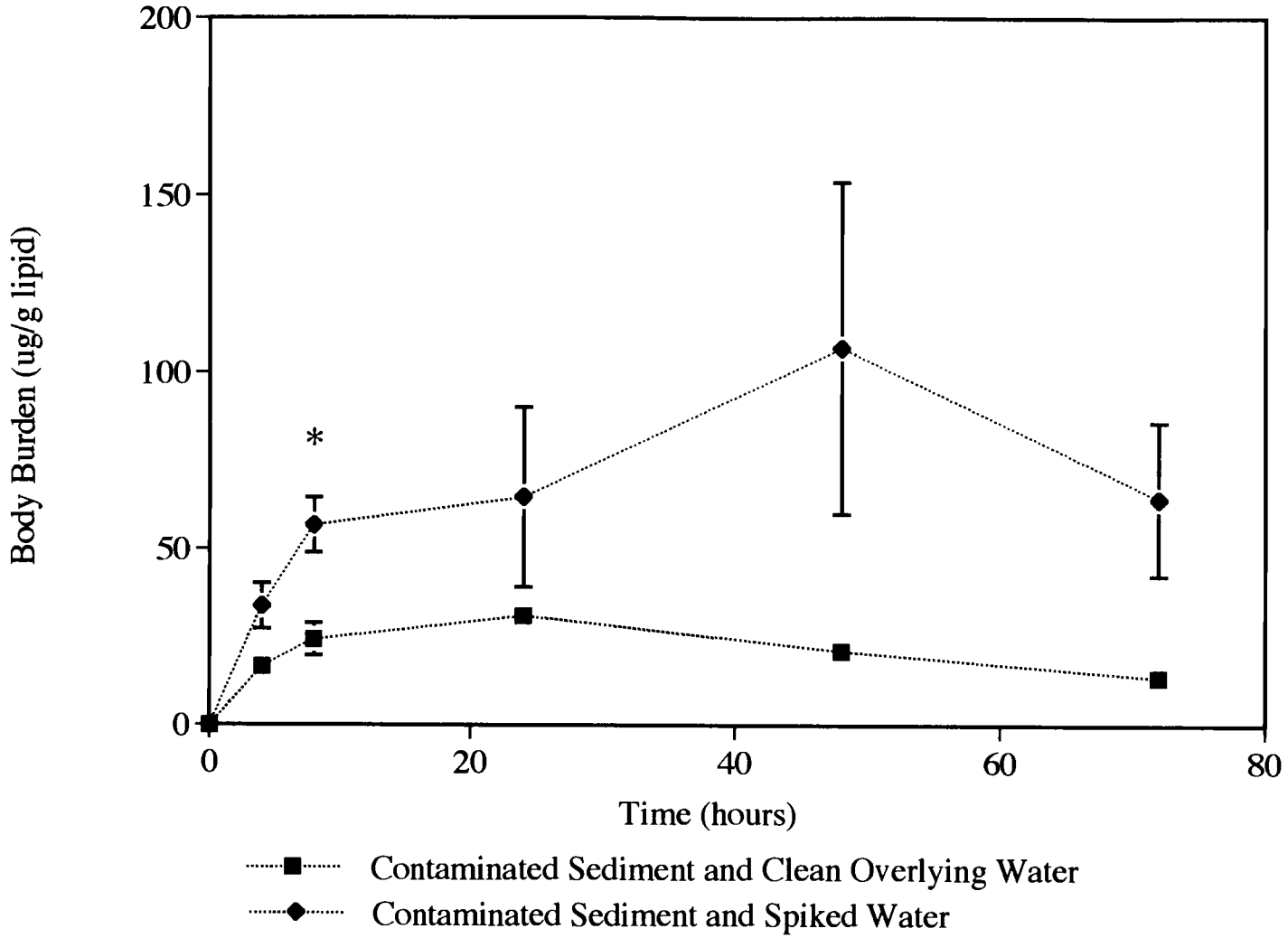


Figure 2.5. Bioaccumulation of $^{14}C$-phenanthrene by Leptocheirus plumulosus from sediment with clean overlying water versus sediment with spiked overlying water. All points± Standard Error of the mean. n = three replicates. * significantly different means.

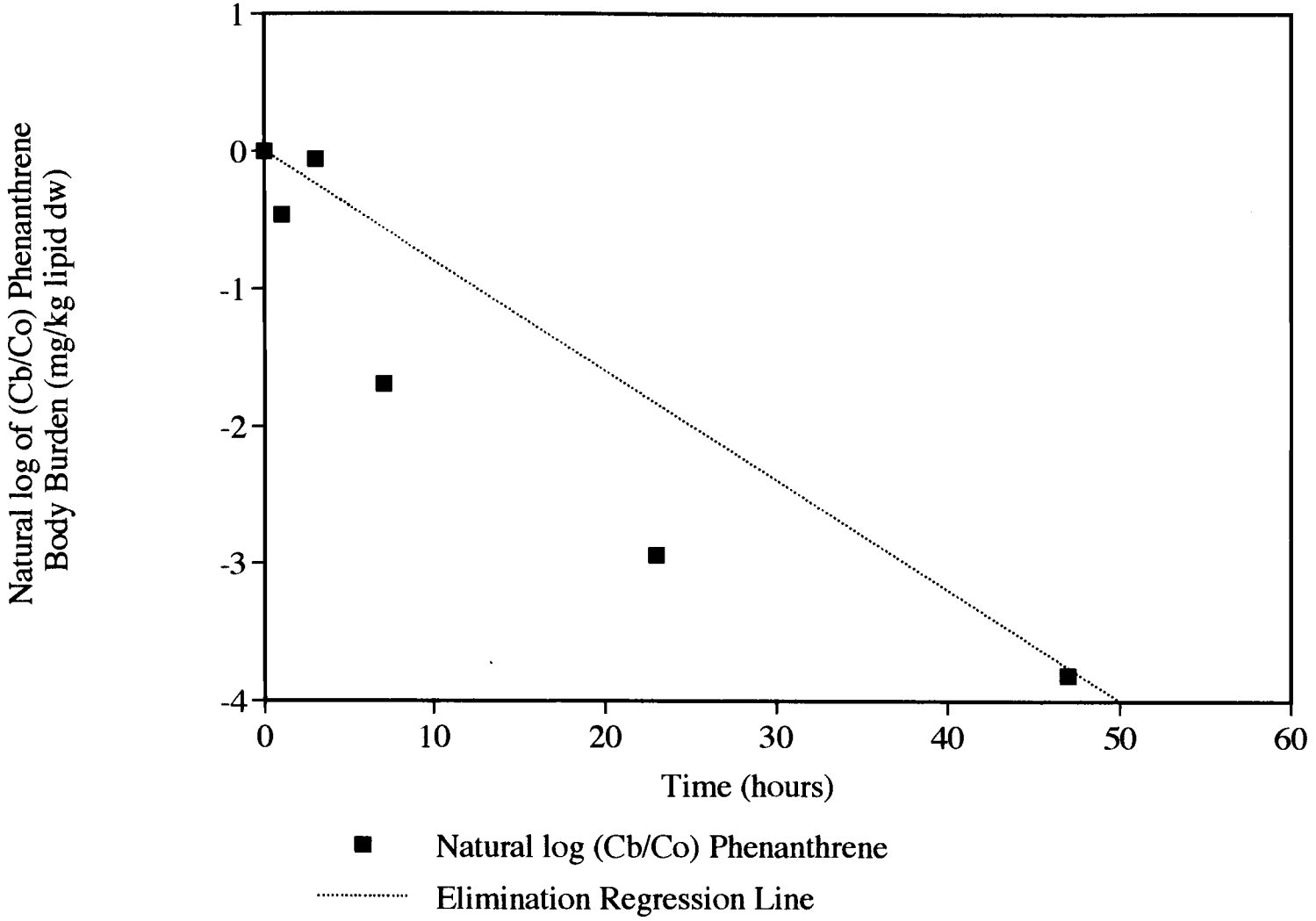


Figure 2.6. Clearance of $^{14}$C-phenanthrene by Leptocheirus plumulosus in Sediment

hours to reach an apparent steady state concentration of 75 ug/g lipid. For *Leptocheirus plumulosus* in both exposure treatments, the uptake curves reached a maximum, at 24 hours in sediment exposure and 48 hours in the sediment and overlying water exposure followed by a decrease in tissue residue concentrations over the remaining time points. This pattern was not observed in the other two species tested and may represent the metabolism and elimination of $^{14}$C-phenanthrene by this species.

The tissue residue levels of $^{14}$C-phenanthrene were compared between the two exposure treatments by comparing the mean body burdens (ug/kg lipid) at each time point between the two treatments using a student's t-test with a p-value of 0.05. For *Leptocheirus plumulosus* there was a statistically significant difference between the mean body burdens between the two treatments at only the 8 hour time point. For *Leptocheirus plumulosus*, the mean concentration of $^{14}$C-phenanthrene was 2-5 times greater in the spiked sediment and spiked overlying water exposure than the spiked sediment only exposure. However due to the high variability within replicates, the differences between treatments were not determined to be statistically significant.

Figure 2.6 shows the elimination data for *Leptocheirus plumulosus* placed in clean sediment. There was a steady decrease in the tissue residue of $^{14}$C-phenanthrene over time and the data from T = 0 hours to T = 48 hours was used to calculate the $k_2$ value of $0.080^{-time}$.

Table 2.5. Uptake and Elimination Rate Coefficients Calculated for Each Species of Amphipod.

| | Linear Regression Model (1) | | Non-Linear Regression Model (1) | | Elimination Rate Coefficients * (2) | |
|---|---|---|---|---|---|---|
| Species | $k_1$ | $R^2$ | $k_1$ | $R^2$ | $k_2$ | $R^2$ |
| *E. estuarius* - (IW) | 0.926 | 0.885 | 0.977 | 0.859 | -0.062 | 0.825 |
| *E. estuarius* (IW+OW) | 3.174 | 0.903 | 3.275 | 0.903 | | |
| *G. japonica* - (IW) | 1.061 | 0.235 | 0.529 | 0.616 | -0.0523 | 0.906 |
| *G. japonica* - (IW+OW) | 4.027 | 0.742 | 3.738 | 0.930 | | |
| *L. plumulosus* - (IW) | 1.509 | 0.386 | 1.006 | 0.653 | -0.080 | 0.834 |
| *L. plumulosus* - (IW + OW) | 2.453 | 0.345 | 3.153 | 0.501 | | |

IW = Contaminated Sediment and Clean Overlying Water Exposure Treatment
IW + OW = Contaminated Sediment and Spiked Overlying Water Exposure Treatment
* Calculated using a linear regression model based on experimentally derived elimination data.
(1) units in mL water/g-tissue per hour
(2) units ($hour^{-1}$)

## 2.4 DISCUSSION:

In this study, the ability of the burrow irrigating behavior used by benthic amphipods to influence the interstitial water concentration of a the PAH phenanthrene was investigated. By using three species of amphipods that exhibit different burrowing behaviors, the effect of different burrowing strategies on the exposure to and bioaccumulation of sediment-associated phenanthrene was also investigated. To assist in this evaluation, the bioaccumulation kinetics for phenanthrene by the three amphipods were examined by modeling in two different ways. Initially, the bioaccumulation uptake rates were calculated by a linear regression model and the elimination rates were also empirically derived from experimental data using an appropriate linear regression model. Later, a non-linear regression method was used to derive the uptake coefficients from the same data set. These model coefficients were used to estimate time to steady state and estimate apparent steady state conditions. The results of the modeling among the two regression models were

compared with experimental data to evaluate the accuracy of each model at estimating the experimentally derived results.

Burrow Irrigating Behavior:

The equilibrium partitioning theory predicts that once the partitioning of a non-polar organic compound between sediment organic carbon and sediment interstitial water is determined, the modeled interstitial water concentration will be the constant exposure point concentration for all benthic organisms. However, benthic amphipods utilize burrow irrigating as a respiratory behavior. Additionally, some species of amphipods use this irrigating behavior as an adjunct to feeding as food particles are brought within the close proximity of an organisms, usually within burrow or tube. The extent to which this behavior, which involves the advection of overlying water into the interstitial pore space surrounding the organism to provide fresh oxygenated water for respiration, can affect equilibrium conditions was investigated.

The presence or absence of $^{14}$C-phenanthrene in the overlying water of the exposure chambers had a significant effect on the accumulation of $^{14}$C-phenanthrene by the amphipods. The body burdens of phenanthrene was higher in all three species when $^{14}$C-phenanthrene was present in the overlying water. This difference in body burdens was evident from the first sampling point to the apparent steady state concentrations exhibited by the individual species. This data suggests that these amphipod species have the ability to ventilate sufficient overlying water into the sediment to effectively disrupt the predicted equilibrium conditions present in the microhabitat surrounding the individual benthic organism. For two of the three species, *Eohaustorius estuarius* and *Grandidierella japonica,* there was a statistically significant difference in the mean tissue levels of $^{14}$C-phenanthrene between the two exposure treatments at each sampling time. For *Leptocheirus plumulosus* the tissue levels of $^{14}$C-phenanthrene were higher in the contaminated overlying water treatment (generally 2-3 times as high) than was observed in the clean overlying water treatment. There was, however, greater variability within replicates which caused the mean differences between treatments to not be statistically significant.

The uptake rate coefficients between the two exposure treatments for each species were statistically compared. The $k_1$'s for *Eohaustorius estuarius* were found to be statistically

different between the two treatments (0.93 vs. 3.17 mL water/g tissue-time linear model and 0.98 vs. 3.28 mL water/g tissue-time non-linear model). The $k_1$'s for the non-linear model for *Grandidierella japonica* was found to be statistically different between the two exposure treatments (0.53 versus 3.28 mL water/g tissue-time). No statistical comparison could be made for the linear regression model as the number of observations included in the coefficient calculation were different between the two exposures, however the $k_1$ for the contaminated sediment clean overlying water (1.06 mL water/g tissue-time) is much lower than the $k_1$ for the contaminated sediment and spiked overlying water treatment (4.03 mL water/g tissue-time). For *Leptocheirus plumulosus*, no statistically significant difference could be found between the non-linear regression $k_1$'s for the two exposure treatments even though the calculated regression coefficients appeared similar to the other two species (1.01 mL water/g tissue-time contaminated sediment, clean overlying water exposure and 3.15 mL water/g tissue-time contaminated sediment, spiked overlying water exposure). Again, no statistical comparison could be made for the linear regression model as the number of observations included in the coefficient calculation were different between the two exposures. However, $k_1$ for the contaminated sediment clean overlying water (1.51 mL water/g tissue-time) is lower than the $k_1$ for the contaminated sediment and spiked overlying water treatment (2.45 mL water/g tissue-time).

This experiment has shown that by varying the concentration of $^{14}$C-phenanthrene in the overlying water, the effective exposure concentration for infaunal organisms is changed. This change was due to biologically mediated processes that are not currently addressed by the equilibrium partitioning model which is strictly a physio/chemical model describing chemical partitioning relationships between various media in a sediment/water/organism system.

This has implications for both laboratory performed sediment bioassays and in the use of equilibrium partitioning to predict exposure in the field. For example, one regulatory implication of this research is that some laboratories are performing marine sediment toxicity tests with amphipods under static conditions while others are performing them under flow through conditions. The static conditions are similar to the spiked sediment and spiked overlying water exposure as over 10-days, the sediment will reach an equilibrium with the overlying water equal to the interstitial water concentration of the sediment. The flow through condition is similar to the clean overlying water and spiked sediment exposures. If the differences in body burdens are in fact maintained over 10-days, some

standardization in the method that these acute toxicity tests are performed would appear warranted.

Additionally, recent EPA guidance on the performance of marine sediment amphipod bioassays recommends that under certain situation the overlying water should be changed during the course of a ten-day acute toxicity test (EPA, 1994b). These water changes are recommended when ammonia in the interstitial and overlying water may cause confounding toxicity results and the guidance recommends a two water volume water change daily until ammonia levels are reduced in the overlying water. Again, the results of this present research indicates that manipulation of the overlying water during the course of an toxicity test can change the exposure point concentration to the benthic organisms used. The implications for sediment bioassay protocols require further investigation.

Comparing Burrowing Behaviors Among Species:

The relative contribution of the burrow-irrigating behavior on the exposure to sediment-sorbed phenanthrene can be investigated by comparing the body burden ratios between the two exposures treatments for a specific species. The types of burrowing behavior exhibited by the three species of amphipods selected for this research are a) tube building by *Grandidierella japonica*, b) burrow building by *Leptocheirus plumulosus*, and c) non-structure forming, free-burrowing by *Eohaustorius estuarius*. As stated by Lee (1991), tubes and burrows facilitate the ventilation or irrigation of overlying water into the sediment by providing an open channel to the sediment surface. Consequently, species that form these structures in the sediment can move a greater volume of overlying water into the sediment than free burrowing species. The greater the amount of overlying water that is pumped into the tube or burrow, the greater the amount of dilution occurring in the microhabitat surrounding the test organisms. Therefore, by evaluating the magnitude of the difference in bioaccumulation between the two exposure treatments; one in which clean overlying water is available to the amphipods to dilute the interstitial water and one in which this process is short circuited by having the overlying water at the equilibrium concentration as interstitial water, the relative ability of each species (and burrowing strategy) to affect its exposure concentration in interstitial water can be compared and contrasted.

In this analysis, the mean body burdens of each individual amphipod species at the 48 and 72 hours time points (after achieving apparent steady-state) for the spiked sediment and spiked overlying water exposure are divided by the corresponding data from the sediment only exposure. The higher the ratio (or difference in body burdens) between these two treatments indicates a greater ability to dilute and decrease exposure due to burrow-irrigating behavior.

As is presented in Table 2.6, the highest ratios of body burdens between exposure treatments are observed for *Grandidierella japonica* (6.78 and 10.09 for the 48 and 72 hour ratios) followed by *Leptocheirus plumulosus* (5.10 and 4.75 for the 48 and 72 hour ratios) and *Eohaustorius estuarius* (3.23 and 3.18 for the 48 and 72 hour ratios). The order of the ratios correlate with the burrowing behavior exhibited by the amphipod species. The highest ratio between exposure treatments was found for the hard tube-building species followed by the soft-burrow building species and the lowest ratio found for the free-burrowing amphipod. As expected, the tube and burrow builders appear to be able to decrease exposure to a greater extent than the non-structure forming species. Therefore, while it is clear that burrow irrigating behavior can disrupt equilibrium conditions and exposure concentrations, the magnitude of this effect will be species and behavior specific.

Table 2.6: Mean Concentration of $^{14}C$-phenanthrene in Amphipod Tissue (Lipid Normalized) at the 48 hour and 72 hour Sampling Times.

| Species | 48 hours (SD) | 72 hours (SD) |
|---|---|---|
| *E. estuarius* - (IW) | 43.17 ug/g lipid (9.09) | 40.12 ug/g lipid (6.13) |
| *E. estuarius* - (IW + OW) | 139.54 ug/g lipid (15.22) | 127.48 ug/g lipid (12.39) |
| *L. plumulosus* - (IW) | 20.91 ug/g lipid (5.84) | 13.45 ug/g lipid (3.49) |
| *L. plumulosus*- (IW + OW) | 106.69 ug/g lipid (79.44) | 63.92 ug/g lipid (37.48) |
| *G. japonica* - (IW) | 25.73 ug/g lipid (9.77) | 16.53 ug/g lipid (3.09) |
| *G. japonica*- (IW + OW) | 174.55 ug/g lipid (24.42) | 166.80 ug/g lipid (2.05) |
| *E. estuarius* - Ratio (IW + OW/IW) | 3.23 Non-structure Forming sp. | 3.18 |
| *L. plumulosus* - Ratio (IW + OW/IW) | 5.10 Burrow Building sp. | 4.75 |
| *G. japonica* - Ratio (IW + OW/IW) | 6.78 Tube Building sp. | 10.09 |

IW = Contaminated Sediment and Clean Overlying Water Exposure Treatment
IW + OW = Contaminated Sediment and Spiked Overlying Water Exposure Treatment

Comparison of Predicted Steady State Concentrations and Time to Steady State:

The body burden of a PAH is determined by the balance between uptake and elimination. Uptake (the movement of a toxicant into or onto an organism) can vary depending on specific species, chemical, and environmental conditions. Elimination is the summation of metabolic, excretory, and physiochemical processes resulting in the decrease of toxicant in the organism (Newman, 1995).

Kinetic models have been increasingly used in aquatic toxicology to assess and predict toxicant effects on aquatic organisms. These kinetic models have been used successfully in pharmacology for decades. Such models permit predictions of the onset of drug action and allows the monitoring of drug clearance and termination of effect. In aquatic toxicology and hazard assessment, kinetic models have been used as a predictive tool to estimate toxicant accumulation, distribution, and, ultimately, effect (Landrum et al., 1992b).

In this study, uptake and elimination rate constants were determined. The rate constants were derived using a simple two-compartment model containing water and organisms

compartments. This model is appropriate for situations in which the exposure by an organisms to the contaminant in questions occurs primarily in water. The water represents the source compartment and the organisms represents the toxicant sink. The toxicant is assumed to be well mixed and homogenous within each compartment. These kinetic models were initially used to predict the accumulation of toxicants by fish from water (Spacie and Hamelink, 1982, Dobbs, 1984). These models are appropriate for use with this data set as Landrum and Robbins (1990) estimated that 88% of phenanthrene taken up by the freshwater amphipod *Diporeia spp.* was through the interstitial water. Meador et al. (1995a) also found that the interstitial water was the primary route of uptake for the infaunal nondeposit-feeding amphipod (*Rhepoxynius abronius*) and a deposit-feeding polychaete (*Armandia brevis*) for low-molecular weight PAHs (LPAH). This study suggests that deposit-feeding and non-deposit feeding infaunal invertebrates will acquire most of their body burden of LPAHs through porewater regardless of feeding strategy; however, ingestion of sediment or food may be the dominate route of uptake when hydrophobic compounds exceed a log $K_{ow}$ of approximately 5.5. Other work in the literature support the hypothesis that interstitial water is the dominant route of accumulation of a variety of sediment associated contaminants (Kemp and Swartz, 1988, Shaw and Connell, 1987, and Knezovitch and Harrison, 1988).

Uptake rate coefficients and elimination rate constants (determined experimentally and by fitting the first-order bioaccumulation model) were used to predict steady state concentrations in each amphipod species and to predict the exposure times necessary to attain these steady state concentrations. These rate constants were initially derived from two different methods: (1) linear regression of the bioaccumulation data only over the linear portion of the uptake curve, and (2) an iterative least-square non-linear method. One value in deriving uptake rate constants by these two different methods is that the rate constants can be used to calculate experimentally derived data to evaluate the accuracy of the modeled predictions. These were then compared with the experimentally observed values to assess the accuracy of the predicted rate constants.

Table 2.7. Comparison of Predicted and Observed Steady State Concentrations and Exposure Times.

| Species | Linear Regression Model (ug/g lipid) | Non-Linear Regression Model (ug/g lipid) | Experimental Steady State Conc. (ug/g lipid) | Predicted Time to Steady State (hr) |
|---|---|---|---|---|
| *E. estuarius* - (IW) | 37.34 | 39.39 | 41.66 | 48.2 |
| *E. estuarius* - (IW+OW) | 127.98 | 132.056 | 133.55 | 48.2 |
| *G. japonica* - (IW) | 47.19 | 23.53 | 21.02 | 53.2 |
| *G. japonica* - (IW+OW) | 179.14 | 166.28 | 170.72 | 53.2 |
| *L. plumulosus* - (IW) | 47.156 | 31.44 | 27.54 | 37.4 |
| *L. plumulosus* - (IW+OW) | 76.66 | 98.53 | 85.32 | 37.4 |

IW = Contaminated Sediment and Clean Overlying Water Exposure Treatment
IW + OW = Contaminated Sediment and Spiked Overlying Water Exposure Treatment

As the results in Table 2.7 show, both the linear and non-linear regression models were able to accurately predict the experimental apparent steady state concentration. For all three species, both exposure treatments, estimates were generally between 10% of predicted and measure values. The two exceptions were *G. japonica* IW and *L. plumulosus* IW. In these cases, the predicted steady state concentrations calculated using the linear regression model were much higher (224% for *Grandidierella japonica* and 171% for *Leptocheirus plumulosus* ). The non-linear model, as it uses a larger proportion of the actual data points, appears to provide a more reliable estimate off steady state concentrations, especially when utilizing experimentally derived $k_2$ values.

The predicted time to reach 95% of the steady state concentration was calculated and the experimentally derived $k_2$ rate constant for each species (Table 2.7). These values were compared against the estimated time to reach steady state tissue concentrations derived from the $^{14}$C-phenanthrene uptake curves. For *Eohaustorius estuarius* and *Grandidierella japonica*, the estimated time to 95% of steady state (48.2 hours and 53.2 hours, respectively) correspond very well to the uptake curves presented in Figures 2.1 and 2.3.

For both of these species, the uptake curves indicate that the concentration of $^{14}C$-phenanthrene continues to increase in these amphipods until the 48 hour sampling point and then remains relatively constant or decreases slightly prior to the 72 hour sampling time. For *Leptocheirus plumulosus* the estimated time to reach 95% of steady state (37.4 hours) falls within the range that the two uptake curves reach their maximum (24 hours for the sediment and spiked water treatment and 48 hours for the sediment and clean water exposure treatment). Overall, the calculated time to steady state corresponded very well with the experimentally produced bioaccumulation data.

Summary:

The objective of this study was to test the assumption of the equilibrium partitioning theory that benthic organisms are exposed to a constant concentration of a contaminant in interstitial water. Specifically, the effect of the irrigation of overlying water into the sediment by marine amphipods was examined as to its ability to disrupt the equilibrium between sediment organic carbon and interstitial water. Additionally, the effect of different burrow or tube building strategies on the exposure to sediment-associated contaminants was investigated. The results of this study indicate that for each of the three species included in this study, the irrigation of overlying water had a significant impact on the exposure concentration.

In addition, it was determined that in situations where the overlying water concentration of a contaminant is less than the interstitial water concentration, there is a behavior dependent reduction in the accumulation of phenanthrene from sediment for different species of marine amphipods. The magnitude of this effect is correlated with the type of burrowing behavior exhibited by the individual amphipod species. This fact has implications for the appropriate selection of amphipod species to use in regulatory bioassay testing. All three of the amphipod species have been recognized by ASTM (1995) as appropriate for use in regulatory sediment bioassay testing. Some guidance does exists to assist in the selection of the amphipod species for a given sediment test depending upon the physical/chemical characteristics of the sediment and interstitial water (e.g. grain size and salinity). However, the results of this research indicate that species utilizing different burrow or tube building behaviors may not be exposed to the equivalent concentration of contaminant in a sediment bioassay. For example, if a tube building species is used in a sediment bioassay and the indigenous species of amphipod at the site being investigated is a

free-burrower, the results of the sediment bioassay may underestimate the magnitude of the adverse biological effects being experienced by the native population of amphipods.

The bioaccumulation kinetics of $^{14}C$-phenanthrene uptake by the three species of amphipods were modeled by both linear and non-linear techniques. The accuracy of both linear and non-linear regression methods were compared as to their capabilities to predict apparent steady state concentrations and the time to reach apparent steady state in the amphipods. Estimates made by both methods were found to correlate well with the experimental bioaccumulation data.

The results of this study reveal a biologically mediated factor that can influence the exposure to sediment-associated contaminants that is not adequately described by standard partitioning relationships such as the equilibrium partitioning theory. These results are particularly pertinent to the standard sediment toxicity bioassay that employs benthic amphipods by indicating the degree of variability in test results which might be introduced by the behavior of the species utilized. The importance of identifying sources of variability and uncertainty associated with current standardized bioassay protocols is underscored by the recent increase in the use of such tests in regulatory decision making. Further research is recommended to determine whether the differences in accumulation of a contaminant observed in this series of 72-hour exposures are maintained over ten days, the length of a standard sediment bioassay. Additional research is necessary to determine whether the differences in body burdens among amphipod species will correlate with differences in response to acute and chronic bioassays. In other words, whether the behavior related differences in exposure among the three amphipod species correlate with differences in species sensitivity to sediment-associated contaminants.

# CHAPTER 3

# A COMPARISON OF THE ACCUMULATION OF PHENANTHRENE BY MARINE AMPHIPODS IN WATER VERSUS SEDIMENT

## 3.1 INTRODUCTION:

Sediment contamination problems have been documented in an increasing number of locations over the past few years. Such contamination has been associated with a variety of biological responses including elevated prevalence of hepatic lesions in certain species of fish (Malins et al., 1982), acute toxicity to benthic invertebrates (Swartz et al., 1982), changes in benthic community structure (Swartz et al., 1986, Chapman et al., 1991), accumulation of contaminants into benthic invertebrates (Meador, 1995a) and bio-magnification or trophic transfer of sediment associated contaminants to fish (Reynoldson, 1987). Contaminated sediments are responsible for a wide variety of deleterious biological responses because for many persistent contaminants, sediments provide an extended form of exposure to aquatic organisms than does the water column. Several characteristics of sediments contribute to the threat that contaminated sediments pose to aquatic communities: (a) various toxic contaminants found only in trace amounts in the water column accumulate in sediments to elevated levels; (b) sediments serve as both sink and source of contaminants to the water column; (c) sediment contaminants may have a greater effect on benthic species because of direct exposure to contaminants; (d) sediments are an integral part of the aquatic environment, and provide habitat, feeding and rearing areas for many aquatic biota (EPA, 1987, Reynoldson, 1987, Shea, 1988).

Regulators have paid considerable attention to developing a methodology to assess the relative hazards posed by contaminated sediments. Central to this issue is determining which ecological or toxicological endpoints should be used in the development of such a sediment management strategy. In other words, defining the theoretical basis for an "unacceptable condition" in sediments. Water resource management has historically used single-species laboratory toxicity tests to set numerical criteria on a chemical-by-chemical basis and such criteria were then used in the field as a threshold concentration not to be exceeded. The assumption being that by protecting the sensitive or surrogate species in the laboratory, the populations and communities in the field would also be protected.

Sediment Quality Criteria:

In the US, the EPA evaluated many different approaches to estimate the toxicity of sediment-associated contaminants to determine underlying factors that could be used to normalize the observed toxicity of contaminated sediments. Two key observations provided insights on quantifying the bioavailability of chemicals in sediments. First, the concentration-response curve for biological effects could be correlated, not to total chemical concentrations, but to the interstitial (pore-water) concentration. Organism mortality, growth rate, and bioaccumulation data appear to support and demonstrate this correlation (DiToro et al., 1991). Second, for a certain class of compounds, the non-polar organics which include a variety of pesticides and polycyclic aromatic hydrocarbons, the movement or partitioning of the chemical from the sediment to the pore-water is controlled by the organic carbon content of the sediment. Therefore, the toxicity or bio-availability of these compounds is controlled by the "Equilibrium partitioning" of the compound between sediment particles and the biologically active phase, the pore water. Thus, if the partitioning coefficients are known for a given contaminant, then measuring its concentration in one environmental phase (e.g. sediment) allows one to predict its concentration in the other (e.g. pore water) (Shea, 1988, Di Toro et al., 1991).
In developing Draft Sediment Quality Criteria (EPA, 1989b), the EPA decided to utilize existing Water Quality Criteria (WQC) as the toxicological benchmark to compare the predicted pore-water concentrations. Briefly, the WQC are based upon a wide range of aquatic water column toxicity data and is currently considered the best available estimate of cause-effect relationships in the water column (EPA, 1989b). The use of the WQC as the toxicological benchmark for pore-water assumes that (a) the sensitivity of benthic species and species tested to derive WQC are similar, and (b) that exposure to the same concentration of a contaminant in interstitial water is equivalent to exposure in the water column. It is this second assumption that will be tested by this research.

Research Objective:

The objectives of my research is to compare the accumulation of the PAH phenanthrene by marine amphipods from sediment and interstitial water versus from a water only exposure system. The null hypothesis for this research is that there is no significant

difference in exposure to benthic amphipods via interstitial water or via the water column. Exposure will be measured by following the bioaccumulation of phenanthrene in tissue over time. The alternative hypothesis is that there is a significant difference in exposure to benthic amphipods via interstitial water or via the water column. If a significant difference is found between these two exposure scenarios, an attempt will be made to determine whether the observed differences are biological in nature (for example, because of differences in metabolism or activity between exposure scenarios) or due to physical/chemical processes that differ between exposures occurring in sediment versus in the water column. Factors that can limit the uptake of phenanthrene by organisms in sediment, such as disequilibrium situations occurring when uptake rates into the organisms are greater than desorption rates from sediment particles, will be discussed.
In this series of experiments, three infaunal marine amphipod species; *Eohaustorius estuarius*, a mobile free-burrowing amphipod, *Leptocheirus plumulosus*, a sedentary, soft-walled burrow building amphipod, and *Grandidierella japonica*, a sedentary tube-building amphipod, were exposed to $^{14}$C-phenanthrene under the following experimental conditions: 1)Sediment spiked at a concentration resulting in an interstitial water concentration of 2.5 ug/L $^{14}$C-phenanthrene with the overlying water maintained at 2.5 ug/L $^{14}$C-phenanthrene, and 2) A water only exposure with the water maintained at 2.5 ug/l $^{14}$C-phenanthrene. The bioaccumulation of $^{14}$C-phenanthrene was followed over 72 hours of exposure and the body burden of $^{14}$C-phenanthrene and uptake kinetic parameters were compared and contrasted between the two exposure treatments to determine whether the exposure to a specific concentration of $^{14}$C-phenanthrene in the water column is equivalent to exposure to EQP predicted concentration of $^{14}$C-phenanthrene in interstitial water.

## 3.2 MATERIALS AND METHODS

Experimental Animals:

*Leptocheirus plumulosus* and *Grandidierella japonica* were obtained from ongoing cultures maintained at the EPA laboratory facility at Newport, OR. *Leptocheirus plumulosus* and *Grandidierella japonica* were obtained prior to the initiation of the bioaccumulation experiments by sieving culture bins with a 0.5-mm mesh-diameter screen to retain suitable adult amphipods. *Eohaustorius estuarius* was manually collected from the upper 10 cm of intertidal, estuarine sands of Yaquina Bay, Newport, OR. The amphipods were collected by trowel at low tide and sieved from their native sediments with a 1.0-mm

mesh-diameter screen. All amphipods were presorted prior to use. Only non-gravid adult amphipods were used to avoid gender specific total lipid content differences due to the presence of eggs or brood pouches in gravid females.

Sediment:

Clean estuarine sediment was collected from McKinney Slough located in Alsea Bay, Waldport, Oregon. The field sediment was washed sequentially through a 1.0-mm mesh-diameter screen and a 0.25-mm mesh diameter screen to remove indigenous macroinvertebrates. The organic carbon content of the sediment was determined by acidifying and drying the sediment and analyzing on a Perkin-Elmer CHON analyzer. Percent total solids was determination was made by measuring weight loss of samples dried at 90°C for 12 hours and 110°C for one additional hour. Based on these data, the sediment was spiked to reach a target whole sediment concentration of 37 ug/g OC dw phenanthrene. This target is the equilibrium predicted concentration of phenanthrene in sediment that would result in an interstitial water concentration of 2.5 ug/L phenanthrene. The sediment was spiked by lining 1 gallon glass jars with $^{14}$C-phenanthrene in an acetone carrier and allowing the acetone to volatilize completely out of the glass jar. This was done to avoid any carrier affect caused by residual levels of acetone in the sediment subsequent to the introduction of the $^{14}$C-phenanthrene. The jars were then filled with the appropriate mass of sediment and placed onto a rolling mill. Sediments were rolled for 2 hours in the dark at room temperature and allowed to settle overnight at 4°C and then rolled an additional 2 hours. The sediments were then allowed to equilibrate for 28 days at 4°C in the dark before use (Ditsworth et al., 1990, Swartz et al., 1990).

Chemicals:

$^{14}$C-Phenanthrene (PHE), labeled on carbon-9, was obtained from Sigma Chemical Company (St. Louis, MO). The specific activity of the $^{14}$C-PHE was 8.3 mCi/mmol and was reported by the manufacturer to be at least 98% radiopure.

Experimental Design - Bioaccumulation Experiments:

For these series of experiments, the exposures were conducted in two separate treatments. In the first, the amphipods were exposed to contaminated sediment with spiked overlying water. In the second, the amphipods were exposed to spiked water containing the same concentration of $^{14}$C-phenanthrene as the sediment interstitial water.

*Exposure Chambers:*

The sediment and water exposures were carried out in precleaned 400 ml glass beakers covered with a pyrex watch glass to reduce contamination of the contents and evaporation of the water and test material over the course of the experiment. The sediment exposure chambers contained approximately 85 g of sediment and 300 ml of overlying water at a salinity of 28 ‰. The water exposure chambers contained 390 ml of spiked water at a salinity of 28 ‰ and contained 15 pieces of 1/3" - 1/2" diameter glass tubing cut to 2" lengths. This glass tubing was added to the beakers to provide refugia for the normally sediment dwelling amphipods. The intent was to decrease the biological activity of the amphipods by reducing swimming behavior in the water column and to decrease cannibalism in the *Grandidierella japonica* .

The overlying water and water was replaced every eight hours in each beaker on an static renewal basis. The old water was carefully siphoned out with a screened siphon to avoid removing any amphipods from the exposure beakers and the new water gently added to avoid resuspension of the sediment in the exposure vessel. For the renewal of overlying water, a fresh batch of spiked water was made up for each water change using the following protocol. A stock $^{14}$C-phenanthrene solution was prepared by adding an appropriate volume of $^{14}$C-phenanthrene in an acetone carrier to a pre-cleaned 2-liter volumetric flask. The acetone was then completely evaporated off, again to avoid any carrier affects in the treatment, and 2-liters of fresh filtered sea water at 28 ‰ added. The flask was then placed on a stir plate with a Teflon stir bar and gently agitated for two hours prior to use. This stock was be diluted down to the target $^{14}$C-phenanthrene concentration in sea water immediately before addition to the exposure chambers.

*Experimental Design:*

Three days prior to the initiation of the exposure treatments, approximately 400 amphipods of each test species were collected and readied for acclimation to experimental conditions. Field collected *Eohaustorius estuarius* were transferred to an aerated plastic tub containing native Yaquina Bay sand, fresh filtered sea water at 28 °/oo salinity, and maintained at 20°C. *Leptocheirus plumulosus* and *Grandidierella japonica* were obtained from ongoing cultures and these amphipods were transferred to an aerated plastic tub containing clean McKinney Slough sediment (same sediment as the test). For *Grandidierella japonica* the salinity in the acclimation tub was maintained at 28 °/oo and the temperature at 20 °C. For *Leptocheirus plumulosus* which is maintained at a salinity of 20 °/oo in culture, the acclimation involved sequentially increasing the salinity in the acclimation tub by a few ppt per day until it reached the test salinity of 28 °/oo. All acclimation tubs were feed daily with a mixture of algae and powdered dry food (DeWitt et al., 1992).

One day before the initiation of the sediment exposure, the test sediment and clean overlying sea water were added to all exposure chambers. This was done to allow suspended sediment particles to settle and to allow time for equilibration of temperature and the sediment-water interface. Also at this time, the water only exposure chambers were set up, including the addition of glass tubes and spiked water at the appropriate contaminant concentration. After the overnight equilibration, the overlying water in each chamber was carefully replaced with sea water containing 2.5 ug/L $^{14}C$ -phenanthrene two hours prior to the initiation of the exposure treatment. At the initiation of exposure, 20 amphipods were distributed to each of the test chambers and spiked sea water added to bring the water level up to the appropriate level.

There were three replicates beakers per species for each sampling time, and three clean control chambers per species were set up to evaluate mortality and the lipid content at the end of the exposure treatment. There were 5 sampling times for each exposure treatment. The amphipods were sampled at 4, 8, 24, 48, and 72 hours after initiation of exposure. At the time of sampling, the amphipods from each exposure chamber were sieved out of the contaminated sediment or water exposure chamber using a 500 um mesh-diameter screen and placed in clean sea water for 15 minutes. The amphipods were then blotted dry and placed in polyethylene tubes and frozen at -80°C until analysis. Table 3.1 presents a summary of the experimental conditions for the bioaccumulation experiments.

Table 3.1: Experimental Parameters for Bioaccumulation Experiments

| | |
|---|---|
| Salinity | 28 °/oo |
| Temperature | 20 °C |
| Exposure Chamber | Covered 400-ml Glass Beakers |
| Overlying Water Renewal Period | 8-Hours |
| Light Cycle | 24-Hour Dark |
| Exposure Time | 72 Hours Total; 5 Sampling Time Points |

Experimental Design - Elimination Experiments:

An additional study was conducted with each species to derive elimination constants ($k_2$'s) for use in the first order uptake model that will be developed for each species. The elimination rate constants are determined from the elimination rate of the pollutant after the organism has been placed in a clean environment. This study was performed in two phases, first an accumulation phase and second an elimination phase which included the sampling and analysis of amphipods.

In the accumulation phase of the study, each species of amphipods was exposed to 2.5 ug/L $^{14}$C-phenanthrene over 72-hours in an water only exposure system. The water in the exposure vessels (400-ml pre-cleaned glass beakers) were maintained at a relatively constant concentration by a static renewal system with 90% water changes every eight hours. For each species, 14 beakers containing 20 amphipods per beaker were used in the accumulation portion of the study. The exposure vessels contained 15 pieces of 1/3" - 1/2" diameter glass tubing cut to 2" lengths. This glass tubing was added to the beakers to provide refugia for the normally sediment dwelling amphipods. The experimental conditions (e.g. temperature, salinity, light regime) were identical to those presented for the bioaccumulation experiments (Table 3.1).

The elimination of $^{14}$C-phenanthrene was determined by following the decrease of tissue residues of $^{14}$C-phenanthrene in the amphipods after being placed in clean sediment. After the exposure period, the amphipods were placed into beakers containing approximately 85 grams of clean McKinney Slough sediment with 300 ml of clean overlying sea water.

Amphipods from each species were placed in triplicate beakers for each sampling time. The sampling times for this portion of the study were 1, 2, 4, 8, 24, and 48 hours after being removed from the exposure water. In the elimination portion of the study, there were triplicate beakers for each time point containing 15 amphipods each. The sampling protocols were identical to those presented for the bioaccumulation experiments.
As in the bioaccumulation study, separate control beakers were set up to evaluate lipid content changes and survival over the course of the experiment. At the beginning of the exposure, four beakers containing 20 amphipods each were set up for each species. During the accumulation phase, these beakers were renewed with fresh clean sea water every eight hours. At the conclusion of the accumulation portion of the study, 2 beakers were sampled for mortality and lipid content to serve as baseline data for the elimination phase. Two beakers with clean sediment were set up for each species and these were sampled at the end of 48 hours for determining overall survival and total lipid content of the amphipods.

Chemical Analysis:

Overlying water and water samples were collected from the exposure beakers by taking 2 ml samples and placing in LSC vials with 18 ml of Optiflour scintillation cocktail for aqueous samples (Packard Instrument Co.). Overlying water samples were taken from duplicate beakers for each species at the start of the exposure and before and after each water change. The activity (dpm) of the water samples were counted on Packard Tri-Carb 2000CA Liquid Scintillation Analyzer with external quench control and the actual concentration or mass of $^{14}$C-phenanthrene was quantified according to sample volume and specific activity of the $^{14}$C-phenanthrene.

Total $^{14}$C in non-aqueous samples were determined using a Packard Tri-Carb sample oxidizer (Model 307A, Packard Instrument, Co.). The $^{14}CO_2$ was trapped as a carbonate salt in Carbo-Sorb (Packard Instrument, Co.) using a volume of 9 ml for sediment and 6 ml for amphipods. The Carbo-Sorb was flushed into a glass scintillation vial with scintillation cocktail (12 ml Permofluor E, Packard Instrument, Co.) and quantified by scintillation counting. The sediment was collected in 3-4 g aliquots removed from the dedicated chemistry beaker at the beginning and end of exposure. Triplicate samples of approximately 0.2 g of sediment were analyzed from each of the sediment samples by thawing and placing in paper combustion cones. Cellulose powder and combust-aide

(Packard Instrument, Co.) were added to each sample to assist in sample combustion and complete oxidation. Recovery and sample memory were quantified prior to each analysis run by conducting performance verification test with Packard $^{14}C$ Spec-Chec (Packard 1990). Recovery of $^{14}C$ exceeded 95% and memory was less than 0.15% in all test runs. A $^{14}C$ standard ($^{14}C$-methyl mathacrylate chip, E.I. du Pont de Nemours and Co.) was combusted at the end of each analysis run to confirm $^{14}C$ recovery.

Chemical analysis of amphipod samples was conducted by taking 0.05 g (wet weight) samples, approximately 4 individual amphipods, and placing in paper combustion cones. Cellulose powder and combust-aide (Packard Instrument, Co.) were added to each sample to assist in sample combustion and complete oxidation. Triplicate samples were analyzed at each sampling time and for each amphipod species.

The interstitial water was collected by placing 35 g of sediment into precleaned 50 ml pyrex centrifuge tubes and centrifuging at 10,000 rpm for 60 minutes. Then, duplicate 2 ml samples were taken of the overlying water in each tube for analysis by LSC. The samples for interstitial water and bulk sediment analysis were collected from triplicate chemistry control beakers (containing no amphipods) that were set up at the beginning of the experiment. For the T=0 hour data, the chemistry control beakers were loaded with sediment as with the experimental beakers and placed with clean overlying water overnight. These beakers were then taken down for sampling sediment and interstitial water prior to the start of exposure. For the T=72 data, the chemistry control beakers were maintained exactly the same as the experimental beakers and were taken down at the conclusion of the experiment.

*Reverse Phase Separation:*

Reverse Phase separation (RPS) of water was performed by passing 2 ml of sample from a pipette through a single, sea water rinsed disposable column, containing 200 mg of $C_{18}$ sorbent (Bond Elut, Analytichem International Inc., and Varian Inc., Harbor City, CA). The columns were held in an evacuated chamber (380 mm Hg), and the column-passed samples collected in 20-ml LSC vials within the chamber (Vac Elut, Analytichem International). The columns were rinsed prior to use with 10 ml of membrane-filtered, laboratory sea water. Following passage through a column, 18 ml of Optiflour scintillation cocktail (Packard Instrument Co.) was added to the elutriate samples and the samples were analyzed directly by LSC.

*Liquid-Liquid Extraction:*

Liquid-liquid extractions were performed in accordance with a method presented by Ozretich (1995). 2 ml of iso-octane were added to 5 ml of sample in a Teflon sealed glass vial and the vial was placed upon its side on an oscillatory table (70 to 80 Hz) in a dark room at room temperature for 12 to 18 hours. The oscillation rate was just below that which would cause waves in the organic layer to break. After liquid-liquid extraction, the organic layer was suctioned out and 2 ml of the aqueous layer added to 18 ml of Optiflour scintillation cocktail (Packard Instrument Co.) and the samples were analyzed directly by LSC to provide the activity remaining in the aqueous fraction.

*Lipid Analysis:*

The percent total lipids were determined for each species of amphipods by first freeze-drying the amphipods and then extracting with chloroform:methanol. The percent lipids were then determined by a microgravimetric technique (Gardner et al., 1985). The total percent lipid (dry weight) were measured for each species and exposure treatment at the beginning and end of exposure. The amphipods collected at the end of the exposure were subject to the same experimental conditions as the test organisms, however they were not exposed to $^{14}$C-phenanthrene.

Three composite samples of 20 amphipods each were selected for each species and frozen at -80°C until analysis. For each species, three separate beakers were set up with uncontaminated McKinney Slough sediment. Twenty amphipods were added to each of these beakers and these beakers served the dual purpose of a negative control (to evaluate non-contaminant related responses to test sediment) and to determine the loss of lipid (if any) in the experimental animals over the course of the 72-hour exposure. The lipid/negative control beakers were maintained in exactly the same manner as the experimental beakers. In determining the percent lipid content for use in lipid normalizing the tissue concentration of phenanthrene for each exposure treatment, the mean lipid content was calculated from the results obtained from the beginning and end of the of the exposure period. The lipid data so collected are presented as percent lipid dry weight (dw).

Wet to Dry Weight Normalization:

Wet weight to dry weight ratios regression models were developed for each amphipod species from a data set including wet weight and corresponding dry weights from individual organisms and from composites of five to ten animals. To develop these regression models, the dry weights and wet weights were plotted and a simple linear regression line drawn through all of the data points. The following regression were used for each amphipod species to convert wet weight data to dry weight.

| | | |
|---|---|---|
| *Eohaustorius estuarius* (n=16) | $y = 0.221x + 0.547$ | $r^2 = 0.987$ |
| *Leptocheirus plumulosus* (n=14) | $y = 0.243x + 0.056$ | $r^2 = 0.995$ |
| *Grandidierella japonica* (n=19) | $y = 0.256x - 0.037$ | $r^2 = 0.978$ |

Where y = wet weight and x = dry weight.

Bacterial Enumeration:

To determine the efficacy of autoclaving sediments to inactivate bacteria living in the sediment, 0.1 ml aliquots of collected interstitial water were plated in triplicate onto Marine Nutrient Agar and Tryptic Soy Agar (TSA). Plates were incubated at 25°C for two weeks and observation of colony forming units (CFU) made daily.

Data Analysis:

The mean body burdens of $^{14}$C-phenanthrene between the two exposure treatments for each species were compared by performing a paired student's *t*-test at each time point. Differences between means were considered significant when $p < 0.05$. Standard deviations (SD) are reported to show the range in the data and the standard error of the mean (SEM) is reported when comparisons of the means are intended.
The regression coefficients generated by the linear and non-linear regression models were compared to determine whether a significant difference exists by Spjotvoll-Stoline T'-method (Sokal and Rolfe, 1981). Differences between regression coefficients were considered significant when $p < 0.05$.

*First Order Accumulation Models:*

A first order bioaccumulation model was used to describe the uptake of $^{14}C$-phenanthrene by amphipods. The first order model has been used to estimate tissue residue resulting from exposure to dissolved (water-borne) contaminants. The objectives of this modeling effort was to compare uptake coefficients between the two exposure treatments conducted in this experiment. In this model, the change in tissue concentration with time is calculated by:

$$dC_t/dt = k_1C_w - k_2C_t \qquad (1)$$

Where:

$C_t$ = tissue residue (ug/g tissue);
$C_w$ = pollutant concentration in water (ug/g water);
$k_1$ = uptake rate coefficient (mL water/g tissue-time);
$k_2$ = elimination rate constant ($time^{-1}$);
t = time.

Initial uptake and elimination rates determined in short-term experiments can be used to predict steady-state bioconcentration factors (BCFs) and the exposure time necessary to reach steady-state.

Elimination of pollutants from tissues was modeled by:

$$C_tt = C_{t0}(e^{-k2t}) \qquad (2)$$

Where:

$C_{t0}$ = tissue residue at the start of the elimination phase ($t_0$).

The $k_1$ and $k_2$ values used in these equations can be estimated by fitting equation (1) to measured tissue residue values using an iterative least-squares nonlinear regression method. Alternatively, $k_1$ can be estimated from the rate of increase in tissue residue measured in short-term uptake experiments during the initial (linear) phase of contaminant uptake when tissue residue concentrations are low and elimination minimal. The elimination rate constant is often determined by depurating contaminated organisms in uncontaminated sediment and determining $k_2$ directly. As the model assumes that $k_2$ is not

a function of tissue residue, it is possible to determine $k_2$ values for test organisms that have been exposed to contaminants in short-term uptake experiments.
Both the linear and non-linear method for deriving uptake rate constant estimates have been reported in the literature (Boese et al., 1997). While both of these methods should give similar results, the non-linear regression method has the advantage of using a greater proportion of the experimental data.

*Linear Regression Accumulation Rates:*

Uptake rate constants ($k_1$) were estimated from short-term uptake experiments with the linear regression of tissue residue (lipid normalized mg/g lipid dry weight) on exposure time (hours). As this method is used only on the linear portion of the uptake curve, the time points to be included in the regression were selected by a visual analysis of the uptake data and an analysis of the $R^2$ values of the regression lines. Generally, only those data in the linear uptake portion of the uptake curve were utilized and the data set with the highest $R^2$ value among those calculated was selected for use.

*Non-linear Regression Accumulation Rates:*

Uptake rate constants ($k_1$) were also estimated by fitting equation (1) to tissue residue data from short-term uptake experiments with the experimentally derived $k_2$ constraining the non-linear regression model. Generally all of the timepoints were included in this method, however in situations where there was a significant decrease in tissue residue at the end of the exposure period, the latter time point data were not included in the $k_1$ estimation. A iterative, nonlinear, least-squares curve fitting technique (SigmaStat, Jandel Scientific Software, San Rafael, CA) was used.

*Elimination Rates Constants ($k_2$):*

The elimination data is modeled as an exponential function. The elimination rate coefficient ($k_2$) is calculated by fitting a linear regression to the natural log of the tissue residue versus time. The elimination coefficient is determined as the absolute value of the slope of this regression line and has the units time$^{-1}$. The elimination rate is determined from a linear regression of the natural log transformed tissue residue data from the elimination study. Generally, all of the data were used in deriving $k_2$ estimates, however,

in situation where the natural log transformed data deviated from linearity, a subset of the data was used to generate $k_2$ estimates by trying to maximize $R^2$ values of the regression lines.

## 3.3 RESULTS:

Exposure Concentrations of $^{14}$C-phenanthrene:

The target interstitial water concentration of phenanthrene for the sediment exposure treatment was 2.50 ug/l. The bulk sediment was spiked with the equilibrium predicted mass of $^{14}$C-phenanthrene and the measured interstitial water concentration of $^{14}$C-phenanthrene after 28-days of equilibration was very close to the target. The amount of radiolabeled compound measured in the interstitial water, compared to the nominal or target concentration was 106.4% (2.66 ug/l T=0). The measured interstitial water concentrations of $^{14}$C-phenanthrene at the beginning and end of the accumulation experiments are presented in Table 3.2. There was no significant change in the interstitial water concentration of $^{14}$C-phenanthrene over the course of the 72-hour exposure.
The target bulk sediment concentration of $^{14}$C-phenanthrene was 37 ug/g OC dw. The sediment concentration of phenanthrene was measured to be 41.87 ug/g OC dw at T=0 and 43.00 ug/g OC dw at T=72. There were slight differences in the sediment concentration of $^{14}$C-phenanthrene between T=0 hours and T=72 hours, however these differences were not statistically significant and there was no apparent trend in sediment concentration over time.

The target water concentration of $^{14}$C-phenanthrene in the water only exposure treatments was 2.5 ug/L. The mean measured concentration of $^{14}$C-phenanthrene was 2.37 ug/L at T=0 and 2.60 ug/L at T=72 hours. The concentration of $^{14}$C-phenanthrene in water are presented in Table 3.2.

Table 3.2. Interstitial Water and Sediment Concentrations of $^{14}$C-phenanthrene.

| Treatment | T=0 (SD) | T=72 (SD) |
|---|---|---|
| Interstitial Water - Sediment Exposure | 2.66 ug/L (0.27) | 2.50 ug/L (0.10) |
| Sediment Conc. - Sediment Exposure | 41.87 ug/g OC dw (1.94) | 43.00 ug/g OC dw (1.01) |
| Water Conc. - Water Exp. | 2.37 ug/L (0.06) | 2.60 ug/L (0.04) |

The water concentrations presented in Table 3.2, for the water only exposure treatment, were the mean concentrations collected from replicate exposure chambers from all three of the test species after the addition of the amphipods at test initiation (T=0) and right before the termination of exposure (T=72). Additional water samples were collected before and after the scheduled water changes and the results were segregated by amphipod species. The measured concentration of $^{14}$C-phenanthrene in the exposure chambers before and after the scheduled water changes are presented in Table 3.3. For each of the three amphipod species, there was a slight decline in $^{14}$C-phenanthrene concentration in the water during the eight-hour intervals between water changes. This decline in water concentration of phenanthrene was most likely caused by uptake of phenanthrene by the amphipods, however, a quantitative mass-balance evaluation was not performed. The concentrations of $^{14}$C-phenanthrene over the 72-hours of exposure were very close to the target of 2.50 ug/L.

Table 3.3. Overlying Water Concentrations of $^{14}$C-phenanthrene Before and After Renewal in ug/L.

| Treatment | Species | Before Renewal (SD) | After Renewal (SD) |
|---|---|---|---|
| Water Exposure Conc. | *E. estuarius* | 2.37 (0.24) | 2.60 (0.12) |
| Water Exposure Conc. | *G. japonica* | 2.41 (0.17) | 2.63 (0.07) |
| Water Exposure Conc. | *L. plumulosus* | 2.43 (0.28) | 2.66 (0.10) |

Lipids:

Total lipids (presented on a percent dry weight basis) were measured for each species at the start and end of the exposure period and the results are presented in Table 3.4. In the sediment exposure, the lipid content for *Eohaustorius estuarius* ranged from 9.50 % - 10.03 % dry weight (dw). In the water exposure, the lipid content ranged from 3.90 % - 5.03% dw. The lipid content for *Grandidierella japonica* ranged from 4.13 % - 4.37 % dw for the sediment exposure and 2.99 % - 3.27 % dw for the water exposure treatment. For *Leptocheirus plumulosus* , the measured lipid content ranged from 3.53 % - 4.23 % dw in the sediment exposure and 3.70 % - 4.47 % dw for the water exposure treatment. The differences in measured lipid content between the two exposure treatments are the result of the fact that these exposure were carried out at different times of the year (sediment exposures conducted in January and water exposures were carried out in March). Additionally, there was slight variability in the measured lipid content over the course of the 72 hour exposure period but the differences were not statistically significant. The mean percent lipid content was calculated for each amphipod species and this value was used to lipid normalize the tissue residue data to ug-phenanthrene/g-lipid.

Table 3.4. Total Lipid Concentrations (percent dry weight (dw)) Measured in the Amphipods.

| | T=0 dw (SD) | T=72 dw (SD) | Mean Lipid Conc. dw |
|---|---|---|---|
| Lipid (*E. estuarius*) - Sediment Exposure | 10.03 % (0.51) | 9.50 % (0.26) | 9.77 % |
| Lipid (*E. estuarius*) - Water Exposure | 3.98 % (0.41) | 5.03 % (1.31) | 4.51 % |
| Lipid (*G. japonica*) - Sediment Exposure | 4.37 % (0.75) | 4.13 % (0.93) | 4.25 % |
| Lipid (*G. japonica*) - Water exposure | 2.93 % (0.50) | 3.27 % (0.31) | 3.10 % |
| Lipid (*L. plumulosus*) - Sediment Exposure | 4.23 % (0.38) | 3.53 % (0.51) | 3.88 % |
| Lipid (*L. plumulosus*) - Water Exposure | 3.70% (0.44) | 4.47 % (0.38) | 4.08 % |

Bioaccumulation Data:

The tissue residue data from the bioaccumulation experiments were converted from wet weight to dry weight using the species specific regression algorithm presented in the materials and methods section of this paper. This data was then lipid normalized to give the final concentration of $^{14}$C-phenanthrene in ug phenanthrene/g lipid dw. The lipid normalized bioaccumulation data (ug/g lipid) for *Eohaustorius estuarius*, *Grandidierella japonica*, and *Leptocheirus plumulosus* are presented in Figures 3.1, 3.2, and 3.3 respectively.

*Eohaustorius estuarius*:

Figure 3.1 presents the tissue residue data for *Eohaustorius estuarius* for both the contaminated sediment exposure and the water only exposure. In the contaminated sediment exposure, the tissue residue of $^{14}$C-phenanthrene increased over the first 48 hours of exposure, reaching an apparent steady state concentration of approximately 133.5 ug/g lipid exhibited during the 48 and 72 hour time points. For the water only exposure, the tissue residue of $^{14}$C-phenanthrene increased over the first 48 hours of exposure, reaching an apparent steady state concentration of approximately 275 ug/g lipid.

The tissue residue levels of $^{14}$C-phenanthrene were compared between the two exposure treatments by comparing the mean body burdens (in ug/g lipid) at each time point using a student's t-test. For *Eohaustorius estuarius* , there was a statistically significant difference between the mean body burdens between the two treatments at all time points, with the tissue residues of $^{14}$C-phenanthrene being higher in the water only exposure.

Uptake coefficients ($k_1$) for *Eohaustorius estuarius* were calculated using a linear regression model and a non-linear regression model. The linear model used data from time points T = 0 through T = 48 hours, which represented the linear portion of the uptake curve. The non-linear regression model utilized all the data points and the model was run with the $k_2$ value (elimination rate coefficient) being constrained to the experimentally derived value of $0.063^{-time}$ ( from Chapter 2 of this thesis). The modeled uptake rate coefficients are presented in Table 3.5.

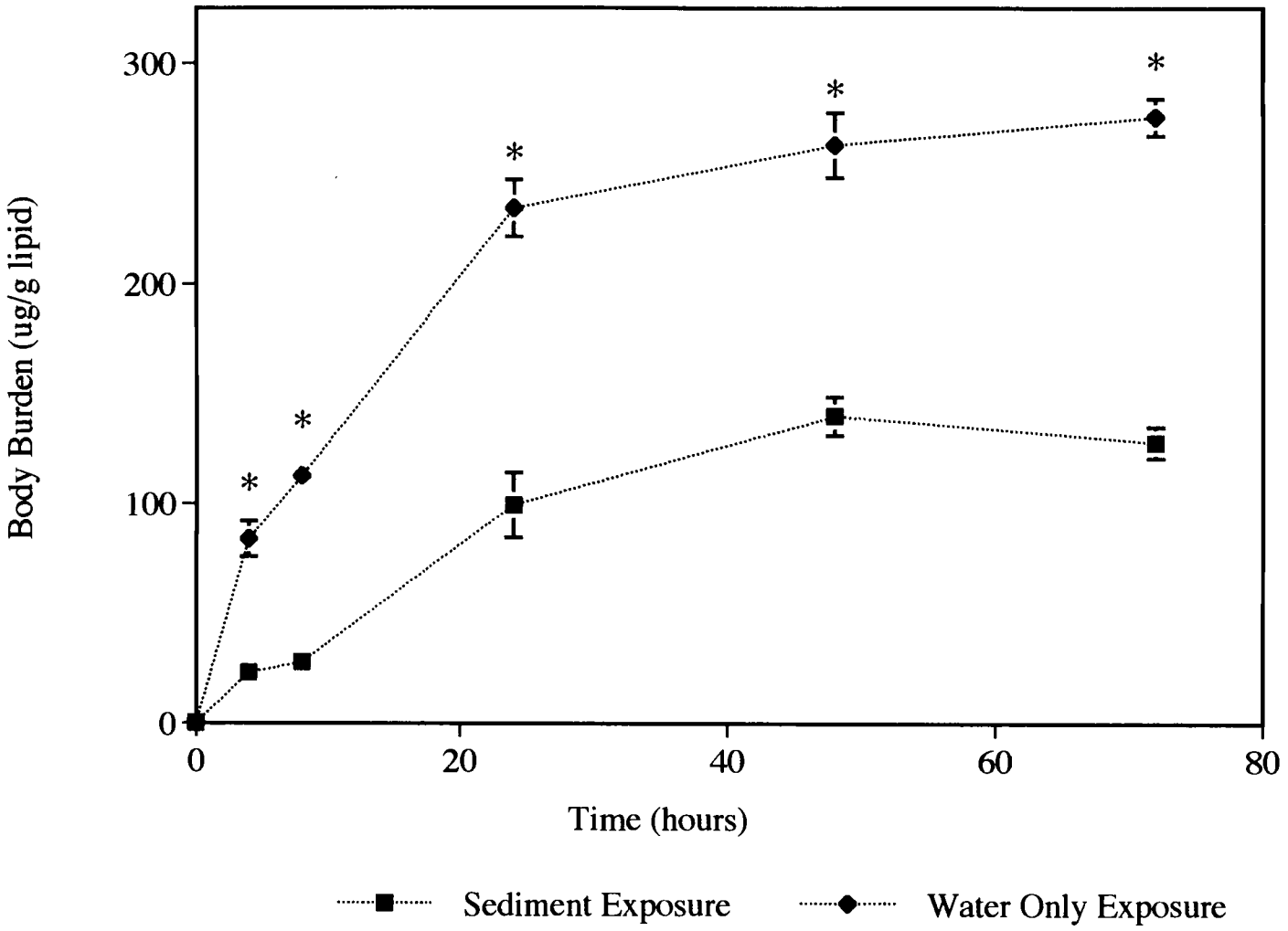


Figure 3.1. Bioaccumulation of $^{14}$C-phenanthrene by Eohaustorius estuarius from sediment and water only exposures. All points ± Standard Error of the mean. n = three replicates. * significantly different means

*Grandidierella japonica:*

Figure 3.2 presents the tissue residue data for *Grandidierella japonica* for both the contaminated sediment exposure and the water only exposure. The tissue residue for $^{14}$C-phenanthrene in the contaminated sediment exposure increased over the first 48 hours to reach an apparent steady state concentration of approximately 170 ug/g lipid. For the water only exposure, the concentration of $^{14}$C-phenanthrene continued to increase over the entire course of the 72-hr exposure reaching a maximum concentration of 607 ug/g.
The tissue residue levels of $^{14}$C-phenanthrene were compared between the two exposure treatments by comparing the mean body burdens (in ug/kg lipid) at each time point between the two treatments using a student's t-test with a p-value of 0.05. For *Grandidierella japonica* there was a statistically significant difference between the mean body burdens between the two treatments at all time points, with the tissue residues being higher in the water exposure.

Uptake coefficients ($k_1$) for *Grandidierella japonica* were calculated using a linear regression model and a non-linear regression model. The linear model used data from time points T=0 through T=48 hours for the sediment exposure and time points T=0 through T-72 hours for the water exposure. These represented the linear portions of the uptake curve for each treatment. The non-linear regression model utilized all the data points for both exposure treatment and the model was run with the $k_2$ value being constrained to the experimentally derived value of $0.052^{-time}$ (from Chapter 2 of this thesis). The modeled uptake rate coefficients are presented in Table 3.5.

*Leptocheirus plumulosus* :

Figure 3.3 presents the tissue residue data for *Leptocheirus plumulosus* for both the contaminated sediment exposure and the water only exposure. The tissue residue for $^{14}$C-phenanthrene in the contaminated sediment exposure increased over the first 48 hours to reach an apparent steady state concentration of 75 ug/g lipid. For *Leptocheirus plumulosus*, the uptake curves reached a maximum at 24 hours in water exposure followed by a decrease in tissue residue concentrations of $^{14}$C-phenanthrene over the remaining time points. The tissue level of $^{14}$C-phenanthrene also declined in the final sampling point under sediment exposure treatment. This pattern was not observed in the other two species tested and may represent heightened metabolism and elimination of $^{14}$C-phenanthrene by this species.

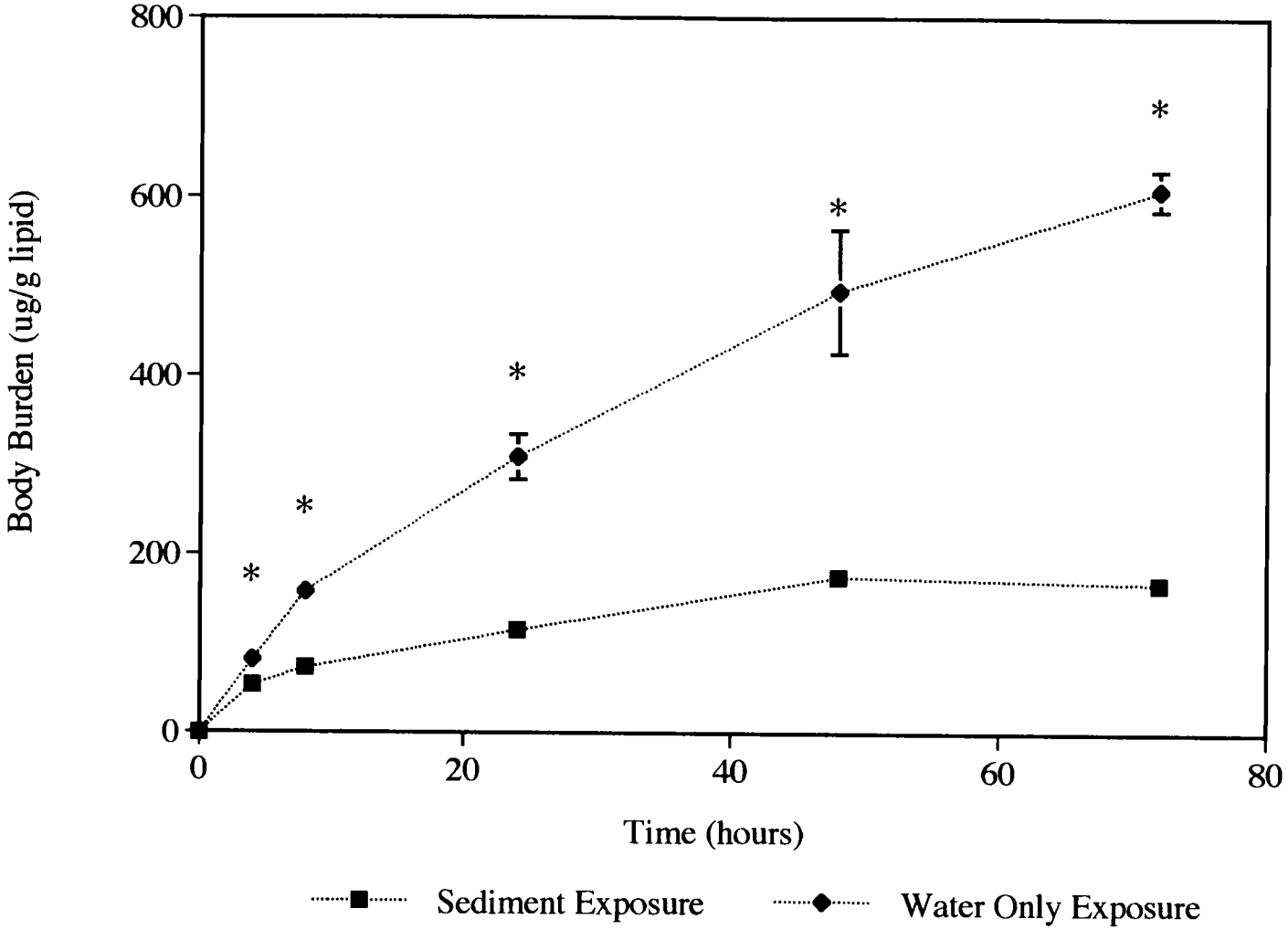


Figure 3.2. Bioaccumulation of $^{14}$C-phenanthrene by Grandidierella japonica from sediment and water only exposures. All points ± Standard Error of the mean. n = three replicates. * significantly different means

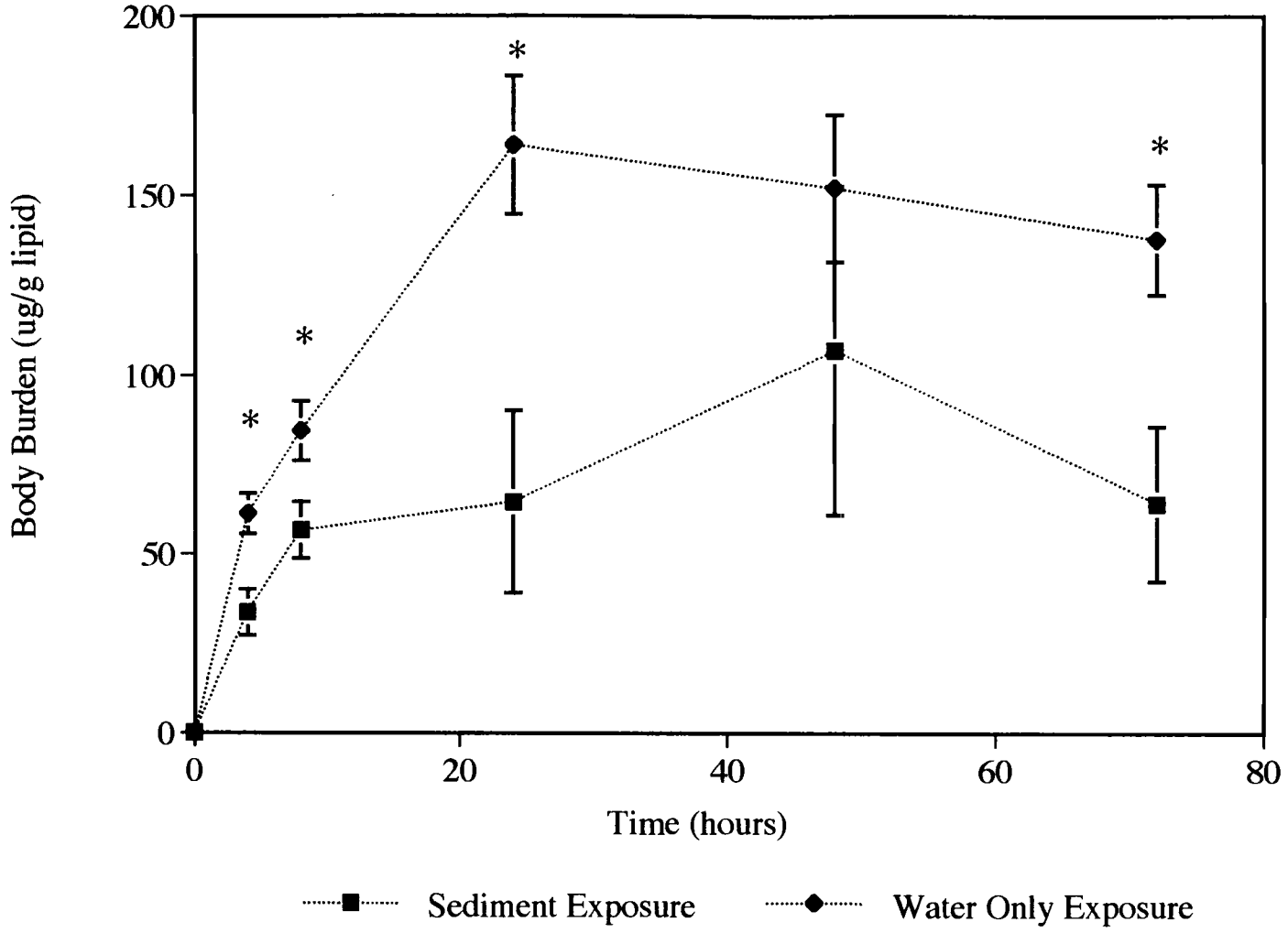


Figure 3.3. Bioaccumulation of $^{14}$C-Phenanthrene by Leptocheirus plumulosus from sediment and water only exposures. All points ± Standard Error of the mean. n = three replicates. * significantly different means.

The tissue residue levels of $^{14}$C-phenanthrene were compared between the two exposure treatments by comparing the mean body burdens (in ug/kg lipid) at each time point between the two treatments using a student's t-test with a p-value of 0.05. For *Leptocheirus plumulosus* there was a statistically significant difference between the mean body burdens between the two treatments at all sampling times with the exception of the 48-hour time point. While the concentration of $^{14}$C-phenanthrene was higher in the water only exposure at this sampling time, the high variability within replicates caused the mean concentration difference between treatments to be not considered statistically significant.

Uptake coefficients ($k_1$) for *Leptocheirus plumulosus* were calculated using a linear regression model and a non-linear regression model. The linear model used data from sampling time points T=0 through T=48 hours for the sediment exposure and time points T=0 through T-24 hours for the water exposure. These represented the linear portions of the uptake curve for each treatment. The non-linear regression model utilized data from time points T=0 through T=48 hours for the sediment exposure and time points T=0 through T=72 hours for the water exposure and the model was run with the $k_2$ value being constrained to the experimentally derived value of $0.052^{-time}$ (from Chapter 2 of this thesis). The modeled uptake rate coefficients are presented in Table 3.5.

Table 3.5. Uptake and Elimination Rate Coefficients Calculated for Each Species of Amphipod.

| | Linear Regression Model | | Non-Linear Regression Model | | Elimination Rate Coefficients * | |
|---|---|---|---|---|---|---|
| Species | $k_1$ | $R^2$ | $k_1$ | $R^2$ | $k_2$ | $R^2$ |
| *E. estuarius* - Sediment | 3.174 | 0.903 | 3.275 | 0.903 | -0.062 | 0.825 |
| *E. estuarius* - Water | 6.571 | 0.66 | 7.135 | 0.972 | -0.062 | 0.825 |
| *G. japonica* - Sediment | 4.027 | 0.742 | 3.738 | 0.930 | -0.0523 | 0.906 |
| *G. japonica* - Water | 9.382 | 0.86 | 11.356 | 0.905 | -0.0523 | 0.906 |
| *L. plumulosus* - Sediment | 2.453 | 0.345 | 3.153 | 0.501 | -0.080 | 0.834 |
| *L. plumulosus* - Water | 7.406 | 0.812 | 5.185 | 0.852 | -0.080 | 0.834 |

* Calculated using a linear regression model based on experimentally derived elimination data (Chapter 2).

Simultaneous Sediment and Water exposures for *Eohaustorius estuarius*:

The initial exposure treatments for this study were run at different times of the year because of limitations in laboratory space and personnel. However, because of the large difference in body burdens observed between the two exposure treatments for all three species and the difference in lipid content observed between exposures (particularly for *Eohaustorius estuarius*), an additional exposure study was conducted. In this study, the sediment and water only exposure treatments were run simultaneously for *Eohaustorius estuarius* . The same protocol described in the materials and methods section was used with the only deviation being that the sampling times were reduced to 24, 48, and 72 hours.

For these exposures, a different batch of $^{14}$C-phenanthrene (supplied by Sigma Chemical Co.) was used which had a higher specific activity than the $^{14}$C-phenanthrene used in the previous experiments (13.3 mCi/mmol vs. 8.3 mCi/mmol). To conserve the amount of the radiolabel, the target activity in the interstitial water and water was kept

constant with the previous studies. For this experiment, the initial target interstitial water concentration of $^{14}$C-phenanthrene was 1.32 ug/L determined by equilibrium partitioning calculation performed at the time of sediment spiking. However, interstitial water collected one day prior to study initiation was found to contain $^{14}$C-phenanthrene below the target concentration by 40% (i.e. 0.80 ug/L). This resulted in a change of protocol for this experiment with the target water and interstitial water concentration of $^{14}$C-phenanthrene being changed to this measured interstitial water concentration of 0.80 ug/L. The interstitial water concentration of $^{14}$C-phenanthrene was checked again at T=0 of the experiment and revealed an interstitial water concentration of 0.80 ug/L.

Table 3.6. Interstitial Water and Sediment Concentrations of $^{14}$C-phenanthrene dry weight (dw).

| | T=0 (SD) | T=72 (SD) |
|---|---|---|
| Interstitial Water Conc. | 0.801 ug/L (0.05) | 1.16 ug/L (0.05) |
| Sediment Conc. | 0.713 ug/g dw (0.007) | 0.691 ug/g dw (0.001) |
| Water Exposure Conc. | 0.863 ug/L | 0.800 ug/L |

The mean body burdens of $^{14}$C-phenanthrene in *Eohaustorius estuarius* (lipid normalized) are presented in Table 3.7. In this experiment, the results again indicated that the concentration of $^{14}$C-phenanthrene was greater in the amphipods exposed in an water only exposure than through a sediment interstitial water exposure. The concentration of $^{14}$C-phenanthrene was higher at all points at which amphipods were sampled though analysis of the data by a student's t-test revealed that, because of the variability within replicates, the body burden of $^{14}$C-phenanthrene, in the water only exposure, was significantly higher (p-value $< 0.05$) only at the 72-hour sampling time.

Table 3.7. Body Burdens of $^{14}$C-phenanthrene for *Eohaustorius estuarius*.

| | 24 hour (ug/g lipid) (SD) | 48 hour (ug/g lipid) (SD) | 72 hour (ug/g lipid) (SD) |
|---|---|---|---|
| Water Exp. | 58.80 (15.55) | 95.91 (19.01) | 98.77 (20.39) |
| Sediment Exp. | 39.51 (9.06) | 68.77 (4.78) | 62.07 (16.55) |

However, the behavior of the $^{14}$C-phenanthrene in the interstitial water of the sediment exposure treatment was substantially different than that exhibited during prior experiments. For example, the interstitial water concentration much lower than that initially predicted by equilibrium partitioning at the time of sediment spiking and the measured concentration of $^{14}$C-phenanthrene in the interstitial water increased over the 72-hours exposure period. Neither of these characteristics were observed in previous sediment spiking and exposure experiments.

Therefore, the collected interstitial water and freshly spiked sea water were subjected to reverse phase extraction to evaluate the chemical characteristics of the $^{14}$C being measured. The results of the reverse phase extraction are presented in Table 3.8. For interstitial water collected at T=0 and T=72 hours, a substantial percent of the total activity was found in the elutriate fraction while for the freshly spiked sea water (T=66 hours) only about 1% of the total activity was found to pass through the $C_{18}$ column. Two possible explanations were available to explain this behavior of $^{14}$C-phenanthrene in interstitial water. First was that the $^{14}$C-phenanthrene was binding to dissolved organic matter in interstitial water which would allow it to pass through the $C_{18}$ Column (Ozretich, 1995, Landrum, 1988). The second was that biotic or abiotic degradation of the $^{14}$C-phenanthrene had caused a change in the chemical characteristics of the compound being measured in interstitial water compared to that in freshly spiked sea water.

Table 3.8 Results of Reverse Phase Analysis of Interstitial and Spiked Sea Water

| Sample | Total Activity (dpm/ml) (SD) | Activity in Elutriate (dpm/ml) (SD) | Percent Activity in Elutriate (SD) |
|---|---|---|---|
| T=0 IW | 133 (8.53) | 66.50 (9.96) | 50.0% |
| T=72 IW | 193 (8.84) | 117.33 (7.77) | 60.8% |
| T=64 Water | 130.8 (2.08) | 1.67 (0.58) | 1.3% |
| Freshly Spiked Interstitial Water | 540 | 4.58 | 0.8% |
| Clean Sea Water | 674 | 5.71 | 0.8% |

To determine whether dissolved organic matter in the interstitial water was causing these unexpected results, 100 ml of interstitial water from an uncontaminated McKinney Slough sediment and 100 ml of fresh sea water were spiked with $^{14}$C-phenanthrene and passed through a $C_{18}$ cartridge after 2.5 hours of equilibration in the dark. If the dissolved organic matter was binding $^{14}$C-phenanthrene, it was expected that the activity present in the elutriate fraction would be much greater for the interstitial water than for the freshly spiked sea water. The results of this evaluation are presented in Table 3.8. As the data in Table 3.8 report, there was no difference in the percent of the total activity measured in the elutriate fraction between sea water and interstitial water which indicate that the anomalous results were not due to adsorption of $^{14}$C-phenanthrene to dissolved organic matter in the interstitial water.

Therefore, additional experiments were conducted to evaluate the changes occurring in the $^{14}$C-phenanthrene spiked sediment. The objective of these additional studies was to determine by reverse phase separation (RPS) and liquid-liquid extraction, the approximate percentage of $^{14}$C-phenanthrene being degraded in sediment over time and by conducting these studies with sterilized and non-sterilized sediment, evaluate the extent of biotic vs. abiotic degradation occurring in the sediment.

In the first set of studies, McKinney Slough sediment was subjected to two sterilization protocols. The sediment that was sterilized was autoclaved under two separate conditions:

1) Auto #1 = Sediment Autoclaved at 121 °C for 15 minutes, and
2) Auto #2 = Sediment Autoclaved at 99 °C for 30 minutes.

Both methods were used as it was unknown which procedure would allow for complete sterilization of the sediment; unautoclaved McKinney slough sediment was used as the control sediment. The efficiency of sterilization was checked at several stages in this experiment by plating an aliquot of the sediment onto Tryptic Soy Agar (TSA) and Marine Agar plates to look for presence of colony forming units. At Day 10 and 24 of equilibration, microbiological analyses showed presence of bacteria in the control sediment but no signs of bacteria in either the Auto #1 nor Auto #2 sediments. Therefore, both sediment sterilization techniques appeared to be effective at sterilizing sediment of bacterial populations.

At two separate times subsequent to sterilization and spiking, the interstitial water from the control and Auto #1 and Auto #2 sediments were subject to reverse phase separation to evaluate whether autoclaving had any effect on the total percent of the activity present in interstitial water that was being observed in the elutriate subsequent to reverse phase separation. As presented in Table 3.9, the percent activity in the elutriate fraction was similar between the two autoclaved sediments and the control, unautoclaved sediment.

Table 3.9 Results of Reverse Phase Analysis of Interstitial Water: Sediment Sterilization Experiment

| Sample | Total Activity (dpm/ml) | Activity in Elutriate (dpm/ml) | Percent Activity in Elutriate |
|---|---|---|---|
| Day 4 - Auto #1 | 261 | 73 | 28% |
| Day 4 - Auto #2 | 263 | 76 | 29% |
| Day 4 - Control | 173 | 67 | 39% |
| Day 24 - Auto #1 | 217 | 69 | 32% |
| Day 24 - Auto #2 | 197 | 61 | 31% |
| Day 24 - Control | 103 | 34 | 33% |

In the second study, McKinney Slough sediment was autoclaved for 30 minutes at 121°C and stored at 4°C until spiking. In this study, the interstitial water was collected after 13 days of equilibration at 4°C and subject to RPS and liquid-liquid extraction. The purpose was to ascertain whether the material passing through a $C_{18}$ Column represented the same fraction of the total activity present in total interstitial water as the fraction that would partition to the aqueous phase in a liquid-liquid extraction procedure. This would lend credence to the theory that the material found in RPS elutriate had been degraded to a more polar metabolite. The results of this analysis is presented in Table 3.10. The percentage of the total initial activity present in the interstitial water that was found in the RPS elutriate was 20.8% which was consistent with the activity found in the aqueous phase of the liquid-liquid extraction of 22.5%. This indicates that the activity present in either the aqueous phase or in RPS elutriate are the same and represents the oxidized or more polar degradation products of the parent phenanthrene compound.

Table 3.10. Results of Reverse Phase Analysis of Interstitial Water: Sediment Sterilization Experiment - II

| Sample | Total Activity (dpm/ml) | Activity (dpm/ml) | Percent Activity in Elutriate or Aqueous Phase |
|---|---|---|---|
| Auto. Sed. RPS | 178.8 | 37.3 in elutriate | 20.8% |
| Auto. Sed. Liq-Liq Extraction | 178.8 | 40.5 in aqueous phase | 22.5% Aq. |

## 3.4 DISCUSSION:

In this study, the bioaccumulation and uptake kinetics of $^{14}$C-phenanthrene by three species of marine amphipods were compared and contrasted between water and sediment exposures. Additionally an attempt was made to evaluate the relative contribution of biotic and abiotic degradation processes in degrading the $^{14}$C-phenanthrene present in the sediment.

The measured concentrations of $^{14}$C-phenanthrene in amphipod tissue were higher for each species and at each sampling time for the amphipods exposed to $^{14}$C-phenanthrene in water versus those exposed to $^{14}$C-phenanthrene in sediment via interstitial and overlying water exposure. Generally, the concentration of $^{14}$C-phenanthrene in tissue increased with increasing exposure time until achieving apparent steady state concentrations. For *Eohaustorius estuarius* and *Grandidierella japonica*, the concentrations of $^{14}$C-phenanthrene in tissue via the water exposure route were statistically greater at each time point and for *Leptocheirus plumulosus*, the concentrations of $^{14}$C-phenanthrene in tissue via the water exposure route was statistically greater for four of the five sampling times.

The concentration of phenanthrene selected for these exposure treatments were well below the reported water-only $LC_{50}$'s for *Eohaustorius estuarius* (131 ug/L phenanthrene) and *Leptocheirus plumulosus* (185 ug/L phenanthrene) (EPA, 1991). No significant increase in mortality or other adverse biological effects were observed in the exposure treatments relative to the negative control beakers for these series of exposure treatments..

The uptake rate coefficients were compared between exposure treatments and between modeling methods. The calculated $k_1$ values were consistently greater in the water exposure treatments than in the sediment exposure treatments. This trend was evident for all three amphipod species examined. The uptake coefficients were statistically compared using the Spjotvoll-Stoline T'-method of multiple comparisons (Sokal and Rolfe, 1981) in the cases where the same number of data points were used in deriving the $k_1$ value between the exposure treatments. For *Eohaustorius estuarius*, this evaluation revealed that the $k_1$ values derived by either the linear or non-linear regression model for the water exposure were greater (p-value $< 0.05$) than those derived for the sediment exposure. For *Grandidierella japonica*, the uptake rate coefficient calculated by the non-linear regression method were compared and again, the $k_1$ value of 11.356 for water was significantly greater than the $k_1$ value of 3.738 calculated for sediment. Because of the different number of data points used in calculating the uptake rate coefficients, statistical comparisons could not be made for *Grandidierella japonica* using the linear regression model and for *Leptocheirus plumulosus* using either regression model. However, the magnitude of the difference between the rate coefficients calculated for sediment and water exposures were consistent for these treatments and species, generally reporting a 2 to 3 fold increase for the water exposures over the sediment exposures.

A comparison of the two regression models revealed that both the linear and non-linear regression models provided relatively consistent estimates of the uptake rate coefficients. However, the non-linear regression model is able to utilize a higher percentage of the available bioaccumulation data and, as evidenced by the higher correlation coefficients ($R^2$) for the non-linear uptake rate estimates, provides for a more accurate estimation of the uptake rate coefficient.

The substantial increase in the value of $k_1$ between the sediment and water exposures was unexpected. Landrum (1991) reported similar patterns of uptake rate coefficient increasing with changes in exposure conditions with modeled uptake or clearance coefficients from sediment ($k_s$). In that study, the sediment clearance coefficient $k_s$ (g dry sediment $g^{-1}$ organism $h^{-1}$) were calculated; which are essentially the same as $k_1$'s measured in this study though what is calculated is the uptake clearance from sediment rather than from water. Landrum measured the bioaccumulation of nine PAHs by the amphipod *Diporeia sp.* under different exposure concentrations and found that the sediment clearance rate coefficients varied by up to four or five fold (for the same PAH and amphipod species) with increasing concentrations of PAHs in sediment. The patterns are similar to those

observed in this present study with increasing uptake rates observed under conditions associated with increased exposure concentrations.

There are several explanations provided in the literature that would try to explain how this could occur. One is that the difference in body burdens observed between exposure treatments is a function of the different physiological conditions experienced by the normally sediment dwelling amphipods in a water only environment and that the increased physiological activity in a water only system would increase respiration and other biological functions which in turn would increase exposure and bioaccumulation (Connell, 1988). However, this appears not to be the case (or entirely the case here) as there were efforts made to minimize the difference in physiological conditions experienced by the amphipods under the two exposure treatments by adding glass tubes to the water-only exposure beakers. The glass tubes were offered as refugia for the amphipods and the organisms were observed inside and under these tubes and were not swimming freely for the majority of the time observed.

The concentration of total lipids measured in the amphipods at the initiation and conclusion of the exposure treatments were conducted, in part, to evaluate whether the amphipods placed in a water only environment were more physically active than those placed in sediment. If this were true, it was expected that the concentration of lipids would have decreased to a greater extent, over the course of the 72-hour exposure interval, in the water only exposure than in the sediment exposure. However, the mean measured lipid concentration increased slightly for all three species of amphipods under water only exposure conditions though these differences were determined not to be statistically significant. In the sediment exposure, the mean lipid content decreased slightly over the course of the 72-hour exposure but again the differences were not statistically significant. It appears that the apparent increase in lipid content seen over the course of the water only exposures is an artifact of the individual variability in lipid content rather than the result of a physiological process that would build up lipid reserves over time. Based on this data, it does not appear that for these three amphipod species, that being in a water only exposure system as designed for this study, caused a significant increase in physiological activity over those placed in a sediment exposure system.

As an alternative explanation, Lee (1991) and Landrum (1991) have suggested that in a sediment exposure system, a localized disequilibrium condition may exist in the microhabitat surrounding the organism that can affect the exposure concentration for that

particular organisms. In theory, because desorption rates from sediment organic carbon for non-polar organic chemicals can be very slow (Borglin, 1996), organisms can rapidly deplete the interstitial water in their immediate vicinity of dissolved contaminants. Subsequently the rate of uptake from interstitial water becomes dependent on the rate of desorption from sediment particles. This would occur in a situation where the uptake of the $^{14}$C-phenanthrene by the amphipod occurs at a greater rate than the desorption of $^{14}$C-phenanthrene from the sediment organic carbon phase resulting in a temporary decrease in the concentration of $^{14}$C-phenanthrene in the interstitial water surrounding the organism. Any process that would result in the reduction of the effective exposure concentration for the organism would decrease the accumulation of $^{14}$C-phenanthrene by the organisms and result in lower uptake coefficients.

The mean total lipid content for *Eohaustorius estuarius*, the only field collected or natural population of amphipods used in this experiment, was significantly different between the two exposure treatments (Table 3.4). This was because the sediment exposures were conducted in January and the water only exposures conducted in March. The mean total lipid concentrations for the other two lab cultured species of amphipods were similar between exposure treatments. This seasonal trend in lipid content has been observed by Landrum (1988) for wild populations of *Diporeia sp.* and seasonal trends in lipid content for *Eohaustorius estuarius* was not unexpected.

However, as the two exposures were conducted at different times of the year, in an subsequent study *Eohaustorius estuarius* was exposed simultaneously to sediment and water to confirm the trends in the bioaccumulation of $^{14}$C-phenanthrene observed between sediment and water exposure. The $^{14}$C-phenanthrene did not behave as predicted in this study with regards to achieving the target interstitial water concentration and also in maintaining a constant concentration over the course of the 72 hours of exposure. The target level of $^{14}$C-phenanthrene for the water only exposure treatment was determined by the interstitial water concentration of $^{14}$C-phenanthrene collected one day prior to test initiation and was found to be 0.80 ug/L. The concentration in the water only exposure were consequently lower than the interstitial water concentration measured at the end of the exposure period (T=72 hours) of 1.16 ug/L. Therefore, it appears that the amphipods in the sediment exposure treatment were exposed to a higher concentration of $^{14}$C-phenanthrene (at least towards the end of the exposure regime) than the amphipods in the water only exposure. This fact could explain why the measured difference in body burdens

between the two exposure treatments were not as great as observed when the exposures were conducted separately.

The behavior of the $^{14}$C-phenanthrene in interstitial water raised the concern some process was changing the chemical characteristics of the radiolabeled parent compound being measured sediment and interstitial water. Such processes could result in changes in the partitioning characteristics of the measured compound between sediment organic carbon and interstitial water or change the bioavailability of the sediment associated contaminant (Landrum, 1988, Ozretich, 1995). One limitation to the use of radiolabeled compounds is that the LSC analysis is only capable of measuring the total radioactivity present in the sample and no information can be collected on whether it is the parent compound or degradation products that are being measured.

As a result of this concern, interstitial water was collected at the initiation and termination of the exposure period and subject to reverse phase separation (RPS) (Table 3.8). It was found that 50% to 60% of the activity present in the interstitial water could be measured in the elutriate passing through the $C_{18}$ column. Elutriates from freshly spiked sea water contained only approximately 1% of the total initial activity prior to reverse phase separation.

Two possible mechanism are available as an explanation for these results: one is that the $^{14}$C-phenanthrene was binding to dissolved organic matter (DOM) in the interstitial water which would allow the complex to pass through a $C_{18}$ column (Ozretich, 1995). Interstitial water has a higher concentration of DOM than the fresh filtered sea water being used in the study as replacement overlying water and as the source water for the water only exposure (R. Ozretich; EPA Newport, OR. personal communication). The second possibility was that biotic or abiotic degradation of the $^{14}$C-phenanthrene had occurred during sediment spiking and equilibration.

These factors were initially not considered important when this study was designed as Ozretich (1995) reported that phenanthrene did not bind in detectable levels to DOM. Additionally, Landrum (1987) reported that the difference in uptake clearance coefficients for phenanthrene and the amphipod *Pontoporeia hoyi* between control and DOM-containing treatments were not significant because of phenanthrene's relatively low binding capabilities to DOM. Landrum (1988) states that the presence of increased levels of DOM do not affect the uptake kinetics of phenanthrene due to its relatively high water solubility

(Landrum, 1988). Thus the total amount of compound in the water appears to be bioavailable. Similar behavior for phenanthrene was observed for binding to DOM in the presence and absence of Aldrich humics (Landrum, 1985). With regards to the degradation of $^{14}$C-phenanthrene in sediment, Landrum (1992a) in a study evaluating the effect of sediment aging on the bioavailability of PAH compounds, reported that for sediment spiked with $^{14}$C-phenanthrene and stored for 150 days at 4°C, there was little or no degradation of the $^{14}$C-phenanthrene (radiopurity 96 $\pm$ 1.8%) and that, therefore, no barriers existed for calculating the concentration of the labeled compound from the measured radio-activity and the specific activity for the compound.

To determine whether the activity found in the RPS elutriate was caused by the DOM in the interstitial water binding the $^{14}$C-phenanthrene, fresh sea water and freshly collected interstitial water from clean sediment were spiked with $^{14}$C-phenanthrene. Similar percentage of activity was present in the elutriate for both spike water samples after equilibration indicating that binding to the additional DOM in the interstitial water was not the cause of the activity found in the RPS elutriate.

In light of these results, an attempt was made to determine the relative contribution of biotic versus abiotic degradation to account for the amount of material found in the elutriate following RPS. Table 3.9 presents data from both autoclaved (sterilized) and control (unsterilized) sediment. The results indicate that after 24-days of equilibration, the same amount of activity could be measured in the RPS elutriate regardless of whether the sediment had been sterilized or not. This indicates that the majority of the degradation of the $^{14}$C-phenanthrene is appears to be occurring via abiotic mechanisms.

A variety of processes, including volatilization, sedimentation, chemical oxidation, photodecomposition, and microbial degradation, are important mechanisms for environmental degradation of PAH (Cerniglia, 1991). Mono- and dioxygenases are two groups of enzymes that are important to the microbial catabolism of PAH. Dioxygenases incorporate both atoms of the oxygen molecule into the PAH. This dioxygenase reaction is the major mechanisms for the initial oxidative attack on PAH by bacteria, which lead to the formation of dihydrodiols that are in the *cis* configuration. Enzymatic fission of the aromatic ring is also catalyzed by dioxygenases. Bacteria are capable of the complete mineralization of phenanthrene and other two and three ring PAHs (Alexander, 1981). In contrast to bacteria, fungi oxidize PAH via a cytochrome P-450 monooxygenase by incorporating one atom of the oxygen molecule into the PAH and the other into water.

Biodegradation of lower-molecular weight PAHs by a wide variety of micro-organisms has been demonstrated and higher biodegradation rates for PAH are observed in PAH contaminated sediments than in pristine sediments indicating the effect of PAH pre-exposure on establishing communities of PAH degrading micro-organisms. Guerin and Jones (1989) reported that phenanthrene degrading bacteria were ubiquitously distributed in the waters and sediments of the Great Bay Estuary, NH. They found that PAH concentration and pre-exposure to the compounds determine the degradative activities of microbial populations. These factors act by selection for species with degradative abilities, induction of enzymes required for degradation, and proliferation of extrachromosomal elements encoding degradative enzymes (Spain and Van Veld, 1983). Similarly, Cullen et al. (1994) found that in the sediments collected from the Kitimat Arm, British Columbia, Canada, that the rate of phenanthrene degradation was dependent on the populations of microorganisms present. While PAH degrading micro-organisms were available in all sediments that were collected, those that were exposed to phenanthrene without prior exposure degraded phenanthrene at relatively slow rates while those previously exposed, the degradation was much more rapid.

In addition to biological oxidation, chemical oxidation and photo-oxidation are important processes affecting the persistence of PAHs in the aquatic environment (Kochany and Maguire, 1994). The chemical oxidation of PAHs depends upon the concentration of singlet oxygen, and other oxidants like organic peroxides, hydrogen peroxide, and peroxyl and hydroxyl radicals. It is believed that these oxidants arise from photochemical processes. Some inorganic salts and oxides, especially those of iron and manganese, can also be involved in the production of oxidants in natural waters and thus influence the chemical oxidation of organic water pollutants. Although there is much information on the redox reactions in natural waters, there is relatively little information on the oxidation of organic pollutants in sediments (Kochany and Maguire, 1994).

The results indicate that bacterial degradation plays only a minor role in the degradation of $^{14}$C-phenanthrene in sediment collected from McKinney Slough, OR. This result makes sense as the ability of bacterial populations to metabolize PAHs are dependent on pre-exposure to PAHs which will selectively cause populations of these PAH metabolizing bacteria to flourish. McKinney Slough is the source of confirmed clean control sediment for the EPA Research Laboratory in Newport, OR and it is unlikely that there has been a substantial amount (if any) contamination with petroleum products in the sediments of this site. However, the results reported here are at odds with Landrum (1987) who found no

appreciable degradation, biotic or abiotic, of $^{14}$C-phenanthrene occurring in sediment spiked and stored under similar conditions. The reasons for this discrepancy are unknown.

These experiments were conducted to test an assumption of the Equilibrium Partitioning Theory that exposure to the same concentration of a contaminant in interstitial water is equivalent to exposure in a water only system. A comparison of the bioaccumulation kinetics and the tissue residue of $^{14}$C-phenanthrene in marine benthic amphipods revealed that there was a greater accumulation and rate of accumulation of $^{14}$C-phenanthrene in a water only exposure system than from a sediment-interstitial water exposure system for each of the three species of marine amphipods evaluated. The data suggest that exposure under these two experimental conditions may not be equivalent. Several potential reasons for the observed differences in accumulation and bioaccumulation kinetics between the exposure treatments are presented in the text.

However, analysis of the sediment and interstitial water revealed that substantial degradation had occurred in the $^{14}$C-phenanthrene with which the sediment had been spiked. Further evaluation revealed that this degradation was primarily via abiotic mechanisms in contrast to bacterial degradation. The degradation of the $^{14}$C-phenanthrene in the spiked sediment may have resulted in a lower exposure concentration to phenanthrene than was calculated via the radioactivity measured in the interstitial water. It is unknown to what extent the $^{14}$C-phenanthrene in the experimental sediments were degraded as RPS and liquid-liquid extraction were only performed on sediment spiked subsequent bioaccumulation experiments. Clearly, if the concentration of phenanthrene was being overestimated by measuring the radioactivity in interstitial water, the magnitude of the difference observed in the accumulation and bioaccumulation kinetics between treatments is suspect.

Further research is needed to determine whether the patterns in uptake and accumulation between exposure treatments observed in these experiments would be repeated with compounds that do not degrade in sediment or in studies conducted using a combination of radiolabel and confirmatory analytical chemistry.

# CHAPTER 4

# SUMMARY

The objectives of this research were to test two assumptions of the "equilibrium partitioning theory" and to evaluate the biotic and abiotic factors influencing the bioavailability of sediment-associated contaminants to benthic invertebrates. The PAH phenanthrene and marine amphipods were used as the model contaminant and model organisms respectively. It is the bioavailability of a contaminant that is fundamental in determining the actual and potential adverse effect posed to the particular target organism. This is because the bioavailability of a chemical represents the total pool of material that is available to the organism and thus incorporates not only the characteristics of the chemical, but also the behavior and physiology of the target organism.

## 4.1 Biotic Factors Influencing Bioavailability

For non-ionic organic contaminants such as phenanthrene, the main route of exposure to benthic invertebrates is via the interstitial water. The biological factors that would affect the bioavailability would be those that could influence the concentration of the contaminant in this interstitial water. In the experiments described in Chapter 2 of this thesis, the effect of the burrow-irrigating behavior exhibited by benthic marine amphipods was investigated along with the species specific differences in burrowing and tube building strategies.

The equilibrium partitioning theory predicts that once the partitioning of a non-polar organic compound between sediment organic carbon and sediment interstitial water is determined, the modeled interstitial water concentration will be the constant exposure point concentration for all benthic organisms. However, benthic amphipods utilize burrow irrigating as a respiratory behavior. Additionally, some species of amphipods use this irrigating behavior as an adjunct to feeding as food particles are brought within the close proximity of an organisms, usually within burrow or tube. The extent to which this behavior, which involves the advection of overlying water into the interstitial pore space surrounding the organism to provide fresh oxygenated water for respiration, can affect equilibrium conditions was investigated.

The results of this research have indicated that burrow-irrigating behavior was a significant factor in determining the accumulation of phenanthrene from interstitial water for all three amphipod species tested. This indicates that the burrow irrigating behavior exhibited by infaunal amphipods can effectively disrupt the predicted equilibrium conditions present in the microhabitat surrounding the individual benthic organism. As discussed in Chapter 2, the mechanism by which burrow-irrigating behavior influenced the accumulation from interstitial water was by diluting the concentration of phenanthrene in interstitial water by the advection of overlying water into the sediment prism. The relative contribution of the burrow-irrigating behavior on the exposure to sediment-sorbed phenanthrene was dependent on the presence or absence of phenanthrene in the overlying water. These results indicate that in situations were the overlying water contains less of the contaminant than does the interstitial water, the actual exposure experienced by benthic amphipods would be overestimated by the "equilibrium partitioning theory".

This research has shown that changes in the overlying water concentration of a contaminant will have an affect on the exposure to the benthic amphipod used in the sediment bioassay. Current accepted methods for conducting marine sediment toxicity tests allow for the manipulation of overlying water either through study design (flow-through versus static designs) or to decrease potentially non-contaminant related confounding factors (ammonia and sulfide toxicity in interstitial water). The effects of such manipulations of overlying water on bioassay responses requires further evaluation.

Additionally, the results showed that the burrowing strategy employed by each species of amphipod also had an affect on the accumulation of phenanthrene from sediment. By comparing the amount of phenanthrene accumulated by each species under conditions where the overlying water was maintained clean versus maintained at equilibrium with interstitial water, the influence of tube and burrow building versus free-burrowing was investigated. The greatest difference in tissue residue between exposure treatments was found for the hard-walled tube building species followed by the soft-walled burrow building species and the least difference found for the free-burrowing amphipod. As expected, the tube and burrow builders appear to be able to decrease exposure by diluting interstitial water borne contaminants to a greater extent than the non-structure forming species. Therefore, while it is clear that burrow irrigating behavior can disrupt equilibrium conditions and exposure concentrations, the magnitude of this effect will be species and behavior specific. The magnitude of this effect is correlated with the type of burrowing behavior exhibited by the individual amphipod species.

Currently, no guidance exists to assist in the selection of specific amphipod species for use in regulatory sediment toxicity testing. If the species specific differences in accumulation and exposure to sediment-associated contaminants have corresponding effects upon the response of the organism in bioassays, additional information will have to be incorporated into the available guidance manuals (EPA, 1994b, ASTM, 1995) to assist in the selection of appropriate species for use under specific sediment testing situations.

## 4.2 Abiotic Factors Influencing Bioavailability

Abiotic factors that could affect the transfer of contaminants from sediment to biota were investigated by evaluating whether exposure to a specific concentration of a contaminant in water was equivalent to exposure to the same concentration in interstitial water. The goal was to determine whether any sediment specific processes may be at work to modulate the exposure and attempts were made to control any confounding biological factors.

In the study reported in Chapter 3, the bioaccumulation and uptake kinetics of $^{14}$C-phenanthrene by three species of marine amphipods were compared and contrasted between water and sediment exposures. The measured concentrations of $^{14}$C-phenanthrene in amphipod tissue were higher for each species and at each sampling time for the amphipods exposed to $^{14}$C-phenanthrene in water versus those exposed to $^{14}$C-phenanthrene in sediment. Several possible reasons for this difference are discussed in Chapter 3. One is that the difference in body burdens observed between exposure treatments is a function of the different physiological conditions experienced by the normally sediment dwelling amphipods in a water only environment and that the increased physiological activity in a water only system would increase respiration and other biological functions which in turn would increase exposure and bioaccumulation. However, this appears not to be the case as there were efforts made to minimize the difference in physiological conditions experienced by the amphipods under the two exposure treatments and no significant differences were reported in the mean lipid content of the amphipods between exposure treatments.

An alternative explanation is that a localized disequilibrium condition exists in the microhabitat surrounding the organism because the uptake of the $^{14}$C-phenanthrene by the amphipod is occurring at a greater rate than the desorption of $^{14}$C-phenanthrene from the sediment organic carbon phase resulting in a temporary decrease in the concentration of

$^{14}$C-phenanthrene in the interstitial water surrounding the organism. Subsequently the rate of uptake from interstitial water by the benthic organism becomes dependent on the rate of desorption from sediment particles. Any process that would result in the reduction of the exposure concentration for the organism would decrease the accumulation of $^{14}$C-phenanthrene by the organisms.

A factor that complicates the interpretation of the results presented in Chapter 3 is the degradation of $^{14}$C-phenanthrene found in sediment and interstitial water spiked in a similar manner as the experimental sediment. The presence of degradation products of phenanthrene in the interstitial water would result in an overestimation of the concentration of $^{14}$C-phenanthrene present when calculated based upon the radioactivity measured in interstitial water. Unfortunately, no confirmatory analytical chemistry was performed on the experimental sediment or interstitial water. The magnitude of the bias introduced that may have been introduced to the study results is unknown.

If further research determines that sediment exposures are not equivalent to water only exposures, the assumption made by the EPA in using Ambient Water Quality Criteria as the basis for developing Draft Sediment Quality Criteria may have to be re-examined.

## 4.3 Recommendations for Future Research

The results of these two experiments indicate that both biological and physical/chemical processes are potentially important in determining the bioavailability of phenanthrene to benthic amphipods. These results provide an important starting point to evaluate whether the trends suggested by these studies can be demonstrated and confirmed under standard bioassay conditions and for compounds exhibiting different chemical characteristics than phenanthrene. Future research remains to be performed in the following areas.

1) Increase the exposure time in the bioaccumulation experiments to ten days, the standard length of acute sediment bioassays. This would allow for the determination of whether the differences in exposure attributed to behavioral process would be maintained over this extended period of time. Additionally, experiments should be conducted with a series of concentrations of a contaminant to determine whether the differences in exposure observed among the species could be correlated with differences in species' response to the bioassay.

2) Conduct the bioaccumulation test with a combination of a radiolabeled and unlabeled compound with confirmatory analytical chemistry. This will eliminate the uncertainty associated with relying solely on the measurement of radioactivity to measure the concentration of the contaminant in question.

3) Conduct the bioaccumulation experiments with compounds of varying $K_{ow}$s to see if the trends observed in these experiments still hold for compounds that are more hydrophobic than phenanthrene. It is reported that the assumptions of "equilibrium partitioning" become less reliable when applied to compounds having high $K_{ow}$s as other exposure pathways, such as the ingestion of sediment particles, become more important than exposure via interstitial water. It will be useful to determine whether the behavior dependent changes in exposure observed in this research are maintained for chemicals with different characteristics than phenanthrene.

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