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# Assessment of the Environmental Effects Associated With Wooden Bridges Preserved With Creosote, Pentachlorophenol, or Chromated Copper Arsenate

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## Abstract

Timber bridges provide an economical alternative to concrete and steel structures, particularly in rural areas with light to moderate vehicle traffic. Wooden components of these bridges are treated with chromated copper arsenate type C (CCA), pentachlorophenol, or creosote to prolong the life of the structure from a few years to many decades. This results in reduced transportation infrastructure costs and increased public safety. However, the preservative used to treat the wooden components in timber bridges is lost to the environment in small amounts over time. This report describes the concentration of wood preservatives lost to adjacent environments and the biological response to these preservatives as environmental contaminants. Six bridges from various states were examined for risk assessment: two creosote-treated bridges, two pentachlorophenol-treated bridges, and two CCA-treated bridges. In all cases, the largest bridges located in biologically active environments associated with slow-flowing water were selected to represent worst-case analyses. Sediment and water column concentrations of preservative were analyzed upstream from, under, and downstream from each bridge. The observed levels of contaminant were compared with available regulatory standards or benchmarks and with the quantitative description of the aquatic invertebrate community sampled from vegetation and sediments. Pentachlorophenol- and creosote-derived polycyclic aromatic hydrocarbons (PAHs) were not observed in the water near any of the selected bridges.

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However, low levels of PAHs were observed in the sediments under and immediately downstream from these bridges. Pentachlorophenol concentrations did not approach toxicological benchmarks. Sediment concentrations of naphthalene, acenaphthylene, and phenanthrene exceeded the probable effect level. Metal levels at the bridges treated with CCA were less than predicted effect levels, in spite of questionable construction practices. Adverse biological effects were not observed in the aquatic invertebrate community or laboratory bioassays conducted on water and sediments sampled at each of the bridges. Results of this study reveal the need to follow the construction information found in *Best Management Practices for the Use of Treated Wood In Aquatic Environments* published by Western Wood Preservers Institute. Regulatory benchmarks used in risk assessments of this type need to be indexed to local environmental conditions. The robust invertebrate communities associated with slow-moving streams over soft bottoms were not susceptible to the concentrations of PAHs that would be expected to affect more sensitive taxa, which typically are located in faster moving water over hard bottoms. Contaminants released from timber bridges into these faster systems (where more sensitive taxa are located) are significantly diluted and not found at biologically significant levels.

Keywords: timber bridges, preservative, CCA, creosote, pentachlorophenol

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# Assessment of the Environmental Effects Associated With Wooden Bridges Preserved With Creosote, Pentachlorophenol, or Chromated Copper Arsenate

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## Introduction

Wood is a renewable resource that can be modified to produce bridge structures that are strong and relatively light weight, compared with steel or concrete, and can be aesthetically pleasing. Wood preservatives are used to increase the life span of wooden bridge structures from a few years to many decades. Wooden bridges, preserved with a variety of chemicals, have been used widely throughout the United States and Canada. They are particularly valuable for small- to moderate-sized pedestrian and vehicular bridges in rural areas.

Timber bridges are typically treated with pentachlorophenol (penta) or creosote. These oilborne preservatives are preferred for long spans where the wood must remain flexible. However, wood treated with waterborne preservatives, represented primarily by ammoniacal copper zinc arsenate (ACZA) and chromated copper arsenate type C (CCA), is also being used in the construction of wooden bridges. A preliminary survey conducted in support of this study indicated that creosote is used primarily in the Northeast and Midwest, penta and creosote in the Pacific Northwest, and CCA throughout the South. These are qualitative trends, and examples of the use of each type of preservative can be found throughout the United States.

This study began with an extensive literature search. That search did not uncover any reports documenting a loss of biological integrity associated with timber bridges constructed of treated wood. However, a perceived environmental degradation is often associated with these structures. Figure 1, a photograph taken in 1995, shows a bridge crossing Narragansett Bay. The bridge was constructed of laminated beams that began dripping creosote during a series of hot summer days. Figure 2 shows the accumulation of dripping creosote on rocks under the bridge.

Krahn (1987) reported the leaching of copper and arsenic from ammoniacal-copper-arsenate- (ACA-) treated wood used as bridge decking and support timbers in British Columbia, Canada. Initial treatment of this wood did not meet Canadian Wood Preservers' Association (CWPA) standards, and the wood was retreated. Krahn's (1987) investigation suggested that the wood was immediately placed in service following the second treatment and that fixation of the metals had not occurred. Krahn (1987) discussed the potential for an avoidance reaction in salmonids at the copper levels observed in the stream but did not document actual environmental degradation.

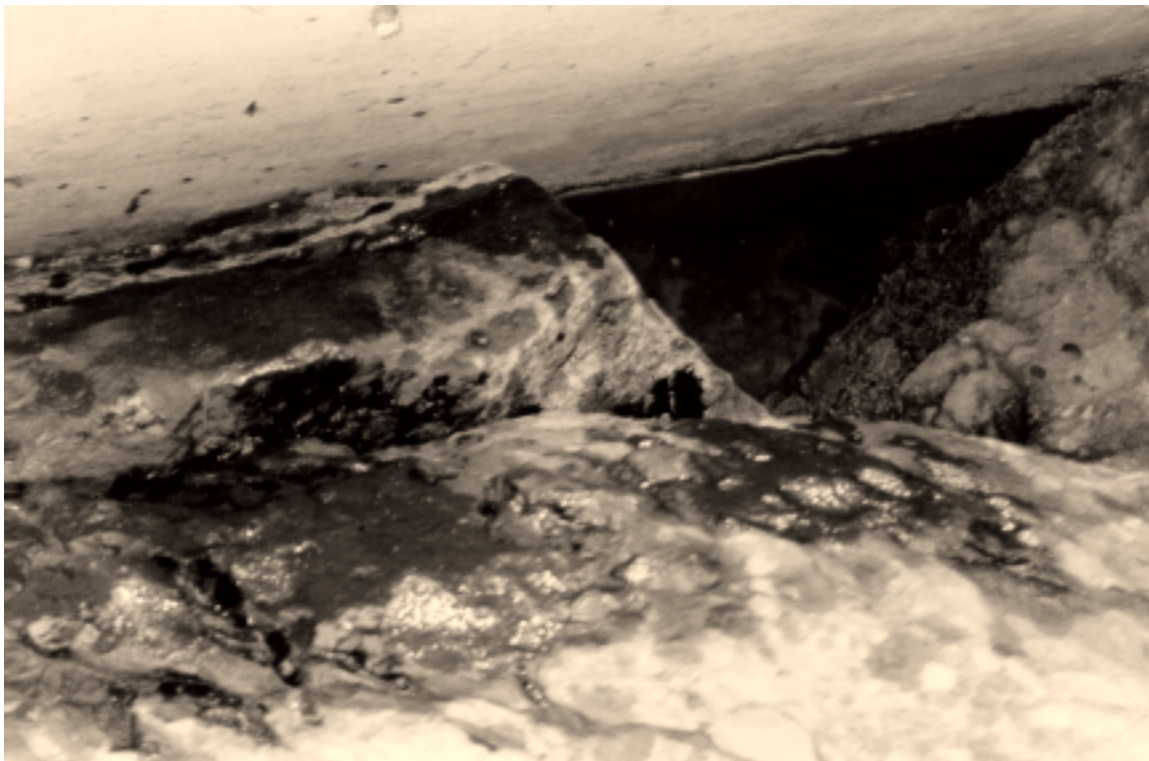
The literature is rich with descriptions of metal and hydrocarbon pollution associated with transportation systems. However, not one document was uncovered that describes the loss of wood preservatives from timber bridges. Therefore, this study breaks new ground in understanding the environmental risks associated with these structures.

In 1997, the USDA Forest Service contracted with Oregon State University and Aquatic Environmental Sciences to conduct a three-phase study (contract RJVA-2828). The purposes of this study were to

- assess the environmental response associated with existing timber bridges,
- determine the loss of various types of preservatives from overhead bridge structures, and
- develop a computer model to assist the Forest Service in understanding the site-specific environmental risks associated with proposed timber bridge construction.



**Figure 1—Creosote loss from treated glulam beams used in constructing a highway bridge across Narragansett Bay.**



**Figure 2—Accumulated creosote from weeping pressure-treated timbers used in bridge construction.**

## Background

Transportation systems are inherently dirty. Polycyclic aromatic hydrocarbons (PAHs) are released from many sources, including asphalt, lubricating oils, and gasoline or diesel engine exhaust. Asphalt contains significant concentration levels of PAH, including the known carcinogen, benzo(a)pyrene at 6 µg/g (Machado and others 1993). In Germany, Muench (1992) observed roadside levels of PAHs at 6.6 to 9.8 µg/g (dry soil weight) and zinc levels of 58 to 330 µg/g. These levels approach those at which adverse biological effects could be anticipated in particularly sensitive aquatic environments.

Elevated concentrations of heavy metals, including zinc and copper, were found within 100 m of roads with traffic volumes exceeding 1,000 vehicles per day (Hoedrejaerv and others 1997). Mean storm water levels of zinc and copper can exceed surface water quality discharge standards (Sasalone and Buchberger 1997). In Moscow, Lepneva and Obukhov (1990) observed copper at 80 µg/g and zinc at 230 µg/g in roadside soils. These levels were four to six times greater than background levels.

Sediment levels of contaminants in aquatic environments adjacent to roadways can be significantly elevated. Faganeli and others (1997) documented the uptake of road surface contaminants, including PAH, zinc, and copper, by plants, bivalves, and gastropods in the Gulf of Trieste. However, the levels of PAHs and metals were not sufficiently high to adversely effect biota (Faganeli and others 1997). The bioavailability of PAHs and their ability to influence biological systems are dependent on a number of environmental physicochemical parameters, particularly the concentration of organic carbon that binds PAH (Johnsen 1987, Brooks 1997b). For example, Paine and others (1996) examined sediment PAH levels and biological responses associated with an aluminum smelter. Levels of 10,000 mg PAH per kilogram of dry sediment were observed in some samples within 1 km of the smelter. However, most samples revealed sediment PAH concentrations of about 150 mg/kg. Despite repeated sampling for several years, the authors found no sediment toxicity associated with four laboratory bioassay test animals and minimal evidence of toxicity in the resident benthic invertebrate community. The authors concluded that the lack of observed toxicity was associated with the reduced bioavailability of PAHs.

Storch and others (1990) found that Chautaugua Lake Bridge runoff was toxic to young-of-the-year sunfish (*Lepomis macrochirus*). The authors suggested that sodium chloride (used to de-ice roads) was a major contributor to the toxicity but that concentration levels of zinc and cadmium were also found at toxic levels. Baekken (1994) compared metal and PAH levels in a small reference lake with those in a similar lake adjacent to a highway. He found that cadmium and zinc concentrations in bivalves were two to three times greater

**Table 1—Metal and PAH levels observed in storm water run-off from the Skyway Bridge in Burlington, Ontario<sup>a</sup>**

Contaminant	Water (µg/L)	Sediments (µg/g)
Zinc	337	997
Copper	136	314
14 PAH	0.015 to 0.500	

<sup>a</sup>Marsalek and others 1997.

next to the highway when compared with the reference lake. The concentration levels of other metals (copper, mercury, nickel, lead) and PAHs in bivalves were low in both lakes, presumably close to background levels. The diversity and abundance of benthic communities were reduced on the highway side of the polluted lake, suggesting effects associated with highway pollutants. Yousef and others (1985) observed elevated levels of metals, including lead, copper, chromium, iron, nickel, cadmium, and zinc, in lake water and sediments associated with a highway bridge. The authors reported that 95% to 98% of the metals associated with the road and bridge were found in the sediments. Marsalek and others (1997) observed high levels of metals and PAHs in runoff from the Skyway Bridge in Burlington, Ontario. The observed levels in water and sediments are summarized in Table 1. The authors noted that sediment levels of metals were considered “grossly polluted” according to the Ontario Ministry of environment and energy guidelines for sediment quality.

A large proportion of the contaminants entering aquatic environments appears to be sedimented at short distances from the point of introduction. Schiffer (1989) found that median zinc levels decreased from 75 µg/L at a storm water inlet to 20 µg/L at a distance of 30.5 m from the inlet. Therefore, the magnitude of the disturbance associated with transportation systems is partially dependent on the size (particularly the width with respect to the roadway) of the water body. Obviously small, stagnant ponds and narrow, slow-moving streams flowing parallel to major transportation routes would likely be more stressed than would a large, swift river flowing under a highway bridge.

O’Malley and others (1996) used molecular abundance and carbon isotope measurements to partition four- and five-ring PAHs by source in Saint John’s Harbor, Newfoundland, Canada. Their mixing model suggested that approximately 50% to 80% of the PAH input to the harbor was of combustion origin, likely dominated by vehicular emissions carried by surface runoff from the city of Saint John’s. In addition, direct petroleum-related contributions, dominated by crankcase oil, accounted for the remaining 20% to 50% of the total PAH input.

Gravel roads can result in the deposition of significant amounts of sharply divided sediments in rivers and streams,



with adverse effects on fish and invertebrate habitat (Cedarholm and Salo 1979, Cedarholm and others 1982). Road traffic tends to break rock into sharp fragments that can cause injury to the gills of aquatic organisms, whereas natural sediments tend to be more rounded by abrasion, thus having a lower propensity to cause physical injury.

The point in this discussion is that transportation systems, including the roads that are carried over water bodies by bridges, are the source of many contaminants, including the metals used to preserve wood (copper, zinc, and chromium) and the PAHs contained in creosote. Unless an attempt is made to control these sources that are not associated with treated wood, they could significantly confound the results of a study intended to evaluate the environmental effects associated with preservative lost from treated wood.

## Previous Treated-Wood Assessments

An extensive literature search did not reveal any reports that discussed environmental effects associated with timber bridges. Brooks (1995a,b, 1996, 1997a–c, 1998a,b) published risk assessment guides and computer models for assessing environmental risks associated with wood treated with creosote, CCA, Ammoniacal Copper Quat, type B (ACQ), and ACZA and used in aquatic environments. These documents focus on the loss of preservative from immersed wood and do not adequately address the loss of preservative constituents from overhead structures.

The magnitudes and characteristics of losses of wood preservatives from overhead bridge structures into underlying aquatic environments are a function of numerous variables, including wood type, preservative, retention rate (how much preservative is in the wood), and production methods. These variables can be controlled by the engineer, specifier, and wood treater. In addition, loss of preservative is a function of rainfall quantity and pH for waterborne preservatives and wood temperature for oilborne preservatives.

Goyette and Brooks (1999) hypothesized that much of the sedimented PAH associated with creosote-treated piling results from solar heating of the wood above the water line. On very hot days, creosote-treated wood acts as a black body, and the temperature of the wood may be much greater than ambient air temperatures. Under these conditions, the creosote may migrate to the surface of the wood where blisters can form and pop, ejecting small particles of creosote away from the piling. Alternatively, the creosote may run down the piling or timber until it forms droplets that fall to the water. Drs. John Simonsen and Jeff Morrell, Oregon State University, are evaluating the magnitude and nature of preservative losses from overhead structures. When these data are available, it is anticipated that a risk assessment computer program will be developed and available in the year 2000.

Several studies examining the environmental response to treated-wood structures have recently been reported. These studies are reviewed in the following sections.

## Creosote Evaluation

Goyette and Brooks (1999) examined biological and physicochemical responses to the presence of two six-piling creosote-treated dolphins, with 2.4- to 3-m-wide bases, placed in a pristine marine environment. The study compared aged (8-year-old) and newly treated piling with an untreated Douglas-fir control and an open control (lacking any structure). Water depths were 8.7 m and currents were slow, with a maximum speed of 1.89 cm/s. Based on the first 535 days of evaluation, the authors concluded the following:

- Creosote contamination occurred primarily as minute tar droplets or particles within surface and subsurface layers of surficial sediments. Water concentration levels of total PAHs immediately adjacent to the down-current piling were in the 0.020 to 0.030  $\mu\text{g/L}$  range, which is a level where no biological effects should be anticipated.
- Sediment levels of PAH reached 18  $\mu\text{g/g}$  dry sediment (range 5.0 to 30.0  $\mu\text{g/g}$ ) within half a meter of the dolphin's perimeter and declined exponentially to levels of about 7.5  $\mu\text{g/g}$  at a distance of 7.5 m. Sediment levels of PAH were close to background levels (0.2  $\mu\text{g/g}$ ) at distances beyond 7.5 m from the dolphin's perimeter.
- The Creorisk Model of Brooks (1997b) predicted about 30% more sedimented PAH than was actually observed during the first 535 days of the study.
- An extensive infaunal community analysis was conducted. This resulted in the identification of PAH-sensitive and PAH-tolerant taxa. However, significant adverse effects were not seen in any compartment of the infaunal community at any distance from the structures.
- Laboratory bioassays included the amphipods *Rhepoxynius abronius* and *Eohaustorius washingtonianus* and solid and liquid phase tests using a marine bioluminescent bacterium (*Vibrio fischeri*), plus echinoderm sperm fertilization tests. The results of laboratory bioassays indicated low-level toxicity associated with Sooke Basin reference sediments. Reduced survival was observed in proximity to the untreated Douglas-fir piling, and increased toxicity was found close to the creosote-treated piling. Taken altogether, bioassay data indicated that sediments located within 0.65 m of the creosote-treated dolphin were toxic in laboratory studies.
- Elevated levels of PAH were observed in blue mussels (*Mytilus edulis*) grown at various distances from the six-piling dolphins during the first 14 days of the study. Tissue levels then declined to background concentration levels.



- Mussels raised in cages within 15 cm of the treated piling grew more slowly than did those grown further away. Survival was excellent in all cohorts (>98%), with no significant differences between stations. All mussel cohorts spawned normally, and no difference was observed in the survival or development of the larvae.
- This site was selected because it was representative of a “worst case” project—slow currents, fine-grained sediments, and low sediment organic carbon, coupled with a healthy infaunal community. The authors concluded that even in these worst-case environments, creosote-treated piling would adversely affect biological resources only in the immediate vicinity (<0.65 m) of the structure. Therefore, they recommended that creosote-treated wood be a product that is managed, particularly in sensitive aquatic environments with very slow currents.
- A comparison of individual PAH compound concentrations with Long and others (1995), Washington Sediment Quality Criteria, and the Canadian Interim Sediment Quality Guidelines suggested that phenanthrene posed the highest level of risk, followed by lower risks associated with fluorene and acenaphthene. Fluoranthene and chrysene posed the most significant risks associated with high-molecular-weight compounds. Only the proposed U.S. Environmental Protection Agency (EPA) sediment quality criteria for acenaphthene, phenanthrene, and fluoranthene were found to be underprotective. The Washington State Sediment Quality Criteria were most efficient in that they were protective of infauna but produced the fewest number (12) of false negatives (instances in which adverse effects were predicted but not actually observed). The threshold effects level (TEL) was least efficient, predicting adverse effects in 52 cases where not one was observed. The probable effects level (PEL) was next most efficient with 21 false positives.

## Creosote Mesocosm Studies

Bestari and others (1998) examined the environmental response of different numbers of newly treated creosote piling placed in 12,000-L freshwater microcosms. The authors concluded the following:

- Water column concentration levels of PAH increased for 7 days following installation of the piling, followed by an exponential decline to approximately background levels within 84 days.
- The loss of PAHs from the water was not reflected in increased sediment concentration levels.
- When these initial concentration levels declined, the authors concluded that “the accumulation of creosote-associated PAHs from impregnated pilings in aquatic environments will likely be minimal and probably not pose a significant risk to aquatic biota” (Bestari and others 1998).

## Metal Uptake

Weis and Weis (1992) examined the uptake of CCA metals from bulkheads on the Atlantic coast of the United States. They found that the green algae *Ulva lactuca* and *Enteromorpha intestinalis* contained elevated levels of metals when compared with the same species collected from reference areas. Significant mortality was observed in snails (*Nassarius obsoletus*) fed an exclusive diet of the algae from CCA treated docks. The snails and algae were housed in small static containers, which exacerbated metal exposure. Unfortunately, water column concentration levels of metal were not reported in this paper. Similarly, Weis and Weis (1993) examined copper levels in oysters residing on CCA-treated piling and bulkheads. They reported American oyster (*Crassostrea virginica*) wet tissue weight copper levels of 12.59 µg/g from reference areas containing no CCA-treated wood, an average level of 27.05 µg/g in oysters growing on CCA-treated piling, and a level of 154.3 µg/g in oysters growing on CCA-treated bulkheads in residential canals. The National Academy of Sciences (NAS 1971) gives a bioconcentration factor of 5,000 for copper in marine mollusks. When coupled with the known metal loss rates from CCA-treated wood (Brooks 1996), elevated levels of copper should be expected in oysters growing on treated wood. To put the observed levels in proper perspective, note that Shuster and Pringle (1969) summarized historical reports of trace metal levels in the American oyster (*Crassostrea virginica*). They found an average of 144.8 µg Cu/g wet tissue weight, with a range of 6.83 to 600 µg/g. The mean oyster tissue copper level reported by Shuster and Pringle (1969) was 5.35 times greater than the level observed in the same species of oysters growing on CCA-treated piling in the Weis and Weis (1993) study. The mean copper level reported by Weis and Weis (1993) for oysters growing on CCA-treated bulkheads (154.3 µg/g) was only 7% greater than the mean for all American oysters reported by Shuster and Pringle (1969). Similarly, Goyette (1975) found whole body copper levels of 2,550 µg Cu/g wet oyster tissue in *Crassostrea gigas* collected from Howe Sound, British Columbia, near a mine site. Reference area tissue copper levels were 380 µg Cu/g wet tissue in this study. The point is that a loss of biological integrity cannot be inferred simply because copper (or any other possible pollutant) is significantly elevated. In this case, the elevated levels of copper observed in oysters growing on CCA-treated wood was well within the normal range of this metal observed throughout the East or West Coasts of the United States.

Weis and Weis (1995) examined the benthic impacts of four CCA-treated wood structures in National Estuarine Research Reserves. Sediment quality benchmarks (Long and others 1995, Suter and Tsao 1996, Jones and others 1997) are based on the fraction of a contaminant in the whole sediment weight, not on the percentage fines as reported by Weis and Weis (1995). Furthermore, the National Oceanic and

Atmospheric Administration (NOAA 1988) cautioned that evaluating the biological risks associated with metal concentration levels in only the fine fraction (<63 µm particle diameter) of sediments can lead to unacceptable bias in the interpretation, especially when fines represent <20% of the sediment matrix. In the Weis and Weis (1995) study, the percentage fines adjacent to bulkheads varied between 1.3% and 3.9% in sediments containing 5.0 to 25.0 µg/g copper in this fine fraction. Converting these levels to total sediment dry weight gives copper concentration levels of <1.0 µg/g at all distances from the bulkhead. These were exceptionally low values that did not approach NOAA's effects range low of 34 µg/g dry sediment weight. Sedimented copper levels observed by Weis and Weis (1995) adjacent to CCA-treated bulkheads were lower by at least an order of magnitude than concentration levels at which any biological effect might be observed. Weis and Weis (1995) found that "Despite the fact that some (piling) were less than 1 year old, no clear accumulation of metals in sediments or organisms was noted; nor were any consistent effects seen on community parameters." The authors concluded "pilings do not appear to produce significant impacts to the nearby environments, at least in well-flushed systems."

Adler-Ivanbrook and Breslin (1999) exposed blue mussels (*Mytilus edulis*) to wood treated to a preservative retention of 40 kg/m<sup>3</sup> CCA for 9 months in 1994 and 3 months in 1995. They found few significant differences in condition index, dry tissue weight, or valve length between mussels grown adjacent to treated wood and control mussels. They found that the tissue levels of copper, chromium, and arsenic in both sea table experiments and field exposures were generally within normal ranges found in mussels from sites within Long Island Sound and around the United States.

Wendt and others (1994) examined the concentration and biological effects of metals (copper, chromium, and arsenic) and organic compounds lost from treated-wood docks in South Carolina macrotidal creek systems. Copper, chromium, arsenic, and PAHs were measured in composite samples of surficial sediments and naturally occurring oyster populations (*Crassostrea virginica*) from creeks with high densities of docks and from nearby reference creeks with no docks. The results indicated that in macrotidal estuarine environments, wood preservative leachates from dock pilings had no acutely toxic effect on four common estuarine species nor did they affect the survival or growth of juvenile oysters for 6 weeks. In some cases, metal leachates accumulated in sediments and oysters immediately adjacent to pilings but were not elevated elsewhere in the same creeks.

## Wetland Boardwalks

Brooks (2000) examined the biological response to an extensive boardwalk system constructed in wetlands associated with the Wildwood Recreational Area on the western slope

of Mount Hood in Oregon. Three spurs constructed of ACZA-, ACQ-, and CCA-treated woods were evaluated. Each spur was extended into aquatic areas of the wetland system. A mechanical control structure, constructed of untreated Douglas-fir, was constructed in a hydrologically isolated part of the wetland for comparison. Metal levels were measured in sediments and water and compared with characteristics of the resident aquatic invertebrate community before construction and at 15, 162, and 336 days following construction. Samples were collected at upstream control stations, at the edge of each structure, and at various distances downstream.

The different structures contributed various levels of copper, chromium, arsenic, or zinc to both the water column and sediments. Based on leaching rates and toxic thresholds in water or sediments, copper lost from CCA-treated wood presented greater environmental risks than did either arsenic or chromium (chromium(III) or (VI)). The U.S. EPA (USEPA 1984) published water quality criteria for copper in freshwater: (1) short-term (acute) exposure and (2) long-term (chronic) exposure. These criteria, which are dependent on water hardness, are described in Figure 3.

$$\begin{aligned} &\text{U.S. EPA acute copper criterion} \\ &\leq 0.960 \exp\{0.9422[\ln(\text{hardness})]1.464\} \end{aligned} \quad (1)$$

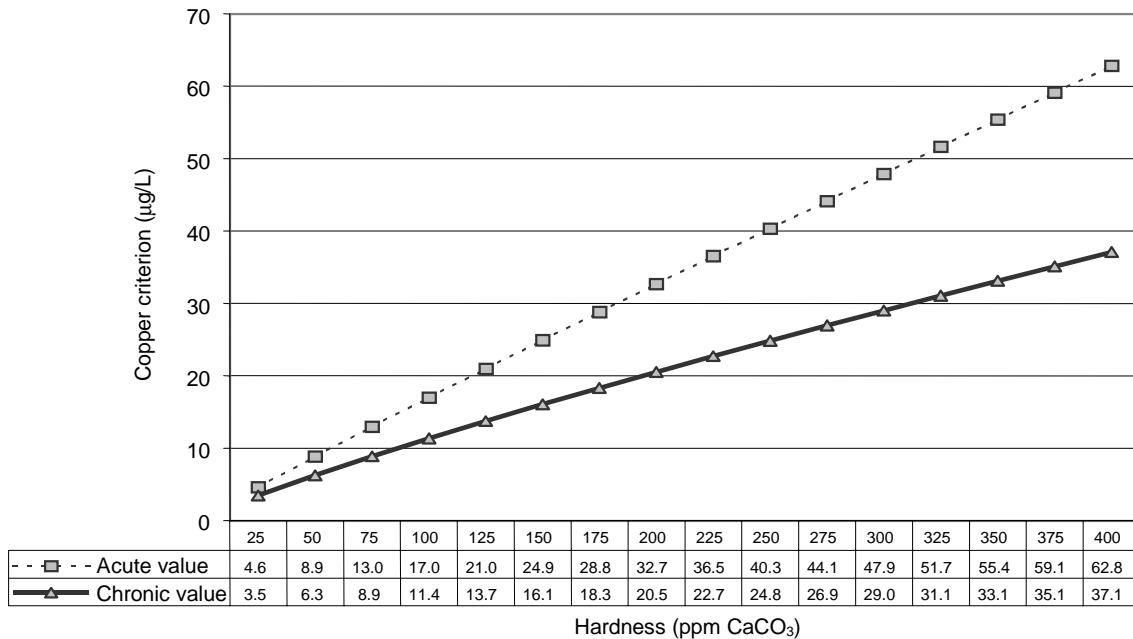
Note: The acute copper criterion is a 1-h average not to be exceeded more than once every 3 years.

$$\begin{aligned} &\text{U.S. EPA chronic copper criterion} \\ &\leq 0.960 \exp\{0.8545[\ln(\text{hardness})]1.465\} \end{aligned} \quad (2)$$

Note: The chronic criterion is a 4-day average not to be exceeded more than once every 3 years.

Freshwater sediment quality criteria have not been established for the metals contained in CCA. Until appropriate freshwater sediment quality standards are developed, the benchmarks given in Table 2 are proposed for evaluating the benthic effects associated with copper. The development of these benchmarks is described in detail by Brooks (2000).

Maximum levels of dissolved or sedimented copper, chromium, or arsenic did not exceed a high no-effect concentration in the aquatic environment adjacent to the CCA-treated bridge and viewing platform in the Wildwood study. Biological endpoints evaluated in that study included species abundance, taxa richness, diversity (Shannon's Index), and community evenness (Pielou's Index). Adverse effects on aquatic invertebrates were not anticipated at the levels recorded in Wildwood, and adverse effects were not observed in invertebrate samples collected from vegetation, from replicated artificial substrate samples, or from sediments. Sediment concentrations adjacent to one of the other two treated-wood platforms reached concentration levels of 200 µg Cu/g dry sediment. However, a reduction in the



**Figure 3—U.S. EPA chronic and acute copper criteria for freshwater. The copper standard is presented in micrograms per liter and hardness values in milligrams of calcium carbonate per liter.**

**Table 2—Recommended benchmarks for assessing environmental risks associated with sedimented copper lost from pressure-treated wood**

Sediment and water column characteristics	Acceptable levels of sedimented copper (µg Cu/g dry sediment)
Coarse-grained sediment (silt and clay <10%) Total organic carbon <0.2% Moderate to low pH (5.5 to 6.5) Low hardness and alkalinity (15 to 25 ppm calcium carbonate)	30
Intermediate sediments (silt and clay between 10% and 25%) Total organic carbon between 0.2% and 1.0% Neutral pH (6.5 to 7.5) Moderate hardness and alkalinity (35 to 100 ppm calcium carbonate)	55
Low-energy, well-buffered streams and lakes (fines >25%) Total organic carbon >1.00% Greater than neutral pH (pH > 7.5) High hardness and alkalinity (>100 ppm calcium carbonate)	100

biological endpoints measured in the study was not observed, even at these levels. This suggests that the benchmarks proposed in Table 2 may be overly conservative from an environmental point of view.

## Materials and Methods

The following protocols were followed in conducting this study.

### Site Selection

Primary producers of timber bridges were identified and interviewed to determine the types of preservatives commonly used. Based on that information, and in consultation with the Forest Products Laboratory (FPL), a decision was made to evaluate two creosote-treated bridges in the Midwest, two penta-treated bridges in the West, and two CCA-treated bridges in the southern tier of the United States. Producers of these bridges were requested to nominate bridges meeting the following site selection criteria:

- CCA-treated bridge candidates should be constructed within 90 days of being evaluated to observe the early loss of metals.
- Creosote- and penta-treated bridges should be 2 to 3 years old to allow time for preservative accumulation in sediments.

- The stream should have healthy riparian (streamside) vegetation.
- Bridges should be substantial in size and should carry vehicular traffic. A large pedestrian bridge would be acceptable but is not a first choice.
- Bridges should be located in rural areas, away from other anthropogenic sources of metals, penta, or PAHs.
- Bridges should have treated wood decks and, if possible, be supported on treated-wood piling that is immersed in water.
- The stream should be relatively slow flowing, preferably  $<10$  cm/s and with substantial flows  $>0.56$  m<sup>3</sup>/s ( $>20$  ft<sup>3</sup>/s).
- The stream should be perennial and contain freshwater, not seawater.
- The stream bottom should be soft (sand, silt, and clay rather than coarse rock and cobble). This bottom structure would be expected where slow currents exist.
- The stream should have a minimum depth of 30 cm.
- Bridges should not be subject to abrasion by commercial boat traffic or barges.
- The treated wood in bridges should be properly treated and should not be painted or wrapped

## Sample Location

The adopted design relied on analysis of variance (ANOVA) and regression analyses to identify differences in sediment physicochemical and biological endpoints at upstream controls, directly under each bridge, and at various distances downstream. Sample station locations are described in Figure 4. Eighteen stations were evaluated at each bridge.

## Data Analyses

The study design allowed for either a *t*-test or ANOVA testing that compared the replicated end points at reference stations located  $>30$  m upstream with those collected under or immediately downstream from the drip line of the bridge. In addition, regression analysis was used to search for clines in biological and physicochemical endpoints from the upstream to downstream sample stations. The significance of the clines was determined by evaluating the statistical significance of coefficients on the various independent variables evaluated in the study. Correlation analysis, including principle components analysis, was used to evaluate the relationship between biological responses and physicochemical endpoints and between the bridge structure (distance) and the concentration of treated-wood preservatives observed in either the water or sediments. Fourth-order polynomial least squares fits to the biological endpoints were constructed. The shapes of these curves provide a qualitative assessment of the biological effects associated with each bridge. A guide to interpreting these graphs is provided in Figure 5.

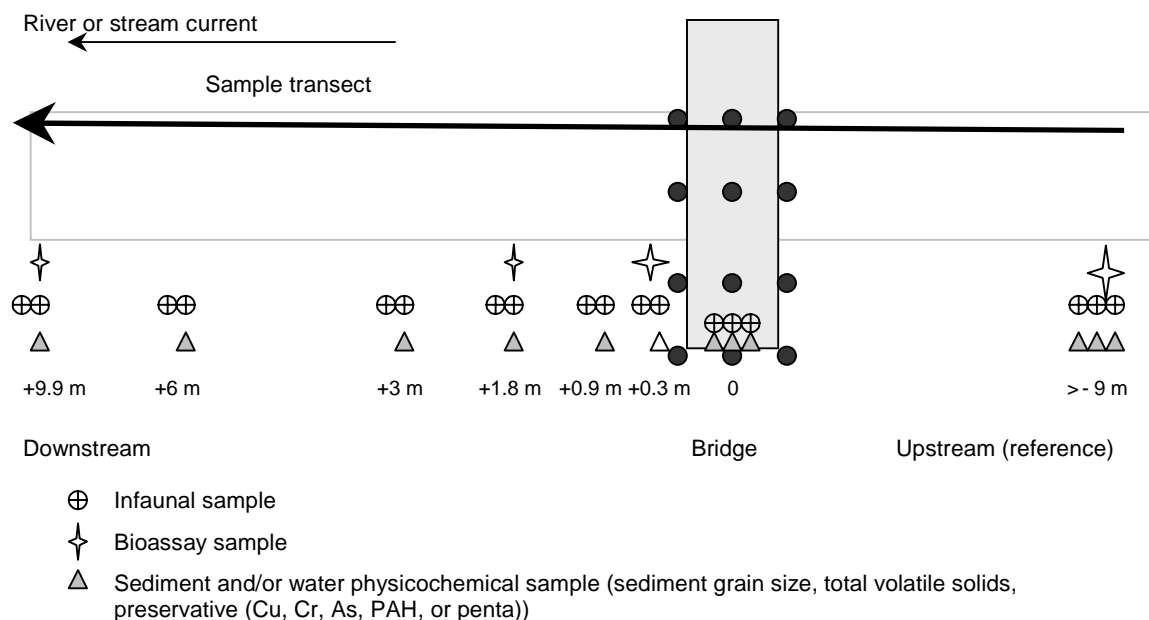
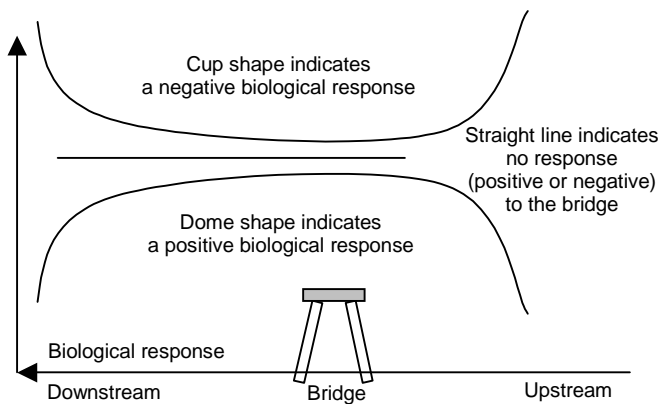


Figure 4—Generalized location of sample stations examined at each timber bridge evaluated in this study.



**Figure 5—Conceptual use of graphical techniques and regression analysis to evaluate the biological response to treated-wood bridges.**

Cause and effect relationships between environmental concentration levels of preservative constituents and biological endpoints were investigated using regression, correlation, and principle components analyses. In some analyses, a variable, such as copper concentration, would be considered independent (with respect to biological endpoints), and in another part of the analysis, the same variable would be dependent, such as the copper concentration with respect to distance from the bridge structure.

The following biological endpoints were evaluated as dependent variables in this study:

- Total species richness (total number of taxa) and Margalef's (1958) Richness Index—Ludwig and Reynolds (1998) discussed the underlying assumption for a functional relationship, of the form  $S = k\sqrt{n}$ , between the number of taxa  $S$  and the number  $n$  of organisms in the sample. This relationship was not found in this database, and valid conclusions were unlikely to follow from the analysis of Margalef's Index. Ludwig and Reynolds (1998) noted that simple species richness  $S$  is a more appropriate endpoint when sample sizes are equal. This analysis evaluated the number of taxa  $S$ .
- Total sample abundance is simply the number of organisms present in a single sample. This metric is useful in identifying environments where stress is severe enough to adversely affect all taxa, including those that are normally tolerant of the stressor. It is not a useful metric for assessing subtle effects that adversely impact intolerant species that are replaced, numerically, by more tolerant taxa.
- Dominant and subdominant species abundance—Dominant taxa were identified as those that represented at least 1% of the total number of organisms in an individual bridge's taxonomic database. Correlation analysis was used to examine the relationship between abundance of dominant and

subdominant taxa and sediment or water column concentrations of copper, chromium, arsenic, zinc, penta, or PAHs as appropriate for the type of treated wood used in constructing the bridge. Highly dominant taxa that were not susceptible to the chemicals of concern were dropped from the database because their numerical strength would have masked effects on more sensitive, but less abundant, taxa. The resulting group of subdominant and more sensitive taxa was considered most likely to reveal adverse effects.

- Shannon's Index (Shannon and Weaver 1949) provides the average uncertainty per species in an infinite community of  $S$  taxa. The form of the index used in this analysis is given by Equation (3). The value of Shannon's Index is zero when a single species is present. The value is maximized when there are a large number of equally represented species and is reduced in communities dominated by a few highly abundant species. The value of Shannon's Index in a sample containing 20 taxa equally represented in a total abundance of 600 animals would be 3.0.

$$H' = -\sum [(n_i/N) \ln(n_i/N)] \quad (3)$$

where the sum is over  $S$  species.

- Pielou's Index (Pielou 1977) is a commonly used measure of community evenness. It expresses the observed value of Shannon's Index relative to the maximum possible value ( $\ln(S^*)$ ). Pielou's Index, given as  $J'$  in Equation (4), varies between 0 and 1, and it generally co-varies with Shannon's Index.

$$J' = H'/\ln(S^*) \quad (4)$$

where  $H'$  is Shannon's Index and  $S^*$  is the number of taxa.

## Physicochemical Characterization of Water Column

The water at each bridge was characterized by measuring temperature, pH, salinity, hardness, depth, total suspended solids, and total volatile solids. A point-in-time assessment of the environment's ability to disperse pollutants associated with each bridge was assessed by measuring current speed and water depth at intervals across the channel, with emphasis along the designated transect line. Note that this point-in-time profile has significant limitations in interpreting sediment data because sediment concentrations are the result of accumulations over time. Obviously, river currents and depths change with each season. River depth and speed are provided to give the reader a sense of the nature of the stream. To obtain an understanding of the hydrodynamics associated with each bridge, this information must be considered in light of the photographic evidence provided and the sediment grain size distribution along the transect.

## Physicochemical Characterization of Sediments

Sediments were characterized by determining the sediment grain size distribution and percentage total volatile solids. Preservative constituents (PAH, penta, copper, chromium, and arsenic) were measured in the water column or sediments, or both, as appropriate at each bridge.

## Characterization of Biological Response

The biological response at each bridge was assessed by characterizing the invertebrate community collected by either a petite Ponar grab or a 0.0309-m<sup>2</sup> sampling device of proprietary design. In addition, four laboratory bioassays were conducted on either the water (for CCA-treated bridges) or sediments (for penta- and creosote-treated bridges).

## Cleaning of Sample Containers and Equipment

Sample bottles were obtained, precleaned, from the laboratory subcontracted to conduct the analyses. Glass bottles were used in all instances. Samples for PAH and penta analyses were washed with a phosphate-free detergent solution, followed by thorough rinses with hot tap water and analyte-free water. This was followed by an acetone rinse and a final rinse using high-purity methylene chloride. Lids were placed on the containers during the final rinse step because the solvent could rinse plastic from the interior screw threads onto the Teflon lining.

Sample bottles for metal chemistry were cleaned in a detergent solution, rinsed with metal-free water, and soaked overnight in a covered bath containing dilute reagent-grade nitric acid. The bottles were then rinsed in metal-free water.

New platinized silicone tubing was used to collect water samples at the CCA- and penta-treated bridges. Separate pieces of tubing were used at each bridge. The tubing was soaked overnight in a 20% nitric acid bath at room temperature, then rinsed thoroughly in distilled water. Each 7.6-m-long piece of tubing was coiled and stored in a sealed bag until opened for use.

## Sample Collection and Field Processing

Water column samples for chemical analysis were collected using a Masterflex LS Sampling Pump (Cole Parmer Instrument Company, Vernon Hills, Illinois) and platinized Teflon tubing with an epoxy-coated lead weight. Samples were filtered in the field across Corning Costar Corporation Nucleopore filters (0.45 μm) (Corning, New York) and acidified. A single piece of tubing was used for each bridge.

Samples were collected at the upstream reference station first, followed by the furthest downstream station, then proceeding upstream to a location under the bridge. Water samples for bioassays were collected in new 2-L bottles by holding the bottle under the water at a depth of about 0.5 m and unscrewing the container's top. A total of 4 L of water was collected for each bioassay. Similar procedures were used to collect water samples in 500-mL bottles to determine total suspended and total volatile solids.

Sediments were sampled using either a stainless steel petite Ponar grab (0.0225 m<sup>2</sup>) or the stainless steel grab described in Figure 6, which samples an area of 0.0309 m<sup>2</sup>. Samplers were washed with detergent in hot water, then rinsed copiously in hot water followed by a rinse in distilled water prior to sampling each bridge. Reference station sediment samples were collected first, followed by the most downstream stations, then proceeding toward the bridge.

The sampler described in Figure 6 was used on rocky substrates and where heavy vegetation cover prevented use of the petite Ponar grab. Separate samples were collected for physicochemical analyses and infaunal analysis. The entire sample was processed to evaluate infauna. In vegetated areas, this included the vegetation growing within the footprint of the sampler. Similarly, this sampler was used in the rocky substrates associated with the penta-treated bridges surveyed in Washington and Oregon. Samples for physicochemical analyses were taken from the upper 2.0 cm of the sediment column.

## Sample Documentation and Handling

Samples were tightly capped in pre-labeled bottles and stored on frozen phase change gel packs to maintain ≤4°C. Samples



**Figure 6—Stainless steel sampler designed for use by scuba divers. The sampler completely encloses a sediment sample covering an area of 0.0309 m<sup>2</sup>, preventing loss of sample as it is returned to the surface.**

were shipped by overnight delivery service to the appropriate analytical laboratory using chain of custody procedures that comply with requirements (ASTM 1988).

## Sediment and Water Analyses for Preservatives

The following summarizes the methods used to evaluate the water and sediments for preservative constituents lost from pressure-treated wood used in constructing timber bridges.

Analyte	Method	Detection limit
Polycyclic aromatic hydrocarbons (PAHs)	HPLC (EPA 8310)	See Appendix A
Penta	GC/ECD (EPA 8151)	2 µg/L or /kg
Copper (water)	ICP-MS (EPA 200.10)	0.030 µg/L
Chromium (water)	GFAA (EPA 200.10)	0.075 µg/L
Arsenic (water)	GFAA (EPA 200.10)	0.310 µg/L
Copper (sediment)	ICP (EPA 6010)	0.100 mg/kg
Chromium (sediment)	ICP (EPA 6010)	0.500 mg/kg
Arsenic (sediment)	GFAA (EPA 7060)	0.200 mg/kg

## Sediment and Water Analyses for Conventional Parameters

The following procedures were used to evaluate conventional parameters in sediments and water:

- Total volatile solids (TVS) analysis was accomplished on 50-g surficial sediment samples. Samples were dried at  $103 \pm 2^\circ\text{C}$  in tarred aluminum boats that had been pre-cleaned by ashing at  $550^\circ\text{C}$  for 30 min. Drying was continued until no further weight reduction was observed. The samples were then ashed at  $550^\circ\text{C}$  for 30 min. or until no additional weight loss was recorded in successive weighings. Total volatile solids were calculated as the difference (percentage) between the dried and ashed weights.
- Sediment grain size (SGS) analysis was accomplished using 100-g surficial sediment taken from the top 2 cm of the sediment column. The sample was wet-sieved on a  $0.64\text{-}\mu\text{m}$  sieve. The fraction retained on the sieve was dried in a  $92^\circ\text{C}$  oven and processed using the dry sieve and pipette method (Plumb 1981). The sieves used for the analysis had mesh openings of 2.0, 0.89, 0.25, and  $0.064\text{ }\mu\text{m}$ . Particles passing the  $0.064\text{-}\mu\text{m}$  sieve during wet sieving were analyzed by sinking rates in a column of water (pipette analysis).
- Reduction–oxidation potential discontinuity (RPD)—A clear, 2.0-cm-diameter corer was inserted into the

undisturbed sediment in samples collected for physico-chemical analysis. The depth at which the sediment color changed from the background color to black (indicative of the precipitation of iron sulfides associated with anaerobic conditions) was measured in centimeters below the sediment–water interface.

- Benthic infaunal analysis—Invertebrate samples were sieved on  $500\text{-}\mu\text{m}$  stainless steel screens using water that was filtered to  $37\text{ }\mu\text{m}$ . The material retained on the sieve was fixed in isotonic water using either 10%, borax-buffered formaldehyde or nontoxic HistoCHOICE (Solon, Ohio) fixative when commercial shipment was necessary. Fixed samples were thoroughly washed in  $10\text{-}\mu\text{m}$ -filtered laboratory water, following 96 h of fixation, and preserved in 70% ethanol. Invertebrates were picked from the background matrix under a microscope at  $10\times$  to  $40\times$  magnification. A second technician re-picked 10% of the samples. When more than 5% additional organisms were obtained during the quality assurance check, all samples picked by the failed technician were re-picked.

Invertebrates were identified to the lowest practical level (generally genus for all orders except Chironomids, which were sometimes identified only to family or tribe). A reference collection containing representative samples for each taxon was developed and archived in 70% alcohol. Following identification, all samples were archived. The reference collection will be retained permanently. Individual samples will be retained for 3 years (until October 2001).

- Current speed and water depth—As a result of the very slow currents at most sites, current speed was assessed by determining the time (seconds) required for a neutrally buoyant drogue to travel a distance of 2 m along a polyvinyl chloride (PVC) pipe fixture designed specifically for this purpose. Water depths were measured using a meter stick. Three replicate measurements were made at each sample station.
- Water total suspended solids (TSS by APHA [n.d.] 2540B)—A representative water sample (approximately 300 mL) was thoroughly mixed, filtered through a  $45\text{-}\mu\text{m}$  glass filter that had previously been ashed at  $550^\circ\text{C}$ , and weighed. The filter was washed three times with 10 mL of distilled water with complete drainage between washings. Suction was continued for 3 min after filtration was complete. The filter was then dried at  $103 \pm 2^\circ\text{C}$  until no weight loss was recorded on successive weighings to 0.1 mg. The difference between the filter's original weight and the weight with dried residue was considered to be the TSS. The value was expressed as milligrams per liter.
- Alkalinity was determined titrimetrically using APHA Method 403.



- Hardness was determined using the EDTA Titrimetric Method (APHA Method 314B.2).
- Water total volatile solids (TVS by APHA Method 2540 B)—The filter, containing the residue used to measure TSS, was ashed at 550°C in a muffle furnace for 1 h and reweighed. The weight loss on ignition, expressed in milligrams per liter, was taken as the TVS. Quality assurance involved weekly calibration of the four-place balance, routine running of blank filters, and triplicate analyses on 5% of the samples (minimum of one).
- Temperature, salinity, dissolved oxygen, and pH—Temperature was measured in the field using both a mercury thermometer and a temperature meter. A dissolved oxygen meter was used to measure dissolved oxygen *in situ* and a microcomputer was used to measure pH. Each meter was calibrated immediately before use at each bridge site. A two-point calibration (pH 7 and 10) was used for the pH meter.
- Bioassay protocols—Four bioassays were conducted at each of the bridges evaluated during this study. As a result of differences in sediment characteristics and the marine environment at the new bridge site in Sandestin, Florida, several types of organisms were used at different bridge sites. Laboratory control sediments and a local upstream reference station at each bridge provided two levels of control in these bioassays. The results at each of these control levels were compared with amphipod survival at three treatment stations located 0.0, 1.8, and 9.9 m downstream from the perimeter of each bridge. Cause and effect relationships were examined using correlation and regression analyses to compare sediment levels of preservative constituents with amphipod survival.

*Hyalella azteca*—Ten-day amphipod sediment bioassays were conducted on sediments from four stations at each of the penta- and creosote-treated bridges. Sediments were evaluated because that was the environmental compartment where maximum concentration levels of either PAH or penta were anticipated and observed. These tests were conducted by MEC Analytical Systems, Inc., in Carlsbad, California, using MEC Protocol P034.0. Methods employed in their program followed the general procedures outlined by ASTM (1997).

*Menidia berylina*—A 96-h acute bioassay was conducted on water from the newly constructed CCA timber bridge in Sandestin, Florida. Water, rather than sediment, was tested at both CCA bridges included in this study because the test species are most sensitive to the dissolved cupric ion (Cu<sup>+2</sup>). This particular test animal was chosen because this was a marine site with salinity measured at 24.9 ppt (parts per thousand). MEC Testing Protocol No. P009.1 was used at

25.5°C. Copper sulfate (CuSO<sub>4</sub>) was used as a reference toxicant with a 96-h LC<sub>50</sub> of 114.0 µg/L.

*Daphnia magna*—A 96-h acute bioassay was conducted on the water from the old CCA timber bridge evaluated in Sandestin, Florida. The protocol given in EPA 600/4-90/027 (4th edition) was used at a test temperature of 25°C. Five ostracods were included in each of four replicated test chambers (20 organisms total). Sodium chloride was used as a reference toxicant.

## Quality Assurance Requirements and Data Qualifying Criteria

The following are quality assurance requirements for the analysis of copper, chromium, arsenic, penta, and PAHs sampled during the timber bridge environmental risk assessment:

Requirement	Data qualifier criteria
Method blank (1 per 20 samples)	No analyte detected in blank
Replicate analyses (1 per 20 samples)	≤20% RPD
Matrix spike (1 per 20 samples)	75% to 125% spike recovery
Surrogate recovery (all samples)	±95% confidence interval

### Sediment Grain Size and Total Volatile Solids

Sediment grain size (SGS) and total volatile solids (TVS) require triplicate analyses on one or a minimum of 5% of the samples. The root square deviation (RSD) should be ≤20% for same sample results.

### Infaunal Analysis

Infaunal analysis standard AES QA/QC procedures were used (PSEP 1986).

## Results and Discussion

### Creosote-Treated Bridges

Since 1865, creosote has been widely used in the United States to protect wood from attack by fungi, marine borers, and insects. Creosote is a distillate derived from coal tar produced by the carbonization of coal and consists primarily of liquid and solid aromatic hydrocarbons.

The treatment of wood products is generally accomplished in accordance with the American Wood-Preservers' Association

(AWPA) C1–92 (AWPA 1992). Average creosote penetration using the “empty cell” process in Douglas-fir is 38.1 mm (1.5 in.), and retention is 320 kg/m<sup>3</sup> (20 lb/ft<sup>3</sup>) for marine applications and 272 kg/m<sup>3</sup> (17 lb/ft<sup>3</sup>) for freshwater (Arsenault 1992, Baechler and Alpen 1962). In 1996, AWPA reduced the required retention in temperate marine waters from 320 to 256 kg/m<sup>3</sup> (20 to 16 lb/ft<sup>3</sup>).

Creosote is a complex mixture of at least 160 detectable hydrocarbon compounds; all 18 major components are cyclic and aromatic. According to Environment Canada (1992), 80% of creosote is composed of PAHs. Some low-molecular-weight creosote compounds, such as the naphthalenes, have a density less than one. However, most components are heavier than seawater and sink in a water column.

The public has voiced concern because creosote contains many of the 16 PAHs known to be acutely and chronically toxic to marine animals. Several of these compounds, most notably benzo(a)pyrene, can degrade to carcinogenic, teratogenic, and mutagenic intermediates during metabolism. As with most potentially harmful substances, pathological responses are a matter of exposure (concentration and length of time).

### Sources of PAH

Polycyclic aromatic hydrocarbons (PAHs) are formed by a variety of processes, including indirect and direct biosynthesis, fossil fuel production and distribution, and incomplete combustion of organic matter. When formed, PAHs can be transported into an aquatic environment through several pathways, including fossil fuel distribution, storm water runoff, sewage effluent, and atmospheric deposition.

- **Biosynthesis**—Indirect biosynthesis of PAH occurs when extended quinones and related polycyclic materials (mostly plant and animal pigments) are exposed to the reducing conditions found in anoxic sediments. The resulting PAHs tend to accumulate in the sediments where they were formed. De novo biosynthesis of PAHs by aerobic and anaerobic bacteria, fungi, and plants is controversial. However, Mallet and others (1972) concluded that both aerobic and anaerobic bacteria can biosynthesize benzo(a)pyrene (B(a)P) and certain other PAHs using fatty acids, sterols, plant pigments, and aliphatic terpenes as substrates. In most cases where PAH biosynthesis has been reported, accumulation of PAH in the organisms purported to have synthesized them could also have been attributed to uptake of PAH from exogenous sources. In light of the literature reviewed, it appears that PAH biosynthesis may occur to a limited extent under special environmental conditions when necessary bacterial growth substrates are present. Eisler (1987) suggested that, on a global scale, biosynthesis annually contributes about  $2.7 \times 10^6$  kg ( $6 \times 10^6$  lb) of PAH to aquatic environments (Table 3).

**Table 3—Global sources of PAHs to aquatic environments<sup>a</sup>**

Source	PAH (kg/year (lb/year))	
Petroleum spillage	168,620,000	(374,782,200)
Atmospheric deposition (from combustion)	49,603,500	(110,230,000)
Wastewater	4,365,108	(9,700,240)
Surface land runoff	2,916,686	(6,481,524)
Biosynthesis	2,678,589	(5,952,420)

<sup>a</sup>Eisler 1987.

- Fossil fuels, including peat, coal, and petroleum, are relatively rich in complex assemblages of PAHs. These compounds reach aquatic environments through surface runoff, in wastewater, and as a result of petroleum spillage. Eisler (1987) estimates that spilled petroleum contributes  $168 \times 10^6$  kg ( $374 \times 10^6$  lb) of PAH to aquatic environments each year (Table 3). This source overwhelms all others in terms of global inputs.
- Pyrolysis of organic matter between 400°C and 2000°C results in the generation of a wide variety of PAHs. Reducing conditions (insufficient oxygen) in the pyrolytic environment favor PAH production. Forest and grass fires, industrial processes, heating, power generation, and petroleum refining release significant amounts of PAH into the atmosphere. These products of combustion are subject to chemical- and photo-oxidation. However, their residence time in the atmosphere is long enough to allow wide dispersal, and they are a major source of PAHs in aquatic environments. According to Eisler (1987), forest and prairie fires and agricultural burning annually release nearly  $32.4 \times 10^6$  kg ( $72 \times 10^6$  lb) of PAHs into the atmosphere. This is three times the amount from all other pyrolytic sources combined.
- Petrolytic sources—Johnston and Harrison (1984) reported that B(a)P deposition along a United Kingdom motorway is  $2.8 \mu\text{g}/\text{m}^2\text{-week}$ . Note that B(a)P is approximately 0.5% to 2.5% of some PAH mixtures, and a direct extrapolation suggests that the total PAH loading along a well-used highway may be  $560 \mu\text{g}/\text{m}^2\text{-week}$ . Winter levels of PAH in coastal areas are greater than summer levels. This is attributed to increased pyrolytic input from the burning of fossil fuels for power generation and heating (Bouloubassi and Saliot 1991). Broman and others (1990) suggested that primary PAH inputs in the Baltic region were from exhaust emissions associated with automobiles, domestic heating, refuse incineration plants, ships, and aircraft. Neff (1979) reported that little-used (224 km) motor oil contained 6.4 ppb (parts per billion) B(a)P, equivalent to nearly 1,280 ppb total PAH. (In this paper, billion is equivalent to  $10^9$ .) Dunn and Stich (1976) found up to

22,000 ppb B(a)P in well-used crankcase oil. This is equivalent to 4.4 ppt total PAH. In 1989, Washington state had 4,179,000 vehicles registered. These vehicles produced 63,000 m<sup>3</sup> (21,000,000 gal) of used crankcase oil each year. That may represent as much as 281,250 kg (625,000 lb) of PAHs available to the environment.

- Industrial and domestic wastewaters are rich in PAHs. Secondary sewage treatment removes some PAHs, but most are released to aquatic environments through sewage treatment plant outfalls. Eisler (1987) notes that untreated, raw sewage contains 100 to 500 ppb total PAHs and sewage sludge contains 200 to 1,750 ppb PAH. Goyette and Boyd (1989) recorded sediment PAH concentration levels of 17 µg TPAH/g dry sediment near a major combined sewage–storm water outfall discharging into Vancouver Harbor. Sediment PAH levels in the central areas of the harbor were consistently in the range of 2 to 5 µg PAH/g dry sediment. Hoffman and others (1984) noted that storm water runoff from urban areas and highways accounted for 71% of the high-molecular-weight PAHs and 36% of the total PAH loading to Narragansett Bay. More than 30% of all pyrolytic PAHs in the coastal sediments of Washington State are supplied by riverine transport of suspended particulate materials, and direct atmospheric input accounts for a maximum of 10% (Prahl and others 1984).

### Observed Levels of PAH

As a result of the many natural sources, PAHs have been present in aquatic environments for thousands of years. However, significantly increased levels have been recorded in ocean sediments since the turn of the 20th century. Neff (1979) reviewed the distribution of PAHs in aquatic environments. He found very low (<1 to 2 ppb) levels of PAHs in the water column of pristine areas and recorded low sediment contamination in pristine areas (< 0.050 µg/g) but higher levels (to 15 µg/g) associated with industrialized areas or human population centers. Eisler (1987) found a similar distribution of sediment PAH levels. Levels in pristine areas of Alaska, Africa, and the Amazon Basin ranged from 0.005 to 0.544 µg/g. Levels in urbanized and industrialized areas ran as high as 791 µg/g in the United Kingdom. Cerniglia and Heitkamp (1991) measured sediment PAH levels ranging from 0.005 µg/g for an undeveloped area in Alaska to 1,790 µg/g at an oil refinery outfall in Southampton, England. Sediment PAH concentration levels in other industrialized areas ranged from 0.198 to 232 µg/g. Transportation systems represent a significant source of PAHs associated with internal combustion engines, lubricants, herbicides, and asphalt and tars used in road surfacing. The following summarizes Eisler's (1987) assessment of PAHs loading to aquatic environments from the most common sources:

Source	PAH loading to aquatic environment	
	kg/PAH/year	lb/PAH/year
Petroleum spillage		374,782,200
Atmospheric deposition (from combustion)		110,230,000
Wastewater	4,365,108	9,700,240
Surface land runoff	2,916,686	6,481,524
Biosynthesis	2,678,589	5,952,420

There is a consistent thread running through research and reviews by Bouloubassi and Saliot (1991), Neff (1979), and Eisler (1987). These authors indicate that PAHs are present in aquatic and terrestrial environments. There are numerous natural sources of PAH, including volcanoes, forest and prairie fires, natural oil seeps, and biosynthesis. According to the sediment record, PAHs have been present in our environment since there was life. Historically, the most natural sources of PAH were either the result of biosynthesis, in which case the PAH remained stable in the anoxic environments where they were formed, or the inputs resulted from volcanoes or forest and prairie fires. These pyrogenic PAHs were widely distributed over large geographic areas and resulted in relatively low background levels (< 0.01 to 0.05 µg/g).

Anthropogenic inputs from oil spills, wastewater, storm water, and petrolysis tend to be concentrated in urban and industrial areas. This may result in concentrating PAHs in aquatic environments and the sediments that underlie them. It is these high concentration levels that are of concern. Petroleum spills represent the major PAH input to our oceans and can have devastating, although probably short-term (2 to 5 years), impacts on aquatic resources.

The literature suggests that sediment PAH levels of less than 0.2 to 0.5 mg/kg may be expected in pristine areas. Worldwide, urban areas have much higher background sediment PAH levels in the range of 0.5 to several milligrams PAH per kilogram of dry soil or sediment. Heavily industrialized areas may have PAH levels of 10 to several hundred milligrams PAH per kilogram.

### Fate of PAH

PAHs form a family of compounds, and the routes of degradation and fates are different for the major classes of PAH. In water, PAHs evaporate, disperse into the water column, become incorporated in bottom sediments, concentrate in aquatic biota, or experience oxidation and biodegradation.

The most important degradative processes for PAHs in the marine environment are photo-oxidation, chemical oxidation,

and biological transformation by bacteria and animals (Neff 1979). Most PAHs in aquatic environments are associated with particulate materials and only about a third are present in dissolved form. Dissolved PAHs will likely degrade rapidly through photo-oxidation (USEPA 1980b). They degrade most rapidly at high concentration levels, at elevated temperatures and oxygen levels, and at high levels of solar irradiation. Different PAHs vary significantly in their relative sensitivity to photo-oxidation.

Because of their low aqueous solubility and hydrophobic character, the higher molecular weight PAHs readily adsorb to particulate materials and solid surfaces in water. The ultimate fate of PAHs that accumulate in sediments is believed to be biotransformation and degradation by benthic organisms (USEPA 1980b). High-molecular-weight PAHs degrade slowly in anaerobic sediments (Neff 1979).

Cerniglia and Heitkamp (1991) discussed microbial degradation of PAHs in aquatic environments. They noted that a wide variety of bacteria, fungi, and algae have demonstrated ability to metabolize PAHs. Low-molecular-weight PAHs, such as naphthalene, degrade rapidly. The higher molecular weight PAHs, such as benz(a)anthracene and benzo(a)pyrene, are more resistant to microbial attack. Cerniglia and Heitkamp (1991) also note that the most rapid biodegradation of PAHs occurs at the water-sediment interface. This is because prokaryotes oxidize PAH as a first step in metabolism. Deeper sediments usually contain little oxygen, thus inhibiting microbial metabolism.

Cerniglia and Heitkamp (1991) summarized the available literature describing the half-life of PAHs in various environments. The results are highly variable and depend on PAH species together with a range of environmental and biological factors. Bacterial communities in polluted areas metabolize PAHs more quickly than do communities in unpolluted areas. Lighter weight PAHs are metabolized more quickly than heavier PAHs. Naphthalene has a short turnover time (days to weeks), whereas the five-ringed benzo(a)pyrene has a long turnover time (years under unfavorable conditions).

Ingram and others (1982) observed that the concentration of creosote in leaching vats increased to greater than 700 ppb in the first 72 h, then decreased to less than 34 ppb at the end of 20 days. They attributed that decrease to bacterial metabolism of the low-molecular-weight PAHs being leached from the pile sections in their study.

Tagatz and others (1983) noted that creosote concentration levels decreased by 42% during 8 weeks in sediments artificially contaminated as part of their mesocosm studies. They attributed the decrease to microbial metabolism.

Neff (1979) attempted to integrate the degradative processes associated with PAH removal from aquatic environments.

He concluded that the residence time of PAH in water is brief. The lower molecular weight aromatics (benzene to phenanthrene) are removed primarily by evaporation and microbial activity. Higher molecular weight PAHs are removed mainly by sedimentation and photo-oxidation. Degradation of PAH by animals in the water column is of minor importance. In nutrient-rich, biologically active, aerobic sediments, the degradation of PAHs is dramatically increased by healthy bacterial and fungal communities. In anaerobic sediments, the higher molecular weight PAHs (4 to 7 rings) may persist for years.

### **Bioconcentration, Bioaccumulation, and Biomagnification**

Bioconcentration and bioaccumulation of contaminants are of special importance because some aquatic species, most notably bivalves, have demonstrated an ability to rapidly bioconcentrate some contaminants in water to high tissue levels. The concern is that persistent contaminants may move up the food chain, biomagnifying to higher concentration levels in each trophic level, until contaminants found at nontoxic levels in the ambient environment reach levels where they do cause stress and disease. For effective biomagnification and movement of contaminants through the food chain, several conditions must be met. First, organisms must have the ability to bioconcentrate low levels of contaminants from the water column or bioaccumulate PAH from sediments or their food. Second, these contaminants, or their toxic metabolic intermediates, must be retained, unaltered, in the tissues of the organism until it falls prey to an animal at a higher trophic level.

Several factors mitigate biomagnification. If contaminants are not absorbed, they cannot accumulate. Numerous organisms, particularly vertebrates, have the ability either to metabolize or excrete organic contaminants. The gut, liver, kidney, and gall bladder are common sites of PAH concentration, metabolism, and excretion in vertebrates. If the contaminants are either rapidly excreted or metabolized to nontoxic compounds, then the chain is broken and biomagnification is not effective in passing contaminants upward through the food chain. DDT is an excellent example of a persistent compound that is bioconcentrated from low levels in the water to higher levels; first in plankton, then in fish, finally in bird populations with devastating consequences.

Neff (1982) reported that most aquatic organisms bioconcentrate PAHs from low concentration levels in the ambient water to higher tissue levels. Bioconcentration factors (BCFs) are predicted by the octanol-water partition coefficients ( $K_{ow}$ ) associated with individual PAH compounds.

Bivalve mollusks, particularly the commercially important mussel (*Mytilus edulis*) and oysters of the genera *Ostrea* and *Crassostrea*, have received far more attention than other aquatic invertebrates, plants, or fish. Bivalve mollusks are

excellent subjects for monitoring pollutants because they filter substantial quantities of water over large and highly permeable gills. For these reasons, mussels have been the subject of numerous studies. Many of these studies have focused on the accumulation of metals and the carcinogenic molecule benzo(a)pyrene.

Benzo(a)pyrene levels recorded by Neff (1979) for uncontaminated areas fall in the undetectable to perhaps 50 µg/L range. Dunn and Stich (1976) recorded tissue levels averaging 59 µg/g in mussels from areas associated with marinas and higher levels, averaging 402 µg/g, in mussels growing on creosote-treated pilings. Dobroski and Epifanio (1980) found that direct uptake of B(a)P from seawater by diatoms was much greater than the rate of trophic transfer from the diatoms to clam larvae.

Eisler (1987) recorded elevated PAH concentrations, especially benzo(a)anthracene, chrysene, fluorene, phenanthrene, and pyrene, in oyster tissues and sediments from the vicinity of marinas. These levels are notably higher in cooler months, when lipids and glycogen are being stored preparatory to spawning (Marcus and Stokes 1985).

For mussels, the general trend towards lower levels of higher molecular weight PAHs relative to the levels in associated sediment suggests an uptake mechanism that involves the solution of PAH in water. Supporting this hypothesis is the observed rapid turnover and shorter half-life of the more soluble, lower molecular weight PAHs (Dunn 1980, Eisler 1987). This suggests that the more soluble (and more bioavailable) PAHs are effectively removed from sediments and metabolized by bivalves. The higher molecular weight PAHs (associated with chronic stress and genetic disorders) remain in the sediments because of their low solubility. However, when absorbed, PAHs are more slowly metabolized by bivalves.

PAH levels in fish are usually low because this group rapidly metabolizes all PAHs (Lawrence and Weber 1984, West and others 1986a,b) or excretes them. High concentration levels of PAH are typically found in the gut, liver, and bile. Raw fish from unpolluted or moderately polluted water seldom contains detectable amounts of PAHs. However, smoking and cooking of fish can increase PAH content to significant levels.

Neff (1982) reported BCFs for several PAHs in the clam *Rangia cuneata*. Note that the BCFs, which range from 6.1 to 32, are for PAHs dissolved in water. Eisler (1987) summarized BCF values from the literature. The BCFs reported in his paper contradict his assertion that bivalves accumulate PAHs more rapidly than fish. For all the values given in his review, the average BCFs are 82 for bivalves ( $n = 8$ ) and 6,844 for fish ( $n = 34$ ). Note that Eisler's (1987) paper reported bioconcentration values from 6 to 236 in the clam, *Rangia cuneata*. Four of the five values were less than 33.

For fish, bioconcentration values range from 44 to 82,916, with most values in the 100 to 1,000 range.

The ultimate fate of most high-molecular-weight PAHs deposited in aquatic environments is sedimentation. Roesijadi and others (1978) examined the accumulation of crude oil in Prudhoe Bay and specific PAHs from oil-contaminated sediments by three infaunal invertebrate species, the sipunculid worm *Phascolosoma agassizii* and the clams *Macoma inquinata* and *Protothaca staminea*. They found that efficiency of PAH uptake from sediments was much lower than from water. Bioconcentration factors for uptake of the four PAHs from contaminated sediments were 0.2 or less, indicating no significant bioconcentration of PAH by this route. However, BCFs for uptake of these four PAHs from seawater were in the 10.3 to 1,349 range, indicating a low to moderate potential for bioconcentration.

Eisler (1987) suggested that bivalves readily take up PAHs from sediments. This hypothesis is contradicted by the results of numerous studies. O'Connor (1991) found that at 117 National Status and Trend Sites where there were both mollusks and fine-grained sediments, the average ratio of mollusk tissue to sediment concentration was only 1.2 for total PAHs. He also noted that mollusks accumulate the low-molecular-weight (and more highly soluble) PAHs to a greater extent (2.0) than the high-molecular-weight PAHs (0.64). Eaton and Zitko (1978) noted that PAH levels in clams and mussels were two orders of magnitude below those detected in sediments. Neff (1979) cites Perdriau's (1964) finding that in no case did benthic animals contain elevated levels of B(a)P when compared with sediment concentration levels. Tissue concentrations in the animals were, on average, 36% of the sediment concentration.

The previously cited studies and Neff (1982) lead to the general conclusion that sediment-adsorbed PAHs are not readily assimilated by benthic animals. Accumulation of PAHs from sediment, when it occurs at all, may be attributed in large part to uptake of PAHs desorbed from sediment particles into the interstitial water. This hypothesis is supported by Swartz and others (1989) who concluded that the concentration of chemicals in interstitial water is the primary determinant of sediment toxicity, not the bulk concentration in the sediment.

### **Depuration of PAH**

Southworth and others (1978) found a half-life of less than 1 h for all PAHs metabolized by *Daphnia pulex*. Jackim and Lake (1978) reported that the half-life of PAHs in most bivalves is about 2 to 16 days. These studies suggest that PAHs are either rapidly metabolized or excreted, at least by these species.

### Biomagnification of PAH in the Food Chain

Neff (1979) reported that the annelid *Neanthes arenaceodentata* had little, if any, ability to accumulate 2-methylnaphthalene from its food. However, the situation is quite different in marine crustaceans and fish, where uptake from food is much more efficient than uptake from water. Arthropods (for example, crabs, amphipods, shrimp) rapidly accumulate the lighter weight PAHs and very rapidly excrete or metabolize these compounds. The half-life of B(a)P in *Callinectes sapidus* is 6 days. Neff (1979) concluded that all results dramatically demonstrated the importance of metabolism in eliminating PAHs from contaminated crustaceans. Broman and others (1990) examined the trophic transfer of PAHs in a study involving seston, the blue mussel (*Mytilus edulis*), and the eider duck (*Somateria mollissima*). Contrary to biomagnification, they observed decreasing PAH concentration levels with increasing trophic levels.

### Bioaccumulation Summary

Aquatic organisms are able to efficiently bioconcentrate PAHs from the water column. It appears that direct transfer from sediments to organisms living within and on those sediments is minimal. Benthic organisms rarely contain higher concentration levels of PAH than are found in the sediments in which they live. PAHs are rapidly metabolized and excreted by vertebrates and arthropods. In bivalves, which do not efficiently metabolize PAHs, the half-life of most PAHs examined was 2 to 16 days. These data suggest that PAHs are not persistent in the tissues of aquatic species and that movement of PAHs through food chains to higher trophic levels is minimal, if it occurs at all.

Neff concluded that

From the limited data available, it would appear that there are large interspecific differences in ability to absorb and assimilate PAH from food. Polychaete worms have a very limited ability to absorb and assimilate PAH, whereas fish absorption of PAH from the gut is limited and variable depending on species of fish, the PAH, and possibly the food matrix in which PAH is administered. Crustaceans, on the other hand, apparently readily assimilate PAH from contaminated food. In all cases where assimilation of ingested PAH was demonstrated, metabolism and excretion of PAH were rapid. Thus, the potential for food chain biomagnification of PAH seems to be limited. For such biomagnification to occur, the material must be readily absorbed from food, and once assimilated, it must be relatively resistant to metabolism or excretion. (Neff 1979)

### Creosote and PAH Toxicity

Aquatic organisms have been exposed to background levels of PAH for eons. The diversity of life in aquatic environments attests to the ability of aquatic species to tolerate these

background levels (1 to 2 µg/L in the water column and 0.010 to 0.50 µg/g in sediments). At what level do PAHs cause pathological responses at the organismal and population levels? In answering that question, we need to consider two types of toxicity: acute and chronic.

Acute toxicity causes observable physiological lesions and is usually measured by mortality. PAH can interact with cells in several ways to cause toxic responses. For example, PAHs may bind reversibly to lipophilic sites in the cell, thereby interfering with cellular processes. Potentially impacted and important intracellular organelles include lysosomes, which contain strong enzymes important in intracellular digestion of complex organic molecules and in the immune response. Increased lysosomal membrane permeability can result in the unregulated flow of these enzymes into the cytoplasm or blood serum with pathological consequences including autophagy. Eisler (1987) noted that the lower molecular weight, unsubstituted PAH compounds, containing two or three rings, such as naphthalene, fluorene, phenanthrene, and anthracene, have significant acute toxicity to some organisms whereas the higher molecular weight, four- to seven-ring aromatics, do not. However, these heavier molecules contain numerous potentially carcinogenic and mutagenic intermediates.

A common measure of acute toxicity is the concentration of a toxicant that causes 50% mortality in a test population within some specified period (often 96 h). This parameter is referred to as the 96-h LC<sub>50</sub>. Borthwick and Patrick (1982) and Neff (1979) reported 96-h LC<sub>50</sub> values for several marine animals. These are summarized in Table 4.

Interestingly, in Neff's (1979) discussion of the effects of PAH on aquatic animals, he cites Caldwell and others (1977) finding that continuous exposure to dissolved naphthalene

**Table 4—Acute toxicity of various PAHs to marine organisms**

Species	96-h LC <sub>50</sub> (µg/L)
Mysids ( <i>Mysidopsis bahia</i> ) <sup>a</sup>	18 to 21
Oysters ( <i>Crassostrea virginica</i> ) <sup>a</sup>	700
Pink shrimp ( <i>Penaeus duorarum</i> ) <sup>a</sup>	240
Sheepshead minnows ( <i>Cyprinodon variegatus</i> ) <sup>a</sup>	3,500
Mosquito fish ( <i>Gambusia affinis</i> ) <sup>a</sup>	150,000 naphthalene
Mosquito fish ( <i>Gambusia affinis</i> ) <sup>b</sup>	1,180,000 toluene
Dungeness crab larvae ( <i>Cancer magister</i> ) <sup>b</sup>	8 naphthalene
Dungeness crab larvae ( <i>Cancer magister</i> ) <sup>b</sup>	170 naphthalene

<sup>a</sup>Borthwick and Patrick 1982.

<sup>b</sup>Neff 1979.

concentrations of 19 to 170 µg/L had no effect on the survival of Dungeness crab larvae. No explanation was given for the low (8 ppb) value reported in Neff's (1979) paper or the differences in the values reported. You might expect that exogenous factors contributed to the discrepancy. The LC<sub>50</sub> values reported in the literature for most organisms and PAH compounds are in the 500 to 5,000 ppb range. Neff (1979) found that in all but a few cases the concentration levels of aromatic hydrocarbons that are acutely toxic to aquatic animals are several orders of magnitude greater than concentration levels found even in the most heavily polluted marine and fresh waters. However, sediments from polluted regions may contain aromatic hydrocarbons at concentration levels similar to or greater than those that are acutely toxic. The limited bioavailability of sediment-adsorbed PAHs undoubtedly renders them substantially less acutely toxic than dissolved PAH. He also noted that PAH-induced stress is cumulative and exacerbated by exogenous stress factors such as abnormal thermal and osmotic conditions.

### **PAH Toxicity to Aquatic Plants**

The effects of various PAHs on aquatic plant growth are highly variable. At low concentrations (10 to 20 ppb), several PAHs act as a stimulant to plant growth. At 300 ppb, chrysene was observed by Boney (Neff 1979) to induce a 58% increase in the growth of the red alga *Antithamnion plumula*. Other PAHs (anthracene and 2-methylnaphthalene) caused declines of -20% and -12% in the same alga at 300 ppb. In general, PAH concentration levels greater than 1,000 ppb inhibit algal growth.

### **Chronic Toxicity**

Neff (1979) addressed chronic stress associated with PAH contamination. He noted that the copepod *Eurytemora affinis* suffered statistically significant reductions in length of life, total number of nauplii produced, and brood size when exposed to 10 ppb naphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, or 2,3,5-trimethylnaphthalene for the duration of their lives. The following discusses documented instances of chronic stress, by effect.

Nearly all PAHs are hydrophobic and lipophilic. Thus, there is a potential for these compounds to become associated with stable lipid pools in aquatic organisms. Energy is generally stored as glycogen in bivalves until gametogenesis, when the glycogen and lipid stores are converted into eggs and sperm. The eggs contain significant lipid reserves and could become a repository for lipophilic PAH. Moore and others (1989) cited Lowe and Pipe's (unpublished) observation that long-term exposure to diesel oil at 30 and 130 ppm caused a decrease in the mass of gametes produced by *Mytilus edulis* and *Macoma balthica*.

Mollusks exhibit reduced ventilation (feeding) rates at PAH levels as low as 30 to 40 ppb in seawater (Moore and others

1989). The feeding inhibition probably resulted from the narcotic effect of hydrocarbons, particularly aromatic hydrocarbons. These compounds have a direct effect on cilia, muscles, and/or the nervous system, which control the mollusk's activity. Reduced feeding rates result in a reduction in "scope for growth," a commonly measured parameter that quantitatively describes the energy available for tissue growth, reproduction, and activity. In bivalves, the major problem caused by reduced scope for growth is poor reproductive capacity. Although this does not have immediate consequences at the organismal level, the long-term consequences of reduced recruitment could be significant for the population.

Neff (1979) concluded his discussion of PAH-induced chronic toxicity by suggesting that although environmentally realistic PAH water column concentrations of 1 to 50 ppb can cause potentially detrimental, sublethal responses in aquatic organisms, in most cases, the PAH concentrations required to elicit significant sublethal responses are greater than those normally encountered in all but the most heavily polluted aquatic environments. This statement was strongly supported by the low levels of creosote-derived PAH (0.030 µg/L) observed by Goyette and Brooks (1999) in the immediate vicinity (15 cm) of a major creosote structure in Sooke Basin.

From the preceding discussion on the uptake of PAH from water, food, and sediments, it appears that PAH concentrations in the water column (including interstitial water in sediments) are the parameters of greatest significance in defining chronic stress. Furthermore, it appears that sustained water column concentrations of 30 to 50 ppb PAH can have subtle, but important, chronic impacts on populations of marine organisms.

### **Neoplasia Associated With PAHs**

Hyperplastic, preneoplastic, and neoplastic lesions have been reported in fish for a number of years. These same types of lesions are far less common in bivalves and other invertebrates.

In vertebrates, enzymes produced by the cytochromium P-450, mixed-function oxidase (MFO), and aryl hydrocarbon hydroxylase (AHH) systems are responsible for initiating catabolism of lipophilic compounds (including PAH). These systems render hydrophobic molecules more water soluble and therefore increase their potential for excretion and detoxification. In the case of certain high-molecular-weight PAHs, the intermediate metabolic products of these enzyme systems can be highly toxic, mutagenic, or carcinogenic. Oxidative metabolism of some PAHs (like B(a)P) results in the production of arene oxides, some of which bind covalently to DNA and RNA (particularly with guanine). The resulting chromosomal lesions can result in unregulated cell growth and division (cancer).



The ability to metabolize high-molecular-weight PAHs varies significantly between the phyla. Among invertebrates, the mollusks have low AHH activity and a limited ability to metabolize high-molecular-weight PAHs. Arthropods and annelids show increased activity and some marine crustaceans have demonstrated significant cytochromium P-450, MFO, and AHH activity.

Vertebrates, including fish, demonstrate high MFO, AHH, and cytochromium P-450 capabilities (Varanasi 1989). The liver is the primary site of MFO activity in fish, and the liver, gut, and gall bladder are primary sites of PAH concentration, metabolism, and excretion. Humans do not normally consume these organs. In Crustaceans, the hepato-pancreas, green gland (excretory organ), pyloric stomach, gills, testes, and eyestalks are major sites of PAH accumulation and AHH enzyme activity. Again, humans do not normally consume these tissues, although the hepato-pancreas is sometimes eaten as "crab butter."

Melanomacrophage centers are an integral part of the teleost immune system. Payne and Fancey (1989) observed that the numbers of melanomacrophage centers increased in the liver of fish exposed to total PAH concentrations in the range of 25,000 to 50,000 ppb. These concentrations are found only in heavily polluted harbors, industrially polluted sites, or oil spills. Payne and others (1988) observed changes in MFO enzyme levels and liver fat content in fish exposed to low dissolved hydrocarbon levels of 1,000 ppb (perhaps even as low as 200 to 300 ppb PAH).

The increased levels of P-450, MFO, and AHH enzymes in fish and crustaceans exposed to very high levels of PAH suggest active catabolism of these molecules. Enzyme induction is not necessarily a sign of stress. However, there is concern because some intermediate products of high-molecular-weight PAH catabolism are carcinogenic, mutagenic, and teratogenic.

### ***Bioindicator Studies***

There is growing interest in enzyme induction and genotoxicity tests as indicators of environmental risk. However, are genotoxicity and enzyme induction tests appropriate indicators of environmental risk? It is important to understand what these tests actually tell us. Effects at the organismal level, associated with external factors, are mediated by numerous levels of protection. Detrimental factors (for example, abnormal temperature, xenobiotics, desiccation, disease organisms, high levels of pollution, UV radiation) are often avoided by mobile animals. Sessile animals (including many bivalves) isolate themselves within tightly closed valves in an attempt to avoid harmful conditions.

At the next level of protection, an animal's integument isolates internal organs and structures from harmful conditions. The skin and gut epithelia are capable of selective absorption

of material. For instance, high-molecular-weight PAHs, adsorbed to sediments, apparently pass through the digestive tract of many annelids without being absorbed through the gut epithelia.

After foreign materials are absorbed into the blood serum through the skin, gills, or gut, organisms respond by sequestering them in vacuoles, metabolizing them in the liver or cleansing them from the serum as it passes through the kidney. Whether or not a molecule is metabolized or excreted depends largely on its ability to penetrate cell membranes. The plasma lemma is highly permeable to essential molecules such as glucose, amino acids, and lipids. These phospholipid bilayers are not very permeable to ions or large charged polar molecules. The four- to seven-ring high-molecular-weight PAHs are generally not charged and therefore do pass across the cell membrane and are actively metabolized by vertebrates.

It is well documented that some metabolic intermediates of high-molecular-weight PAHs, particularly arene oxides, can bind covalently to guanine, producing DNA lesions, which may result in unregulated cell growth (cancer). These metabolic intermediates are frequently found in the digestive gland (liver or hepatopancreas) where metabolism is most active. The literature contains many citations regarding hepatic lesions (including hepatic carcinomas) in demersal fish associated with PAH-contaminated sediments. However, the levels of contamination observed at Eagle Harbor, the Duwamish River, and Elizabeth River, at which significant increases in hepatic carcinomas were observed, are generally greater than 25 to 50 mg/kg. In some cases, Eagle Harbor sediments contained as much as 6,000 mg/kg PAH.

Mixed function oxidases (MFO), cytochromium P-450, ethoxy resofurin-o-deethylase (EROD), and aryl hydrocarbon hydroxylases (AHH) are important enzyme systems for the metabolism of high-molecular-weight PAHs. There are numerous reports in the literature suggesting that PAH-metabolizing enzyme systems are activated at sediment PAH levels as low as 1.0 ppm (Johnson and others 1994)

As previously stated, intermediate PAH metabolites, such as arene oxides, can covalently bind to DNA, resulting in lesions. However, DNA contains numerous mechanisms that repair miscoded or damaged sequences. This repair is achieved by a suite of enzymes capable of recognizing damaged or mismatched base pairs and excising them. Environmental or random damage to DNA is not unusual, and the presence of nicks or double-stranded breaks in nuclear (or ribosomal) DNA does not often lead to unregulated cell growth. Increased DNA damage obviously increases the risk for failure of these repair mechanisms, resulting in a number of diseases.

The point is that numerous levels of protection are involved in maintaining the biological integrity of an organism.

In evaluating environmental risks, we must recognize the importance of these cellular safeguards. The questions we ask must recognize that different levels of biological organization will respond differently to the same level of insult. Therefore, our questions must be posed carefully, and caution should be exercised when extrapolating biological responses at one level of organization to responses at another level. Consider a simple analogy: The sun feels good on our skin and it is necessary for the synthesis of vitamin D. Peel away the skin, expose the underlying tissue to the same beneficial sun, and the underlying cells die. Almost anyone would recognize that evaluating sunlight, based on the response of a naked cell, has nothing to do with an organism's response to the same level of light. This may seem simplistic, but these same principles must be applied to genotoxicity tests.

What question is asked by genotoxicity tests? Ernst (1994) reported the results of genotoxicity tests using subtidal sediments collected at various distances from a wharf constructed of creosote-treated wood. PAHs were extracted from the sediments, dried, and re-dissolved in dimethylsulfoxide (DMSO). Trout hepatocytes were exposed to various concentrations of the PAH preparation and genotoxicity was assayed using the nick translation assay (NTA) of Gagne and others (1995) and a modified version of the alkaline precipitation assay (APA) described by Olive (1988). The results were quantified by defining a toxicity threshold (TT) as the geometric mean of the lowest observed effect concentration (LOEC) and the no observed effects concentration (NOEC). This test measured the response of DNA in naked digestive gland cells to isolated PAHs suspended in a material, which is an exceptionally powerful solvent for both polar and non-polar compounds. DMSO is often used as a reaction medium for bimolecular nucleophilic reactions in which the attacking nucleophile (arene oxide) bears a negative charge. Its use in these genotoxicity studies greatly facilitates transfer of PAHs across the plasma lemma and arene oxides into the nucleus.

Based on this discussion, it appears that the question being asked is "How many DNA nicks and breaks occur when we eliminate, or impair, many of a cells nuclear defense mechanisms and expose DNA to PAH and their intermediate metabolic products?" This is an interesting question, and as expected, we find that the degree of DNA insult is proportional to the PAH exposure. In other words, this study revealed a quantifiable dose-response relationship. The dose is isolated PAHs and the response is from naked cells whose nuclear and cell membranes have been compromised in the presence of DMSO.

Does our current understanding of bioindicators allow their use in assessing environmental risks? There are numerous weaknesses in our current understanding. Consider the following:

<b>Genotoxicity test environment</b>	<b>Organismal environment in open aquatic systems</b>
PAHs are desorbed and extracted from sediments. They are made very available to the test cells.	PAHs are bound to sediments. They are not readily available in the water column.
No organismal epithelium is present.	After desorption from sediments, PAHs must cross an external epithelium (skin, gills, gut) before entering the blood stream for delivery to the digestive gland.
No kidney is present to clear PAHs.	Kidney functions to clear some xenobiotics. Fish rapidly excrete most PAHs.
Plasma lemma is compromised by DMSO.	Cell membrane selectively restricts movement of PAH into the cell. This increases the probability of excretion and decreases the probability of metabolism.
Lysosomal membranes are compromised by DMSO.	Lysosomal membranes help contain intermediate metabolites during metabolism.
Nuclear membrane is compromised by DMSO.	Nuclear membrane provides another level of protection for DNA.
DNA lesions are assumed to result in unregulated cell growth.	DNA repair mechanisms reduce the probability of unregulated cell growth.

Creosote and PAHs do result in disease in demersal fish at sufficiently high concentration levels. The review presented here is intended to provide insight to the mechanisms leading to the observed hepatic carcinomas. However, before these genotoxicity tests can be used to establish environmental criteria, we need to correlate the observed cellular responses with those at the population or organismal levels of organization. The response of a naked cell, with at least seven layers of protection stripped away, to isolated PAH, does not describe the response of whole organisms living in close association with sedimented PAH.

Other bioindicator tests (primarily enzyme induction tests) suffer from the same weakness. The response of an enzyme system to an appropriate substrate has little to do with the response of the organism to that substrate. Bioindicators certainly have a future in environmental studies. However, adequate correlations between cellular or genetic responses and organismal or population responses to pollutant levels have not been made.

Payne and others (1988) reported a study supporting the hypothesis that many point sources of hydrocarbon contamination could be harmful to fish health. They found that MFO enzyme levels were altered at hydrocarbon levels as low as 1.0 mg/kg. The authors noted that PAH levels in this range are encountered over a broad range of aquatic environments, many of which are not associated with pollution. They suggested that hydrocarbons often occur in sufficient concentrations to affect biological responses in fish. Consistent with the discussion presented here, Payne and others concluded that meaningful bioindicators must distinguish between effects per se and either chronic or acute effects.

The development of simple, timely, and effective tests to evaluate the risks posed by pollutants to aquatic organisms is important work. However, until a better understanding of the correlation between effects observed in bioindicator studies and the response of organisms and populations of organisms living in open environments is achieved, bioindicators have little value as either regulatory or environmental health assessment tools.

Vogelbein and others (1990) described hepatic neoplasms in the Mummichog (*Fundulus heteroclitus*) from a site with high levels (22 mg/kg) of PAHs in sediments. Of the Mummichogs collected at this site, 93% had gross hepatic lesions and 33% had hepatocellular carcinomas. Note that fish from a site of lower contamination (0.063 mg/kg) did not show signs of hepatic lesions or carcinomas. Similar cellular lesions have been described in fish from a highly urbanized area (Duwamish River estuary) in Puget Sound (Pierce and others 1977).

Colwell (1986) examined mussels and seawater associated with creosoted marine pilings at the Roosevelt Roads Naval Station Complex in Puerto Rico. She employed *Salmonella typhimurium* in the familiar Ames test (Ames and others 1975) for mutagenicity and found no detectable mutagenic activity in bacteria from either the water or mollusks associated with the creosote. She concluded that the creosote did not exhibit appreciable leaching into the surrounding water.

### **Effects of PAHs on Aquatic Organisms**

Mesocosm studies by Stekoll and others (1980) and Widows and others (1982,1985) reported similar community responses to petroleum and PAH contamination. Significant, long-term reductions in the abundance and diversity of invertebrate fauna were reported when ambient water levels contained as little as 130 µg/L dissolved diesel oil for prolonged periods. Less significant population effects were observed on a rocky shore community exposed to 30 ppb diesel oil for 2 months.

Tagatz and others (1983) examined the effect of creosote-contaminated sand on macrofaunal communities. They found that the lowest creosote (in sediment) concentration at either

of their sites that affected the number of individuals or species was 844 mg/kg for mollusks and 177 mg/kg for echinoderms, annelids, and arthropods.

The adaptation of microbial communities in the gut of *Limnoria tripunctata* and in sediment was well documented and discussed in Neff (1979). Similar adaptations were described by Wade and others (1989) in Gulf of Mexico hydrocarbon seep communities, including numerous species of annelids, crustaceans, bivalves, and fish. Tissue PAH concentrations indicate that these organisms were chronically exposed to high levels of PAH. The seep organisms were able to survive and thrive in an environment of high PAH exposure. The apparent ability to cope with these elevated PAH levels may involve specially adapted or evolved enzyme systems.

### **Aquatic Toxicity Summary**

The low-molecular-weight PAHs, such as naphthalene and acenaphthene, produce acute toxic effects in marine animals because they are more soluble than the higher molecular weight compounds. Acute intoxication in the sensitive larval stages of marine invertebrates may occur at water column concentrations as low as 8 to 10 µg/L. However, for most species, the literature suggests that water column concentrations of greater than 20 µg/L are required for significant responses. Low-molecular-weight PAHs are more soluble than the high-molecular-weight compounds, and bacteria and other aquatic organisms more rapidly metabolize them. The potential for their accumulation to toxic levels is small except when introduced in large quantities (such as petroleum spills). However, laboratory (including mesocosm) studies have demonstrated photo-enhanced toxicity associated with fluoranthene and anthracene at levels as low as 3 µg/L in the water column.

Because of their low biological availability, sedimented PAHs have a low potential to cause acute pathological responses at either the organismal or population levels in aquatic species. However, sediment levels of creosote exceeding 177 mg/kg have been shown to cause significant impacts on populations of the most sensitive taxa (Tagatz and others 1983). Furthermore, bacteria and eukaryotes have demonstrated a remarkable ability to adapt to relatively high levels of background PAHs.

Chronic toxicity is more difficult to measure than acute toxicity. This review suggests that chronic stress can occur in organisms, including bivalves, at concentrations as low as 30 to 40 µg PAH/L. Chronic stress causes reduced scope for growth and reduced reproductive capacity, which can have long-term consequences for populations of aquatic species.

In addition to direct physiological stress (Sunda 1987), there is a potential for the high-molecular-weight PAHs (particularly B(a)P) to form carcinogenic, mutagenic, and teratogenic compounds during metabolism by crustaceans and

vertebrates. Neff (1979) summarized his section on neoplasia by noting that although carcinogenic PAH can produce cancer-like growths and cause teratogenesis and mutagenesis in some aquatic invertebrates and vertebrates, there are no reports of the induction of cancer by exposure of aquatic animals to environmentally realistic levels of carcinogenic PAHs in water, food, or sediments. However, more recent work points out increases in the number of hepatic lesions and carcinomas with sediment PAH burdens as low as 10 mg/kg.

### Potential for Human Pathology Associated With PAHs

Neff (1979) and Stegeman (1981) stated that consumption of PAH-contaminated mollusks probably constitutes a minor source of human dietary PAH compared with PAHs in smoked foods, charcoal-broiled meats, and even many vegetables. Moore and others (1989) agreed, with the caveat that “except possibly where animals have been exposed to very high concentrations of PAHs such as those occurring following an oil spill.”

Average concentrations of 402 ppb B(a)P were reported by Neff (1979) in mussels from creosoted piling in harbors and marinas, where there are numerous sources of PAHs. Eisler (1987) listed human health criteria proposed by the EPA and others for various PAH compounds. The proposed maximum human consumption of benzo(a)pyrene is 1.6 µg/day. Goyette and Brooks (1999) compared tissue levels of PAH (TPAH) in mussels (*Mytilus edulis*) grown on and at various distances from new and used creosote-treated piling. Tissue levels of PAH peaked in samples collected 14 days following construction and then declined to background levels. Based on the highest PAH concentrations observed in these mussels, the authors determined that a person would have to eat 408 g of mussel tissue (about 1.35 kg (3 lb) of live mussels in the shell) every day to exceed the U.S. EPA dietary limit for carcinogenic PAHs.

Note that Mallet and others (1963) observed a B(a)P concentration of 55 µg/kg in *Mytilus edulis* collected from a pristine area on the coast of Greenland. The authors attributed this high level to biosynthesis of B(a)P by sediment-dwelling anaerobic bacteria. It was also possible that B(a)P could be leaching from peat deposits, which are extensive in that part of Greenland. Neff (1979) reported high levels of perylene (3.01 mg/kg) in Glastonbury peat from Avalon, England. This observation again points to the ubiquitous nature of PAHs and the multitude of natural and anthropogenic sources.

The American Wood-Preservers’ Association recognizes the potential toxicity of creosote (AWPA 1996). The association provides a list of precautions to be taken in the use and handling of creosote-treated wood. This list includes precautions against using creosote in human or animal habitations where

direct contact occurs. AWPA recommends that creosote-treated wood used above grade in homes be well sealed and that it not be used in close association with either human or animal feedstuffs. However, the EPA label for creosote does allow its incidental use, such as for piers and floats, in association with human drinking water supplies.

Table 5 lists benchmarks for PAHs in drinking water established by the Council of European Communities (CEC), the Canadian Council of Ministers of the Environment (CCME), the British Columbia Ministry of the Environment, and the U.S. EPA. Of these, benzo(a)pyrene is the compound of most concern.

The CEC directive relating to the quality of water intended for human consumption sets a maximum total PAH level of 200 ng/L based on the amount of fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene. Eisler (1987) suggested that the concentration of these carcinogenic PAHs should be maintained at less than 0.028 µg/L to reduce the increased cancer risk to less than 10<sup>-5</sup>. The concentration of the previously listed carcinogenic PAHs observed by Goyette and Brooks (1999) at a distance of 15 cm from a large creosote-treated wood structure was 0.000051 µg/L—less than Eisler’s suggested benchmark by a factor of 550. The sum of all PAHs observed by Goyette and Brooks (1999) was 0.031 µg/L—less than the CEC benchmark by a factor of 6.5. This suggests that drinking water intakes could safely be placed within 15 cm of creosote-treated piling in drinking water supplies. However, that placement would not be recommended because it would represent unnecessary risk.

### Sediment Quality Benchmarks For PAHs

Goyette and Brooks (1999) examined the effects of creosote-treated piling in Sooke Basin, British Columbia. The extensive physicochemical and biological database included infaunal community analysis and seven *in situ* and laboratory

**Table 5—Canadian and U.S. regulatory limits for drinking and ground water**

Compound	Authority	Remediation level
Total carcinogenic PAH	Council of European Communities	0.200 µg/L
Benzo(a)pyrene	British Columbia	0.100 µg/L
Benzo(a)pyrene	Canadian Council of Ministers of the Environment	0.010 µg/L
Benzo(a)pyrene	U.S. EPA	1.6 µg benzo(a)pyrene/day

bioassays. Physicochemical analyses included a detailed description of sediment and water column concentrations of alkylated and parental PAHs. This database allowed for an examination of the efficacy of existing and proposed sediment quality benchmarks in predicting adverse biological response. The U.S. EPA draft sediment quality criteria for acenaphthene (130 µg/g organic carbon), phenanthrene (180 µg/g organic carbon), and fluoranthene (620 µg/g organic carbon) were found to be underprotective in that they failed to predict observed adverse biological effects in three database samples. False negative responses (adverse effects observed but not predicted by the benchmark) were not observed for any of the other benchmarks. Goyette and Brooks (1999) found that 60 individual PAH compounds exceeded the threshold effects level (TEL) (Jones and others 1997) in seven samples where no toxicity was observed. These false positive indications associated with the TEL were observed for every PAH compound except naphthalene. The Washington State Sediment Quality Criteria (WAC 173–204) were most efficient in predicting adverse effects (12 false positive responses), and the probable effects level (PEL) resulted in 21 false positive responses. The mean of the TEL and PEL ((TEL + PEL)/2) resulted in 30 false predictions of adverse effects where none was observed.

Swartz (1999) examined existing and proposed sediment quality guidelines and proposed consensus guidelines that appear to resolve some of the current inconsistency. He describes a ΣPAH toxicity threshold that is consistent with the effects range low (ER-L) of Long and others (1995) and a ΣPAH mixture LC<sub>50</sub> that is similar to the effects range median (ER-M) described by the same authors. In the report herein, sediment PAH concentrations are compared with the ΣPAH toxicity threshold, the ΣPAH mixture LC<sub>50</sub>, and the mean of these two values in predicting biological risk at creosote-treated bridges. Table 6 summarizes these benchmarks.

### Site Selection

Nine creosote-treated bridges were evaluated on the West Coast of the United States during the site selection process. Not one of these bridges was considered appropriate, primarily because the structures involved the use of both creosote- and penta-treated wood. Two bridges crossing Pipe Creek in Cass County, Indiana, were selected because they best met the site selection criteria (Fig. 7). These bridges carry cars, commercial vehicles, and farm equipment across a slow-moving, but substantial, stream. Vehicle use statistics were not available; however, the rural nature of the environment suggested relatively light traffic compared with urban roadways or interstate highways. Both bridges sit on 20 Class A piling treated to a nominal retention of 272 kg/m<sup>3</sup> (17 lb/ft<sup>3</sup>) in the treated zone. Support beams, crossbeams, decking, and guardrails were all similarly treated with creosote oil to a retention of 128 or 160 kg/m<sup>3</sup> (8 or 10 lb/ft<sup>3</sup>) in the treated zone.

**Table 6—ΣPAH toxicity threshold, ΣPAH mixture LC<sub>50</sub>, and mean of these two values for 17 parental PAHs<sup>a</sup>**

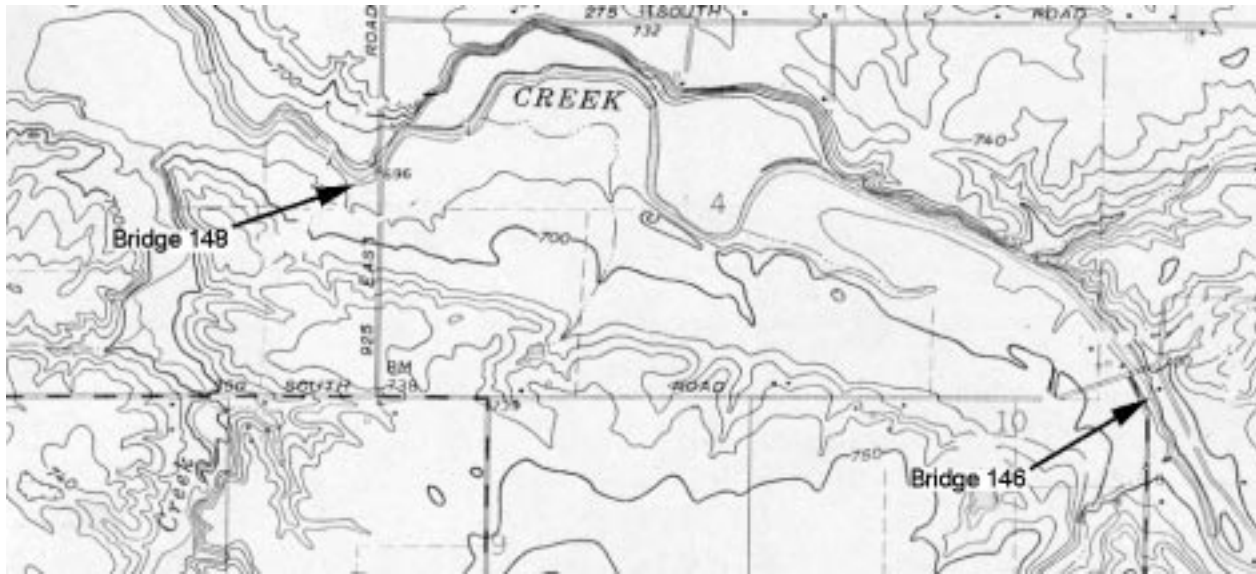
PAH compound	ΣPAH toxicity threshold (µg/g organic carbon)	ΣPAH mixture LC <sub>50</sub> (µg/g organic carbon)	Mean (µg/g organic carbon)
Naphthalene	13	71	42.0
Acenaphthylene	3	15	9.0
Acenaphthene	4	23	13.5
Fluorene	17	90	48.5
Phenanthrene	29	155	92.0
Anthracene	21	114	67.5
Fluoranthene	69	371	220.0
Pyrene	90	481	285.5
Benz(a)anthracene	21	111	66.0
Chrysene	31	169	100.0
Benzo(b)fluoranthene	33	180	106.5
Benzo(k)fluoranthene	29	155	92.0
Benzo(a)pyrene	33	179	106.0
Low-molecular-weight PAH	87	468	277.5
High-molecular-weight PAH	306	1,646	976.0
Total PAH	393	2,114	1,253.5

<sup>a</sup>All values, except mean, are from Swartz (1999).

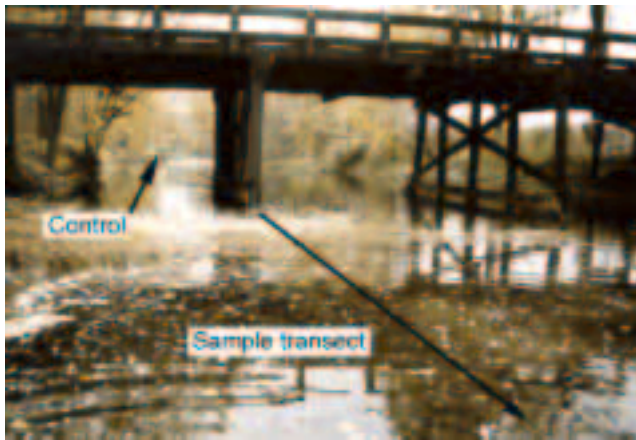
The area is intensively used for agriculture, and Pipe Creek appeared to carry a significant load of topsoil (sand and silt). In addition, agricultural pesticides may have an effect on aquatic invertebrates. However, because agricultural activity (corn production) covers much of the landscape both upstream and downstream, it seems reasonable to suggest that those effects would equally influence all areas of the aquatic environment, including control and treatment stations. Industrial sources of PAHs were not apparent within 5 miles of either bridge. This qualitative assessment suggested that any observed differences in the invertebrate community between upstream controls and downstream treatment stations would be associated primarily with the physical presence of the structure or PAHs released from the creosote-treated wood, or both.

### Bridge 146

Figure 8 is a photograph taken from a downstream location, looking upstream along the sampling transect, on November 3, 1997, during the collection of physicochemical and biological samples at Bridge 146. The weather was overcast, with light wind from the north, and it was snowing. The water temperature was 5.5°C, and the air temperature was 4.5°C at 0900.



**Figure 7—Location of creosote-treated bridges on Pipe Creek in Cass County, Indiana, that were subjected to an environmental risk assessment.**



**Figure 8—Bridge 146 over Pipe Creek viewed from downstream looking upstream along the sample transect. Water depths varied between 10 and 31 cm. Water flow along the transect was <1.0 cm/s.**

Conventional physicochemical characteristics of the water and sediments are described in Table 7. The Indiana State Division of Water provided flow data for Pipe Creek. Records collected since 1960 indicated that the mean annual flow in Pipe Creek was 1.9 m<sup>3</sup>/s (67.6 ft<sup>3</sup>/s). The lowest daily mean was 0.09 m<sup>3</sup>/s (3.3 ft<sup>3</sup>/s) recorded February 1, 1977. Current speeds along the chosen transects were <1.0 cm/s, and water depths along the sampling transect varied between 10 and 31 cm on November 3, 1997.

The sampled transect was characterized by slow currents (<1.0 cm/s) and fine sediments dominated by sand with less than 10% silt and clay. Brooks (unpublished data) found that total organic carbon averaged 60% of the total volatile solids (TVS) measured in marine sediments. Extrapolating to this freshwater environment suggests that Pipe Creek sediments contained between 0.43% and 0.52% organic carbon. The corresponding sediment quality benchmarks for PAHs at this level of organic carbon are listed in Table 8. These values were corrected to dry sediment weight, based on the observed mean organic carbon content of 0.48%, and represent site-specific sediment benchmarks for this evaluation.

Bridge 146 was constructed in 1995 and was more than 2 years old at the time of this evaluation. The creosote model of Brooks (1997b) predicted continued PAH accumulation in sediments for about 1,000 days after construction. At the time of this evaluation, Bridge 146 was approximately 900 days old, and absent any bedload movement and dispersal of PAH, the sediment concentrations should have been nearing their maximum concentration. However, water flow in Pipe Creek varies significantly with the season and it is likely that sediments and sediment contaminants were dispersed and carried downstream during periods of high flow. During this examination, local scouring was evident within 30 cm of individual piling, and these areas were avoided in sampling.

#### ***Sedimented Concentrations of PAHs***

Figure 9 compares the observed sediment concentrations of total PAHs with the benchmarks defined in Table 8.

**Table 7—Physicochemical characteristics observed at Bridge 146 on Pipe Creek, Cass County, Indiana, November 3, 1997<sup>a</sup>**

Station	Depth (cm)	RPD (cm) <sup>b</sup>	Total PAH (µg/g)	TVS <sup>c</sup> (%)	Sand (%)	Silt and clay (%)
-22.8 m (-76 ft) upstream control	10.0	>3.0	0.2288 ND <sup>d</sup>	1.98 ± 1.22	81.03 ± 10.22	14.37 ± 3.21
0.0 under bridge	24.0	>3.0	1.2801 ± 0.8612	0.86 ± 0.22	83.08 ± 26.19	5.84 ± 0.63
0.45 m (1.5 ft) downstream	21.0	>3.0	2.1396	0.77	72.91	6.31
0.9 m (3.0 ft) downstream	15.0	>3.0	2.8426	0.72	90.88	7.67
1.8 m (6.0 ft) downstream	17.0	>3.0	5.5376	1.01	91.39	4.59
3 m (10.0 ft) downstream	16.0	>3.0	1.7476	0.80	89.59	7.15
6 m (20.0 ft) downstream	10.5	>3.0	0.5402	0.83	48.03	7.00
22.8 m (33.0 ft) downstream	20.0	>3.0	0.2288 ND	0.72	60.70	3.36

<sup>a</sup>All values are the mean of two replicates except the -22.8 m (-76 ft) upstream control and samples taken under the bridge, which are the mean of three replicates. Where appropriate, values include 95% confidence limits on the mean.

<sup>b</sup>RPD is the reduction-oxidation potential discontinuity. It is an indication of the depth in sediments at which sufficient oxygen is present to support aerobic metabolism.

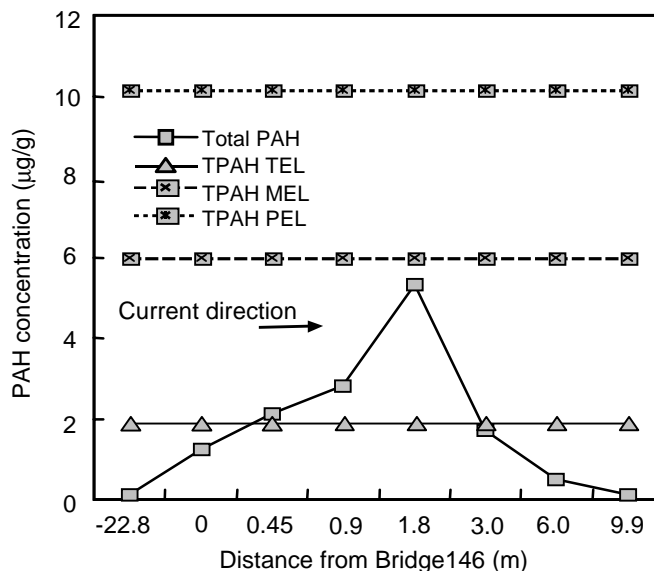
<sup>c</sup>TVS is total volatile solids measured as a percentage of sediment dry weight.

<sup>d</sup>PAHs were not detected. The value is the sum of the detection limits.

**Table 8—Consensus sediment quality benchmarks specific to total organic carbon observed at Bridge 146 over Pipe Creek in Cass County, Indiana<sup>a</sup>**

PAH compound	ΣPAH toxicity threshold	ΣPAH Mixture LC <sub>50</sub>	Mean
Naphthalene	0.06	0.34	0.20
Acenaphthylene	0.01	0.07	0.04
Acenaphthene	0.02	0.11	0.06
Fluorene	0.08	0.43	0.23
Phenanthrene	0.14	0.74	0.44
Anthracene	0.10	0.55	0.32
Fluoranthene	0.33	1.78	1.06
Pyrene	0.43	2.31	1.37
Benz(a)anthracene	0.10	0.53	0.32
Chrysene	0.15	0.81	0.48
Benzo(b)fluoranthene	0.16	0.86	0.51
Benzo(k)fluoranthene	0.14	0.74	0.44
Benzo(a)pyrene	0.16	0.86	0.51
Low molecular weight PAH	0.42	2.25	1.33
High molecular weight PAH	1.47	7.90	4.68
Total PAH	1.89	10.15	6.02

<sup>a</sup>Swartz (1999). All values are µg PAH/g dry sediment weight.



**Figure 9—Sediment concentrations of total PAH measured upstream (controls) and downstream from Bridge 146 crossing Pipe Creek in Cass County, Indiana. Sediment PAH concentrations are in micrograms per gram (dry sediment weight). The threshold effects level (TEL), mean effects level ((TEL + PEL)/2), and probable effects level (PEL) are computed at the observed level of total organic carbon (0.48%).**



Goyette and Brooks (1999) provided a detailed history of individual PAH compounds present in new creosote oil, treated piling, the water column, and sediments at Sooke Basin as a function of time following installation. They found that much of the low-molecular-weight compounds were lost during the treating process or were dissolved in the water column and diluted. The intermediate weight compounds, phenanthrene through chrysene, presented the greatest risk to aquatic organisms, and the high-molecular-weight compounds were found only in very low concentrations. Table 8 summarizes the  $\Sigma$ PAH toxicity threshold (TEL),  $\Sigma$ PAH mixture LC<sub>50</sub> (PEL), and the mean of these two values ((TEL + PEL)/2) for 17 parental PAHs adjusted to the mean sediment organic carbon (0.48%) determined November 3, 1997. These were site-specific benchmarks for predicting biological response at Bridge 146.

Observed PAH concentrations in surficial sediment samples (upper 2.0 cm of the sediment column) are described in Table 9 for Bridge 146. Concentrations exceeding the TEL ( $\Sigma$ PAH toxicity threshold) were observed at distances between 0.45 and 1.8 m (1.5 and 6.0 ft) from the downstream perimeter of the bridge structure. Nearly all the low and

intermediate molecular weight compounds exceeded their individual TELs at these same distances. Not one of the concentrations of high-molecular-weight compounds was appreciably elevated, and those for which Swartz published data were not elevated above their TEL. All three replicate sediment samples collected under the bridge (0.0 m) contained significantly elevated ( $\alpha = 0.05$ ) levels of naphthalene and acenaphthene compared with the upstream control. These levels exceeded both the (TEL + PEL)/2 level and the PEL. Acenaphthene and phenanthrene concentrations exceeded the PEL at the station located 1.8 m (6.0 ft) downstream from the bridge. If the PAHs were bioavailable, these levels could result in significant acute biological effects in sensitive species. The low-molecular-weight PAHs (naphthalene and acenaphthene) have higher water solubilities than the higher molecular weight compounds, are therefore more biologically available, and are generally not associated with chronic effects. Their catabolism does not result in the production of arene oxides and other cancer-promoting intermediate metabolites. These results suggest that acute effects may be present in aquatic organisms under the bridge and to distances of 1.8 m (6.0 ft) downstream.

**Table 9—Concentrations of sedimented PAHs observed in the vicinity of Bridge 146, Cass County, Indiana<sup>a</sup>**

Compound	Reporting limit (µg/g)	Concentrations (µg/g dry sediment weight) for various sampling stations							
		-22.8 m (-76 ft)	0-0.3 m (0.0-1)	+0.45 m (+1.5)	+0.9 m (+3.0)	+1.8 m (+6.0)	+3 m (+10)	+6 m (+20)	+9.9 m (+33 ft)
Naphthalene	0.020	0.010	<b>XX 0.617</b>	<b>0.042</b>	0.061	0.180	0.066	0.010	0.010
Acenaphthylene	0.020	0.010	0.014	0.010	0.010	0.010	0.010	0.010	0.010
Acenaphthene	0.020	0.010	<b>XX 0.163</b>	0.051	<b>0.065</b>	<b>XX 0.310</b>	<b>0.096</b>	0.026	0.010
Fluorene	0.010	0.010	0.060	0.066	0.083	0.360	0.099	0.022	0.010
Phenanthrene	0.020	0.010	0.120	0.330	0.330	<b>XX 1.600</b>	0.460	0.092	0.010
Anthracene	0.020	0.010	0.023	0.079	0.067	0.010	0.059	0.024	0.010
Fluoranthene	0.020	0.010	0.095	0.560	0.900	<b>1.200</b>	0.390	0.110	0.010
Pyrene	0.020	0.010	0.077	0.500	0.770	0.950	0.340	0.097	0.010
Benzo(a)anthracene	0.003	0.001	0.032	0.170	0.170	0.270	0.078	0.063	0.001
Chrysene	0.020	0.010	0.010	0.120	0.170	0.190	0.010	0.010	0.010
Benzo(b)fluoranthene	0.004	0.002	0.002	0.067	0.077	0.083	0.040	0.002	0.002
Benzo(k)fluoranthene	0.003	0.002	0.002	0.033	0.043	0.054	0.015	0.002	0.002
Benzo(a)pyrene	0.005	0.002	0.002	0.063	0.052	0.086	0.030	0.002	0.002
Benzo(ghi)perylene	0.010	0.010	0.010	0.024	0.010	0.010	0.010	0.010	0.010
Ideno(1,2,3-cd)pyrene	0.009	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Dibenzo(a,h)anthracene	0.006	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Total PAH	0.209	0.114	1.236	2.122	2.815	5.320	1.710	0.487	0.114

<sup>a</sup>Reported concentrations are from the top 2 cm of the sediment column. Sediment PAH concentrations exceeding the threshold effects level, defined by Swartz (1999) as the  $\Sigma$ PAH toxicity threshold, are in bold type. Sediment PAH concentrations exceeded the probable effects level, defined by Swartz (1999) as the  $\Sigma$ PAH mixture LC<sub>50</sub>, are preceded by XX. Cells containing sediment PAH concentrations exceeding the (TEL + PEL)/2 benchmark used in this assessment have a gray background.

However, Long and others (1998) examined the efficiency of TEL and PEL in predicting toxicity. They found that values between TEL and PEL did not predict increased toxicity (over controls) until the number of levels exceeding TEL was more than 20. A similar analysis using PEL suggested that 53.3% of the samples were judged not toxic when one to five PEL were exceeded. The nontoxic samples decreased to 37% when 6 to 10 PELs were exceeded, and the benchmark's efficiency further increased to 10% nontoxic responses when 11 to 20 PELs were exceeded in a mixture. In this case, two PELs were exceeded under the bridge and at +1.8 m (+6.0 ft) downstream. For this number of exceedances, Long and others (1998) found that 52% of the samples were nontoxic, 12% were marginally toxic, and 36% were highly toxic.

To put these values in better perspective, Long and others (1998) reported that 65% of the sediment samples in which TELs were not exceeded tested nontoxic. However, 26% of these relatively clean sediments were found to be marginally toxic and 9% were highly toxic. Goyette and Brooks (1999) found that the Sooke Basin Reference Station was consistently toxic in a suite of bioassay tests compared with reference sediments from Esquimalt Lagoon. Similarly, Deniseger and Erickson (1998) found that 8 of 12 reference sites evaluated using *Eohaustorius washingtonianus* in the Broughton Archipelago were toxic when the results were compared with sediments from which the test animals were collected. There are numerous site-specific physicochemical and biological factors that determine the ultimate toxicity of contaminants. The benchmarks (TEL, PEL, (TEL + PEL)/2) against which Pipe Creek sediment concentrations of PAHs were compared are only as good as the screening tools. Based on the results of this analysis, there is a reasonable likelihood that sedimented PAHs under Bridge 146 and at a distance of 1.8 m (6.0 ft) downstream could be associated with moderate adverse biological effects. The information displayed in Table 9 is provided in graphical format in Figure 9. It is apparent that PAHs were either being transported downstream prior to being deposited in sediments or that the PAHs were deposited under the bridge, then moved downstream with the sediment bedload. In the latter case, the PAH profile presented in Figure 9 represents a time history of PAH lost (after degradation) from the bridge. This study was not designed to assess PAH transport from creosote-treated structures, and this observation is simply a hypothesis worthy of additional testing. The observed spatial profile is more likely a result of both factors.

### Biological Response—Infauna

A total of 8,187 invertebrates were identified in 18 sediment samples collected in the vicinity of Bridge 146. Each sample represented an area of 0.0309 m<sup>2</sup>. These samples contained 51 taxa. Taxonomic codes are defined in Appendixes B and C. Dominant taxa were defined as those representing 1% of the total infaunal abundance (count = 82). The invertebrate community was dominated by annelids (4,579 total) and

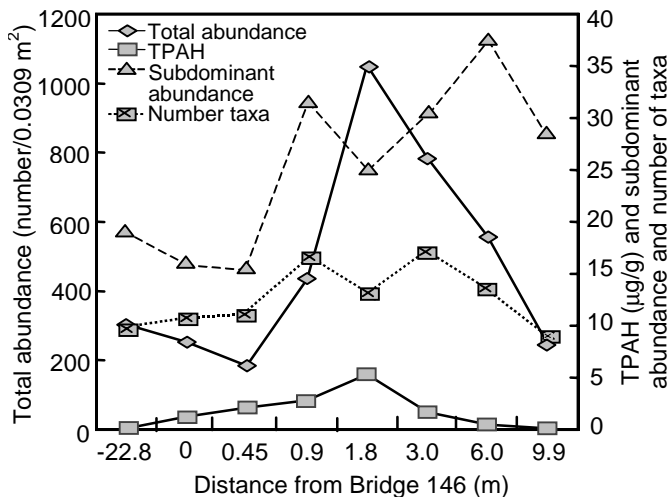
chironomids in the genus *Chironomus* (3,107). Subdominant taxa were defined as those representing 1% of the abundance of all taxa except the two dominants (count = 5.0). In addition, to be included in the subdominant database, a taxon must have been found in at least 10% of the samples (2.0 samples). Eighteen taxa met these criteria. Dominant and subdominant taxa represented 99.2% of the total taxa and are identified in Table 10.

This was a robust community dominated by annelids and chironomids. Few mayflies (Order Ephemeroptera) or caddisflies (Order Trichoptera) and no stoneflies (Order

**Table 10—Dominant and subdominant invertebrate taxa observed in sediment samples collected in the vicinity of Bridge 146 crossing Pipe Creek in Cass County, Indiana<sup>a</sup>**

Taxa	Code	Found in number of samples	Total abundance
<b>Dominant Taxa</b>			
Annelids	ANNE	18	4,579
<i>Chironomus</i> sp.	AICHSS	18	3,107
<b>Sub-dominant Taxa</b>			
<b>Gastropods</b>			
<i>Pleurocera</i> sp.	MGPLS	10	36
<i>Ferrisia</i> sp.	MGFRS	7	9
<i>Physella</i> sp.	MGPHLS	9	54
Ostracods	ACOST	2	11
<b>Order Ephemeroptera</b>			
<i>Caenis</i> sp.	AICAE	14	46
<i>Ephemera</i> sp.	AIEPHA	8	24
<b>Order Hemiptera</b>			
Family Corixidae	AICOR	6	12
<b>Order Coleoptera</b>			
<i>Berosus</i> sp.	AIBERS	5	7
<i>Dubiraphia</i> sp.	AIDUBS	13	49
<b>Order Trichoptera</b>			
<i>Polycentropus</i> sp.	AIPOSS	4	5
<b>Order Diptera</b>			
<i>cf. Probezzia</i> sp.	AIPBEZ	7	30
Family Chironomidae	AICHR	9	40
<i>Procladius</i> sp.	AIPRS	5	7
<i>Dicrotendipes</i> sp.	AIDTS	16	79
<i>Eukiefferiella</i> sp.	AEUKS	7	13
<i>cf. Chrysops</i> sp.	ACHS	6	14
Total abundance of dominants and subdominant taxa			8,122

<sup>a</sup>Eighteen samples, each covering 0.0309 m<sup>2</sup>, were collected.

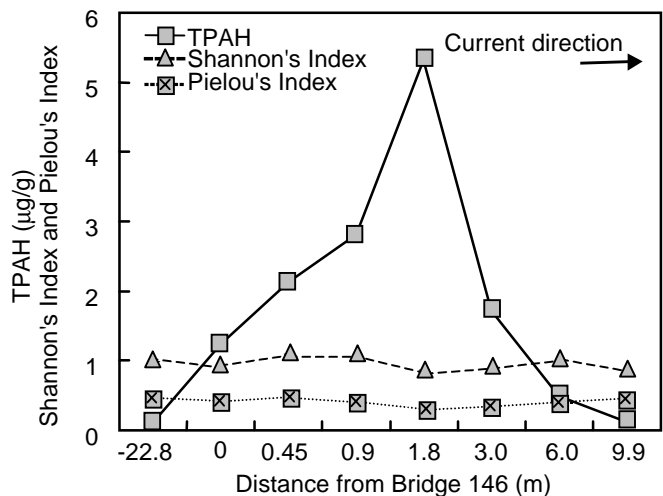


**Figure 10—Abundance of all taxa, subdominant taxa, and sediment levels of total PAH ( $\mu\text{g/g}$  dry sediment weight) observed in the vicinity of Bridge 146 crossing Pipe Creek in Cass County, Indiana, November 3, 1997.**

Plecoptera) were observed in the fine-grained sediments characteristic of this part of Pipe Creek. The composition of the infaunal community is undoubtedly influenced by soil loss from adjacent agricultural areas and subsequent movement of this bedload of sand, silt, and clay down the watershed. In Figure 10, total abundance, subdominant abundance, and the number of taxa observed in 0.0309- $\text{m}^2$  samples are compared with sediment PAH concentrations. More taxa were observed under and downstream from the bridge than at either the upstream control or the downstream-most station, where PAHs were not detected in sediments above the analytical detection limit. The abundance of all taxa was slightly reduced under the bridge and a distance of 0.45 m (1.5 ft) downstream. Total abundance was highest at the 1.8-m (6.0-ft) station where TPAH concentrations peaked at 5.3  $\mu\text{g/g}$ . Subdominant taxon abundance decreased at stations located under the bridge and 1.8 m downstream. However, subdominant taxa were more abundant at all downstream stations than at the upstream control.

Figure 11 does not suggest significant differences in either Shannon's Index or Pielou's Index as a function of either sediment PAH concentration or downstream distance from the bridge. The simplest interpretation of Pielou's Index is that it represents the proportion of the maximum value of Shannon's Index observed in a sample. All calculated values of Shannon's Index were low (about 1.0), indicating the stressful nature of the environment. Similarly, Pielou's Index was less than 0.5 in all samples.

PAH data (Table 9) suggest two areas where adverse biological effects might be observed. The area under the bridge contained high levels of the low-molecular-weight compounds naphthalene and acenaphthylene. In addition, the

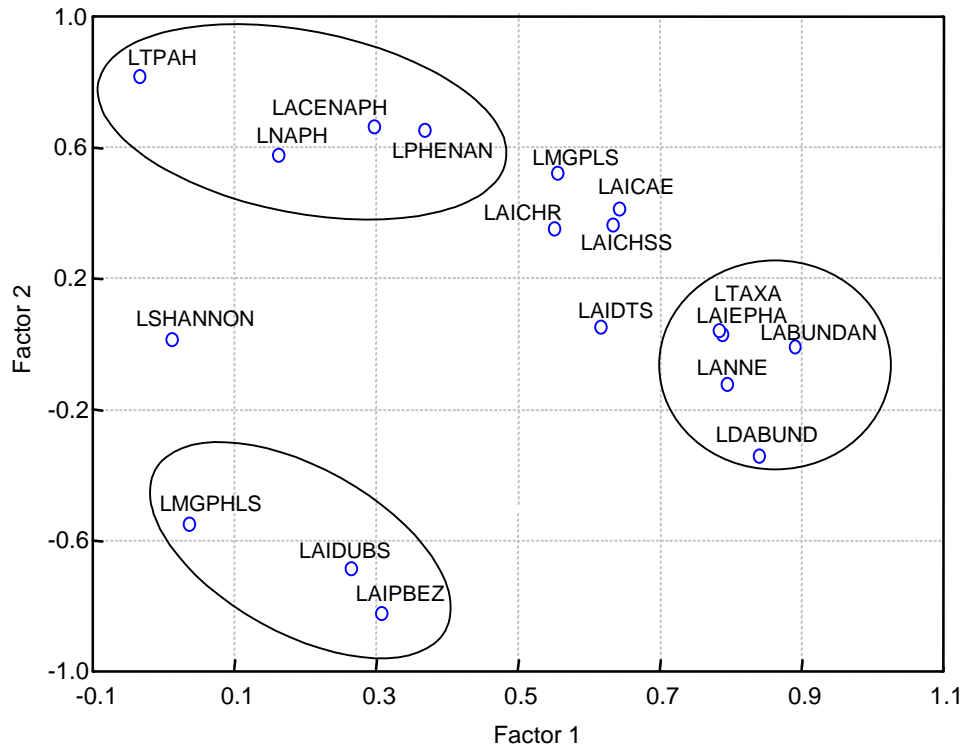


**Figure 11 —Comparison of Shannon's and Pielou's Indexes with total PAH (TPAH) observed in sediments at Bridge 146 crossing Pipe Creek in Cass County, Indiana, November 3, 1997.**

station located 1.8 m downstream from the downstream perimeter of the bridge contained levels of acenaphthene and phenanthrene exceeding the PEL.

The significance of biological effects was explored using  $t$ -tests on  $\log(n + 1)$  transformed count data and log transformed variables with continuous distributions. These transformations were needed to meet the underlying assumptions for parametric testing. Significant ( $\alpha = 0.05$ ) differences in biological response variables (number of taxa, abundance, subdominant abundance, and Shannon's Index) were not observed between the upstream control and either the 0.0- or 1.8-m downstream stations where adverse effects might be associated with elevated concentrations of creosote-derived PAH. The null hypothesis that response variables at the upstream control compared with samples from under the bridge or at a distance of 1.8-m downstream were significantly different was not rejected with the probability of being equal, ranging from  $p = 0.58$  to 0.76.

The biological response to sedimented PAHs was further explored using principal components analysis (PCA). The factors were rotated using varimax normalization, and the results are presented in Figure 12. Factor loadings on factor 1 were all positive except total PAH, which was close to zero but negative ( $-0.033886$ ). Annelids (LANNE), the mayfly, *Ephemera* sp. (AIEPHA), and the biological metrics, taxa (LTAXA), abundance (LABUNDAN), and subdominant abundance (LDABUND) were significantly loaded on factor 1. Total PAH (LTPAH) was significantly loaded on the positive end of factor 2; many individual taxa were negatively loaded on this axis, but only the dipteran, *Probezzia* sp., was a significant factor. Subtle ecological



**Figure 12—Principal components analysis of  $\log(n + 1)$  transformed biological response variables and  $\log$  transformed PAH concentrations in samples collected from Pipe Creek in the vicinity of Bridge 146 in Cass County, Indiana, November 3, 1997.**

relationships and possible effects associated with sedimented PAHs are shown in Figure 12. These include the following:

- The number of taxa and both total abundance (LABUNDAN) and subdominant species abundance (LDABUND) group together with annelids (LANNE) and mayflies in the genus *Ephemera* (LAIEPHA). This suggests that more abundant and diverse communities were found in association with annelids and mayflies. Shannon's Index describes the evenness of the distribution of animals within species in a community. Note that Shannon's Index (LSHANNON) is located well to the left of the other biological endpoints, suggesting that a few species tend to dominate Pipe Creek infauna and that increases in the more abundant taxa resulted in decreases in Shannon's Index.
- All the PAH group in the upper left corner of the chart (Total PAH = LTPAH, naphthalene = LNAPH, acenaphthene = LACENAPH, and phenanthrene = LPHENAN). The concentration of total PAH and/or naphthalene may have been more stressful than either acenaphthene or phenanthrene.
- The presence of significant quantities of naphthalene is unexpected with creosote contamination. Goyette and Brooks (1999) found that most of the naphthalene was lost during the treating process from piling produced using *Best Management Practices* (WWPI 1996). This process requires the use of "clean creosote" containing less than 0.5% xylene-insoluble material (<1.5% when treating ponderosa or southern yellow pine). In addition, these procedures require heating of the treated wood for 1 h in an expansion bath under 74 kPa (22 inHg) of vacuum for 2 h following impregnation. This is followed by a final steaming at <116°C for 2 h (timber) or 3 h (piling). Following these steps, the treated wood is placed under a minimum 74-kPa (22-inHg) vacuum for 4 h. The *Best Management Practices* process releases excess preservative from the treated wood and results in a product with a minimum amount of surface residue. Naphthalene has a relatively low vapor pressure and is more soluble in water than the other PAHs. It is likely that the *Best Management Practices* process caused the significant loss of naphthalene observed by Goyette and Brooks (1999). Because of its age, it is unlikely that the wood used to construct these bridges was treated using *Best Management Practices* procedures.

- The taxa present in Pipe Creek were not particularly sensitive to PAHs in the vicinity of Bridge 146. Correlation analysis on the log-transformed data was used to investigate the relationship between total PAHs, naphthalene, acenaphthene, phenanthrene, and biological endpoints. All significant correlations with individual PAHs were positive, indicating increased abundance in the presence of higher sediment concentrations of naphthalene, acenaphthene, and phenanthrene. Significant negative correlations were found between the gastropod *Physella* sp. (MGPHLS,  $r = -0.59$ ) and the dipteran *Bezzia* sp. (LAIPBEZ,  $r = -0.71$ ). A significant positive correlation was found between LTPAH and the mayfly *Caenis* sp. (LAICAE,  $r = 0.68$ ). Chironomids in the Family Chironomidae (AICHR,  $r = 0.27$ ) and the dominant genus *Chironomus* sp. (AICHSS,  $r = 0.17$ ) were also tolerant of elevated PAHs, but the correlations were not significant. The correlation analysis is summarized in Table 11.

### Sediment Bioassay Response

Ten-day sediment toxicity tests were completed using the amphipod *Hyaella azteca* in accordance with ASTM E1706 (ASTM 1997) as modified by MEC Analytical Systems Protocol P034.0. Five tests were rejected during the initial testing series as a result of low control survival. MEC attributed this to stressed amphipods used for that series.

**Table 11—Summary of Pearson correlation coefficients between individual taxa (Column 1) and total PAH (LTPAH), naphthalene (LNAPH), phenanthrene (LPHENAN), and acenaphthene (LACENAPH)<sup>a</sup>**

	LTPAH	LNAPH	LPHENAN	LACENAPH
LANNE	-0.26	0.27	0.46	0.50
LMGPLS	0.40	0.21	0.73	0.52
LMGPHLS	-0.59	-0.14	-0.19	-0.11
LAICAE	0.68	0.52	0.61	0.49
LAIEPHA	0.10	0.29	0.52	0.40
LAICOR	0.41	0.39	0.24	0.17
LAIIDUBS	-0.56	-0.12	-0.49	-0.26
LAIPBEZ	-0.71	-0.53	-0.31	-0.49
LAICHR	0.27	-0.09	0.65	0.29
LAICHSS	0.17	0.18	0.28	0.32
LAIIDTS	-0.01	0.27	0.25	0.23
LAEUKS	0.32	0.68	0.39	0.61
LACHS	-0.44	-0.04	-0.32	-0.07
LTAXA	0.25	0.12	0.54	0.30
LABUNDAN	-0.19	0.23	0.48	0.49
LDABUND	-0.38	0.06	0.25	0.14
LSHANNON	0.12	0.19	0.10	0.03

<sup>a</sup>Taxa codes are listed in Table 10.

New sediments were collected at B146 and B148 in August 1998, and the tests were repeated. Two levels of control were provided in these bioassays. Standard MEC laboratory test sediments were used as the first level of control. A second level of control was provided by evaluating the results of treatment stations located at +0.45, +1.8, and +9.9 m (+1.5, +6.0, and +33 ft) downstream with sediments from the local control station located 22.8 m (76 ft) upstream at B146. Survival was excellent in all tests, and significant differences were not observed in *t*-tests on log-transformed proportions between any of the test stations. There were no significant differences in survival, and the null hypothesis that survival was equal between any pair of stations was not rejected.

### Risk Assessment Summary

Moderately elevated PAH levels were observed in sediments under and immediately downstream from Bridge 146. Sediment levels of naphthalene and acenaphthylene exceeded the PEL given by Swartz (1999) in sediments collected under the bridge. Sediment levels of PAH peaked 1.8 m (6.0 ft) downstream from the downstream perimeter of the bridge. The sum of all priority pollutant PAHs exceeded the TEL of Swartz (1999) but did not exceed the PEL or the intermediate value ((TEL + PEL)/2) invoked as a benchmark for this evaluation. Sediment concentrations of acenaphthene and phenanthrene exceeded the PEL at station 1.8 m (6.0 ft). The weight of evidence suggested that minor adverse effects could be observed under these conditions.

Pipe Creek flows through an agricultural landscape devoted primarily to the production of corn. The stream carries a significant bedload of sand, silt, and clay. The resulting invertebrate community is dominated by annelids and chironomids. This community did not include significant numbers of larvae in the more sensitive Orders Ephemeroptera, Plecoptera, or Trichoptera. Pipe Creek invertebrates constituted a robust community living in a stressful environment. Significant differences in the abundance of organisms, the number of taxa or their diversity, as measured by Shannon's Index, were not observed. However, the number of taxa and the abundance of subdominant taxa were observed to decline under the bridge and at the +1.8-m (+6.0-ft) station where exceedances of the PEL were observed. These subtle effects were further examined and their reality substantiated using principle components analysis. However, it must be emphasized that the observed differences were small and not statistically significant. The observations could have been simply the result of random sampling.

Ten-day sediment bioassays using the amphipod *Hyaella azteca* did not reveal significant survival differences in sediments from stations located downstream from the bridge when compared with those located upstream or with laboratory control sediments.

## Bridge 148

The photograph in Figure 13 was taken November 4, 1997, from a downstream location, looking upstream. The photograph in Figure 14 was taken from a position under Bridge 148 looking downstream along the sampling transect. The weather was overcast, with light wind from the northwest, and it was snowing.

The water temperature was 5.5°C, and the air temperature was 5.5°C at 1200 hours. Conventional physicochemical characteristics measured in water and sediments are described in Table 12. The Indiana State Division of Water provided flow data for Pipe Creek. Records collected since 1960 indicate that the mean annual flow in Pipe Creek is 1.91 m<sup>3</sup>/s (67.6 ft<sup>3</sup>/s). The lowest daily mean was 0.09 m<sup>3</sup>/s (3.3 ft<sup>3</sup>/s) recorded on February 1, 1977. Current speeds along the sampling transect were too slow to be measured with either a drogue or a current meter equipped with a magnetic head. Current speeds in the main channel varied between 0.16 and 0.27 m<sup>3</sup>/s (5.83 and 9.67 ft<sup>3</sup>/s) in water depths of 20 to 50 cm.

### Sediment Concentrations of PAHs

Mean total volatile solid (TVS) at stations 0.0 to 20.0 was 1.48 ± 0.66. Using the conversion factor of 0.6 (Brooks, unpublished data) suggests a total organic content of 0.89%. Table 13 defines sediment PAH benchmarks for Bridge 148 derived from Swartz (1999) at 0.89% total organic carbon. Figure 15 compares sediment concentrations of PAHs observed at Bridge 148 with these benchmarks; Table 14 summarizes PAH data. Not one of the benchmarks was exceeded,



Figure 13—Bridge 148 over Pipe Creek viewed from downstream.

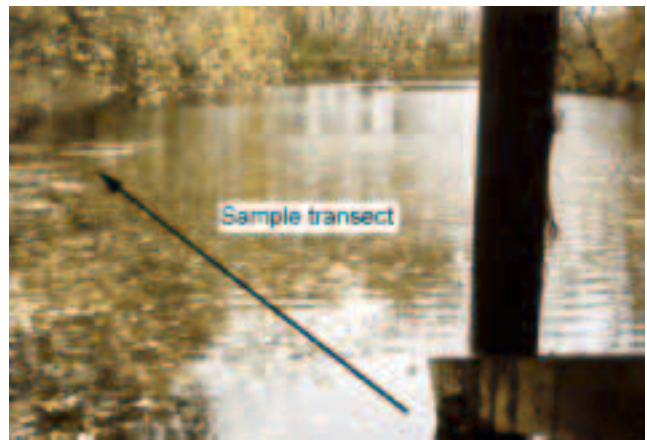


Figure 14—Bridge 148 looking downstream along the chosen sample transect

Table 12—Physicochemical characteristics observed at Bridge 148 on Pipe Creek, Cass County, Indiana, on November 4, 1997<sup>a</sup>

Station	Depth (cm)	RPD <sup>b</sup> (cm)	Total PAH (µg/g)	TVS <sup>c</sup> (%)	Sand (%)	Silt and clay (%)
–9 m (–30 ft) upstream control	17	1.5	0.1144 (ND <sup>d</sup> )	0.91 ± 0.21	72.17 ± 16.67	8.01 ± 3.14
0.0 under bridge	29	>3.0	0.9813 ± 3.1781	1.15 ± 1.05	89.46 ± 7.52	8.48 ± 5.52
0.45 m (1.5 ft) downstream	19	>3.0	1.9573	1.14	88.37	7.83
0.9 m (3.0 ft) downstream	14	>3.0	0.6414	1.10	89.04	9.83
1.8 m (6.0 ft) downstream	12	>2.0	2.2563	2.22	85.71	13.09
3 m (10 ft) downstream	23	>3.0	0.5654	1.71	88.74	9.80
6 m (20 ft) downstream	24	>3.0	0.3194	1.56	85.68	9.21
9.9 m (33 ft) downstream	14	>3.0	0.5160	5.91	27.50	70.40

<sup>a</sup>All values are the mean of two replicates except the –9 m (–30 ft) upstream control and samples taken under the bridge, which are the mean of three replicates. Where appropriate, values include 95% confidence limits on the mean. Total PAH values include half the detection limit when individual PAHs were not observed.

<sup>b</sup>RPD is the reduction–oxidation potential discontinuity. It is an indication of the depth in sediments at which sufficient oxygen is present to support aerobic metabolism.

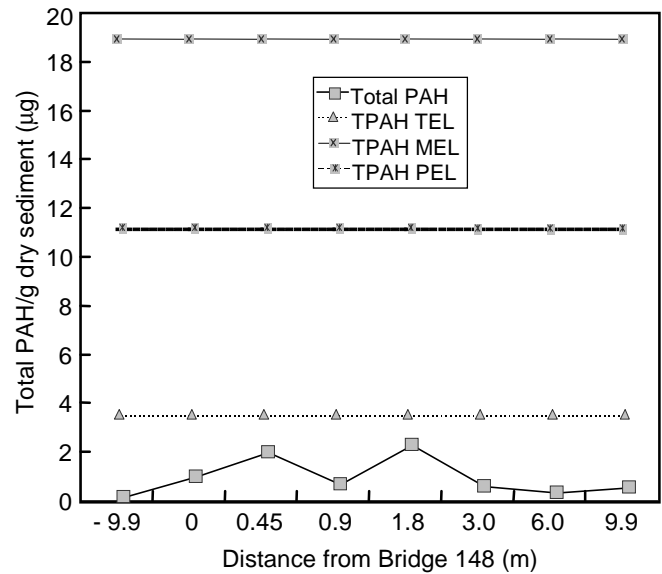
<sup>c</sup>TVS is total volatile solids measured as a percentage of dry sediment weight.

<sup>d</sup>PAHs were not detected. The value is the sum of these detection limits.

**Table 13—Consensus sediment quality benchmarks specific to the total organic carbon observed at Bridge 148 over Pipe Creek in Cass County, Indiana<sup>a</sup>**

PAH compound	ΣPAH toxicity threshold	ΣPAH mixture LC50	Mean
Naphthalene	0.12	0.63	0.37
Acenaphthylene	0.03	0.13	0.08
Acenaphthene	0.04	0.20	0.12
Fluorene	0.15	0.80	0.48
Phenanthrene	0.26	1.38	0.82
Anthracene	0.19	1.01	0.60
Fluoranthene	0.61	3.30	1.96
Pyrene	0.80	4.28	2.54
Benz(a)anthracene	0.19	0.99	0.59
Chrysene	0.28	1.50	0.89
Benzo(b)fluoranthene	0.29	1.60	0.95
Benzo(k)fluoranthene	0.26	1.38	0.82
Benzo(a)pyrene	0.29	1.59	0.94
Low-molecular-weight PAH	0.77	4.17	2.47
High-molecular-weight PAH	2.72	14.65	8.69
Total PAH	3.50	18.81	11.16

<sup>a</sup>Swartz (1999).



**Figure 15—Sediment concentrations of total PAH measured upstream (controls) and downstream from Bridge 148 crossing Pipe Creek in Cass County, Indiana. Sediment PAH concentrations are in micrograms per gram (dry sediment weight). The threshold effects level (TEL), mean effects level ((TEL + PEL)/2), and probable effects level (PEL) are computed at the observed level of total organic carbon (0.89%).**

**Table 14—Concentrations of sedimented PAHs observed in the vicinity of Bridge 148, Cass County, Indiana<sup>a</sup>**

Compound	Reporting limit (µg/g)	Concentrations (µg/g dry sediment weight) for various sampling stations							
		-9.9 m (-33 ft)	0-0.3 m (0-1 ft)	+0.45 m (+1.5 ft)	+0.9 m (+3.0 ft)	+1.8 m (+6.0 ft)	+3 m (+10 ft)	+6 m (+20 ft)	9.9 m (+33 ft)
Naphthalene	0.020	0.010	<b>0.0260</b>	<b>0.0100</b>	0.0520	0.0610	0.0100	0.0220	0.0230
Acenaphthylene	0.020	0.010	0.0100	0.0100	0.0100	0.0100	0.0100	0.0100	0.0230
Acenaphthene	0.020	0.010	0.0100	0.0100	0.0380	<b>0.0930</b>	0.0210	0.0100	0.0230
Fluorene	0.010	0.010	0.0150	0.0690	0.0100	0.1300	0.0260	0.0100	0.0630
Phenanthrene	0.020	0.010	0.2367	<b>0.6100</b>	0.1300	<b>0.5900</b>	0.1000	0.0590	0.0040
Anthracene	0.020	0.010	0.0283	0.0700	0.0220	0.0890	0.0240	0.0100	0.0040
Fluoranthene	0.020	0.010	0.3450	0.4900	0.1200	0.4800	0.1300	0.0750	0.0055
Pyrene	0.020	0.010	0.2690	0.4200	0.0970	0.4600	0.1400	0.0890	0.0230
Benzo(a)anthracene	0.003	0.001	0.0082	0.1300	0.0490	0.1400	0.0540	0.0013	0.0230
Chrysene	0.020	0.010	0.0100	0.0100	0.0100	0.0640	0.0100	0.0100	0.0070
Benzo(b)fluoranthene	0.004	0.002	0.0018	0.0420	0.0018	0.0460	0.0018	0.0018	0.1300
Benzo(k)fluoranthene	0.003	0.002	0.0017	0.0250	0.0820	0.0260	0.0190	0.0017	0.0230
Benzo(a)pyrene	0.005	0.002	0.0023	0.0440	0.0023	0.0500	0.0023	0.0023	0.0100
Benzo(ghi)perylene	0.010	0.010	0.0100	0.0100	0.0100	0.0100	0.0100	0.0100	0.0230
Ideno(1,2,3-cd)pyrene	0.009	0.004	0.0043	0.0043	0.0043	0.0043	0.0043	0.0043	0.0230
Dibenzo(a,h)anthracene	0.006	0.003	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.1400
Total PAH	0.209	0.114	0.9813	1.9573	0.6414	2.2563	0.5654	0.3194	

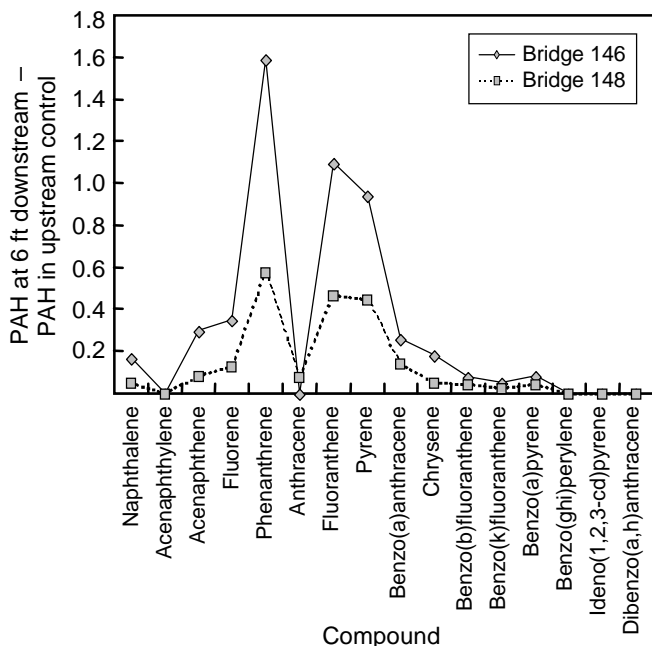
<sup>a</sup>Reported concentrations are from the top 2 cm of the sediment column. Sediment PAH concentrations exceeding the threshold effects Level, defined by Swartz (1999) as the ΣPAH toxicity threshold, are in bold type. Cells containing sediment PAH concentrations exceeding the (TEL + PEL)/2 benchmark used in this assessment have a gray background.



including the threshold effects level, and no biological effects should be associated with sediment levels of PAHs observed at this bridge. As with Bridge 146, highest sediment concentrations of PAHs were observed in the sample collected 1.8- m (6.0-ft) downstream from the downstream drip-line of the bridge. This suggests that PAHs from the bridge are deposited in this area as a function of water depth, PAH particle size, and current speed.

Goyette and Brooks (1999) observed a shift toward increasing proportions of high-molecular-weight PAHs in sediments at the Sooke Basin Creosote Evaluation Study site. Unlike Pipe Creek, currents in Sooke Basin were very slow and there was no evidence suggesting that PAHs, when sedimented, were redistributed by storms or tides. The low and intermediate weight compounds (through pyrene) appeared to be fairly rapidly degraded, likely by bacteria, in Sooke Basin sediments. The higher molecular weight compounds are more refractory to microbial degradation, and their proportion likely increased as a result of the hypothesized differential catabolism.

Bridge 148, constructed in 1980, was 17 years old during this evaluation. In contrast, Bridge 146, constructed in 1995, was only 2 years old. If PAHs lost from these bridges remained undisturbed in Pipe Creek sediments, then a similar shift to a higher proportion of high-molecular-weight compounds would have been expected. In fact, as depicted in Figure 16,



**Figure 16—Differences in upstream and 1.8 m (6 ft) downstream concentrations of various PAHs in sediments associated with 17-year-old Bridge 146 and 2-year-old Bridge 148 crossing Pipe Creek in Cass County, Indiana.**

the suite of sedimented PAH compounds are remarkably similar at the two bridges. Characteristic of creosote, phenanthrene, fluoranthene, and pyrene were dominant compounds at both bridges. The high-molecular-weight compounds remained at relatively low concentrations. This suggests that sedimented PAHs observed in this study were recently deposited (within the past year) and that historic deposits, in which low and intermediate compounds were degraded leaving a dominance of high-molecular-weight compounds, were likely redistributed and diluted as sediments moved downstream during winter and spring high water flows. If this hypothesis is correct, then reduced PAH concentrations would be expected each spring. PAHs would be released from the bridges during hot summer weather and they would accumulate in downstream sediments, reaching a maximum during the fall and early winter before high flows reduce their concentration levels. This study was conducted in the late fall while stream flows were still low. If this hypothesis is correct, then the PAH concentrations observed in this study probably represent the peak in an annual cycle.

### Biological Response—Infauna

A total of 11,505 invertebrates were identified in 18 sediment samples covering 0.0309 m<sup>2</sup> each collected in the vicinity of Bridge 148. These samples contained 69 taxa. Taxonomic codes used in the database are defined in Appendix C. Dominant taxa were defined as those representing 1% of the total infaunal abundance (count = 115). As with Bridge 146, the invertebrate community was dominated by annelids (ANNE = 2,383) and chironomids in the genus *Chironomus* (AICHSS = 7,396). In addition, mayflies in the genus *Caenis* (AICAE = 324), dipterans in the genus *Bezzia* (AIBEZ = 267), and chironomids in the genus *Dicrotendipes* (AIDTS = 145) also met the criteria. Dominant and subdominant taxa are listed in Table 15. These 19 taxa accounted for 99% of the animals recovered at Bridge 148.

In Figure 17, total abundance, subdominant abundance, and the number of taxa observed in 0.0309-m<sup>2</sup> samples are compared with sediment PAH concentrations. Both measures of abundance and the number of taxa are depressed under the bridge and at a distance of 20 m downstream. It also appears that there is a mixed correlation between biological endpoints and the concentration of PAH in sediments. PAH levels are low and biological endpoints high at upstream and downstream stations. Between the bridge and the 6-m (20-ft) downstream stations, it appears that biological endpoints are positively correlated with sediment PAH concentrations.

In Figure 18, Shannon's and Pielou's Indexes are compared with sediment concentrations of total PAHs. The values of these endpoints are low, suggesting a community dominated by a few opportunistic species, as has already been noted. The infaunal community has even lower diversity from under the bridge to a distance of 6 m (20 ft) downstream. The mixed response just noted suggests that there may be other



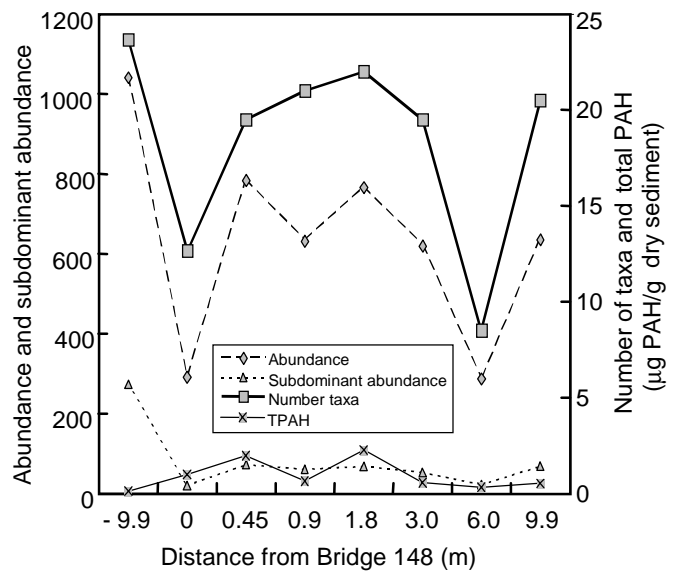
**Table 15—Dominant and subdominant invertebrate taxa observed in sediment samples collected in the vicinity of Bridge 148 crossing Pipe Creek in Cass County, Indiana<sup>a</sup>**

Dominant taxa	Code	Found in number of samples	Total abundance
Annelids	ANNE	18	2,383
<i>Chironomus</i> sp.	AICHSS	18	7,396
<i>Caenis</i> sp. (Ephemeroptera)	AICAE	16	324
cf. <i>Probezzia</i> sp. (Diptera)	AIPBEZ	8	267
<i>Dicrotendipes</i> sp. (Diptera)	AIDTS	16	145
Sub-dominant taxa			
Gastropods			
<i>Pleurocera</i> sp.	MGPLS	15	55
<i>Stagnicola</i> sp.	MGSTS	9	93
<i>Physella</i> sp.	MGPLS	10	96
Bivalvia			
<i>Sphaerium</i> sp.	MPSPH	10	43
Order Hemiptera			
Family Psyllidae	AIPSY	9	70
Order Coleoptera			
<i>Oreodytes</i> sp.	AIORES	6	22
<i>Berosus</i> sp.	AIBERS	6	16
<i>Dubiraphia</i> sp.	AIDUBS	12	65
Order Diptera			
<i>Cryptolabis</i> sp.	AICRLS	3	20
cf. <i>Bezzia</i> sp.	AICBEZ	4	21
Family Chironomidae	AICHR	14	112
<i>Procladius</i> sp.	AIPRS	10	58
<i>Eukiefferiella</i> sp.	AEUKS	12	59
<i>Micropsectra</i> sp.	AIMPS	15	95
Total abundance of dominants and subdominant taxa			11,340

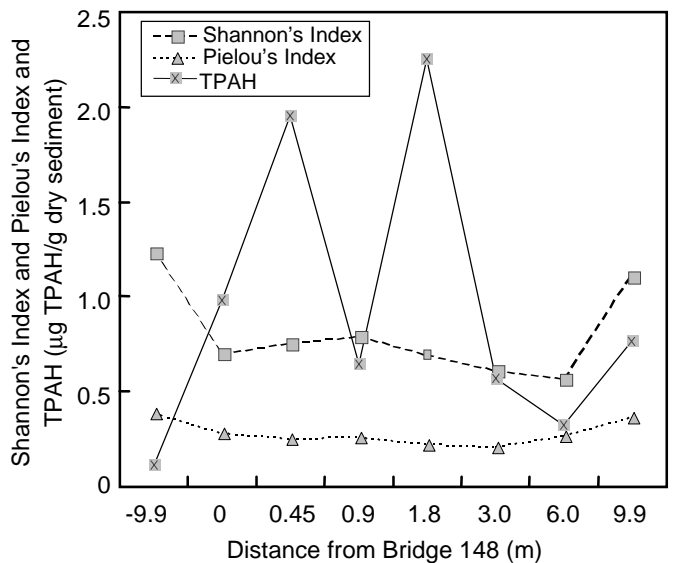
<sup>a</sup>Eighteen samples, each covering 0.0309 m<sup>2</sup>, were collected.

factors influencing the invertebrate community in this area of Pipe Creek. These factors were investigated through correlation analysis in Table 16 and the principle components analysis described in Figure 19. Total volatile solids and percentile fines (silt and clay) were included in the analysis but excluded from Table 16 because significant correlations were not observed.

Sediment concentrations of both total PAHs and phenanthrene were at or below the TEL defined by Swartz (1999). However, 12 of the 14 biological endpoints were negatively correlated with phenanthrene, and five of those were significantly negative at  $\alpha = 0.05$  (Table 17). Only chironomids and specifically those in the genus *Eukiefferiella* sp. were significantly positively correlated with PAH. All other taxa were negatively correlated. That correlation suggests that either

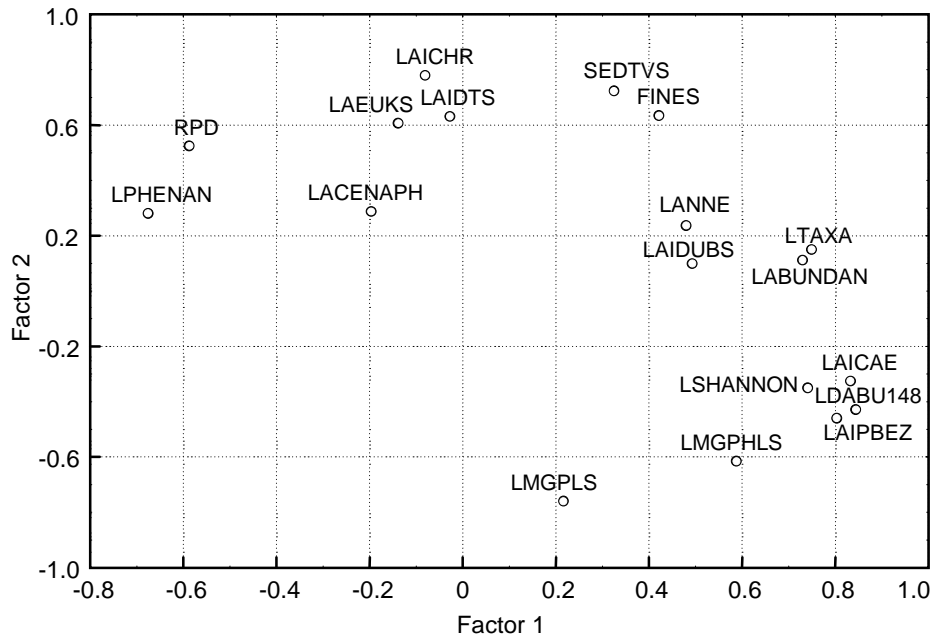


**Figure 17—Number of taxa, total abundance, subdominant species abundance, and sediment concentrations of total PAH (TPAH) (micrograms TPAH per gram dry sediment) observed in the vicinity of Bridge 148 crossing Pipe Creek in Cass County, Indiana, November 4, 1997.**



**Figure 18—Comparison of Shannon's and Pielou's Indexes with TPAH observed in sediments at Bridge 148 crossing Pipe Creek in Cass County, Indiana, November 4, 1997.**

this robust community was moderately sensitive to PAH, particularly phenanthrene, or that sediment PAH concentrations were correlated with some other environmental factor responsible for the observations.



**Figure 19—Principal components analysis of  $\log(n + 1)$  transformed biological response variables and log transformed PAH concentrations in samples collected from Pipe Creek in the vicinity of Bridge 148 in Cass County, Indiana, November 4, 1997.**

**Table 16—Pearson correlation matrix, describing the relationship between selected biological endpoints and independent factors that may have affected the invertebrate community<sup>a</sup>**

Dependent variable	Code	Distance	Depth	RPD	LTPAH	Lphenanthrene
Annelids	ANNE	0.28	0.38	-0.01	-0.05	-0.29
<i>Pleurocera</i> sp.	LMGPLS	<b>0.80</b>	<b>-0.76</b>	<b>-0.74</b>	<b>-0.60</b>	-0.50
<i>Physella</i> sp.	LMGPHLS	<b>0.86</b>	<b>-0.67</b>	<b>-0.75</b>	<b>-0.62</b>	<b>-0.60</b>
<i>Caenis</i> sp.	LAICAE	<b>0.90</b>	-0.59	<b>-0.88</b>	-0.52	<b>-0.65</b>
<i>Dubiraphia</i> sp.	LAIDUBS	0.22	0.40	-0.15	0.12	-0.32
<i>Bezzia</i> sp.	LAIPBEZ	-0.58	-0.35	<b>-0.71</b>	<b>-0.64</b>	<b>-0.85</b>
Family Chironomidae	LAICHR	<b>-0.67</b>	0.57	0.49	<b>0.71</b>	0.57
<i>Chironomus</i> sp.	LAICHSS	0.44	-0.27	-0.51	0.07	-0.08
<i>Dicrotendipes</i> sp.	LAIDTS	-0.36	0.28	0.32	-0.06	-0.14
<i>Eukiefferiella</i> sp.	LAEUKS	<b>-0.65</b>	0.59	<b>0.66</b>	<b>0.71</b>	<b>0.60</b>
Total number taxa	LTAXA	0.56	-0.26	<b>-0.63</b>	-0.13	-0.37
Total abundance	LABUNDAN	0.58	-0.22	-0.58	-0.03	-0.25
Shannon's Index	LSHANNON	<b>0.73</b>	0.23	0.56	<b>-0.63</b>	<b>-0.79</b>
Subdominant abundance	LDABU148	<b>0.96</b>	-0.52	<b>-0.87</b>	<b>-0.61</b>	<b>-0.78</b>

<sup>a</sup>Significant ( $\alpha = 0.05$ ) correlations are in bold type.

**Table 17—Summary results of Bridge 148 sediment bioassays using the test amphipod *Hyalella azteca***

Replicate	Percentage of surviving amphipods with an initial count of 20 per replicate				
	Labo- ratory control	Up- stream control	+0.45 m (+1.5 ft)	+1.8 m (+6.0 ft)	+9.9 m (+33.0 ft)
1	100	100	90	100	100
2	100	90	100	100	100
3	90	100	100	100	100
4	100	100	100	100	100
5	100	100	100	100	100
6	100	90	100	100	100
7	100	100	100	100	100
8	100	100	100	100	100
Mean	98.8	97.5	98.8	100	100

The Bridge 148 sample transect was chosen because of its slow currents and depositional characteristics that would allow for the accumulation of PAHs lost from the bridge. Large quantities of maple leaves (*Acer* sp.) had accumulated along the sample transect, particularly between the downstream perimeter of the bridge and the 6-m (20-ft) downstream sample station (Fig. 14).

The reduction–oxidation potential discontinuity (PRD) is an indication of the depth to which sediments are aerobic. At Bridge 148 in the fall of 1997, the depth of the RPD appeared most influenced by the accumulation of maple leaves. These leaves had recently fallen and had not yet started to decompose. However, numerous aquatic insects were found in the leaf mats (as expected). Note in Table 16 that the depth of the RPD is negatively correlated with the abundance of most taxa. This indicates that where an abundance of organic material (maple leaves) was found, the RPD was reduced, but the abundance of most taxa was increased. As shown in Figure 19, the depth of the RPD and sediment concentrations of phenanthrene were positively correlated ( $r = 0.52$ ). This suggested that greater sediment concentrations of phenanthrene were found in areas where the RPD was deeper and the leaf mat was reduced. Leaf matting was not quantified during the survey, and no direct measure of this factor was possible. Therefore, this discussion remains purely hypothetical. The value of the triad approach (physicochemical analysis + invertebrate community analysis + laboratory bioassay analyses) to environmental risk assessment is clearly evident in this instance.

### **Biological Response—Sediment Bioassays**

Results of 10-day sediment bioassays using the amphipod *Hyalella azteca* are presented in Table 17. Significant differences were not observed in the 10-day survival of test animals between stations. Survival at downstream stations

was equal to or greater than survival in laboratory test sediments and greater than survival in sediments collected at the upstream control. Results from these bioassays suggest that sediment toxicity was not responsible for the differences observed in the resident invertebrate community.

### **Risk Assessment Summary of Bridges 146 and 148**

Results of these surveys are consistent with our existing knowledge regarding the physics and chemistry of PAHs, the loss of creosote from pressure-treated wood, and the biological response to sedimented PAHs. The following summarizes our findings:

- Creosote does bleed from pressure-treated wood. In freshwater, at 17°C, the rate is approximately 37 µg/cm<sup>2</sup>-day. For a Class A piling that is 30 cm in diameter and submerged in 60 cm of water, the total surface area is 5,652 cm<sup>2</sup>. The piling loses 0.213 g of PAHs per day during the first year, equivalent to a drop of crankcase oil. That amount decreases exponentially with time and by the mid-point of piling life (about 35 years), PAH loss is reduced to about 3% of these initial losses. This temporal reduction was evident in the Pipe Creek data. Sediment concentrations of PAH downstream from the 17-year-old Bridge 146 were much less than those observed at the 2-year-old Bridge 148.
- PAHs observed in the vicinity of both Bridges 146 and 148 were rich in acenaphthylene, fluorene, phenanthrene, and fluoranthene. These intermediate weight compounds are consistent with new creosote contamination, and the bridge structure appears to be the source. As sedimented creosote ages, the low and intermediate weight compounds are metabolized by microbes, leaving a deposit rich in the high-molecular-weight compounds. This shift in PAH species was not observed at these two bridges, despite the 15-year difference in their ages. High summer temperatures likely exacerbate the loss of creosote oil from the elevated portions of the bridge. These compounds accumulate in sediments during low summer flows and likely reach a peak during the late summer and fall (when this survey was completed). Before these accumulations can weather (as indicated by a preferential loss of the intermediate weight compounds), the deposits are diluted and redistributed downstream during high winter and spring flows.
- Elevated sediment concentrations of PAH were observed downstream from both bridges. Concentrations of naphthalene, acenaphthylene, and phenanthrene exceeded the PEL defined by Swartz (1999), and adverse biological effects could have been expected in very sensitive taxa at the new Bridge 148 but not at the older Bridge 146.
- Sediment concentrations of PAH downstream from Bridge 146 were low, and acenaphthene and phenanthrene barely exceeded the TEL defined by Swartz (1999). Adverse effects were not expected in the invertebrate community, but

significant reductions in most biological endpoints were observed between the downstream perimeter of the bridge and 6 m (20 ft) downstream. In contrast, no sediment toxicity was observed in laboratory bioassays when survival in downstream sediments was compared with either laboratory or upstream control sediments. These results and the analysis suggest that the invertebrate community was more affected by the presence of various degrees of coarse particulate organic matter (CPOM) in the form of heavy mats of recently dropped maple leaves than on the low concentrations of PAH found in adjacent sediments.

- Adverse effects were not observed in either the resident invertebrate community at Bridge 146 or laboratory bioassays. Pipe Creek was chosen for this assessment because of the slow current speeds that would allow PAHs lost from the bridge to accumulate in the immediate vicinity of the bridge. The landscape surrounding Pipe Creek is devoted to agriculture (primarily corn), which likely contributes to the significant sand, silt, and clay bedload carried by the stream. The combination of slow currents and fine-grained sediments results in a stressful environment that does not support a sensitive invertebrate community. The invertebrate community in Pipe Creek is dominated by annelids and chironomids, both considered robust taxa. That is likely the reason that adverse effects were not observed when they could have been expected in a more sensitive community. It could be argued that a similar bridge placed over a faster flowing stream with a more diverse and stable benthic habitat (more cobble and boulder with less sand) would result in the observation of adverse biological effects. However, habitats characteristic of more sensitive communities with large numbers of EPT taxa include higher current speeds. These higher current speeds would result in significantly reduced concentrations of PAH. Modeling of PAH concentration as a function of current speed suggests that in water bodies where current speeds exceed 25 to 30 cm/s, PAHs would be dispersed to the point of not being detectable in sediments.
- The point is that sediment quality criteria are frequently based on the most sensitive taxa. These criteria do not take into account the relationships between water body physicochemical characteristics (for example, current speeds), community composition as a function of those physicochemical characteristics, and the transport and deposition of potential contaminants as a function of those same factors. These results are similar to those observed by Brooks (2000) in an examination of the biological response to handicapped access boardwalk construction in a series of beaver ponds in the Wildwood Recreational Area of Oregon.
- Both of these creosote-treated bridges appeared relatively clean and dry with little evidence of preservative weeping from overhead components. However, preservative does



**Figure 20—Creosote weeping from pressure-treated piling at Bridge 148.**

migrate out and run down the surface of piling (Fig. 20). It is also likely that some preservative drips from overhead structures during hot summer days. Oregon State University is evaluating losses from overhead structures as a function of preservative retention and ambient temperature. That work will significantly improve our knowledge of creosote loss rates from treated bridges and its transport in aquatic environments.

## Penta-Treated Bridges

Penta-treated wood was identified during the site selection survey as the second most commonly used preservative in the construction of timber bridges.

### Sources

The U.S. Department of Agriculture (USDA 1980) noted that penta is “ubiquitous in aquatic environments and its sources are unclear.” Observable levels of penta can result from direct contamination, degradation of other organic compounds, or chlorinated drinking water. The USDA report notes that circumstantial evidence, including the detection of penta in rainwater, indicates that penta may occasionally be present in ambient air.

Historically, penta has been used extensively in agriculture and industry as an insecticide, fungicide, herbicide, algacide, and disinfectant. However, the major commercial application of technical grade penta is in the wood preservation industry. Eisler (1989) reported that 80% of the  $23 \times 10^6$  kg of technical penta produced annually is used for wood preservation. He noted that penta is found at levels from  $<1$  to  $7.3 \mu\text{g/L}$  in some British Columbia waters. In general, Eisler’s (1989) data imply a correlation between industrial-urban centers and increasing penta concentrations in the water column.

Matsumoto (1982) noted that vascular plants and their detritus naturally produce many phenolic acids. However, he stipulated that he found no evidence of naturally occurring penta in his study of the polluted Tokyo River water and pristine river or reservoir and pond waters on the Bonin Islands. No information was obtained that suggested that penta is biosynthesized, and there are no known significant natural sources of penta. However, Lampi and others (1992) observed up to 30 to 70 µg penta/kg dry sediment in cores dating back to the 17<sup>th</sup> century. They concluded that this may have been due to wood burning and aerial transport, because the lake is about 8 km (5 miles) from the nearest settlement and tens of kilometers from the nearest industrial center.

### Environmental Chemistry

Penta (C<sub>6</sub>Cl<sub>5</sub>OH) has a relative molecular mass of 266.34, a melting point of 190°C, a boiling point of 310°C, and a density of 1.98 g/cm<sup>3</sup> at 22°C. The vapor pressure is 0.00415 Pa at 20°C. Penta has an octanol–water partition coefficient (log *K<sub>ow</sub>*) of 3.3 at neutral pH, 5.1 at pH 4, and 1.9 at pH 8. Penta readily dissolves in most organic solvents. Its solubility in water is also pH dependent and varies between 10 mg/L at pH 6 to 20 mg/L at pH 8 (Mackay and others 1995).

In the 1970s and 1980s, scientific recognition of the toxicity of some dioxins led to increased public concern. The dioxin 2,3,7,8-tetrachlorodibenzo, commonly called 2,3,7,8-TCDD, was recognized as an exceptionally toxic compound, at least to some test organisms. This fact, coupled with the knowledge that this compound was present in Agent Orange (the defoliant used in Vietnam), heightened concern. Eisler (1989) noted that many commercial samples of technical grade penta were heavily contaminated with a large number of potentially toxic compounds including dibenzofurans, dioxins, and hexachlorobenzenes. The relative toxicity and accumulation potential of some of these contaminants exceeded that associated with the parent penta by several orders of magnitude (Huckins and Petty 1981). For example, Eisler (1986) reported that some isomers of hexachlorodibenzodioxin, present in technical grade penta at concentrations of 1,000 to 17,300 µg/kg during the 1970s were lethal to guinea pigs at doses of 60 to 100 µg/kg body weight. The dioxins in penta of greatest concern are the hexachlorodibenzo-p-dioxins (HxCDD). The U.S. EPA has limited the concentration of this compound to 2 mg/kg in commercial penta sold in the United States. A maximum concentration in any one batch of 4 mg/kg is allowed.

Penta is not dissociated from its hydroxyl ion in aqueous solutions at pH < 5.0. However, as the pH increases, the bioavailability and toxicity of penta decrease because less of the compound is found in the undissociated form. The solubility of penta and potential for adsorption to suspended inorganic particulate matter (particularly clay) is positively correlated with the dissociated fraction, which increases significantly at pH > 6.5.

### Fate

Penta may be dissolved in water or sorbed to suspended matter or bottom sediments. In addition, penta is taken up by fauna and flora that metabolize the compound at various rates. Penta readily degrades in the environment by chemical, microbiological, and photochemical processes (USDA 1980, Eisler 1989). Photochemical degradation is a function of the spectrum and intensity of incident light and appears to proceed rapidly in natural environments with half-lives of 0.15 to 15 days (Smith and others 1987). The degradation of penta in sediments depends on a number of environmental factors that are discussed in the following sections. Half-life in sediments can range from days to years, depending on environmental conditions. The ultimate fate of penta appears to be burial in anaerobic sediments under infrequently encountered conditions or mineralization to carbon dioxide and water.

### Dissolved Penta

Penta that is dissolved in water may be removed by volatilization, photodegradation, absorption, or biodegradation. Penta is subject to rapid photodegradation under laboratory conditions. Boyle and others (1980) examined the degradation of penta in a two- by two-array of microcosms that did and did not contain natural lake sediments held under aerobic and anaerobic conditions. At the end of the 131-day experiment, the authors determined the penta half-life under each of the four conditions. Their results are summarized in Table 18. Fisher (1990) concluded that in aerobic and organically rich environments, the half-life of dissolved and sedimented penta would be about 1 week. Middaugh and others (1993) determined that the gram-negative bacterium *Pseudomonas* sp. (strain SR3) was able to degrade penta and that it provided an adequate sole carbon source sustaining growth. Nearly complete degradation of 39,000 to 40,000 µg

**Table 18—Pentachlorophenol remaining in water and sediments at the end of 131 days in each of four aquaria<sup>a</sup>**

Test conditions	Total penta in water (mg)	Water half-life (days)	Penta in sediments (mg)	Total penta (mg)
Aerobic without mud (lighted)	0.95	18.6	0.950	
Aerobic with mud (lighted)	0.21	13.9	0.03	0.240
Anaerobic without mud (dark)	16.00	79.8		16.000
Anaerobic with mud (dark)	0.005	12.8	0.04	0.045

<sup>a</sup>Penta (100 mg) was originally added to each of the aquaria. Water column half-life is given for penta as determined in this experiment.

penta/L was accomplished by acclimated *Pseudomonas* sp. Penta half-lives in freshwater streams reported in McAllister and others (1996) varied between 40 and 120 h.

Boyle and others (1980) also partitioned  $^{14}\text{C}$  at the end of the experiment. The bulk (99%) of the sedimented  $^{14}\text{C}$  was observed in the non-biogenic clay fraction. They also found  $^{14}\text{C}$  in algae, floating flocculent material, and other biogenic material in the water column. Minimal  $^{14}\text{C}$  was observed elsewhere in the microcosms (including the aquarium sides and cover). The authors concluded that penta degradation was positively correlated with incident light levels, pH, oxygen, and the presence of sediment. They concluded that penta is likely most persistent in the deoxygenated hypolimnion water of lakes. Several environmental factors affect the rate at which penta is degraded in natural aquatic environments.

### **Effects of pH and Water Temperature on the Degradation of Penta**

Valo and others (1985) found that the metabolism of penta was inhibited at less than 8°C or greater than 50°C. Optimum degradation occurred at 28°C. Jarvinen and Puhakka (1994) and Jarvinen and others (1994) found that 99% of the penta present in contaminated groundwater was degraded at 5°C to 10°C. Trevors (1982) documented no penta degradation by acclimated *Pseudomonas* at 0°C. Penta degradation rates at 4°C were dependent of the specific *Pseudomonas* strain used. However, an average of 28.2% of the initial 50,000 µg penta/L substrate was metabolized in 80 days at 4°C, and 50.2% of the same concentration was metabolized in 8 days at 20°C.

Valo and others (1985) observed penta degradation at pH values between 5.6 and 8.0. A neutral or slightly acidic pH was found to be optimum. Wong and Crosby (1981) observed penta half-lives of approximately 100 h at pH 3.3 and 3.5 h at pH 7.3 in sterile solutions containing 100,000 µg penta/L. Penta degradation was not observed in flasks maintained in the dark.

It appears that penta degradation is optimum at pH values between 6.5 and 8.0 and at temperatures between 10°C and 30°C. These are conditions expected in much of North America during all seasons except winter, when low temperatures at northern latitudes can be expected to decrease penta degradation rates. In addition, penta is expected to degrade more slowly in areas subjected to low pH.

### **Microbial Community Adaptation**

Larsson and others (1993) and Larsson and Lemkemeier (1989) observed significantly higher penta degradation by unacclimated microbial communities inhabiting brown water lakes containing high levels of humic acid when compared with clear water lakes. These authors concluded that the microbe communities inhabiting brown water lakes had

adapted to the higher phenol levels naturally present in the water and therefore were pre-acclimated to metabolize penta.

McAllister and others (1996) reviewed the literature pertaining to the microbial degradation of penta. They confirmed that sediment penta levels exceeding 300 µg/kg inhibit microbial degradation until a period of acclimation has passed. When acclimation began, it appears that the higher the initial concentration of PCP, the longer the maximum number of viable cells, capable of degrading PCP, was maintained. Gonzalez and Hu (1991) observed that lag phases of 10 h occurred at 10 mg/L, 30 h at 20 mg/L, 55 h at 44 mg/L, 80 h at 80 mg/L, and 200 h at 200 mg/L.

In summary, it appeared that microbial communities were not generally preadapted to metabolize penta. Initial exposure of naïve communities to penta concentrations as low as 300 µg penta/L can reduce growth. An adaptation of several hours to perhaps 2 weeks is necessary for community adaptation to penta. Following this time, adapted communities can tolerate much higher concentrations of penta and rapidly metabolize it.

### **Effects of Additional Carbon Sources on Penta Metabolism**

Topp and others (1988) studied the response of penta-degrading *Flavobacterium* sp. to high levels of penta with and without the addition of sodium glutamate as a cometabolite. They found that the specific activity of penta-degrading cells in the absence of supplementary carbon was  $1.51 \times 10^{-13}$  g penta/cell-h. They showed that the form and amount of alternative substrates were important in determining the metabolism of penta. For instance, optimal stimulation of penta removal required the addition of 3.0 g sodium glutamate/L. However, glutamate in combination with glucose or cellobiose partially repressed pentametabolism. *Flavobacterium* removed 2.5% of the penta from a 25,000 µg/L initial concentration. The addition of 4 g sodium glutamate/L increased metabolism, resulting in the removal of 61.9% of the penta in 3 h. However, when the mixture was amended with 4 g sodium glutamate and 5 g/L glucose, penta metabolism was reduced to 15.5% in 3 h. It was the combination of the two substrates that reduced penta metabolism, because in a separate experiment the authors found that the addition of 0.5 g of glucose to the medium in the absence of sodium glutamate resulted in the complete degradation of an initial 61,000 µg penta/L solution by *Flavobacterium* sp. The amount of penta removed decreased when incremental amounts of sodium glutamate were added to the glucose. Topp and others (1988) did not discuss the possibility that *Flavobacterium* sp. acclimated and degraded penta when it represented a sole carbon source but preferentially shifted to alternate substrates when available. Perhaps rather than acting antagonistically, the combination of sodium glutamate and glucose acted synergistically, reducing the dependence of the bacteria on penta. Yu and Ward (1994) observed

maximum penta degradation in medium supplemented with glucose and peptone.

Topp and others (1988) found that amendment with supplementary source of carbon reduced the lag time required before significant penta metabolism commenced. These authors noted that penta concentrations greater than 20,000 µg/L inhibited *Escherichia coli* and that *Pseudomonas* was inhibited at concentrations greater than 500,000 µg/L. They also reported that 50,000 µg penta/L was degraded as a sole source of carbon after a lag phase of 90 h.

Penta half-lives in freshwater streams reported in McAllister and others (1996) varied between 40 and 120 h. Consistent with Liu and others (1981), McAllister and others (1996) noted that additional substrates tend to reduce penta degradation rates and hypothesized that adsorbed penta is less bioavailable. McAllister and others (1996) also noted that the most widely studied penta degrading microorganisms are the pure culture bacterial strains, *Flavobacterium* and *Rhodococcus chlorophenolicus*. The enzymes responsible for initiating the catabolism of penta by *Flavobacterium* sp. have been isolated and characterized. Furthermore, the genes encoding these enzymes have been characterized and cloned into *E. coli*, which then demonstrated the ability to degrade penta.

These reports suggest that penta will be degraded more rapidly in organically rich environments. However, some caution is necessary because there is evidence that some combinations of organic substrates appear to result in slightly reduced degradation rates.

### **Effects of Water Hardness on Dissolved Penta**

Brockway and others (1984) studied the fate of penta in static and continuous-flow hard and soft water mesocosms. They observed no significant effect on the fate or effects of penta associated with water hardness.

### **Sedimentation**

Penta is moderately persistent in soil. Published data indicate that penta can persist in soils for various times, ranging from weeks (21 days) to 5 years. Under most conditions, penta will seldom persist in the soil for more than 9 months and its half-life will frequently be far less. Numerous studies have identified soil microorganisms capable of penta degradation. In most studies of penta biodegradation, acclimated populations of microorganisms have been utilized. The current major use of penta is for wood preservation; therefore, the likeliest source of soil contamination is leaching or bleeding of the preservative from treated wood. Such phenomena may result in low levels of penta contamination within meters of a treated pole.

Fisher (1990) constructed microcosms with water at pH 4, 6, and 8 and sediments with 0.0% and 3.0% total organic

carbon (TOC). She found that more penta was partitioned to the sediments at lower pH than at higher pH values. In addition, she reported a positive correlation between sediment TOC and penta concentration. The organisms in the high TOC microcosms accumulated significantly less penta than did those in the 0.0% TOC systems. This work is consistent with Eisler's (1989) observation that at low pH, penta is fully protonated and lipophilic, whereas at high pH, it is ionized and unlikely to adsorb to organic ligands.

Shimizu and others (1992) determined the adsorption coefficients of penta in aquatic environments with various organic carbon contents (0.72% to 2.38%) and clay contents (10.1% to 60.8%). They concluded that at pH values between 6 and 8, the adsorption coefficient was not influenced significantly by organic carbon content (correlation coefficient = 0.12) but was positively correlated with clay content (correlation coefficient = 0.94). This work suggests that clay particles (which frequently carry an electrical charge), rather than particulate or dissolved organic carbon, form a more likely adsorption nucleus in aquatic environments.

In summary, it appears that the potential for sedimentation of penta is a complex problem driven by at least the following parameters:

- At reduced pH values, penta tends to be fully protonated and more lipophilic. Therefore, it would be expected to adsorb to dissolved or particulate organic matter. In contrast, at higher values of pH, more penta is expected to be ionized, with a higher potential for binding to polar adsorption nuclei represented by particulate inorganic matter (silt and clay).
- It appears that more penta will be partitioned from the water column to sediments having increased organic carbon content. This can have a major effect in removing penta from the water column.

### **Degradation of Sedimented Penta**

Bryant and Rogers (1990) described the degradation of penta in anaerobic sediments from diverse locations around the world. They observed that dechlorination did not occur for at least the first 15 days of exposure in unadapted sediments. However, following that adaptation, penta was completely degraded by 33 days. A second addition of 70 mg penta/kg sediment on day 33 was rapidly dechlorinated to about 25 mg/kg in 2 days. In contrast, they observed no biotransformation within 40 days after penta was added to unadapted Cherokee Pond sediments. The point made in this study is that not all microbes have the ability to dechlorinate penta as a first degradative step. Their study is consistent with other reports, indicating that unacclimated, but biological rich, sediments require approximately 2 weeks for development of suitable microbial communities before aerobic or anaerobic degradation of penta begins. However, when established,

these communities rapidly catabolize penta. Interestingly, these authors found no degradation of penta in autoclaved sediments, further emphasizing the microbial nature of the observed degradation.

Van Gestel and Ma (1988) determined penta half-lives in low pH and organically rich Holten (pH 5.6; 6.1% organic matter) and Kooyenburg (pH ~5.0; 3.7% organic matter) soils of 23.2 to 47.9 days, respectively. There was no significant difference in the half-lives of penta in these two sediments.

Smith and Novak (1987) found that penta concentrations as high as 25,000 µg/L in saturated soils were degraded to nondetectable levels in less than 3 months. They found that chlorophenol degradation rates were linearly related to the initial concentration and varied between 100 µg/L-day at 200 µg/L initial concentration to greater than 10,000 µg/L-day at an initial concentration of about 800,000 µg/L.

### ***Effects of Sediment Oxidation–Reduction on Penta Degradation***

Delaune and others (1983) examined the degradation of penta in estuarine sediments following a major accidental spill in a Louisiana Gulf Coast estuary. They completed a series of laboratory experiments in which pH was manipulated between 5.0 and 9.0 and sediment redox between –250 and +500 mV. The authors found maximum degradation at pH 8.0, with declines at either lower or higher values. Penta was observed to degrade at all values of redox. However, significantly higher degradation rates were observed under aerobic (+250 and +500 mV) than with reducing (0.0 and –250 mV) conditions. A half-life of about 24 days was apparent at pH 6.5 and a redox potential of +500 mV. However, at this pH, minimal degradation was observed at any other value of redox. At pH 8.0, significant degradation was observed at +250 and +500 mV redox potentials and a half-life of 26.5 days was apparent at +500 mV. The authors found that penta was more tightly bound to oxidized sediment solids than to reduced sediments. Therefore, they concluded that there was a tendency for penta to become preferentially associated with the thin oxidized surface sediment horizon as well as with suspended colloidal particulates, which would also tend to be oxidized. Under either condition, penta would be retained in the photic zone of shallow estuaries where the potential for photodegradation would be enhanced. The authors concluded that although tidal transport and photodegradation in the water column could play a role in the removal of residual penta from a spill area, their laboratory studies suggested that degradation under either aerobic or anaerobic conditions could account for the disappearance of residual penta left in the immediate vicinity of the spill or that which was transported from the spill site and deposited onto the bottom in adjacent water bodies.

Guthrie and others (1984) studied the anaerobic degradation of penta as a component of sewage sludge during treatment. They found that methanogenic bacteria were unaffected by penta concentrations less than 200 µg/L. Acclimation of the bacterial flora to penta increased the inhibition threshold to about 600 µg/L. They found that penta was biodegradable anaerobically and that removal was so complete that the soluble concentrations in the Phase II reactors were below detection limits of 5 µg/L. Sorption appeared a minor mechanism of penta removal, and volatilization was considered insignificant. The authors concluded that penta undergoes extensive anaerobic biodegradation, especially by acclimated microbial communities. Anaerobic degradation of penta was confirmed by Kudo (1989).

Liu and others (1981) observed similar results in their comparison of aerobic and anaerobic degradation of sedimented penta at pH 7.0. They observed an increase in the aerobic half-life of 0.36 days to 190 days in anaerobic conditions. They also found that the inclusion of either sodium chlorophenolate or glucose as a second substrate inhibited rather than enhanced the anaerobic degradation of penta. In contrast, Valo and others (1984) found that penta degradation was enhanced by the addition of 0.4 or 40 mM ammonium chloride (NH<sub>4</sub>Cl).

Bryant and Rogers (1990) examined the degradation of penta in anaerobic sediments from Georgia, Florida, New York, and the Soviet Union. They observed an adaptation of about 15 to 20 days during which little penta degradation occurred. Following microbial adaptation, degradation in anaerobic sediments was rapid with an unstated half-life of 2 to 7 days. Penta half-lives taken from the literature are summarized in Table 19.

Data provided in Baker and others (1980), DeLaune and others (1983), and Boyle and others (1980) were interpreted to produce half-lives as a function of initial penta concentration, reduction oxidation potential, pH, and temperature. Sedimented half-life was estimated assuming that microbes required 15 days to adapt to penta and that degradation rates remained linear at all times. This is a small database, and the methodology is not precise. However, the results are consistent with the remainder of the literature and appear to reasonably predict sedimented penta half-lives that are important in understanding its accumulation in sediments. Data from these studies are summarized in Table 20.

These data were analyzed using linear and nonlinear regression analyses. Within the ranges of data at hand, sedimented penta half-life was not a function of the initial concentration ( $p = 0.10$ ) or temperature ( $p = 0.40$ ). In addition, the constant term was not significant ( $p = 0.42$ ). Redox potential and pH were significant factors ( $p = 0.001$  and  $p < 0.000$ , respectively). The final regression was highly significant ( $p < 0.00015$ ), and it explained 76% of the variation in the database. The underlying assumptions requiring normally



**Table 19—Summary of pentachlorophenol half-lives and the effects of various environmental parameters on degradation rates**

<b>Reported penta half-life in water</b>	<b>Half-life (days)</b>	<b>Conditions</b>
Boyle and others (1980)	18.6	Aerobic in the laboratory
Boyle and others (1980)	79.8	Anaerobic in the laboratory
Crossland and Wolff (1985)	2.0 to 4.7	Outdoor mesocosms
Liu and others (1981)	0.36	Aerobic in the laboratory
Liu and others (1981)	190.0	Anaerobic in laboratory
Wong and Crosby (1981)	2.0	pH 7.3 (natural sun-light)
Yu and Ward (1994)	~1.5	Mixed bacterial cultures
<b>Effects of pH (7.3) on penta half-life</b>	<b>Half-life (h)</b>	<b>Conditions</b>
Wong and Crosby (1981)	3.5	Laboratory F40BL lamps
Wong and Crosby (1981)	100.0	Laboratory F40 BL lamps
Wong and Crosby (1981)	48.0	Natural sunlight
<b>Effects of ambient temperature on penta half-life</b>	<b>Half-life (days)</b>	<b>Conditions</b>
Topp and others (1988) 4.0 °C	>80	<i>Pseudomonas</i> cultures
Topp and others (1988) 20 °C	<12	<i>Pseudomonas</i> cultures
<b>Anticipated half-life of penta in soils or sediments</b>	<b>Half-life (days)</b>	<b>Conditions</b>
DeLaune and others (1983)	24 to 26	Natural estuarine sediments
McAllister and others (1996)	10 to 70	Flooded soils
McAllister and others (1996)	<2 to >5	Freshwater streams
Neary and others (1990)	Average 30	Southern ecosystems
Smith and Novak (1987)	<5	9 to 13 mg PCP/L unsaturated soil
Smith and Novak (1987)	~15	Average 3 mg PCP/L unsaturated soil
<b>Effects of sediment reduction–oxidation potential</b>	<b>Half-life aerobic (days)</b>	<b>Half-life anaerobic (days)</b>
Boyle and others (1980)	13.9	12.8
Bryant and Rogers (1990)		2 to 5
McAllister and others (1996)		144
<b>Penta half-life in plants and animals</b>	<b>Species</b>	<b>Penta half-life (h)</b>
Benner and Tjeerdema (1993)	<i>Atherinops affinis</i>	52.7
Glickman and others (1977)	<i>Onchorhynchus mykiss</i>	6.2 to 6.9

distributed residuals and homoscedasticity were met. The resulting predictive equation is

$$\text{Sedimented penta half-life} = 18.19448 \times \text{pH} - 0.29284 \times \text{redox (mV)} \quad (5)$$

At pH 7.5, this equation predicts a penta half-life of 63 days in reasonably well-oxygenated (+250 mV) sediments. In reducing sediments (–100 mV), the half-life is increased to 165.7 days. Figure 21 provides a detailed representation using a quadratic smoothing routine in the Statistica software package (StatSoft, Tulsa, Oklahoma). Under normal environmental conditions, the redox potential in surface

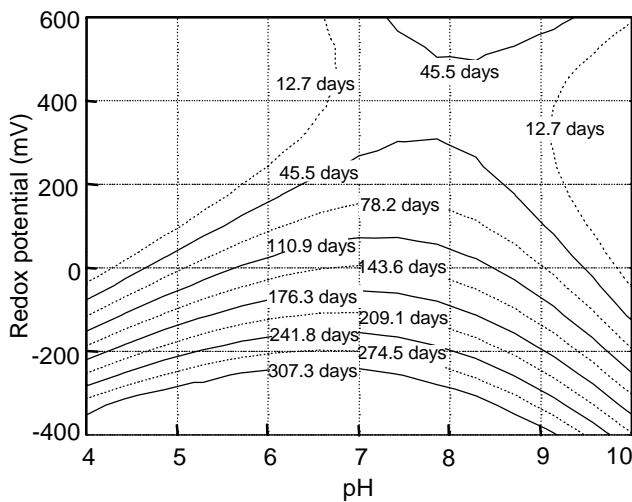
sediments will vary between –100 and + 400 mV, and the pH is expected to vary between 6 and 8.5. Figure 1 suggests that under these conditions, sedimented penta half-lives will vary significantly between 12.7 and 176.4 days. The linear equation used to make predictions in this model predicts a half-life of 24.8 days at pH 7.8 and +400 mV and 156.6 days at pH 7.0 and –100 mV.

A three-dimensional display of sedimented penta half-life as a function of redox and pH is shown in Figure 22. This figure was constructed using the distance weighted least squares routine in the Statistica software package.

**Table 20—Estimated sedimented pentachlorophenol half-lives as a function of initial penta concentration, reduction–oxidation potential, pH, and temperature<sup>a</sup>**

Source	Initial penta concentration (µg/kg)	Redox potential (mV)	pH	Temperature (°C)	Half-life (days)
Baker and others 1980	100.0	250	7.1	0.0	47.5
Baker and others 1980	100.0	250	6.9	20.0	36.7
Boyle and others 1980	1,400.0	0	4.5	15.0	13.0
Boyle and others 19080	1,400.0	250	6.8	15.0	7.5
DeLaune and others 1983	20.0	500	6.5	33.1	6.0
DeLaune and others 1983	20.0	250	6.5	33.1	103.5
DeLaune and others 1983	20.0	0	6.5	33.1	178.8
DeLaune and others 1983	20.0	500	8.0	33.1	12.0
DeLaune and others 1983	20.0	250	8.0	33.1	32.5
DeLaune and others 1983	20.0	0	8.0	33.1	81.0
DeLaune and others 1983	20.0	-250.0	8.0	33.1	290.0
DeLaune and others 1983	20.0	500.0	9.0	33.1	50.0
DeLaune and others 1983	20.0	250.0	9.0	33.1	50.0

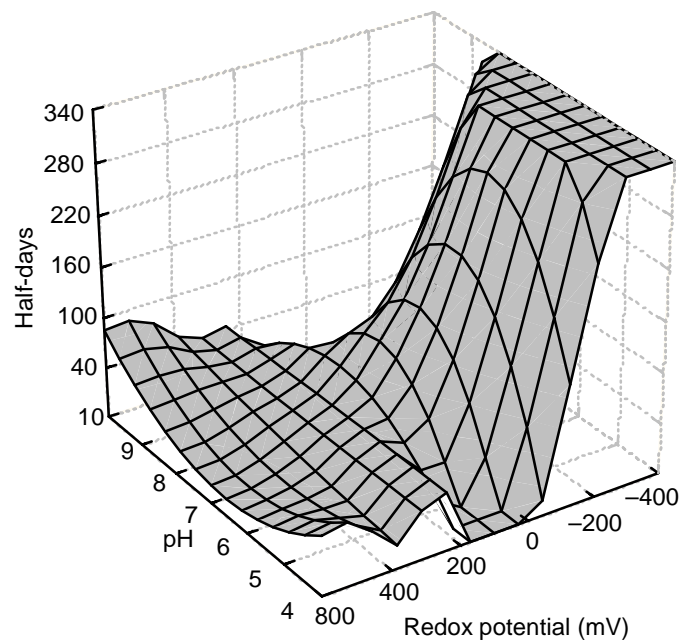
<sup>a</sup>Sedimented penta half-lives were estimated from data.



**Figure 21—Quadratic solution to the penta half-lives in Table 3, with sediment reduction–oxidation potential (millivolts) and pH as independent factors. ( $z = -715.685 + 243.79x - 1.042y - 17.252x^2 + 0.073xy + 0.001y^2$ ).**

### Case Studies

Seidler and others (1986) examined the transport and fate of penta-contaminated wastewater as it passed through two estuarine ponds being used to grow shrimp in Florida. Penta was added to the test ponds by broadcasting 500 mg of sodium-pentachlorophenate in acetone on day zero, followed by the addition of 250 mg on alternate days thereafter to provide a theoretical concentration of 10 µg penta/L. Penta concentrations of 3 to 5 µg/L were observed in the treatment



**Figure 22—Half-life of sedimented penta in days on the Z axis as a function of sediment reduction–oxidation potential and pH. The three-dimensional plot was developed using a distance-weighted least square routine.**

ponds during the first 22 days of the study. At pond pH 8.0, the authors predicted that 99.9% of the added penta would be dissolved in the phenolate form, suggesting that photolysis would be the most significant degradation process. They calculated a penta half-life of 2 days for the ponds being treated.

Seidler and others (1986) claimed that sediments contained 54 times more penta than was found in the water column. However, data presented in their paper suggest that sediment levels mimicked water column levels with a concentration factor of 1 to 10. Sediment concentrations of penta averaged about 5 µg/kg with a maximum of about 18 µg/kg. The authors concluded that their results suggested that a chronic influx of penta at a concentration of 10 µg/L to an estuarine environment resulted in elevated concentrations in the water, sediment, and shrimp. Penta concentrations in shrimp (maximum of about 18 µg/g on day 20) were positively correlated with water column concentrations rather than with sediment concentrations. This suggests direct uptake from the water rather than biomagnification from infaunal and epifaunal prey. When the source of penta was removed, the compound rapidly disappeared from the water, shrimp, and sediments (in a matter of hours or days).

Crossland and Wolff (1985) determined the half-life of penta in outdoor ponds repeatedly dosed to maintain a concentration of 50 to 100 µg penta/L. The authors hypothesized that evaporation, sorption, hydrolysis, biodegradation, and indirect phototransformation of penta would be of minor importance under the environmental conditions at the ponds. The partition coefficient for penta between water and sediments was predicted to be near unity. The authors used the SOLAR mathematical model to calculate a direct photodegradation rate constant for the transformation of penta in the ponds where pH varied between 7.3 and 10.3, with a mean of 8.3. Several bioassays were conducted, and pond invertebrates were enumerated at levels of taxonomy exceeding Order. The results appeared consistent with the general body of literature describing the toxicity of penta. The observed half-lives of penta in the three treatment ponds varied between 2.0 and 4.7 days and were in good agreement with the predicted half-lives based on results of the SOLAR analysis. The authors concluded that direct phototransformation was responsible for nearly all the penta degradation observed in this study. In addition, they noted that at the end of the study, sediment concentrations of penta were very similar to water column concentrations. They hypothesized that insufficient time had elapsed for development of a microbial community capable of efficiently metabolizing penta in the sediments.

Robinson–Wilson and others (1983) examined the degradation of penta in a series of experimental ponds contaminated by a single high dose of penta (1,000 µg/L) followed by a series of small doses (0.2, 0.2, 0.4, and 0.4 µg/L) at monthly intervals. Two of the replicated sets of ponds held only

phytoplankton, whereas the third set of ponds contained rooted macrophytes. The authors found increased metabolism of penta in the ponds containing rooted macrophytes. The increased degradation of penta in the macrophyte ponds resulted in lower body burdens in channel catfish, bluegill, and largemouth bass. These fish species survived the highest dose of penta (1,000 µg/L) in the pond containing macrophytes but succumbed in the ponds containing only phytoplankton. The authors suggested three hypotheses as possible explanations for these results. However, only two of those hypotheses appear different from each other: (1) the presence of the macrophytes resulted in a different chemical or physical environment in the ponds that increased penta degradation, and (2) the macrophytes or aufwuchs community associated with the macrophytes were incorporating penta as a conjugate or within the cell structure.

Fisher (1990) observed that the concentration of dissolved penta increased with increasing pH but that the uptake by both organisms and sediments decreased. She also observed that increasing sediment organic carbon was associated with higher sediment levels of penta and reduced concentrations in the water column.

Delaune and others (1983) studied the fate of penta following a major spill in a Louisiana Gulf Coast estuary. They found that the degradation of penta was strongly influenced by sediment pH and redox potential. Degradation rates decreased with decreasing sediment redox and were maximized at pH 8.0, with reduced degradation at either higher or lower pH values. In addition, these authors found that penta was more tightly bound to oxidized sediment than to reduced sediment. They observed that all the penta had essentially disappeared from the spill area within 18 months and hypothesized that observed microbial degradation could account for the degradation. Sediments in the study area contained 3% to 5% carbon, and the sediment pH was essentially neutral at 6.8.

It is apparent that a variety of microorganisms are able to degrade penta. In general, it appears that aerobic degradation is more efficient than anaerobic degradation and that increased degradation occurs at moderate temperatures (between 10°C and 35°C). In most instances, it appears that penta degradation by microbial communities is enhanced by the presence of alternate carbon substrates.

### **Metabolism**

Fisher (1990) noted that algae, invertebrates, and vertebrates rapidly metabolized penta. Biotransformation rates were significantly greater in algae, especially at higher pH levels and in association with high-TOC sediments. Biotransformation rates in snails and fish varied between 0.52 and 3.88, with no significantly different rate between the two phyla.

Kukkonen and Oikari (1988) examined the metabolism of penta in the cladoceran *Daphnia magna*. The authors found

that neonate (<24 h old) and adult daphnia were equally able to metabolize penta and that humic content in the aquaria (dissolved organic carbon up to 23.5 mg/L) had no effect on the metabolic rate. The concentration of penta in these experiments was 20 µg penta/L, and the pH was low at 5.5. The authors did not determine a metabolic half-life. However, their data indicated that the concentration of free penta in the water column declined to 50% of the initial value in about 10 h and that 50% of the penta taken up by *Daphnia magna* was metabolized in approximately 24 h. They concluded that the principle metabolic pathway in *D. magna* involved sulfate conjugation.

Trujillo and others (1982) found the half-life of penta in midges to be 4.7 days. However, data in Lydy and others (1994) suggest that midge tissue concentrations of penta peaked at about 12 to 14 h, then declined rapidly to 50% of the maximum in about 24 h. The authors calculated a half-life of 15 h for penta in the midge.

Glickman and others (1977) determined penta half-lives in a variety of rainbow trout (*Oncorhynchus mykiss*) tissues. The values varied from 6.2 h in blood to 23.7 h in fat. Penta is lipophilic, and these results suggest that there is short-term sequestration in body lipids. The authors found high penta levels in the bile of these fish and concluded that the penta was being conjugated with bile and excreted.

Stehly and Hayton (1989) examined the metabolism of penta in rainbow trout, fathead minnows, sheepshead minnows, firemouth, and goldfish that had been exposed to penta for 64 h. They found that penta metabolism was species specific. Consistent with other studies, Stehly and Hayton found that biliary excretion accounted for less than 30% of the total penta metabolites and that 76% of the penta metabolites in bile consisted of penta-sulfate or penta-glucuronide. All metabolites excreted into the water were sulfate conjugates, and bile was enriched in glucuronide conjugates.

Similar results were demonstrated by Cravedi and others (1995) in Arctic char (*Salvelinus alpinus*) eleutheroembryos (end of yolk sac resorption or 50 to 100 mg wet weight). Test pH was 7.9 and duration was 48 h. They observed that penta-glucuronide accounted for 24.2% of the <sup>14</sup>C found in water at the end of 48 h. The parent penta accounted for 29.5%, and pentachlorophenylsulfate represented 49.4% of the <sup>14</sup>C present in the water column.

Benner and Tjeerdema (1993) studied the toxicokinetics and biotransformation of penta in a marine species of fish (*Atherinops affinis*). The fish were exposed to 50 µg penta/L for 24 h to determine the BCF, the elimination rate constant, and the elimination rate half-life. The absorption rate constant was 0.012/h, leading to a BCF of 278. The elimination rate constant was higher at 0.014/h, and an elimination half-life of 52.7 h was determined. During 24 h of exposure to clean seawater, topsmelt depurated 32.9% of the retained penta and

residues. Most (64.9%) of the penta was excreted unaltered. However, penta metabolites pentachlorophenylsulfate (18.9%) and pentachloro-β-D-glucuronide (16.2%) were also observed. The authors note that these same compounds have been identified as intermediates in the metabolism of penta by goldfish, fathead minnows, rainbow trout, firemouths (*Cichlasoma meeki*), sheepshead minnows, and striped bass. Benner and Tjeerdema concluded that topsmelt rapidly absorbed and more slowly depurated penta after short-term exposure through excretion and/or detoxification by sulfation and glucuronidation.

### Bioconcentration and Bioaccumulation

Bioconcentration and bioaccumulation of contaminants are of special importance because some aquatic species, most notably bivalves, have demonstrated an ability to rapidly bioconcentrate contaminants in water to high tissue levels. The concern is that persistent contaminants may move up the food chain, biomagnifying to higher concentrations in each trophic level, until contaminants found at nontoxic levels in the ambient environment reach concentrations where they cause stress and disease. For effective biomagnification and movement of contaminants through the food chain, several conditions must be met.

First, organisms must have the ability to bioconcentrate low levels of contaminants from the water column or their food. Second, these contaminants, or their toxic metabolic intermediates, must be retained, unaltered, in the tissues of the organism until it falls prey to an animal at a higher trophic level.

Several factors mitigate biomagnification. If contaminants are not absorbed, they cannot accumulate. Numerous organisms, particularly vertebrates, have the ability either to metabolize or excrete organic contaminants. The gut, liver, kidney, and gall bladder are common sites of penta concentration, metabolism, and excretion in vertebrates. If the contaminants are either rapidly excreted or metabolized to nontoxic compounds, then the chain is broken and biomagnification is not effective in passing contaminants upward through the food chain.

DDT is an excellent example of a persistent compound that was bioconcentrated from low levels in the water to higher levels—first in plankton, then in fish, and finally in bird populations, with devastating consequences.

### Bioconcentration of Penta From Water

The uptake of penta is a function of pH (Fisher 1990, Fisher and Wadleigh 1986) and not so much of water concentration. For example, at pH 4.0 penta is fully protonated and therefore highly lipophilic, resulting in higher bioconcentration potential. Conversely, penta is completely ionized at pH 9.0 with lower bioconcentration potential and significantly reduced toxicity. In general, Fisher (1990) observed a negative

correlation between the uptake of penta by algae, snails, and fish with pH. Highest bioconcentration factors (BCFs) were found at pH 4 (BCF = 117.2 to 681.9). Bioconcentration factors at environmentally realistic pH 6.0 and 8.0 ranged from 3.8 (algae at pH 8.0) to 271.1 (fish at pH 6.0).

Maekelae and Oikari (1990, 1995) determined penta BCFs from 145 to 342 in adult (55-mm valve length) freshwater mussels (*Anadonta anatina*). Tests were conducted at pH 6.5 in penta concentrations of 7 and 14 µg/L. However, an equilibrium penta concentration of only  $1.8 \pm 0.1$  µg/L was reached in 4 and 16 h in the two experiments. They found higher penta concentrations in the digestive gland and kidney compared with whole body soft tissues. In a more recent experiment using (<sup>14</sup>C) penta, these same authors (Makela and Oikari 1995) found steady state BCFs averaging 100 in *Anadonta anatina* and 73 in *Pseudanodonta complanata*. The pH in these experiments was 6.5, and the penta concentration was 9.7 µg/L. The BCFs were determined as the body burden of penta (measured in a number of ways) divided by the final concentration of penta. It must be recognized that when determining BCFs for labile chemicals, such as penta, the average exposure affecting uptake is likely much greater than the final concentration, which tends to inflate the BCF. It would appear that the development of biologically meaningful BCFs should use some intermediate concentration of the contaminant. An appropriate protocol would necessarily require consideration of the dynamics of degradation in the ambient water integrated over time compared with the depuration and metabolism of the contaminant in the organism at question.

Niimi and Cho (1983) suggested that penta is rapidly accumulated and eliminated by trout in the natural environment. Uptake from water appeared to be the most important pathway, and accumulation through food was thought to represent only a minor contribution. Consistent with Glickman and others (1977) and Rogers and others (1990), Niimi and Cho observed highest penta levels in the liver and bile of fish fed penta-contaminated feed to satiation for 40 days. Biliary excretion appeared to be a major route of depuration. They noted that the residence time of penta in water was short and suggested that its impact on fish would be most evident in localized areas that receive a continuous input of penta from a point source. Furthermore, Niimi and Cho (1983) concluded that bioaccumulation (biomagnification) of penta through the food chain is minimal and the observation that penta levels in smelt and alewife, the primary forage species for many Lake Ontario salmonids, were similar to concentrations found in the predators.

### **Bioaccumulation of Penta From Sediments**

The ultimate fate of penta deposited in aquatic environments is either decomposition in the water column or sedimentation. Fisher (1990) concluded that "Thus, for the organic sediment system, bioaccumulation will be determined by

interactions between pH, available sorption sites, degree of ionization of PCP, and levels of sediment ingestion."

Midges are deposit feeders and are known to rework sediments by feeding and burrowing. Therefore, midges are not only exposed to penta in interstitial water, but they also ingest contaminated particles. Fry and Fisher (1990) compared the bioaccumulation of penta by *Chironomus riparius* allowed to burrow in penta-contaminated sediments with similar midges held in suspension directly above the sediments. A third experiment exposed dead midge larvae to contaminated sediments to evaluate the passive uptake of penta. The authors documented BCFs of 229 from water and 7.3 from sediment in which the midges were burrowing.

The bodies of dead midges exposed to contaminated sediment yielded a BCF of 13.3. Fry and Fisher (1990) noted that Trujillo and others (1982) found the half-life of penta in midges to be 4.7 days. They hypothesized that the lack of metabolic degradation in the passive uptake experiment contributed to the higher ultimate penta body burden in the dead chironomids. However, they noted that ionized penta appeared to have a significant affinity for the body wall of the dead larvae and concluded that passive uptake from pore water appeared to be important to the accumulation of penta from sediments.

Fry and Fisher (1990) noted that penta has a log  $K_{ow}$  of 5.01. They concluded that penta does not behave like a neutral lipophilic compound and therefore its activity and fate are not predictable from its octanol–water partition coefficient. In contrast, Lydy and others (1994), found that BCFs in the midge *Chironomus riparius* (BCF = 458) were reasonably well predicted by a much lower octanol–water partition coefficient ( $K_{ow} = 0.758$ ) when the water concentration was 9 µg penta/L.

Fisher (1990) found that the sorption of penta to organic sediment significantly reduced its concentration in the water column at all pH levels, thereby reducing accumulation of penta in the microcosm's organisms. At environmentally realistic pH 6 to 8, BCFs were lower in microcosms having 3% TOC sediments compared with microcosms having sediments lacking any TOC.

Haque and Ebing (1988) examined the bioconcentration of penta from water and soil. They determined BCFs from soil of 6.3 for *Allolobophora caliginosa* and 22.2 for *Lumbricus terrestris*. They found that penta was rapidly bound to the soil and concluded that ingestion of contaminated particles was a significant pathway.

Van Gestel and Ma (1988) investigated the toxicity and bioaccumulation of penta in the earthworms *Eisenia fetida andrei* and *Lumbricus rubellus* in organically rich sandy soils (3.7% to 6.1% organic matter) with low pH (5.0 to 5.6). The LC<sub>50</sub> values for *Lumbricus rubellus* were 883 mg

penta/kg dry soil in Holten soil (pH 5.6 and 6.1% organic matter) and 1,094 mg penta/kg dry soil in Kooyenburg soil (pH ~5.0 and 3.7% organic matter). The differences in these LC<sub>50</sub> values were not significant. Bioconcentration factors were based on the average of the sediment values on day 0 and day 14. This appears a more reasonable approach than using the values on the last day, which was done by Maeke-lae and Oikari (1990). The BCF, based on bulk sediment penta concentration, varied between 3.4 and 8.0. In contrast, the BCFs varied between 426 and 996 when based on pore-water penta concentrations. The authors suggest that the porewater BCF was consistent with BCFs of 475 observed in fish.

### Biomagnification of Penta in Food Chains

Schuytema and others (1993) fed mealworms contaminated with 64.8 to 2,604 µg penta/g to African clawed frogs (*Xenopus laevis*) for 27 days. They observed no mortality in the frogs and no significant bioaccumulation of penta. Highest concentrations of penta were found in the frog's liver. However, these levels were inversely proportional to the level of penta in the mealworms. Penta was found in frog liver at <4.6 µg/g in frogs fed mealworms contaminated to 64.8 µg penta/g, whereas the liver of frogs fed mealworms with 2,604.6 µg penta/g contained penta at <0.6 µg/g.

Niimi and Cho (1983) determined the uptake from food and half-life of penta in rainbow trout (*Oncorhynchus mykiss*). Trout fed diets containing 40 µg penta/kg food attained whole body levels of 2 µg/kg during the 3-month study. Trout fed with penta-contaminated feeds with levels 75 times greater (3,000 µg/kg) accumulated 40 µg penta/kg at the end of 40 days. This level declined to 20 µg/kg by the end of the study. The biological half-life of penta in trout was estimated to be approximately 7 days. This observation is consistent with the literature describing the depuration and metabolism of penta in vertebrates and invertebrates. It appears that penta clearance in all tested organisms is fast enough to minimize any potential for the compounds to biomagnify in food chains.

### Toxicity

Penta is known to uncouple oxidative phosphorylation, inhibiting adenosine triphosphate (ATP) pathways important to respiration in both animal and plant cells. In addition, Moreland and Hilton (1976) described penta as a general inhibitory uncoupler, suggesting that it has several sites of action, including photophosphorylation, protein synthesis, and lipid biosynthesis (Morrod 1976). All the mechanisms of penta's toxicity have not been precisely defined but may generally involve the disruption of cellular membranes (Jayaweera and others 1982, Senger and Ruhl 1980, Smejtek and others 1983).

Acute toxicity causes observable physiological lesions and is usually measured by mortality. Penta interferes with the

**Table 21—Acute toxicity of freshwater fish to penta (96-h LC<sub>50</sub>)<sup>a</sup>**

Species	Concentration (µg penta/L)
<i>Oncorhynchus mykiss</i> (rainbow trout)	34 to 121
<i>Oncorhynchus nerka</i> (sockeye salmon)	63 to 68
<i>Oncorhynchus tshawytscha</i> (chinook salmon)	68 to 78
<i>Salmo salar</i> (Atlantic salmon)	500
<i>Salvelinus fontinalis</i> (brook trout)	128
<i>Lepomis macrochirus</i> (bluegill)	120 to 350
<i>Micropterus salmoides</i> (largemouth bass)	136 to 287

<sup>a</sup>Reported in Eisler (1989).

oxidative phosphorylation by uncoupling the production of adenosine triphosphate from adenosine diphosphate. Because this process provides the energy source for cellular metabolism in most organisms, penta is a broad-spectrum biocide.

A common measure of acute toxicity is the concentration of a toxicant that causes 50% mortality in a test population within some specified period (often 96 h). This parameter is referred to as the 96-h LC<sub>50</sub>. Eisler (1989) summarized 96-h LC<sub>50</sub> concentrations for aquatic organisms. For most freshwater species, the 96-h LC<sub>50</sub> varied between 100 and 2,000 µg penta/L. In general, Eisler's (1989) data suggest that freshwater vertebrates (fish) are more sensitive than invertebrates. Table 21 summarizes the lower LC<sub>50</sub> values provided by Eisler (1989) for salmonids and centrarchids. Salmonids of the genus *Oncorhynchus* appear most sensitive. In contrast, invertebrate LC<sub>50</sub> values are typically greater than 100 µg/L.

### Chronic Toxicity

Toxicants can have subtle effects that are important to the competitiveness of individuals in natural environments and the sustainability of populations of organisms. Numerous endpoints are evaluated in assessing chronic effects. Most commonly, these endpoints involve reproduction and/or growth. The lowest contaminant concentration in a bioassay that does not produce an observable effect is referred to as the no observed effect concentration (NOEC). The lowest concentration at which the effect being evaluated is observed is referred to as the lowest observed effect concentration (LOEC). If an effect is observed at 200 µg/L but not at 100 µg/L, the first value would be reported as the LOEC and the second as the NOEC. The actual effects threshold would be somewhere between the two values. ENVIRON (1996) summarized a number of chronic endpoints for aquatic fauna and flora exposed to penta. In Table 22, data are further summarized to include only those that included the pH test.

**Table 22—No observed effects level (NOEL) and lowest observed effects level (LOEL) associated with penta in freshwater environments<sup>a</sup>**

Endpoint	Duration	Species	NOEL	LOEL	pH	°C	EPA
Reproduction; number viable eggs	16	<i>Lymnaea stagnalis</i> (snail)	50	NR	8.0	18.3	46.5
Larval survival and reproduction	10 – 28	American Flagfish	55	102	6.95	25	5.4
Biomass & mortality, eggs @ 10°C; alevins @ 15°C; fry @ 20°C	>28	<i>Oncorhynchus mykiss</i>	10.9	25	8.0	10 – 20	15.6
Hatchability, survival, & growth	32	<i>Pimephales promelas</i>	16.5	34.6	6.5	25	3.5
Survival & growth (fry & juveniles)	90	<i>Pimephales promelas</i>	6	13	7.4	25	8.6
Survival & growth (fry & juveniles)	90	<i>Pimephales promelas</i>	36	85	7.4	25	8.6
Survival & growth (fry & juveniles)	90	<i>Pimephales promelas</i>	>130	>130	9.4	25	63.9
Early life stage hatchability, survival, & growth	32	<i>Pimephales promelas</i>	44.9	73	7.55	25	10.0
Hatchability, survival, & growth	32	<i>Pimephales promelas</i>	63.7	125	8.5	25	25.9
Hatchability, survival, & growth	32	<i>Pimephales promelas</i>	27.6	58.2	7.5		9.5
Hatchability, survival, & growth	32	<i>Pimephales promelas</i>	32	75	8.0		15.6
Growth	56	<i>Chaetogammarus marinus</i>	100	NR	8.0	NR	15.6
Number of viable oocytes	18	<i>Oncorhynchus mykiss</i>	11	19	7.4	12	8.6
Number of viable oocytes	18	<i>Oncorhynchus mykiss</i>	12	22	7.5	12.5	9.5
Reproduction	21	<i>Daphnia magna</i>	180	320	8.0	20	15.6
Survival and reproduction	7	<i>Daphnia magna</i>	100	500	8	20	15.6
Inhibition of cell growth	5	<i>Skeletonema costatum</i>	11	20	8.1	19 – 22	17.3
Inhibition of cell growth	5	<i>Selenastrum capricornutum</i>	12	17	7.5	24 – 25	9.5
Inhibited cell growth	5	<i>Anabaena flos-aquae</i>	7.8	18	7.5	24 – 25	9.5
Growth, reduction; biomass	21	<i>Elodea canadensis</i>	230	380	7.95	22	14.9
Inhibited cell growth	5	<i>Inavicula pelliculosa</i>	40	77	7.5	25	9.5
Inhibited cell growth	5	<i>Anabaena flos-aquae</i>	7.8	18	7.50	24 – 25	9.5
Fron density & biomass	14	<i>Lemna gibba</i>	32	72	5.0	23 – 27	0.8

<sup>a</sup>All penta values are in µg penta/L.

Roszell and Anderson (1994) examined the effect of penta on nonspecific immune function in two phagocytic cell populations isolated from the estuarine fish, *Fundulus heteroclitus*. They found that phagocytosis of yeast particles was significantly inhibited at penta concentrations greater than 5,000 µg/L. A comparison of phagocytic response between controls and a penta level of 1,000 µg/L did not reveal significant differences.

Brown and others (1987) observed a reduced number of feeding acts in the young of largemouth bass exposed to penta concentrations of 67 and 88 µg/L but not at concentrations less than 67 µg/L. Endpoints measured included the number of feeding attempts and the number of misses and mistakes. The NOEL was 45 µg penta/L. Hardness (65 mg/L calcium carbonate) and pH (7.7) were not measured directly in this experiment but were assumed equal to that observed in a 1985 study using the same water supply.

Keller (1993) noted that in 1993 more than 40 species of freshwater unionid mussels were listed as endangered or

threatened under the Endangered Species Act. Keller determined a 48-h LC<sub>50</sub> value in juvenile *Anadonta imbecilis* at pH 7.0 and compared the results with concurrent bioassays on *Daphnia magna* and *Lepomis macrochirus*. Results indicated that the 48-h LC<sub>50</sub> for the juvenile mussels (610 µg penta/L) was greater than the 48-h LC<sub>50</sub> for the daphnid (330 µg/L) or the 96-h LC<sub>50</sub> for bluegills (240 µg/L). At pH 7.0, the EPA chronic penta water quality standard is 5.73, giving a safety factor of 106.

### Temperature Effects

Fisher and Wadleigh (1986) examined the toxicity of penta to the midge *Chironomus riparius* at 15°C, 25°C, and 35°C. The endpoint she examined was a flight response following stimulation with a pair of forceps during exposure to PCP in soft water at pH 7.0. Fisher observed that EC<sub>50</sub> values increased from 1,176 µg/L at 15°C to 1,556 at 25°C, then declined to 631 µg PCP/L at 35°C. She concluded that midge metabolism increased at the higher temperatures and that penta's interference with respiration (phosphorylation of

ADP to ATP) at the higher metabolic rate was responsible for the increased EC<sub>50</sub> at 35°C. Similar results were reported by Fisher (1991) in an experiment conducted at pH 4, 6, and 8. The EC<sub>50</sub> values for penta in this study varied between 253 µg/L at pH 4 and 35°C to 2,052 µg penta/L at pH 8 and 25°C. Eisler (1989) cites similar results from Hedtke and Arthur (1985) who reported positive correlations between EC<sub>50</sub> and temperature for fathead minnows (*Pimephales promelas*), the isopod *Asellus racovitzai*, and the snail *Physa gyrina*.

In summary, it appears that the toxicity of penta to aquatic species increases with increasing temperature. Similar increases in the toxicity of penta to *Notopterus notopterus* with increasing temperature (16 °C, 23 °C and 36°C) were observed by Gupta and others (1983).

### **pH Effects**

Fisher (1991) demonstrated that the toxicity of penta is inversely related to pH. The effective concentration (EC<sub>50</sub>) of penta resulting in failure of *Chironomus riparius* to execute an appropriate flight response increased from 384 µg/L at 25°C and pH 4 to 2,052 µg penta/L at the same temperature and pH 8.0.

Smith and others (1987) examined the toxicity of penta to *Selenastrum capricornutum* and found that culture media equilibrium pH and 96-h EC<sub>50</sub> were strongly correlated ( $r = 1.00$ ) between pH 7.3 and 8.5. The authors concluded that the toxicity of penta is due primarily to the concentration of the undissociated compound.

The dependence of penta toxicity on pH was further elucidated by Spehar and others (1985) in fish and amphipods. They found that acute exposures in all three species showed that penta toxicity was decreased with increasing pH. Kaila and Saarikoski (1977) observed a similar response in crayfish (*Astacus fluviatilis*). The 8-day LC<sub>50</sub> decreased from 53 mg/L at pH 7.5 to 9 mg penta/L at pH 6.5.

Stehly and Hayton (1990) described the uptake and clearance of penta in goldfish (*Carassius auratus*) as a function of environmental pH (7.0, 8.0, and 9.0). The authors found reduced uptake and clearance at increasing pH. They concluded that pH-related changes in the pharmacokinetics of penta resulted in a decrease in its BCF with increasing pH and suggested that this could account for both the decreased capacity of fish to accumulate penta and its reduced toxicity at higher pH values.

Early life-stage exposures of fathead minnows showed that chronic penta toxicity and bioaccumulation were similarly decreased when pH values were increased. Stehly and Hayton developed a relationship describing bioconcentration as a function of pH. Reported bioconcentration values ranged from 1,066 at pH 6.5 to 281 at pH 8.5:

$$\text{Fathead minnow BCF} = 10^{[4.80 - 0.28(\text{pH})]} \quad (R^2 = 0.94)$$

The authors concluded that the decrease in chronic penta toxicity appeared to be due to reduced bioaccumulation and toxicity as a direct result of the increased dissociation at higher pH values.

### **Water Hardness Effects**

Inglis and Davis (1972) examined the effects of water hardness (13.0, 52.2, 208.7, and 365.2 mg calcium carbonate/L) on six species of fish, including rainbow trout. Reported values of pH ranged from 7.8 at a hardness of 13.0 mg/L to pH 8.0 at all other values of hardness. The authors concluded that water hardness had no significant effect on the toxicity of penta to any of the tested species.

### **Dissolved Organic Carbon Effects**

Lee and others (1993) examined the acute toxicity of penta to zebrafish (*Brachydanio rerio*) and the cladoceran (*Daphnia magna*) at TOC concentrations varying between 0.0 and 50 mg/L. They observed no significant difference in the 96-h EC<sub>50</sub> (zebrafish bioassay) or 48-h EC<sub>50</sub> (*Daphnia magna*) as a function of TOC at any of the tested values.

### **Toxicity to Aquatic Plants**

Smith and others (1987) investigated the toxicity of penta to *Selenastrum capricornutum* as a function of pH and found that the 96-h EC<sub>50</sub> was given by the relationship

$$\begin{aligned} 96\text{-h EC}_{50} (\text{Selenastrum capricornutum}) \\ = \exp[0.847(\text{pH}) - 4.28] \end{aligned}$$

The regression coefficients in this relationship are similar to the current EPA penta standard and demonstrate reduced toxicity at elevated levels of pH where more penta is in the dissociated form (that is, having lost the OH radical). The authors suggested that the toxicity of penta is primarily associated with the undissociated species of the compound.

### **Regulatory Criteria for the Protection of Aquatic Resources**

Section 304 of the Clean Water Act requires the EPA to publish and update ambient water quality criteria (USEPA 1980a). These criteria reflect the latest scientific knowledge of the identifiable effects on the health and welfare of aquatic resources including fauna, flora, and human uses.

The brief review provided in this document is consistent with U.S. EPA (USEPA 1986) in predicting that penta acute and chronic toxicity are inversely correlated with water pH and dissolved oxygen concentration and directly correlated with temperature. At pH 6.5, the U.S. EPA found that acute values ranged from 4.4 to greater than 43,920 µg penta/L. Chronic values ranged from less than 1.835 to 79.66 µg penta/L. The mean acute-chronic ratios ranged from 0.89 to 15.79 µg/L. Freshwater algae were affected by concentrations as low as 7.5 µg/L, whereas vascular plants were adversely affected at



greater than 89 µg/L. Bioconcentration factors ranged from 7.3 to 1,066 in three species of fish.

Procedures described in the *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (USEPA 1985) indicate that except where a locally important species is highly sensitive to penta, freshwater aquatic organisms and their uses should not be affected unacceptably if the average short-term concentrations (µg penta/L) do not exceed the numerical values given by

Acute criterion:

$$1\text{-h average concentration} < \exp[1.005(\text{pH}) - 4.830] \mu\text{g/L}$$

Chronic criterion:

$$4\text{-day average (once every 3 years)} < \exp[1.005(\text{pH}) - 5.290] (\mu\text{g/L})$$

Note that these are not appropriate concentrations for continuous exposure, and they are not no-observed-effect levels or threshold effects levels. For instance, at pH 6.8, the chronic criterion for penta is 4.68 µg/L. At this pH, the U.S. EPA (USEPA 1986) notes that a penta concentration of 1.74 µg penta/L caused a 50% reduction in growth of yearling sockeye salmon in a 56-day test. This may seem inconsistent with the chronic EPA value. However, remember that the EPA criterion is for a maximum 4-day exposure, not a 56-day exposure.

These criteria are not rules, and they do not have regulatory impact. However, several states have used the U.S. EPA (USEPA 1986) as the basis for setting regulatory standards for potentially toxic compounds, including penta:

Washington State (WAC 173-201), Texas (30 TAC 307.6), Utah (UAC R317-2-14), Florida (FAC 62-302.530), Indiana (327 IAC 2-1-6), and Oregon (OAR 340 41).

Different criteria have been proposed or adopted by other jurisdictions. The most prominent of these were reviewed by ENVIRON (1996). Data for continuously distributed criteria provided in ENVIRON (1996) were subjected to nonlinear regression analyses. The results are presented in Table 23 and Figure 23.

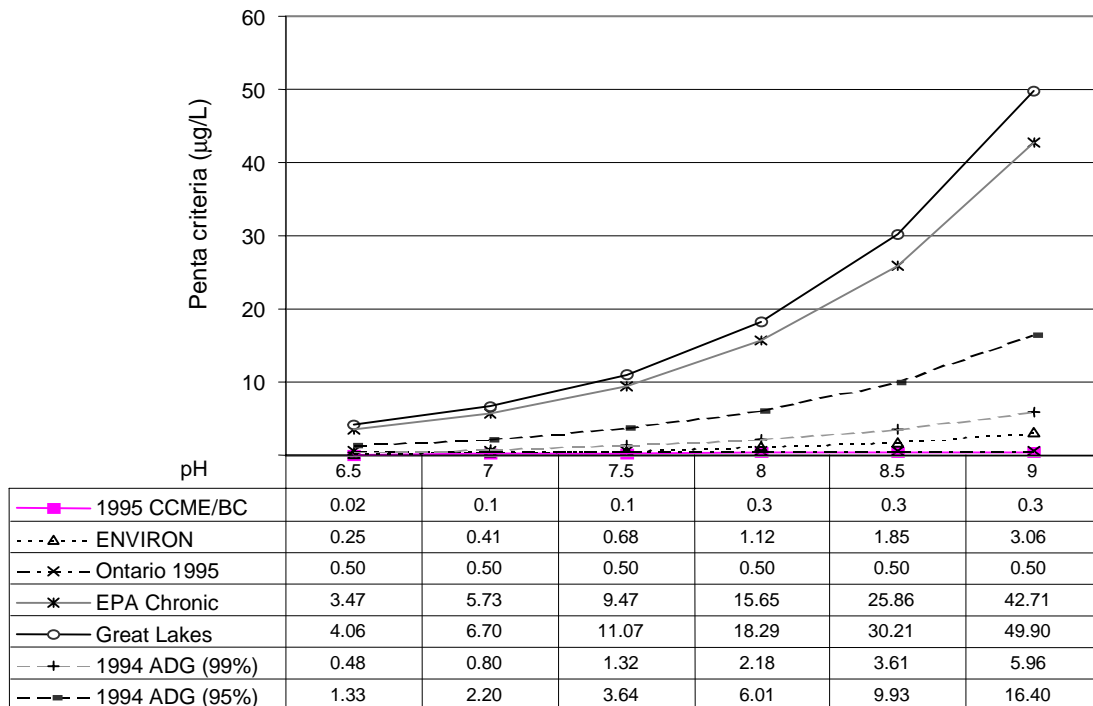
The Draft 1995 CCME criteria and the 1995 Ontario guidelines (ENVIRON 1996) do not adequately reflect the effect of pH on the toxicity of penta. These criteria are either under protective at low pH or over protective at high pH. The 1994 Aquatic Risk Assessment and Mitigation Dialogue Group (ADG) Level of Concern Approach (ENVIRON 1996) produces two sets of criteria. The lower values are sufficient to protect 99% of the species, and the upper values are sufficient to protect 95% of the species. These are continuous criteria; therefore, it is inappropriate to compare their recommendations with those of the 1-h and 96-h U.S. EPA criteria.

In response to recommendations made by an EPA task force, the ADG was sponsored by the U.S. EPA in 1992 and the North American Chemicals Association in 1993. The ADG included representatives of the U.S. EPA, agrochemical companies, academia, and environmental and agricultural interest groups. The Society of Environmental Toxicology and Chemistry (SETAC) acted as a facilitator for meetings of the group and published a final report with recommendations (SETAC 1994). The ADG recommended an “integrated probabilistic risk assessment approach that included both the

**Table 23—Various jurisdictional criteria for penta<sup>a</sup>**

Jurisdiction	Criterion	Basis for criterion
Proposed 1995 interim CCME (1997) guidelines	0.02 at pH 6.5; 0.1 at pH = 7.0 or 7.5; 0.30 at pH ≥ 8.0	Extrapolation from acute guppy data; safety factor = 0.1
ENVIRON (1996) calculated	Chronic criterion = $\exp[1.002(\text{pH}) - 7.90]$	Application of 0.1 safety factor to lowest values using 1991 CCME chronic LOEC in the database
Ontario Canada ENVIRON (1996)	0.5 at all values of pH	Application factor of 0.01 applied to the lowest mean species-specific acute LC <sub>50</sub> independent of pH
U.S. EPA (1986)	4-day chronic = $\exp[1.004(\text{pH}) - 5.28]$	Statistical approach using all relevant acute toxicity data; set to protect 95% of species; ACR = 3.166
Great Lakes Water Quality Initiative (ENVIRON 1996)	4-day chronic = $\exp[1.004(\text{pH}) - 5.12]$	Similar to U.S. EPA approach except the ACR = 2.608
SETAC (1994)	Continuous chronic = $\exp[1.005(\text{pH}) - 7.26]$	Graphical approach using entire database of Group Level of Concern primary chronic NOECs

<sup>a</sup>The U.S. EPA and Great Lakes chronic criteria are for 4-day exposures. Others listed are continuous criteria. All values are in µg/L.



**Figure 23—Various jurisdictional criteria defining allowable concentrations of penta in freshwater as a function of average pH. All values are in micrograms penta per liter.**

probability of exposure and effects.” ENVIRON (1996) applied the ADG methodology to derive penta chronic toxicity guidelines sufficient to protect 90%, 95%, and 99% of species. The results for the protection of 95% and 99% of species are included in Table 23 and Figure 23.

### **Carcinogenicity**

Jorens and Schepens (1993) note that penta is not classified as a human carcinogen but suggest that historical contaminants in commercial penta products, such as chlorodibenzo-p-dioxins, are known carcinogens. The EPA has classified penta as a B2 carcinogen (that is, no evidence of carcinogenic response in humans, but evidence in animals is sufficient to cause the compound to be suspect). Similarly, Health Canada (1994) and IARC (1991) have classified penta as a Group III.B compound that is possibly carcinogenic to humans.

### **Mutagenic and Teratogenic Effects**

Venegas and others (1993) examined the teratogenic effects of penta in an amphibian (*Caudiverbera caudiverbera*). They examined embryonic development in water containing 15, 150, 300, and 1,500 µg/L penta. Results of the micronucleus test on premetamorphic larvae indicated higher micronucleus formation rates in the controls than in the various penta treatments. Chromosomal aberration tests in anaphase and telophase provided a second endpoint. The authors concluded that the highest penta dose resulted in inhibition and

delays in development of normal growth of embryos of this amphibian. However, the study found no clear mutagenic effects. CCME (1997) concluded that although chlorophenols may have reproductive and fetotoxic effects, they do not appear to be teratogenic or mutagenic.

### **Toxicity Summary**

For the purpose of this risk assessment, we used the U.S. EPA chronic criteria as a benchmark against which to compare levels of penta migrating into the water column during the first 4 days. The 1994 ADG, necessary to protect 99% of species, was used as a second benchmark against which to assess long-term water column concentrations of penta within a few centimeters of the immersed wood on days  $\geq 5.0$  following immersion of the treated wood.

### **Penta Sediment Quality Benchmarks**

Washington State (Washington Administrative Code, Chapter 173-204-320) has developed an apparent effects threshold (AET) penta standard for marine sediments at 360 µg/kg. The AET is the lowest concentration of a chemical above which adverse effects are always observed in Puget Sound sediments. Adverse effects are determined from laboratory bioassays on a variety of test animals and on the paired analysis of infaunal communities and sediment chemistry. These standards are considered sufficient to protect most marine organisms.

The New York State Department of Environmental Conservation (NYSDEC 1993) has established a freshwater sediment criterion for penta of 100 µg penta/g sedimented organic carbon for acute toxicity and 40 µg penta/g sedimented organic carbon to prevent chronic toxicity. These criteria are based on an equilibrium partitioning model and state water quality criteria for penta and are intended for use in screening contaminated sediments. The NYSDEC criteria are expressed as a concentration in sediment by prescribing an organic carbon content. For example, in a sediment with 1% organic carbon, the corresponding chronic criterion value is 400 µg penta/kg dry sediment ( $40 \mu\text{g/g TOC} \times 0.01 \text{ TOC} = 0.4 \mu\text{g/kg}$ ), or 800 µg/kg at 2% TOC, 1,200 µg/kg at 3% TOC, etc.

The Washington State marine standard of 360 µg penta/kg dry sediment weight was used as a benchmark in assessing marine environmental risks associated with the use of penta-treated wood, and the NYSDEC criteria was used for freshwater sediments. The risk assessment program automatically computed the sediment penta criteria based on a user input for TOC.

### Loss of Penta

Southern yellow pine piling was treated to 8.0 kg/m<sup>3</sup> (0.5 lb/ft<sup>3</sup>) of penta (in the treated zone) and immersed in freshwater and salt water at a variety of pH values. Penta loss rates used in this assessment were developed data from a 1994 study, *Pentachlorophenol – Leaching From Utility Poles Exposed to the Aquatic Environment*, prepared by Springborn Laboratories for the Pentachlorophenol Task Force as part of data call-in for re-registration of penta by the U.S. EPA (Connor 1994). The study was produced and compiled in accordance with all pertinent U.S. EPA Good Laboratory Practice regulations.

The utility poles chosen for testing were selected from a kiln-dried stock of southern yellow pine. Two utility pole sections, approximately 3.3 m (11 ft) long and 25.4 cm (10 in.) in diameter were treated to 8.0 kg/m<sup>3</sup> (0.5 lb/ft<sup>3</sup>) of penta in the treated zone using the Lowry Process. An additional 3.3-m (11-ft) untreated utility pole section was selected from the same kiln lot as a control. The three poles were cut into 30 segments.

The pole segments were leached in tanks containing unbuffered reagent water, reagent water buffered at pH 5, 7, and 9, seawater, and 0.1 N hydrogen chloride solutions. Triplicate tanks of each solution contained a treated pole segment, with an additional tank for each solution containing an untreated control. In each tank, the pH was measured daily and adjustment made with either acid or base to maintain the pH within ±0.1 units. Leachate temperature varied between 18.3°C and 22.0°C during the experiment, with a mean and standard deviation of  $20 \pm 0.2^\circ\text{C}$ . The air-exposed cut end of the pole sections was sealed with epoxy, and TENAX traps (TENAX SpA, Italy) were installed in the

sealed tanks to capture any volatilized penta. The traps did not reveal evidence of the volatilization of penta during the study.

The immersed, cut ends of the poles were not sealed. Haloui and others (1995) determined the diffusivity of penta along three orthogonal axes in treated wood. They found that the longitudinal diffusivity (parallel to the grain structure) was seven times greater than either the radial or tangential diffusivity. This has implications for leaching studies, because if the end of the pole is exposed to water, as it was in this case, it can represent a significant fraction of the total leaching surface area. Penta released from the cut end of a pole or piling that is driven into sediments about 3 m will not be available to the water column. Therefore, in estimating the preservative lost to the water, only the surface grain should be considered. A pole diameter of 25.4 cm (10 in.) would result in a cut-end surface of 506.7 cm<sup>2</sup>. If the length of the pole was 27.8 cm (11 in.), then the horizontal grain surface area exposed to the water would be 2,218.2 cm<sup>2</sup>. If preservative is lost seven times faster at the cut end, then the loss rates determined in this study should be reduced to 47% of the observed rate [ $(506.7 + 2218.2) \text{ Observed Rate of Loss} \div (7 \times 506.7 + 2218.2) = \text{Longitudinal Surface Loss Rate} = 0.47 \times \text{Observed Loss Rate}$ ].

There is a second problem associated with conducting static leaching tests of highly degradable organic compounds such as penta. The study did not state that the tanks were held in the dark. However, no information was given describing the lighting conditions (intensity or wavelength) under which the glass aquariums were held. The literature describing the degradation of penta clearly demonstrates that in normal sunlight, penta half-lives can be several days, certainly less than the 30 days over which this experiment was conducted. Therefore, some photolysis of the penta should be anticipated during the experiment. Secondly, both fungi and bacteria have demonstrated an ability to degrade penta (McAllister and others 1996). Connor (1994) noted the presence of fungi in the pH 5 and 7 treatments after approximately 1 week of immersion. Bacterial growths were reported in all tanks in this study. It is likely that these growths were metabolizing penta following the first week or two of immersion.

The antagonistic effects associated with increased loss rates from exposed end grain and decreased loss rates associated with the metabolism of penta by microbes and degradation by light were not quantifiable in this experiment, and no correction was attempted. However, note that the increases as a result of not sealing the end grain would be observed immediately as would decreases associated with photodegradation. The decreases in calculated loss rate associated with microbial catabolism would not be observed for probably 5 to 10 days. As shown in the following paragraphs, this risk assessment focuses on the first 5 days of immersion because that is the period in which maximum penta concentrations are anticipated in the water column. Therefore, it appears that the

assessment may be somewhat conservative in that greater loss rates associated with the exposed end grain were not being balanced by microbial degradation in the near term.

The leachate was analyzed following 1, 3, 7, 14, 21, and 30 days of immersion using high performance liquid chromatography (HPLC). As previously noted, the study followed EPA Good Laboratory protocols. Note that between 99.9% and 108% of the penta added to spiked samples was recovered in this study.

Data provided by Connor (1994) were analyzed in the Statistica Non-Linear Estimation algorithm and results were used to develop loss rates per square centimeter per day. The sampling times were converted to the mean day during the sample period rather than the day on which samples were collected. The resulting predictive equation is provided. The regression explained 73% of the variation in the database, and each of the final coefficients were significant at  $\alpha = 0.05$ .

$$\text{Penta loss} = 10.9 \exp[-0.255(\text{day}) + 0.355(\text{pH}) + 0.01(\text{salinity})] \mu\text{g}/\text{cm}^2\text{-day} \quad (6)$$

This expression was used to estimate the loss of penta from poles treated to  $8.0 \text{ kg}/\text{m}^3$  ( $0.5 \text{ lb}/\text{ft}^3$ ) in the treated zone.

### Dioxins in Penta

Penta does not contain 2,3,7,8 tetrachloro-dibenzo-dioxin (2,3,7,8 TCDD). The U.S. EPA has established water quality criteria for this compound of  $<0.01 \mu\text{g}/\text{L}$  maximum and  $0.00001 \mu\text{g}/\text{L}$  continuous concentrations.

Penta does contain less toxic hepta- and hexa-dioxins (HxCDD) for which no water quality standards were

available. It is possible, using the toxic equivalency factors (with respect to TCDD) for these compounds and summing over their concentration in commercial penta to express the sum in terms of a toxic equivalency quotient (TEQ). The toxic equivalency for penta is  $2.84 \text{ mg}/\text{L}$ . This risk assessment assumes that the hepta- and hexa-dioxins found in penta are lost to aquatic environments in proportion to their relative proportion in the preservative ( $2.84 \text{ mg dioxin}/\text{L}$  of preservative). The resulting value can then be compared with the EPA standards for 2,3,7,8 TCDD in assessing risk.

### Results From Penta-Treated Cougar Smith Bridge

A total of 11 penta-treated bridges were considered as candidates for this risk assessment. All but four of these were eliminated because they also contained creosote-treated components. The Cougar Smith Bridge (Washington state) crossing the West Fork of the Satsop River is an HS20 structure with a clear span of 60.96 m (200 ft). This bridge was constructed in 1996. All wood components of this bridge were preserved to a retention of  $8.0 \text{ kg}/\text{m}^3$  ( $0.5 \text{ lb}/\text{ft}^3$ ) (in the treated zone), with penta dissolved in light solvent oil. The bridge sits on concrete footings, and the treated wood is not immersed in water. The Satsop River is used extensively by several species of salmon. Salmon were observed spawning in the gravel under and just downstream from the bridge in the fall of 1997.

The Cougar Smith Bridge was originally scheduled for survey in the fall of 1997. However, an early series of storms raised the Satsop River above flood stage, and the monitoring was rescheduled for August 4, 1998, to allow for possible preservative loss associated with migration of the solvent oil during summer heating. The location of the bridge is

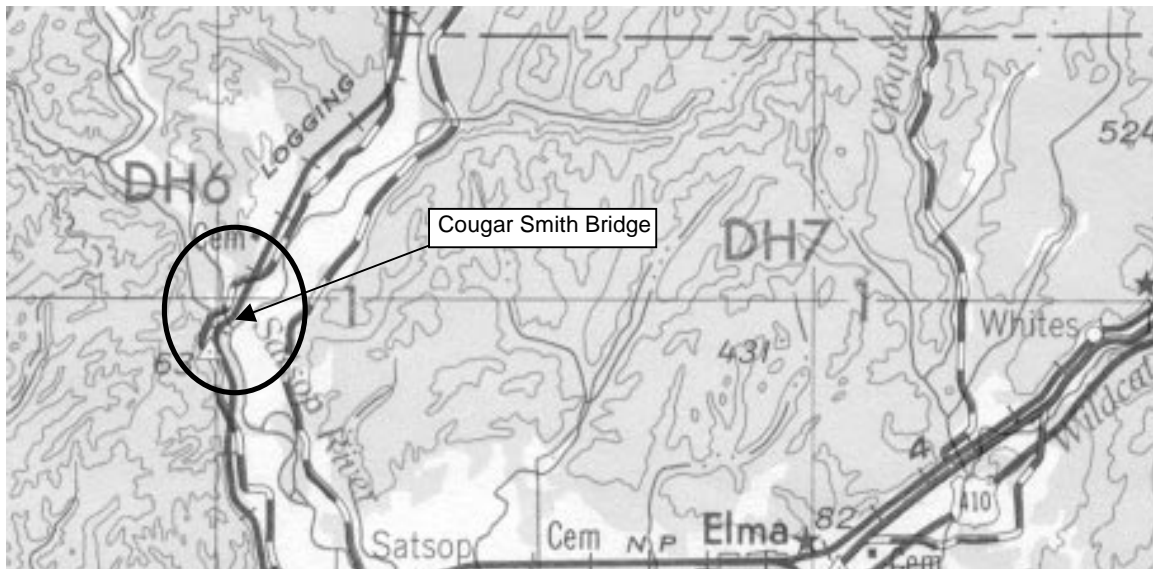


Figure 24—Location of the Cougar Smith Bridge crossing the West Fork Satsop River in Southwestern Washington.



**Figure 25—Cougar Smith Bridge (a) as viewed from the north showing the treated-wood arches and (b) underside of bridge showing the treated-wood deck and support beams.**



**Figure 26—West Fork Satsop River flowing under the Cougar Smith Bridge in southwestern Washington.**

described in Figure 24. Figure 25a depicts the entire structure, and Figure 25b is included to illustrate the large quantity of treated wood involved in the bridge decking and support beams.

As shown in Figure 26, the Satsop River was experiencing moderate to low flows during the August 4, 1998, survey. An area of the river with slowest flows and relatively sandy sediments was chosen for the sampling transect because this area appeared most likely to contain elevated levels of the preservative. Current speeds averaged 27 cm/s along the sampling transect. Currents in the main channel averaged 63.7 cm/s in water averaging a 36-cm depth.

During this survey, the weather was clear and sunny with no wind. The water temperature was 13.5°C, and the air temperature was 23.9°C at 1100 hours. Water pH was essentially neutral, averaging  $6.90 \pm 0.1$  (three measurements). Water column alkalinity was 33.25 and hardness was 28.16. Conventional physicochemical characteristics of sampled sediments are described in Table 24. Sediments along the sampled transect were dominated by gravel and cobble with minor amounts of sand, silt, and clay. Sediment total volatile solids (TVS) averaged  $1.74 \pm 0.16\%$ , equivalent to 1.04% TOC.

#### ***Sediment and Water Column Concentrations***

Penta was not detected in the water column at the upstream control station or under the bridge at the detection limit of 0.25 µg/L (three samples each station) (Table 24). Penta was detected in one sediment sample located 0.45 m (1.5 ft) downstream from the downstream perimeter of the bridge at a level of 19.0 µg/kg. The sediment concentration at the 1.8 m (6.0 ft) downstream station was estimated at 6.6 µg/kg, which was less than the calculated detection limit. Penta was not detected in other samples at this bridge.

#### ***Sediment Benchmark***

As previously noted, the New York State Department of Environmental Conservation (NYSDEC 1993) has established a freshwater sediment criterion for penta at 40 µg penta/g organic carbon to protect aquatic organisms from chronic effects. Total organic carbon in sediments under the Cougar Smith Bridge was calculated at 1.04%. At this level of TOC, the New York State benchmark would be 420 µg penta/kg dry sediment weight. The maximum level observed was 4.5% of this benchmark, and adverse biological effects were not anticipated.

#### ***Biological Response—Infauna***

The Satsop River supported a complex and abundant mix of megafauna that were not quantitatively sampled. These included numerous schools of juvenile salmon and hundreds of sculpins (Family Cottidae) observed under the bridge along with dozens of crayfish (*Pacifastacus* sp.).

**Table 24—Physicochemical characteristics observed at the Cougar Smith Bridge on the West Fork Satsop River in southwest Washington State on August 4, 1998<sup>a</sup>**

Station	Depth (cm)	RPD (cm)	Penta <sup>b</sup> (µg/kg)	TVS (%)	Sand (%)	Silt and Clay (%)
-9.9 m (-33 ft) upstream control	16.0	>10.0	7.5 U	1.56	5.99	1.86
0.0 under bridge	25.3	>10.0	7.4 U	2.00	8.52	1.90
0.45 m (1.5 ft) downstream	30.0	>10.0	9.0	1.91	1.06	1.69
0.9 m (3.0 ft) downstream	28.0	>10.0		1.44	8.27	3.61
1.8 m (6.0 ft) downstream	22.0	>10.0	6.6 J	1.89	11.21	4.02
3 m (10 ft) downstream	15.5	>10.0		1.70	14.35	4.03
6 m (20 ft) downstream	15.5	>10.0	7.4 U	1.66	9.25	5.19
9.9 m (33 ft) downstream	16.0	>10.0	7.2 U	1.73	9.58	3.83

<sup>a</sup>All values are the mean of two replicates except the -9.9 m (-33 ft) upstream control and samples taken under the bridge, which are the mean of three replicates.

<sup>b</sup>J indicates an estimated value when that result is less than the calculated detection limit. U indicates the compound was not detected at the given detection limit.

A total of 12,787 invertebrates in 69 taxa were identified in 18 samples at the Cougar Smith Bridge. Taxonomic codes used in the database are defined in Appendix C. Dominant taxa were defined as those representing 1% of the total in-faunal abundance (count = 128). The community was fairly evenly distributed, with 18 taxa meeting the 1% criterion. Dominant taxa are identified in Table 25. These 18 taxa (26% of total taxa) represented 90.2% of the total abundance.

The dominant taxa included many recognized as intolerant of metals (particularly copper) and other pollutants (Orders Ephemeroptera, Plecoptera, and Trichoptera) and sensitive chironomids (including *Tanytarsus* sp. and *Cricotopus* sp.). The number of taxa, total species abundance, and dominant species abundance are displayed in Figure 27 as a function of distance upstream and downstream from the Cougar Smith Bridge. Figure 28 summarizes Shannon's and Pielou's Indexes at these same stations.

Figures 27 and 28 indicate that all measures of biological response at distances downstream from the bridge were either equal to or exceeded those found at the upstream control. There was a significant increase in species richness (number of taxa) and diversity (Shannon's Index) observed at those stations, ranging from under the bridge (0 distance) to 3 m (10 ft) downstream. As previously noted, penta levels were 4.5% of the New York State sediment quality criterion for this compound, and penta was not observed in water. Biological effects were not expected at these low preservative levels, and adverse biological effects were not observed in association with this bridge.

### Laboratory Bioassays

A series of 10-day amphipod (*Hyalella azteca*) tests were completed on sediments collected upstream and at distances of +0.45, +1.8, and +9.9 m (+1.5, +6.0, and +33.0 ft) downstream from the perimeter of the Cougar Smith Bridge (Table 26).

The sediment grain size distribution in West Fork Satsop River sediments contained predominantly gravel and cobble. This distribution contained more coarse material than desired for *Hyalella azteca* bioassays. However, survival was generally good in all tests. Survival at the upstream control was 93.8%, and this result provided an appropriate basis for judging toxicity associated with downstream treatment stations. Survival data was log transformed, and *t*-tests were used to evaluate the significance of differences in amphipod survival between stations.

Penta was detected in sediments at the +0.45 and +1.8 m (+1.5 and +6.0 ft) downstream stations. Ten-day amphipod survival was not significantly reduced at either of these stations when compared with survival at the upstream control. Survival at the 9.9 m (33.0 ft) downstream station was significantly less than at the control, but penta was not detected in these sediments and the results are likely associated with some other factor.

### Risk Assessment Summary

Creosote-treated bridges discussed previously in this report were sited over a slow-flowing stream carrying a large

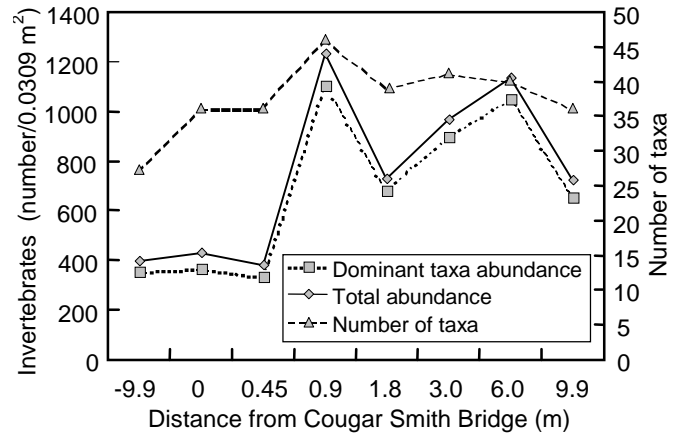


**Table 25—Dominant invertebrate taxa observed in sediment samples collected in the vicinity of Cougar Smith Bridge crossing the West Fork Satsop River in Mason County, Washington<sup>a</sup>**

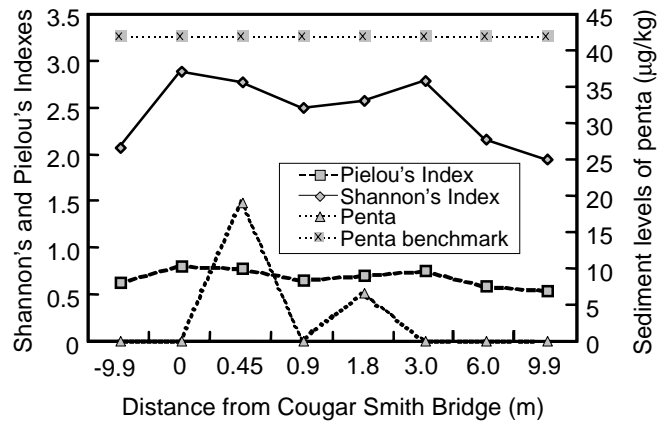
Dominant taxa	Code	Found in number of samples	Total abundance
Phylum Annelida	ANNE	18	757
Order Acarina	AACAR	18	801
Order Ephemeroptera			
<i>Cynigma</i> sp.	AICYNA	17	157
<i>Baetis</i> sp.	AIBAESS	15	354
Order Plecoptera			
Family Nemouridae	AINEM	14	295
<i>Sweltsa</i> sp.	AISWE	18	1,899
Order Coleoptera			
<i>Cleptelmis</i> sp.	AICLEP	18	531
Order Trichoptera			
<i>Glossosoma</i> sp.	AIGLOS	12	154
<i>Brachycentrus</i> sp.	AIBRAS	12	224
<i>Apatania</i> sp.	AIAPAS	16	212
Order Diptera			
<i>Pedicia</i> sp.	AIPEOS	16	252
<i>Pericoma</i> sp.	ACPERS	15	147
Family Chironomidae	AICHR	18	454
<i>Trissopelopia</i> sp.	AICTRIS	18	246
<i>Synendotendipes</i> sp.	AISYET	18	493
<i>Tanytarsus</i> sp.	AITYS	18	3,539
<i>Cricotopus</i> sp.	AICCRIC	17	701
<i>Synorthocladus</i> sp.	ASYNS	16	313
Total abundance of dominants			11,529

<sup>a</sup>Eighteen samples were collected, each covering 0.0309 m<sup>2</sup>.

bedload of sand and silt and clay. The invertebrate community associated with Pipe Creek was dominated by annelids and robust species of chironomids—both tolerant of the naturally stressful conditions found in this environment. The levels of PAHs found in sediments at Pipe Creek exceeded the TEL and PEL values for several compounds. However, adverse effects on the invertebrate community or in laboratory bioassays were not observed. It was hypothesized that adverse effects would be associated with these PAH concentrations found in more sensitive lotic communities that generally are found in faster flowing water with stable substrates. It was also hypothesized that at speeds greater than 20 to 25 cm/s, where these more sensitive communities were likely to be found, that preservatives lost from bridge structures would be greatly diluted.



**Figure 27—The number of taxa, the abundance of all taxa, and only dominant taxa observed in 0.0309-m<sup>2</sup> samples collected in the vicinity of the Cougar Smith Bridge crossing the West Fork Satsop River, in Washington State, August 5, 1998.**



**Figure 28—Shannon's and Pielou's Indexes plotted against sediment penta levels as a function of distance upstream and downstream from the Cougar Smith Bridge.**

The Cougar Smith Bridge presents an example of this second type of environment. Current speeds in the West Fork Satsop River were moderately fast at >27 cm/s, and the bottom was very stable, being composed of primarily sand, gravel, and cobble. A diverse community of pollution-sensitive species was observed under and downstream from this bridge. Penta was not detected in the water and was detected at very low levels in sediments in only two samples. In neither case did the sediment levels reach even 5% of an appropriate chronic benchmark. Adverse biological effects were not anticipated, and none was observed. In general, the invertebrate community under and immediately downstream from the bridge was more abundant and diverse than was found either upstream or downstream at a distance of 9.9 m (33 ft).

## Results From Upper Dairy Creek Bridge

The Upper Dairy Creek Bridge (Oregon) was chosen as the second penta-treated bridge in this risk assessment (Fig. 29). The bridge carries a live load of HS20, including a 76.2-cm

(3-in.) asphalt surface. It has a clear span of 17.4 m (58 ft) and sits on concrete abutments. All wooden components were treated to a retention of 96 kg/m<sup>3</sup> (0.6 lb/ft<sup>3</sup>) Type A penta. Figure 30 shows the general configuration of the bridge, and Figure 31 shows the wooden decking and support timbers.

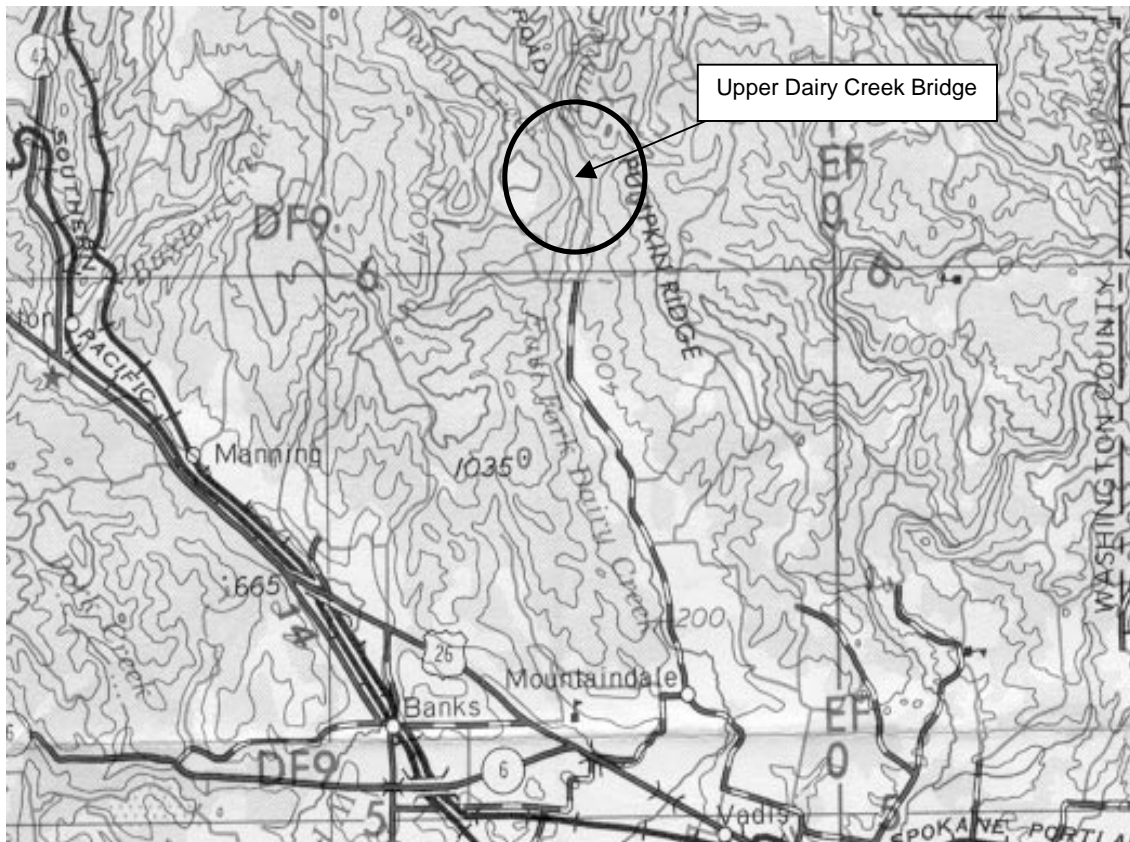
**Table 26—Summary results of Cougar Smith Bridge sediment bioassays using the test amphipod *Hyalella azteca*<sup>a</sup>**

Replicate	Surviving amphipods (%)				
	Laboratory control	Up-stream control	0.45 m (+1.5 ft)	1.8 m (+6.0 ft)	9.9 m (+33.0 ft)
1	100	100	90	90	60
2	100	100	100	90	100
3	100	100	90	100	80
4	100	100	100	100	90
5	100	90	100	90	60
6	100	80	90	80	90
7	100	80	90	100	70
8	100	100	50	90	60
Mean	100	93.8	88.8	92.5	76.3

<sup>a</sup> Initial count of 20 per replicate.

As shown in Figure 30, the Upper Dairy Creek was in a low to moderate flow state during the November 6, 1997, survey. The sampling transect represented the area of the stream bed with finest sediments and slowest flows. Current speeds along this transect were less than 3 cm/s. However, much faster currents were present throughout much of the stream channel, particularly in the middle of the stream where currents were >100 cm/s.

The weather was overcast, and a light rain was falling. The water temperature was 10.8°C at noon on December 7, 1997, and the air temperature was 14.5°C. Water pH was essentially neutral, averaging 6.89 ± 0.05 (three measurements). Water column alkalinity was 37.5 mg/L, and hardness was 32.8 mg/L. Conventional physicochemical characteristics of sampled sediments are described in Table 27. Sediments along the sampled transect were dominated by sand and gravel, with minor amounts of silt and clay.



**Figure 29—Location of the Upper Dairy Creek Bridge in Washington County, Oregon.**





**Figure 30—Upper Dairy Creek Bridge (Washington County, Oregon, Bridge Number 1366) as it appeared November 6, 1997, during this biological evaluation.**



**Figure 31—Bridge deck and support structure under the Upper Dairy Creek Bridge.**

### ***Sediment and Water Column Concentrations***

Penta was not detected in the water column at the upstream control station or under the bridge at the detection limit of 0.25 µg/L (three samples each station) (Table 27). Penta was detected in all three samples collected under the bridge at concentrations ranging from 15 to 28 µg penta/kg dry sediment. A concentration of 17.0 µg/kg was also detected in sediments collected 0.9 m (3.0 ft) downstream from the downstream perimeter of the bridge. Penta was not detected in other sediment samples at this bridge.

### ***Sediment Benchmark***

As previously noted, the New York State Department of Environmental Conservation (NYSDEC 1993) has established a freshwater sediment criterion for penta at 40 µg penta/g organic carbon to protect aquatic organisms from chronic effects. The mean of TVS in sediments from the vicinity of Upper Dairy Creek Bridge was  $5.88 \pm 0.59$ , indicating a mean TOC content of about 3.53%. At this level of TOC, the New York State benchmark would be 1,412 µg penta/kg dry sediment weight. The maximum observed level (28 µg/kg) was 1.98% of this benchmark, and adverse biological effects were not anticipated in association with preservative loss from the bridge.

### ***Biological Response—Infauna***

A total of 9,158 invertebrates in 74 taxa were identified in 18 samples at the Upper Dairy Creek Bridge. Taxonomic codes used in the database are defined in Appendix C. Dominant taxa were defined as those representing 1% of the total infaunal abundance (count = 92). A total of 16 taxa met the 1% criterion. Dominant taxa are identified in Table 28. These 16 taxa (22% of total taxa) represented 90.2% of the total abundance.

**Table 27—Physicochemical characteristics observed at the Upper Dairy Creek Bridge, in Washington County, Oregon, November 7, 1997<sup>a</sup>**

Station	Depth (cm)	RPD (cm)	Penta <sup>b</sup> (µg/kg)	TVS (%)	Sand (%)	Silt and clay (%)
–9.9 m (–33 ft) upstream control	21.0	>10.0	9.7 U	$5.1 \pm 0.7$	$21.9 \pm 9.6$	$5.2 \pm 2.9$
0.0 under bridge	40.0	>10.0	$20.0 \pm 7.9$	$7.9 \pm 1.3$	$62.2 \pm 3.5$	$12.7 \pm 3.5$
0.45 m (1.5 ft) downstream	29.0	>10.0	11.0 U	5.1	57.4	13.5
0.9 m (3.0 ft) downstream	31.0	>10.0	17.0	4.6	30.9	5.9
1.8 m (6.0 ft) downstream	34.0	>10.0	10.0 U	5.3	3.9	1.6
3 m (10 ft) downstream	33.0	>10.0	8.6 U	3.7	20.4	5.7
6 m (20 ft) downstream	29.0	>10.0	9.5 U	6.3	10.6	3.2
9.9 m (33 ft) downstream	24.0	>10.0	11.0 U	6.6	26.0	4.4

<sup>a</sup>The upstream control and samples taken under the bridge are the mean of three replicates. Where appropriate, values include 95% confidence limits on the mean.

<sup>b</sup>U indicates that the compound was not detected at the given detection limit.

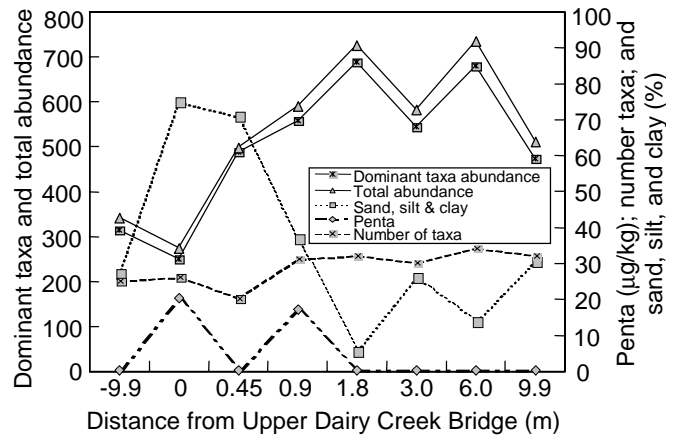
**Table 28—Dominant and subdominant invertebrate taxa observed in sediment samples collected in the vicinity of Bridge 146 crossing Pipe Creek in Cass County, Indiana<sup>a</sup>**

Dominant taxa	Code	Found in number of samples	Total abundance
Phylum Annelida	ANNE	16	137
Order Ephemeroptera			
<i>Ephemerella</i> sp.	AIEPHS	18	702
<i>Paraleptophlebia</i> sp.	AIPAR	15	167
<i>Epeorus</i> sp.	AIEPES	18	580
cf. <i>Heptagenia</i> sp.	AICHEPS	15	202
<i>Rhithrogenia</i> sp.	AIRHIS	14	112
cf. <i>Baetis</i> sp.	AIBAE	17	313
<i>Zapada</i> sp.	AIZAPS	17	318
<i>Sweltsa</i> sp.	AISWE	14	93
Family Perlodidae	AIPER	15	93
Order Trichoptera			
<i>Glossosoma</i> sp.	AIGLOS	18	4,771
cf. <i>Hydropsyche</i> sp.	AIHYHS	18	194
Family Brachycentridae	AIBRAD	17	165
Family Lepidostomatidae			
<i>Lepidostoma</i> sp.	AILPS	17	127
Order Diptera			
Family Chironomidae			
<i>Polypedilum</i> sp.	AIPPS	11	279
<i>Eukiefferiella</i> sp.	AEUKS	18	313
Total abundance of dominant taxa			8,566

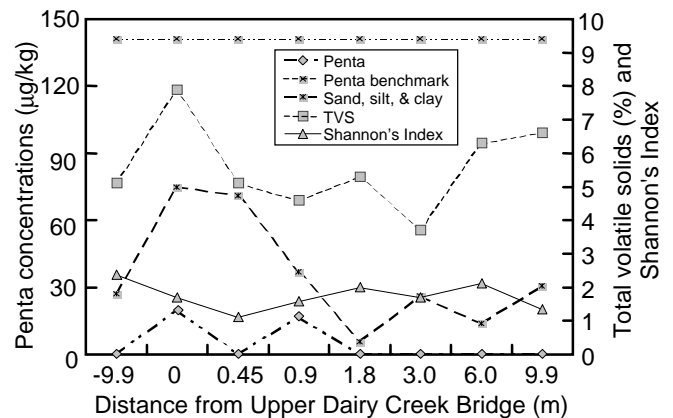
<sup>a</sup>Eighteen samples were collected, each covering 0.0309 m<sup>2</sup>.

Dominant taxa represented 93.5% of the total invertebrate abundance in these samples. The invertebrate community was dominated by sensitive taxa in the orders Ephemeroptera and Trichoptera. Annelids and chironomids made up only 8.5% of the number of dominant taxa. The remaining 91.5% were members of the orders Ephemeroptera or Trichoptera. The EPT are recognized as sensitive indicators of pollution. The number of taxa, total species abundance, and dominant species abundance are displayed in Figure 32 as a function of distance upstream and downstream from the Upper Dairy Creek Bridge. Figure 33 summarizes Shannon's and Pielou's Indexes at these same stations.

The sensitive species dominating the invertebrate community in this section of Upper Dairy Creek generally prefer hard and stable substrates. Figures 32 and 33 suggest that TVS and proportion sand, silt, and clay were higher at stations 0 and 0.45 m (1.5 ft) than at other stations examined in this



**Figure 32—The number of taxa, the abundance of all taxa, and only dominant taxa compared with the percentage of sand, silt, and clay in sediments and penta concentrations observed in 0.0309-m<sup>2</sup> samples collected in the vicinity of the Upper Dairy Creek Bridge in Washington State, November 7, 1997.**



**Figure 33—Shannon's Index plotted against sediment physicochemical parameters, total volatile solids, and percentage of sand, silt and clay as a function of distance upstream and downstream from the Upper Dairy Creek Bridge.**

assessment. Table 29 summarizes the results of a series of *t*-tests on log transformed data comparing the upstream control with downstream treatment stations. Few significant differences were observed. The proportion of sand, silt, and clay was only significantly greater ( $\alpha = 0.05$ ) in sediments from under the bridge compared with the upstream control. Table 29 indicates that Shannon's Index is the only biological metric that was significantly different in downstream samples compared with the upstream control.

The maximum observed sediment concentration of penta was low, being less than 1.98% of the New York benchmark at the calculated level of organic carbon. The variation in

**Table 29—Statistical significance of biological and sediment physicochemical endpoints between the upstream control and downstream stations at the Upper Dairy Creek penta-treated bridge<sup>a</sup>**

	Under bridge	0.45 m (1.5 ft)	0.9 m (3.0 ft)	1.8 m (6.0 ft)	3 m (10 ft)	6 m (20 ft)	9.9 m (33 ft)
Number taxa	0.58	0.24	0.25	0.22	0.42	0.13	0.22
Total invertebrate abundance	0.97	0.28	0.10	0.06	0.10	0.055	0.44
Abundance of dominant taxa	0.90	0.24	0.11	0.06	0.11	0.07	0.44
Shannon's diversity index	<b>0.01</b>	<b>0.00</b>	0.06	0.21	<b>0.03</b>	0.43	0.91
Sediment penta	0.055	1.00					
TVS	0.09	0.95					
Sum percent sand, silt, and clay	<b>0.01</b>	0.21					

<sup>a</sup>The probability that the mean value at the downstream station equals the mean value at the upstream control is provided for each endpoint. Values that are significantly different ( $\alpha = 0.05$ ) are in bold type.

sediment concentrations of penta observed under the bridge was high enough to avoid significance at the 0.05 level. However, at  $\alpha = 0.055$ , the differences would be significant.

The relationship between sediment physicochemical characteristics and biological response was explored using varimax normalized principle components analysis (Fig. 34). The first two factors, displayed in Figure 34, explained 47.5% of the variation. The next two factors explained an additional 31.9%, giving a total of 79.42% for the four factors considered in this analysis.

Factor 1 was significantly positively correlated with the mayflies (LAIEPHS = *Ephemerella* sp., LAIPAR = *Paraleptophlebia* sp., LAICHEPS = *Heptagenia* sp., and LAIZAPS = *Zapada* sp.). It was significantly negatively correlated with the percentage sediment sand, silt, and clay (LFINES).

Factor 2 was significantly correlated with the caddisflies *Glossosoma* sp. (LAIGLOS), Family Brachycentridae (LAIBRAD), total abundance (LABUND), and dominant species abundance (LDABUND). *Glossosoma* sp. was the most abundant invertebrate in this study; therefore, its abundance is highly correlated with the metrics total abundance (LABUND) and dominant species abundance (LDABUND). There were no significant negative correlations associated with factor 2.

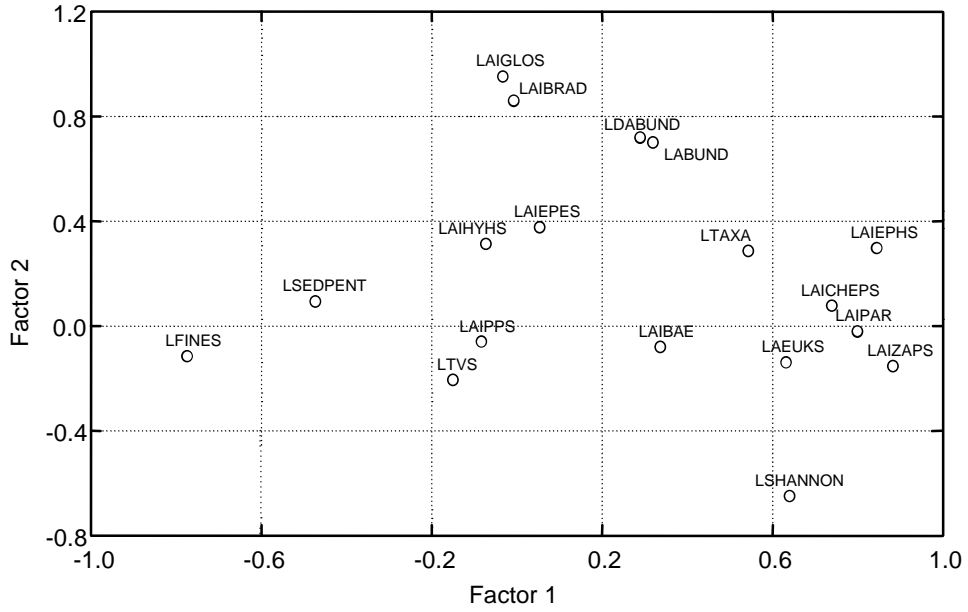
Factor 3 was significantly correlated only with the chironomid, *Polypedilum* sp. (LAIPPS).

Factor 4 was significantly positively correlated with the mayflies *Epeorus* sp. (LAIEPES) and *Baetis* sp. (LAIBAE) and the caddisfly *Hydropsyche* sp. (LAIHYHS). Factor 4 was significantly negatively loaded with the physicochemical variable total volatile solids (TVS). Sediment concentrations of penta were not significantly correlated with any of the four

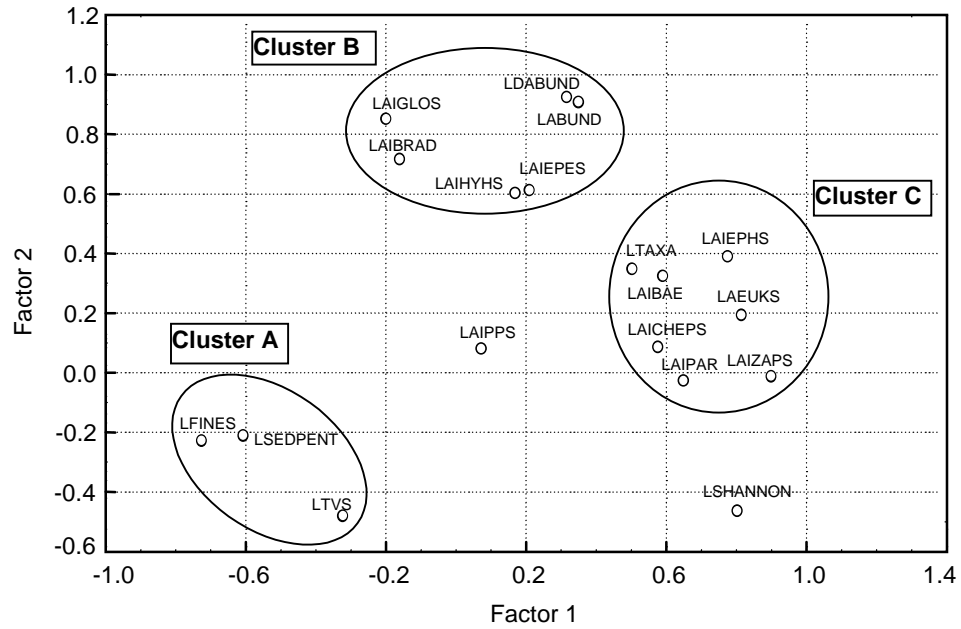
factors. However, it was moderately negatively correlated (−0.47) with factor 1 and fairly strongly negatively correlated (−0.61) with factor 4. This is likely because factor 1 was negatively correlated with fines (sand, silt and clay) and factor 4 was negatively correlated with TVS. These last two physicochemical parameters increased in depositional environments where penta could also be expected to accumulate. This analysis suggests that the sensitive community of mayflies and caddisflies resident in Upper Dairy Creek may have been reduced in areas containing more sand, silt, and clay (LFINES) and increased concentrations of organic matter (TVS). Sediment concentrations of penta were well below the New York State chronic sediment quality criterion, and the position of LSEDSENT was probably due to its accumulation in depositional areas with increased fines and TVS.

This analysis is presented in a more intuitive manner in Figure 35, which summarizes the results of a principal factors (MINRES) extraction followed by Varimax normalization using the same data.

Factor 1 is defined significantly by the abundance of the mayflies *Ephemerella* sp. and *Zapada* sp., the chironomid *Eukiefferiella* sp., and Shannon's Index in the positive direction, and by the proportion of fines (LFINES) in the negative direction. Significant correlations with factor 2 involve the abundance of invertebrates (*Glossosoma* sp., Family Brachycentridae, total abundance, and dominant taxa abundance). Three variable clusters are evident in Figure 35. Cluster A consists of the physicochemical variables, TVS, sand, silt and clay (LFINES), and sedimented penta (LSEDSENT). Increased values of these variables are associated with depositional environments that are obviously somewhat unfavorable to the sensitive taxa resident in this part of Upper Dairy Creek.



**Figure 34—Varimax normalized principal components analysis displaying the relationship between factors 1 and 2 in a four-factor analysis. Variable codes are given in Appendix B.**



**Figure 35—Principal factors (MINRES) extraction and varimax normalization of independent physicochemical variables and biological response variables observed in sediments at the Upper Dairy Creek Bridge in Washington County, Oregon, November 7, 1997.**

Cluster B represents species and biological metrics contributing to measures of invertebrate abundance. These variables included dominant species abundance (LDABUND), total abundance (LABUND), and the most abundant taxon *Glossosoma* sp. (LAIGLOS). Note that values of Shannon's Index are far from and negatively correlated with Clusters A and B, as expected. Cluster C contains a group of taxa that are important in determining the total number of taxa (LTAXA) observed in Upper Dairy Creek sediments. In addition, taxa in cluster C appear to be unadapted to the depositional environments characterized by cluster A.

### Laboratory Bioassays

The preceding analysis hypothesized that the low levels of penta observed in Dairy Creek sediments are not associated with adverse biological effects but rather that they are correlated with sediment fines and TVSs characteristic of depositional areas. In addition, it has been hypothesized that it is the increased fines and TVS found in depositional samples that are responsible for the minor decreases observed in some biological endpoints.

A series of 10-day amphipod (*Hyalella azteca*) tests were completed on sediments collected upstream and at distances of 0, +1.8, +9.9 m (0, +6.0, and +33.0 ft) downstream from the downstream perimeter of the Cougar Smith Bridge. The results are summarized in Table 30.

**Table 30—Summary results of Upper Dairy Creek Bridge sediment bioassays using the test amphipod *Hyalella azteca*<sup>a</sup>**

Replicate	Surviving amphipods (%)				
	Laboratory control	Upstream control	+0.0	+1.8 m (+6 ft)	+9.9 m (+33 ft)
1	100	100	100	95	100
2	100	95	85	95	100
3	95	100	100	95	90
4	100	95	100	95	100
5	100	90	95	100	95
Mean	99.0	96.0	96.0	96.0	97.0
95% CI	1.96	3.67	5.71	1.96	3.92
$p^{1b}$	1.00	0.331	0.498	0.070	0.221
$p^{2c}$	0.331	1.00	0.671	0.810	0.679

<sup>a</sup>Initial count of 20 per replicate.

<sup>b</sup> $p^1$  is the probability that amphipod survival at the Dairy Creek station is equal to survival in the laboratory control sediment.

<sup>c</sup> $p^2$  is the probability that amphipod survival in downstream sediments at the Dairy Creek Bridge is equal to survival in sediments collected at the upstream control station.

The sediment grain size distribution in Upper Dairy Creek sediments contained significant quantities of gravel and cobble but held more sand than those at the Cougar Smith Bridge. This distribution is still coarser than desired for *Hyalella azteca* bioassays. However, survival was excellent in all tests. Survival at the upstream control was 96.0%, and this result provides an appropriate basis for judging toxicity associated with downstream treatment stations. Survival data were transformed using the arcsin (square root (proportion surviving)) transformation, and *t*-tests were used to evaluate the significance of differences in amphipod survival between the upstream control and downstream treatment stations.

Penta was detected in sediments at the +0 and 0.9 m (+0.0 and +3.0 ft) downstream stations. Ten-day amphipod survival was not significantly reduced at any downstream station compared with survival in either laboratory control sediments or in sediments from the more appropriate upstream control station. These results support the previous hypothesis that there was no adverse biological response to the use of penta in treating the wooden components of the Upper Dairy Creek Bridge.

### Risk Assessment Summary

Creosote-treated bridges discussed previously in this report were sited over a slow-flowing stream that carried a large bedload of sand and silt and clay. The invertebrate community associated with Pipe Creek was dominated by annelids and robust species of chironomids—both tolerant of the naturally stressful conditions found in that stream. The level of PAHs found in sediments at Pipe Creek exceeded the TEL and PEL values for several compounds. However, no adverse effects on the invertebrate community or in laboratory bioassays were observed. It was hypothesized that adverse effects would be associated with these PAH concentrations in more sensitive, lotic communities that generally are found in faster flowing water with stable substrates. It was also hypothesized that at current speeds greater than 20 to 25 cm/s, where these more sensitive communities were likely to be found, preservatives lost from bridge structures would be greatly diluted.

The Dairy Creek Bridge presents an intermediate example lying between the fast flows found at the West Fork Satsop River Bridge and the slow flowing waters of Pipe Creek. Current speeds in the West Fork Satsop River were moderately fast (>27 cm/s) and the bottom was very stable, being composed of primarily gravel and cobble. Flows along the sampling transect at Upper Dairy Creek were significantly less (about 3 cm/s), particularly under the bridge. A diverse community of pollution-sensitive mayflies and caddisflies was observed under and downstream from the Upper Dairy Creek Bridge. Penta was not detected in the water and was detected at levels representing less than 2% of the New York State sediment quality criteria at two stations.

A small decrease in several biological endpoints, particularly Shannon's Index, was observed directly under the Dairy Creek Bridge. This decrease appears associated with the significantly greater proportion of sand, silt, and clay found in under-bridge sediment samples than was collected downstream. This part of the study could be criticized for not collecting samples further into the channel under the bridge where the substrate was more characteristic of the upstream control and downstream treatment stations. However, it was the author's opinion that collecting samples further in the channel would not represent a worst case because it was very unlikely that penta would have been detected in sediments underlying the swift currents. All samples collected in support of this study were taken from areas representing worst-case (that is, slow flow) conditions.

The weight of evidence presented in this analysis, including the low levels of penta observed in sediments, lack of penta in water, lack of toxicity in laboratory bioassays, and the relationship between the larval insect community and sediment grain size and TVS all support the conclusion that no adverse biological effects were associated with the use of penta in constructing the Upper Dairy Creek Bridge. Note that the biological effects discussed in this analysis were minor and generally not statistically significant between the upstream control and treatment stations. There were no adverse effects to document on November 7, 1997.

## CCA-Treated Bridges

Chromated copper arsenate (CCA) is a formulation of copper, chromium, and arsenic dissolved in an aqueous solution. CCA was first developed in 1933 and has been widely used throughout the world as a wood preservative for 60 years. The metals in CCA-treated wood undergo chemical change after impregnation, resulting in their being bonded or fixed to wood fibers. These formulations combine the fungicidal properties of copper with the insecticidal properties of arsenic pentoxide. In CCA, the fixation of arsenic and copper is dependent on the presence of chromium. Several formulations, using different forms of the metals and marketed under a variety of trade names, are available. The American Wood Preservers' Association (AWPA) classifies these formulations as CCA Types A, B, and C. Each type may exhibit slightly different behavior in aquatic environments. However, these differences are small and only the most commonly used formulation of CCA was considered in this review.

Ammoniacal copper arsenate (ACA) has recently been improved for the replacement of some of the arsenic with zinc. The current formulation of ammoniacal copper zinc arsenate (ACZA) contains 25% zinc as zinc oxide, 50% copper as cupric oxide, and 25% arsenic as arsenic pentoxide. Ammonia in this formulation catalyzes the fixation of copper, arsenic, and zinc to the wood fibers (Lebow and Morrell 1993).

Both arsenic and chromium are heavy metal poisons. Both have chronic and acute environmental health risks associated with them. Copper does not generally constitute a human health risk. However, low concentrations of copper, in certain ionic forms, are highly toxic to fauna and flora. The known toxicity to humans of arsenic and chromium has resulted in concern about introducing them into the environment.

Several reviews assessing the environmental risks associated with treated wood have been compiled (Konasewich and Henning 1988, Ruddick and Ruddick 1992, Stranks 1976, and USDA 1980). The conclusion reached in these papers was that the use of treated wood causes no significant hazard to the environment. However, all these reviews suffer from lack of quantitative analysis, leaving some doubt about the risks associated with using treated wood in aquatic environments. Cooper (1990) discusses the considerable confusion created by contradictions in the technical literature regarding the leaching of metals from CCA-treated wood products. Brooks (2000) reviewed the toxicity of copper, chromium, and arsenic in freshwater ecosystems. The following is intended to expand that discussion to include marine environments.

## Background Levels and Sources

The metals used in wood preservatives are naturally occurring elements. The purpose of this discussion is to gain an understanding of the background concentrations of these elements and the natural and anthropogenic sources that contribute to present environmental levels.

### Arsenic

Arsenic is found in rocks and soil from concentrations of less than 1.0 to several hundred mg/kg. Andreae (1978) found background levels of arsenite (arsenic(III)) to be less than 0.9 µg/L in seawater and total inorganic arsenic (primarily arsenate or arsenic V) to be less than 1.5 µg/L. Neff (1997) reported that the average concentration of total arsenic in the ocean is about 1.7 µg/L. He found that uncontaminated near-shore sediments contained between 5 and 15 µg As/g dry sediment weight. Arsenic concentrations in deep-sea sediments were greater, at about 40 µg/g<sup>1</sup>. Contaminated sediments in urban and industrial areas may contain several thousand micrograms arsenic per gram of dry sediment. Finally, Neff (1997) reported that river sediments in England contained between 7 and 950 µg As/g dry sediment. Penrose and Woolson (1974) reported arsenic levels of 2.3 to 8.3 µg/L in the Caribbean and 0.8 to 4.5 µg/L in the Gulf of Mexico. The U.S. EPA (USEPA 1985) reported arsenic levels of 1.5 µg/L in Puget Sound water. Brooks (unpublished data) found arsenic levels of 19 µg/L in marine water samples in Little Skookum Inlet, South Puget Sound. There is a consensus among other authors (Waslenchuk 1977, 1978,

Sanders and Windom 1980) that total arsenic concentrations in marine waters generally range between 1 and 1.5 µg/L.

Andreae (1978) found significantly more reduced arsenic (arsenite) than would be expected in highly oxygenated water where chemical equilibrium models suggest that most of the arsenic should be in the less toxic arsenate form. The ratio of arsenite to arsenate is correlated with chlorophyll  $\alpha$  production, suggesting that the speciation of arsenic in natural waters is highly influenced by biological activity. Sanders and Windom (1980) estimated that as much as 15% to 20% of total arsenic is reduced to arsenite by phytoplankton during spring and fall blooms on the continental shelves.

Woolson (1983) suggested that the natural arsenic cycle is not greatly disturbed, on a global basis, by human activities. He reported that volcanoes constitute the major natural source and estimated their contribution at 70,000 metric tons/year. All other natural sources contribute only 8,000 metric tons/year. Anthropogenic sources add nearly 25% to the total global loading. Iron, steel, lead, zinc, and copper production contribute 82% of the anthropogenic emissions of  $23.6 \times 10^9$  g/year. Pesticides account for  $0.20 \times 10^9$  g/year, or less than 1% of total anthropogenic input. Neff (1997) reported total natural emissions of arsenic to the atmosphere at about 45,000 metric tons/year and anthropogenic emissions at 28,000 metric tons/year for a total atmospheric loading of 73 metric tons/year.

Woolson discussed the arsenic cycle and suggested that the final environmental fate of all arsenic is incorporation into oceanic sediments. Characteristic levels of arsenic in Washington State marine sediments are presented in Table 31. Values range from less than 10 mg/kg in reference areas to greater than 70 mg/kg in highly impacted areas like Elliott Bay. Carpenter (Penrose and Woolson 1974) reported arsenic levels of 3 to 15 mg/kg in sediments of Puget Sound. He also reported levels of 290 to 980 mg/kg in sediments near a Puget Sound smelter.

The USDA (1980) reported extreme variation in the amount of arsenic found in fresh surface and ground waters of the world (undetectable to 276,000 µg/L). Table 32 provides a cross section of these data. Whanger and others (1977) found similar levels in Oregon waters. Oregon spring water typically contained 133 to 900 µg/L arsenic, and lake water was significantly less (<1 to 9 µg/L).

These data suggest that on a global scale, little arsenic is contributed to aquatic environments by pesticide use. However, local arsenic levels may be highly influenced by anthropogenic inputs. Typical arsenic levels in marine water are in the low microgram per liter range. The report herein assumes that marine levels of total arsenic are 1.7 µg/L and that the ratio of arsenite to arsenate is approximately 1:4. Total arsenic concentrations of 10 to 30 mg As/kg appear reasonable for sediments in reference areas. Additional analysis in

**Table 31—Metal levels in sediments of Puget Sound<sup>a</sup>**

Area	Arsenic (ppm)	Copper (ppm)
Straight of Georgia	<10	<50
San Juan Islands	<10	<50
Bellingham Bay	<30	>350
Strait of Juan De Fuca	<10	<50
Penn Cove	<10	<50
Everett	<70	>350
Dyes Inlet	<70	<150
Elliott Bay	>70	>350

<sup>a</sup>Puget Sound Environmental Atlas (1992)

**Table 32—Typical arsenic levels observed in fresh water**

Location	Arsenic level (µg total arsenic/L)
Glacial ice in Sweden	2.0 to 3.8
Thermal waters in western United States	20.0 to 3,800
Columbia River, Washington State	0.2 to 86.9
Yellowstone River	4.5
California well water	10.0 to 2,000
Washington well water	5.0 to 6.0
Oregon well water	0.0 to 1,700
California lakes	0.0 to 100
Wisconsin lakes	4.0 to 117
English rivers	7.0 to 950

this report assumes that sedimented levels in industrial areas are about 100 mg/kg. Arsenic levels in freshwater appear to be greater than in marine waters. For purposes of additional analysis, an average total arsenic loading of 50 µg/L is assumed for lotic and lentic fresh waters.

This review suggests that the state of arsenic in aquatic environments is controlled by a variety of parameters that are independent of the presence of treated wood. Therefore, the form of arsenic leached from treated wood probably has little effect on species of arsenic found in the water. These processes are rapid with first-order rate constants for arsenite oxidation reported in the range of 0.5 to 2.2/day. This analysis suggests that regardless of the arsenic species released from treated wood (most is in the form of arsenate), between 1% and 20% will be converted to arsenite and between 80% and 99% will be in the form of arsenate.

### Chromium

Chromium is the 21<sup>st</sup> most abundant element in the earth's crust, with a mean concentration in U.S. soils of about 40 mg/kg (Barnhart 1997). Eisler (1986) reviewed chromium

hazards to aquatic species. He reported that the earth's crust contains a mean of 125 mg/kg chromium. Natural-weathering processes release an estimated 32,000 metric tons of chromium per year. The annual world production of chromium is estimated at  $7 \times 10^6$  metric tons, and industrial inputs can be significant. Storm water, sewage treatment plants, metal plating, leather tanning, and mining industries contribute significant amounts of chromium to aquatic environments. Untreated industrial effluents contain up to  $5 \times 10^6$  µg/L, and electroplating waste streams contain up to  $1.29 \times 10^6$  µg/L.

Background levels of 0.0 to 5 µg/L chromium were reported by Eisler (1986) for uncontaminated seawater. Freshwater levels were somewhat higher at 1.0 to 10 µg/L. The U.S. EPA (USEPA 1983) summarized chromium levels in surface waters of the United States. Values ranged from undetectable in some California well water to 2,790 µg/L for water near a cooling tower. Average values were between 4 and 20 µg/L with a range of <1 to 112 for U.S. surface waters. The EPA did not list chromium as a metal of concern in Puget Sound.

Turekian and Scott (1967) reported suspended loads of chromium in North American rivers ranging from 37,000 to 460,000 µg/L. They estimated that 870 tons of chromium are transported by the Susquehanna River each year. Rivers located east of the Mississippi have higher concentrations of many metals than do western rivers. However, all these lotic systems transport significant amounts of chromium to the estuaries at their mouths. Eisler (1986) reported that marsh sediments receiving fertilizers containing sewage sludge for 7 years contained between 2,150 and 4,750 mg/kg of chromium. Sediments associated with industrial outfalls had as much as 54,300 mg/kg of chromium. Sediments from reference areas contained 50 to 54 mg/kg of chromium.

In summary, uncontaminated seawater contains very low levels of chromium. This review finds that anthropogenic inputs of chromium are significant. This is particularly true in localized areas near urban or industrial centers because the metal is readily incorporated into sediments. Additional analysis in this report assumes a background level of 3 µg/L in marine water and 12 µg/L in freshwater. Based on values in the literature, sediments in unpolluted areas are assumed to have 52 mg/kg chromium and industrialized areas are assumed to have a level of 1,000 mg/kg.

### **Copper**

Copper levels of 1 to 10 µg/L were reported by Boyle (1979) from unpolluted waters of the United States. However, concentrations downstream of municipal and industrial outfalls may be much higher (Hutchinson 1979). The U.S. EPA (USEPA 1985) reported low levels (0.25 µg/L) of copper in the waters of Puget Sound.

Copper levels in Washington sediments (Puget Sound Environmental Atlas 1992) are summarized in Table 32. Lu and

Chen (1976) reported reference area sediment copper levels in San Pedro Bay at 5 to 10 mg/kg.

Based on available information, this report assumes that background water column levels of copper are <2.0 µg/L in marine environments and 2 µg/L in lotic and lentic freshwaters. Values of 10 to 30 mg/kg are assumed for unpolluted sediments and 350 mg/kg in sediments near industrialized areas.

### **Cycling and Fate**

#### **Arsenic**

The chemistry of arsenic in water is complex, and the form present in solution is dependent on such environmental parameters as pH, organic content, suspended solids, and sediment characteristics. Neff (1997) provides the most recent review of the chemistry of arsenic in aquatic environments. Thermodynamic considerations predict that at neutral pH and relatively high levels of dissolved oxygen, most arsenic should be oxidized to arsenate. Johnson (1972) found that marine bacteria can reduce arsenate to arsenite. Neff (1997) noted this same pathway but found that in aerobic water, arsenite is oxidized rapidly to the less toxic arsenate form, both abiotically and by bacteria. When oxygen is deficient, as may occur in association with the upwelling of deep ocean water, arsenite concentrations can significantly increase. However, arsenate is always the most abundant form of arsenic in oxygenated, biologically productive waters of the ocean.

Onishi (Andrae 1978) reports that arsenic(III) (arsenite) represents only about 20% of the total arsenic found in seawater. Neff (1997) concluded that arsenite (the toxic form of arsenic) usually represents less than 1% to no more than about 10% to 20% of the total arsenic in marine environments.

In addition to inorganic arsenic, several authors (Andrae 1978, Johnson 1972, Lunde 1977, Penrose and others 1977) have demonstrated that bacteria, phytoplankton, marine invertebrates, and vertebrates can biotransform arsenic into relatively less toxic organic compounds. These reactions involve methylation and reduction to produce methylarsinic acid and dimethylarsinic acid. The low toxicity of these organic compounds allows high body burdens of arsenic in healthy organisms, which are eventually incorporated into the sediments. However, significant amounts of arsenate may be regenerated in the water column from phytoplankton that sink below the photic zone and perish. Thus, there is an arsenic cycle, which involves a cycling of arsenic through its various inorganic and organic forms. The relatively high levels of arsenic found in sediments, compared with levels in the water column, suggest that the ultimate fate of arsenic in aquatic environments is incorporation into sediments.



## Chromium

Two species of chromium are prevalent in aquatic environments: chromium(III) is less toxic than chromium(VI). Most chromium(VI) found in nature is a result of domestic and industrial emissions (Steven and others 1976). Interaction of chromium(VI) molecules with organic compounds can result in reduction to a comparatively less toxic trivalent form. However, in aerobic marine environments, chromium(VI) is the more abundant species. Chromate, hydrochromate, and dichromate are soluble in water and are therefore mobile in aquatic environments.

The ultimate fate of chromium(VI) appears to be incorporation into fine-grained sediments with high organic and iron content. Adsorption of chromium(VI) onto sediments is dependent on salinity and is greatest at salinity of 0.1 to 1.0 parts per thousand (Mayer and Schick 1981). However, its fairly high solubility allows easy migration in and out of the water column over aerobic sediments. Observed concentrations in European estuaries ranged from 3.9 mg/kg in intertidal sands to 162 mg/kg in anaerobic mud (Rehm and others 1984).

Chromium(III) forms stable complexes with negatively charged inorganic and organic compounds. It is rarely found uncomplexed in waters, with decaying plant or animal tissues, or with silt and clay particles. Precipitated chromium hydroxides remain in the sediments under aerobic conditions. With low pH and anoxic conditions, chromium(III) hydroxides may solubilize as ionic chromium(III). However, Lu and Chen (1976) found that chromium was not significantly released from sediments into seawater under either oxidizing or reducing conditions.

## Copper

Copper is found in natural water as a free ion or complexed with humic acids, carbonate, and other inorganic and organic molecules. Copper is an essential element in the normal metabolism of both plants and animals. Therefore, a significant portion of the copper found in both fresh and marine systems may be taken up by the biota. The ultimate fate of much of this copper is sedimentation.

Harrison and others (1987) found very low copper levels (<12 mg/kg) in sandy substrates associated with power plant effluents. He suggested that the lack of organic matter in these sediments was responsible for the low copper content. Clarke (1974) noted that iron sulfide renders copper insoluble in anaerobic sediments. These reports suggest that copper accumulation in sediments is highly influenced by sediment chemistry and physical characteristics. Fine sediments, coupled with poor water circulation, could be expected to accumulate more copper than coarse sediments in highly oxygenated areas. Copper accumulations in fine-grained, anaerobic sediments are probably not biologically available. Therefore,

these environments may serve as an important mechanism for the removal of excess copper from aquatic environments.

The cycling of copper from sediments to the water column is a function of the sediment reduction–oxidation potential. Lu and Chen (1976) examined the release of copper from sediments as a function of sediment grain size and oxygen availability. Three oxidizing conditions were examined: oxidizing, 5 to 8 ppm dissolved oxygen; slightly oxidizing,  $\leq 1$  ppm dissolved oxygen; and reducing,  $S(-II)_T = 15$  to 30 mg/kg. Small amounts of bound copper were released to the overlying water in the reducing and slightly oxidizing environments, resulting in concentrations of 0.2 to 0.5  $\mu\text{g/L}$ . Copper releases in the oxidizing environment resulted in significantly higher interfacial seawater concentrations (3.2  $\mu\text{g/L}$ ). This effect was slightly more pronounced in the coarsest sediment tested (silty-sand sediment). These data imply higher copper releases from sediments in aerobic (healthy) environments. There are two ways to look at this phenomenon.

In more coarse-grained, highly oxygenated sediments, bound copper is more easily lost to the water column and dispersed over greater distances, until the copper finds anaerobic sediments, where it will likely be buried and eventually incorporated into the lithosphere. These anaerobic sediments support reduced infaunal and epifaunal communities of organisms. Therefore, we would expect reduced environmental impacts from copper incorporated into anaerobic sediments.

In enclosed bodies of water with coarse grained, aerobic sediments, this study suggests that copper will not be as tightly bound to the sediments and will migrate into the interstitial and surficial water where it is bioavailable. Data were not provided on the copper species released from the sediments; therefore, it was difficult to assess the toxicity of the released copper.

The work of Lu and Chen (1976) suggests that caution is appropriate when dealing with copper materials in poorly flushed embayments with aerobic ( $> 2$  to 3 mg/L dissolved oxygen) sediments. These arguments suggest that anaerobic sediments are a more efficient trap for released copper and that reduced environmental risks should be anticipated from copper releases associated with anaerobic sediments compared with those associated with aerobic sediments.

Data presented in Lu and Chen (1976) are not sufficient to develop an expression describing copper releases from sediments at a variety of sediment concentrations. These effects appear to be subtle, and their exclusion should not significantly flaw the risk assessment. This discussion is provided as background for project proponents and permit writers. Consideration of these factors may be important when considering the relative risks associated with different wood treatments.

## Bioconcentration, Bioaccumulation, and Biomagnification

Chromium and copper are essential micronutrients for plants and animals. Their uptake and metabolism is a normal biological process. The pentavalent form of arsenic (arsenate) is chemically similar to phosphate, and arsenate may be readily taken up by plants and animals in their efforts to sequester phosphate for normal cellular metabolism. This section discusses the potential for the bioaccumulation of each of these metals by aquatic plants and animals.

### Arsenic

Arsenic(III) is a potent toxicant in mammals (including humans), and there are considerable data describing its bioaccumulation.

Penrose and others (1977) examined the arsenic budget in a sea urchin–alga system and concluded that organic arsenic is rapidly excreted by most organisms. Therefore, while there may be significant bioconcentration of arsenic from surrounding waters, there is no apparent biomagnification in food chains. Organisms containing high levels of arsenic in their tissues tend to be those that are prone to incidental ingestion of sediment particles while feeding.

Arsenic concentration from ambient water was also reported by Schroeder and Balassa (1966), Lunde (1970, 1972), and Fowler and others (1975). High levels of arsenic in marine animals were reported by USDA (1980) from around the world. Reported levels of arsenic, expressed as a proportion of wet tissue weight, for some typical marine species are provided in Table 33, excerpted from USDA (1980). Woolson (1975) reported that arsenic concentrations are 10 to 100 times greater in marine fish and shellfish than in freshwater species. However, as seen in previous sections, reported arsenic concentrations in marine waters are typically less

(1.5 µg/L) than in freshwater (see Table 32). No plausible explanation for this apparent contradiction was offered in the literature.

Penrose and Woolson (1974) reviewed studies by Fernandez del Riego, Seydel, and Lunde, who suggest that arsenic is not biomagnified in food chains. Boothe, Knauer, Black, and Penrose (Penrose and Woolson 1974) suggested that marine organisms rapidly excrete arsenic ingested in food. Woolson (1975) summarizes his review of arsenical bioaccumulation by noting that

Arsenic is bioconcentrated by aquatic organisms but not biomagnified. Plants usually accumulate more arsenic than fish, and Crustacea accumulate intermediate amounts. Marine organisms normally contain more arsenic than their freshwater counterparts. However, the arsenic contained in the organisms is apparently not toxic to animals or humans, and is readily excreted. (Woolson 1975)

Neff (1997) reviewed the literature describing the bioaccumulation of arsenic in marine environments and humans and noted that inorganic arsenic is poorly bioconcentrated from water but that organic arsenic compounds have much higher BCFs. The inorganic arsenic that is bioconcentrated is rapidly converted to organoarsenic compounds, which are not toxic. Arsenobetaine is the most abundant form of arsenic in tissues of most marine animals examined to date. It usually represents 50% to more than 95% of the total arsenic in crustaceans and fish. Neff (1997) concluded that inorganic arsenic, at concentrations typically found in productive, oxygenated seawater and brackish waters, does not represent a significant hazard to marine organisms and ecosystems.

The available evidence indicates that arsenic does not biomagnify in food chains. It appears that arsenic ingested at lower levels of the food web is converted to organic molecules, which are rapidly excreted at the next trophic level. For purposes of the analysis in this report, it will be assumed that levels of arsenic are dependent on ambient water levels and they are not biomagnified in higher trophic levels.

### Chromium

Eisler (1986) reported that algae and higher plants accumulate chromium from seawater by factors of up to 8,600 and from solutions containing 50 mg/L chromium by a factor of 18 in 48 h. Although chromium is abundant in primary producers, there is little evidence of biomagnification through marine food chains. Baptist and Lewis (Eisler 1986) followed the transfer of chromium through an experimental food chain and observed a decline in the concentration of chromium through each of four trophic levels. A comparison of the results of this food chain study with measurements of direct chromium uptake from seawater suggests that direct uptake is a far more important pathway than assimilation

**Table 33—Arsenic content of aquatic animal life**

	Arsenic (µg arsenic/g dry tissue weight)
Marine	
Crab	27.0 to 52.5
Clams (all species)	90.0 to 127.2
Oysters ( <i>Crassostrea virginica</i> )	0.6 to 42.8
Lobster ( <i>Panulirus borealis</i> )	0.003 to 9.6
Tuna	0.7 to 4.6
Fresh water	
Trout	0.069 to 0.149
Perch ( <i>Perca fluviatilis</i> )	0.6
Bass ( <i>Micropterus salmoides</i> )	0.070 to 0.930
Channel catfish ( <i>Ictalurus punctatus</i> )	0.0 to 3.100

through the food chain. Bioconcentration factors (BCFs) for numerous aquatic species are given by U.S. EPA (USEPA 1983). The reported BCF for chromium(VI) in fish muscle is less than 1.0. EPA obtained BCF values of 125 and 192 for chromium(VI) in oysters and blue mussels. The EPA document also gives values for chromium(III) and concludes that they are similar to those given for chromium(VI). The EPA conclusion was that mean BCF values of 0.5 and 130 are appropriate for fish muscle and bivalve mollusks, respectively. These are both relatively low BCFs. It appears that chromium is not biomagnified in food chains and that chromium concentration levels at all trophic levels are primarily a function of background levels in the water.

### Copper

The National Academy of Sciences (NAS 1971) provides copper BCFs for numerous taxa. These values range from 100× for benthic algae to 30,000× for phytoplankton. Mollusks concentrate copper by a factor of 5,000 in muscle and soft parts. No information was reviewed on the bioaccumulation of either copper or zinc by aquatic organisms. For the purposes of this paper, it is assumed that copper and zinc accumulation in aquatic organisms is primarily a function of metal concentration in the ambient water, and although many organisms may bioconcentrate copper, there is inadequate information describing the biomagnification of copper through food webs.

### Toxicity to Aquatic Fauna and Flora

To assess the potential impact of CCA-treated wood used in aquatic environments, it is necessary to determine the minimum levels of these metals causing acute or chronic stress in populations of marine organisms.

### Arsenic

Arsenic is a common environmental metal whose toxic properties have been known for centuries. The toxicology of arsenic may be divided into three general areas: direct inhibition of cellular respiration, mutagenic effects, and hemolysis. Baroni and others (1963) and Penrose and Woolson (1974) noted that controlled attempts to attribute carcinogenic properties to the arsenicals failed. Ferm (1977) demonstrated the teratogenic nature of sodium arsenate injected into a variety of experimental animals.

Arsenical toxicity is dependent on the oxidation state, chemical form, and route of exposure. In general, arsenic acids are least toxic, followed by inorganic arsenate, arsenoxides, inorganic arsenite, and the trivalent organic and inorganic arsines are the most toxic.

Eisler (1988) reported acute toxicity levels for a variety of freshwater and marine plants and animals associated with several species of arsenic. Lethal concentrations, which killed 50% of the invertebrate test organisms (LC<sub>50</sub>), are

**Table 34—Lethal levels of arsenite in fresh-water plants and animals**

Taxa	Arsenite (As <sup>+3</sup> )
Fresh water	
Algae	1,700 to 4,000 (LC <sub>100</sub> )
Cladocerans	1,300
Amphipod	960 (28-day LC <sub>100</sub> )
Goldfish	490 (7-day LC <sub>50</sub> )
Fish	15,000-35,000
Marine water	
Red algae	300 (LC <sub>100</sub> )
Copepods	510
Dungeness crab	230
Oyster (eggs)	7,500 (48 h LC <sub>50</sub> )
Blue mussels	16,000 (LC <sub>100</sub> )
Pink salmon	3,800 (10-day LC <sub>50</sub> )

<sup>a</sup>Unless specified, values are for the LC<sub>50</sub> expressed in µg As/L.

provided in Table 34. In marine water, it appears that arsenic levels in excess of 200 µg/L may result in the mortality of juvenile Dungeness crab and an unspecified species of red algae. NTIS (1986) reported acute values of arsenic(III) for 12 saltwater animals. The range of sensitivity was from 232 to 16,030 µg/L. Chronic stress was observed at about half these values, or 116 µg/L. Arsenic(V) was less toxic for the two invertebrates examined, with acute values of 2,000 and 3,000 µg/L. None of these animals was as sensitive to arsenic as were some algae, which showed toxic responses to either arsenic(III) or (V) at values as low as 19 µg/L.

Eisler's (1986) data suggest that in freshwater, arsenic levels associated with acute toxicity appear to be somewhat higher, in the neighborhood of 900 µg/L. NTIS (1986) reported acute toxicity associated with arsenic(III) in 16 species of freshwater animals. An acute value of 812 µg As/L was found for cladocerans. At the other end of the range, the acute level for a midge was 97,000 µg/L. From these papers, it appears reasonable to assume an LC<sub>50</sub> of 800 µg/L for the more sensitive freshwater species. NTIS (1986) suggested that chronic stress is encountered by all freshwater species at about 21% of their acute values. For the most sensitive species, this value would be 168 µg/L total arsenic.

This review indicates that arsenite can cause chronic stress in several marine animals at levels as low as 168 µg/L in freshwater systems and 230 µg/L in marine systems. For most aquatic organisms, arsenate is far less toxic. However, for the most sensitive marine algae, this review indicates no difference in toxicity between the two primary valence states of arsenic (+3 and +5) and toxicity thresholds, which may be as low as 19 µg/L.

## Chromium

The toxicity of chromium to aquatic species can vary by an order of magnitude, or more, depending on a variety of biological and physical factors. These include differences associated with species, age, developmental state, temperature, pH, salinity, length of exposure, and interaction with other contaminants. Chromium(VI) is most toxic to the developmental stages of aquatic species in soft, freshwater, with low pH. Eisler (1986) reported a 96-h LC<sub>50</sub> of 200 µg/L for salmon fingerlings and 495 µg/L for rainbow trout (*Oncorhynchus mykiss*) eggs. Most species of fish tolerate >10,000 µg/L, and Bluegills (*Lepomis macrochirus*) demonstrated a high toxic threshold at 213,000 µg/L in water with hardness of 120 mg/kg calcium carbonate.

In marine water, chromium(VI) toxicity also varies by orders of magnitude, depending on the taxa. The range is about the same as for freshwater species. Polychaetes and larval crabs (*Callinectes sapidus*) are the most susceptible organisms at 200 and 320 µg/L, respectively.

## Copper

Copper is an essential element for most living organisms. It is added at a concentration of 2.5 µg/L in Guillard's Medium F/2 to seawater for the optimum culture of marine algae (Strathman 1987). At concentration levels slightly above those required as a micronutrient, copper can be highly toxic, especially to the larval stages of marine invertebrates. A single copper fitting in a seawater system may destroy most invertebrate embryos being cultured in the laboratory.

U.S. EPA's (USEPA 1984) Ambient Water Quality Criteria reports that copper toxicity in aquatic environments is related to the concentration of cupric (Cu<sup>2+</sup>) ions. The cupric ion is highly reactive and forms various copper complexes and precipitates, which are significantly less toxic than the cupric ion (Knezovich and others 1981). Sunda (1987) has proposed a basic mechanism to explain the observed relationship between free ion activities and the bioavailability of metals such as copper. He observed that the complexed species of copper are charged or polar and cannot pass directly across the lipid bilayer of the cell membrane. Thus, transport of these metals across the membrane would require that they interact with specific metal transport proteins. Because the free ion activity is a measure of the potential reactivity of a metal, it reflects the ability of that metal to interact with these transport proteins. The many chemical forms of copper in aquatic environments are maintained in a dynamic state of equilibrium that depends on salinity, temperature, pH, alkalinity, dissolved oxygen, sediment physicochemical characteristics, and the presence of other inorganic and organic molecules.

The dual nature of copper as an essential trace element and a potential toxin at low concentrations demands that organisms strictly regulate copper at internal levels suitable for

metabolic requirements. Roesijadi (1980) reported that copper is normally present at relatively high levels in the tissues of marine animals (>1.0 µg/g). Roesijadi (1980), Harrison and others (1987), and Harrison and Lam (1985) reviewed both the environmental detoxification of copper and the physiological detoxification of copper by *Mytilus edulis*, *Protothaca staminea*, *Patella vulgata*, *Ostrea edulis*, and *Littorina littorea*. Copper detoxification and metabolic regulation were associated with copper binding by low- and high-molecular-weight metallothionein-like proteins in the digestive gland and sequestering of copper in lysosomes.

Costlow and Sanders (1987) used a metal-chelate buffer system to regulate the free ion concentration of copper in seawater. They exposed crab larvae to a range of free cupric ion concentrations and monitored survival, duration of normal development, and growth. The authors reported significant reductions in growth correlated with copper accumulation and concluded that when exposed to cupric ion concentrations in seawater that are below ambient concentrations, crab larvae are able to regulate their bioconcentration of copper. At higher concentrations of the cupric ion, copper bioconcentration increases and larval growth was inhibited.

Harrison and others (1987) reported that copper discharged from the San Onofre power plant cooling system was mostly in bound forms under normal operating conditions. Their study found sufficient organic ligands available in ambient seawater to complex most of the copper and expected little or no impact from the discharges. Harrison and others (1987) conducted copper bioassays on a number of aquatic invertebrate and vertebrate species. They found that *Crassostrea gigas* embryos were most sensitive (48-h LC<sub>50</sub>=10 µg/L) and larval herring the least sensitive. The range of 48-h LC<sub>50</sub> values for copper was 10 to 2,000 µg/L. Dinnel and others (1983) published the results of copper toxicity bioassays on various life stages of several marine organisms. They reported a very low LC<sub>50</sub> (1.9 µg/L) for the sperm of the red sea urchin (*Strongylocentrotus franciscana*). This value seems suspect because it falls within the range normally expected in unpolluted seawater. Other values from the Dinnel and others (1983) study are presented in Table 35.

Gametes and the embryos of marine organisms are most sensitive to copper. Based on the previous discussion regarding the metabolic regulation of copper, it seems reasonable to suggest that the susceptibility of embryos to even low copper concentrations is associated with their inability to regulate cellular exposure to the cupric ion. Copper concentrations maintained at levels low enough to protect embryos are sufficient to ensure that toxic effects are not imposed on larvae and adult organisms. With the exception of the sperm of the red sea urchin, environmental levels less than 6 µg/L appear reasonable for the protection of aquatic life in marine waters. In areas where red urchins spawn, additional restrictions should be considered.

**Table 35—Total copper toxicity measured in controlled bioassays**

Taxa	EC <sub>50</sub> or LC <sub>50</sub> (µg Cu/L)
Sperm	
Purple sea urchins	34.0
Oysters	12.1
Salmon	44.2
Embryo	
Purple sea urchins	6.3
Oysters	6.1
Mussels	21.0 to 35.0
Larvae	
Crab zoea	95.7
Squid	309.0
Cabezon	95.3
Adults	
Sand shrimp	898.5
Shiner perch	417.7
Coho salmon smolt	601.0

Because of the variety of molecular structures containing copper in aquatic environments, and a lack of definitive information about their relative toxicity, no single analytical measurement is ideal for expressing copper concentrations with respect to their potential toxicity to aquatic life. Baldwin (1989) advises that active copper (operationally defined by acidifying the aqueous sample to pH 4 with nitric acid and measuring the concentration of copper that passes through a 0.45-µm membrane filter) is probably the best available measurement.

This review revealed few copper toxicity data that included an analysis of the form of copper used in the bioassay. Most toxicity data are reported on the basis of total or dissolved copper. If bioassays are conducted in distilled water with low complexing capacity, there is significant potential to overestimate the toxicity of copper in the natural environment. If 2-mg copper sulfate is added to a liter of distilled water, much of this may become available in its toxic cupric ion form. However, the same amount of copper added to organically rich estuarine water could result in only a small fraction being present in the toxic form, the majority of the copper being detoxified by adsorption to sediments and precipitation or complexation with organic molecules. These comments indicate the difficulty in accurately assessing the impact of copper in natural environments. However, because of the potential for detoxification, water quality criteria based on total copper will result in conservative criteria.

### Summary

This review of metal toxicity to aquatic organisms clearly demonstrates that copper is the metal of most concern in

association with CCA-treated wood used in aquatic environments. From a purely biological point of view, the cupric ion should be maintained at less than 6 µg/L in marine environments. No observed effect levels (NOEL) for copper in embryos were not found in this review. However, it seems reasonable that these levels would be at least half the 48 h-LC<sub>50</sub> level, or 3 µg/L. Chromium is less toxic, and susceptible species have LC<sub>50</sub> ≥ 200 µg/L. Arsenic, which is notoriously toxic to humans and other mammals in some valence states, is highly tolerated by marine animals at levels up to 230 µg/L. However, the susceptibility of some species of red algae impose an upper limit of 19 µg/L in marine environments where they are present. The most susceptible freshwater species, examined to date, tolerate up to 168 µg/L arsenic.

Most potentially toxic substances are regulated at the Federal and State Levels. Therefore, it would be presumptuous to suggest that these values should be adopted as appropriate benchmarks when considering the use of treated wood in aquatic environments. This review is intended to provide insight to the regulatory standards, which are discussed in the following section.

### Regulatory Benchmarks

Numerous states have adopted U.S. EPA water quality criteria for metals. Washington State Administrative Code (1992) defines water quality standards for surface waters. The code states that toxic substances shall not be introduced above natural background levels in waters of the state, which have the potential either singularly or cumulatively to adversely affect characteristic water uses, cause acute or chronic toxicity to the most sensitive biota dependent upon those waters, or adversely affect public health, as determined by the Department of Ecology. Table 36 lists criteria established for the protection of aquatic life in Washington State.

The U.S. EPA marine water quality criteria are presented in Table 37. Direct comparison of the U.S. EPA criteria for copper and zinc with the Washington State Standards is not appropriate because the latter are variable and depend on water hardness. Table 37 also compares U.S. EPA criteria with existing average metal concentrations in unpolluted areas of Puget Sound.

With the exception of the reported copper toxicity to red sea urchin sperm, both the State and Federal criteria are consistent with biological considerations and reflect significant safety factors for the protection of aquatic life.

### Marine Sediment Standard

Washington is the only jurisdiction that has developed Marine Sediment Quality Standards for metals (Washington State Administrative Code 1991). These standards are based on apparent effects thresholds (AETs). These sediment standards are summarized in Table 38 together with the TEL and

**Table 36—Water quality standards for surface waters in Washington state, based on EPA criteria<sup>a</sup>**

Contaminant	Water quality (µg/L)			
	Fresh acute	Fresh chronic	Marine acute	Marine chronic
Arsenic	360	190	69	36
Chromium(VI)	16.0	11.0	1,100.0	50.0
Copper	10.4	7.3		2.5

<sup>a</sup>A hardness of 59 mg calcium carbonate/L was used for values requiring computation.

**Table 37—Marine water quality criteria and ambient concentrations of dissolved metals in Puget Sound seawater<sup>a</sup>**

Metal	Ambient concentrations of dissolved metals (µg/L)	
	EPA water quality criteria	Puget Sound ambient levels
Arsenic	36.0	1.50
Copper	2.5	0.25
Zinc	58.0	0.50

<sup>a</sup>U.S. EPA 1985.

**Table 38—Comparison of marine sediment quality criteria (AET) and benchmarks (TEL and PEL)**

Metal	Value (µg metal/g dry sediment)			
	AET <sup>a</sup>	TEL <sup>b</sup>	PEL <sup>b</sup>	(TEL + PEL)/2
Copper	390	18.7	108	63.35
Chromium	260	52.3	160	106.15
Arsenic	57	7.24	41.6	24.42

<sup>a</sup>Apparent effects threshold (AET) based marine sediment quality criteria defined by Washington State.

<sup>b</sup>Threshold effects level (TEL) and probable effects level (PEL) marine sediment quality benchmarks (Jones and others 1997).

**Table 39—Freshwater sediment benchmarks<sup>a</sup>**

Metal	Threshold effects level (TEL)	Probable effects level (PEL)	(TEL+PEL)/2
Copper	28	100	64
Chromium	36	120	78
Arsenic	11	48	30

<sup>a</sup>Ingersol and others (1996). These are not regulatory criteria. They are benchmarks for screening sediments based on amphipod and midge bioassays.

PEL developed by the Florida Department of Environmental Protection. These are marine sediment quality benchmarks reported in Jones and others (1997). They are not sediment quality criteria. British Columbia has recommended sediment criteria based on the mean of the TEL and PEL. That value is included in Table 38. The last value ((TEL + PEL)/2) was used in this assessment to predict adverse biological effects in marine environments.

### Freshwater Sediment Benchmarks

Regulatory criteria have not been developed for metals in freshwater sediments. However, Ingersol and others (1996) published benchmarks for evaluating freshwater sediments based on amphipod and midge bioassays. Benchmarks provided by these authors are summarized in Table 39.

### Summary of Sources and Toxicity in Aquatic Environments

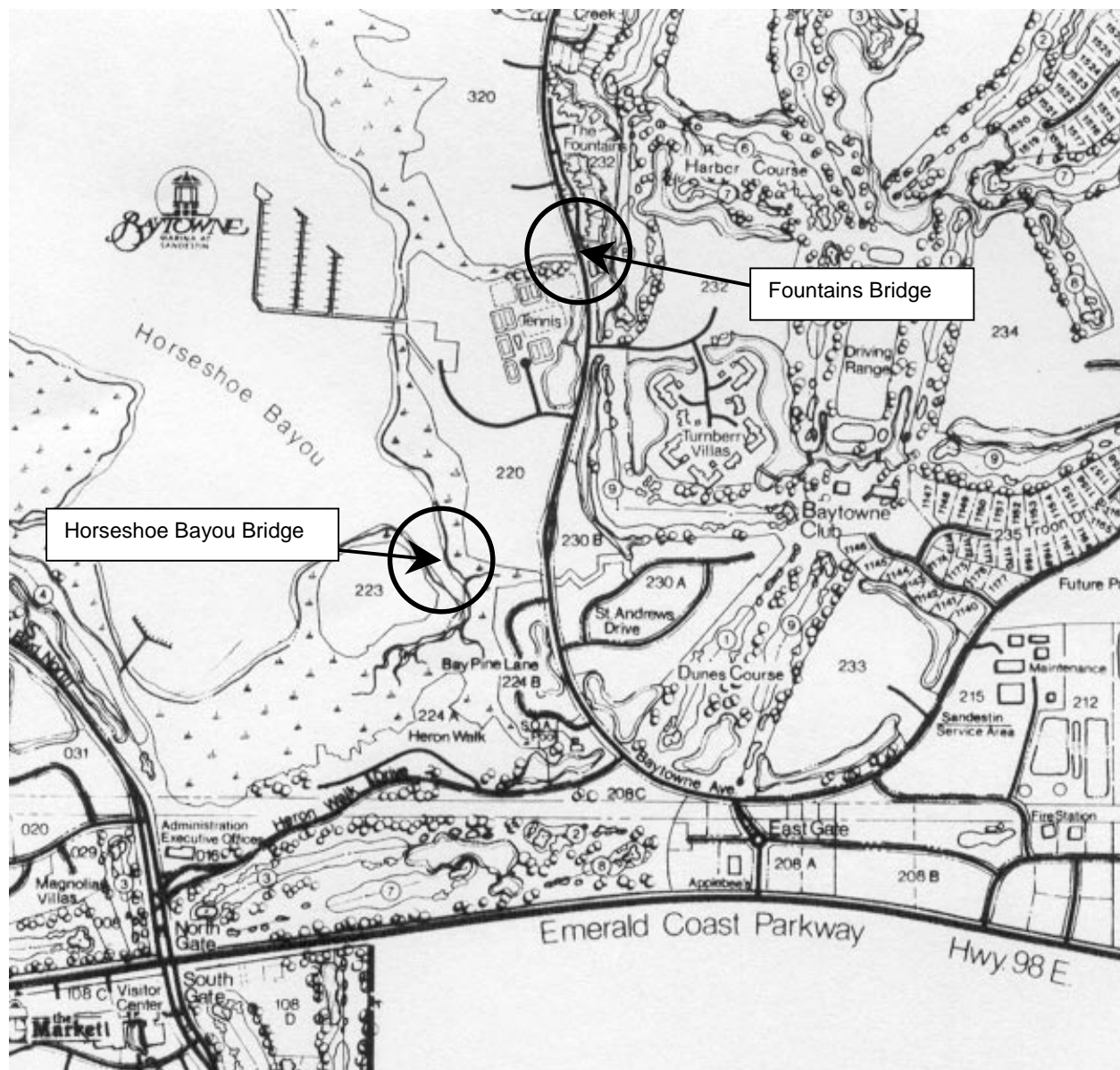
The metals of concern when considering the use of CCA-treated wood in aquatic environments are arsenic, chromium, and copper. These metals are natural components of the earth's crust and are found at various concentrations in both freshwater and marine environments. All three are essential micronutrients (Neff 1997). However, high concentrations of arsenic, chromium, and copper are known to be toxic. Arsenic and chromium are tolerated at moderately high levels by aquatic species. Buchanan and Solomon (1990) concluded that chromium and arsenic concentrations in CCA leachate were deemed sufficiently low as to have little or no effect on LC<sub>50</sub> determinations.

With respect to CCA-treated wood, copper is the most toxic metal to aquatic organisms, particularly in marine environments. Copper toxicity, associated with the uncomplexed cupric ion, is most detrimental to the early life stages of marine invertebrates. These stages are not readily visible and therefore this toxicity is not necessarily apparent to casual observation.

This risk assessment used regulatory criteria for dissolved metals in freshwater and marine environments. The recommendations of Brooks (2000) and the mean of TEL and PEL was used in lieu of freshwater sediment quality criteria, which have not yet been adopted. The Washington State Sediment Quality Criteria and the mean of the TEL and PEL were used to evaluate marine sediment metal concentrations associated with CCA-treated timber bridges.

### Results From Horseshoe Bayou Bridge

More than 20 CCA-treated bridges were evaluated during the site selection process. Most existing CCA-treated bridges are pedestrian in nature and were excluded for that reason. Because metal losses decline exponentially with time from CCA-treated wood (Brooks 1996, 1997c), it was considered important to evaluate at least one CCA-treated timber bridge



**Figure 36—Site plan showing the location of the Horseshoe Bayou Bridge (marine) and the Fountains Bridge (freshwater) located in Sandestin Resort, Sandestin, Florida.**

within a week of completing construction. An HS20 bridge, treated entirely with CCA and scheduled for completion in March 1998 was located in Sandestin, Florida, at the Sandestin Resort. The location of the bridge is identified in Figure 36 along with the second CCA-treated bridge examined in this evaluation. The Horseshoe Bayou Bridge crosses Choctawhatchee Bay and provides access to Jolee Island. The total length is 84.6 m (282 ft). A copy of the bridge elevation plan is provided in Figure 37. The Horseshoe Bayou Bridge (HBB) sits on 87 Class A southern yellow pine piling treated to a retention of  $40 \text{ kg/m}^3$  ( $2.5 \text{ lb/ft}^3$ ) with CCA type C. Piling cross bracing was treated to  $12.8 \text{ kg/m}^3$

( $0.8 \text{ lb/ft}^3$ ) with CCA-C; the bridge deck, support beams, and railings were treated to  $6.4 \text{ kg/m}^3$  ( $0.40 \text{ lb/ft}^3$ ) CCA. The bridge was nearing completion on March 22, 1998, when the risk assessment evaluation was conducted.

#### ***Rainfall in Sandestin, Florida***

February and March of 1998 were wetter than normal in Sandestin, Florida. Table 40 describes rainfall patterns prior to the time of evaluating the new Horseshoe Bayou CCA-treated bridge. The weather was clear and sunny during this evaluation. However, 38 mm (1.5 in.) of rain fell on February 16, 1998, and a total of 110 mm (4.23 in.) fell during the

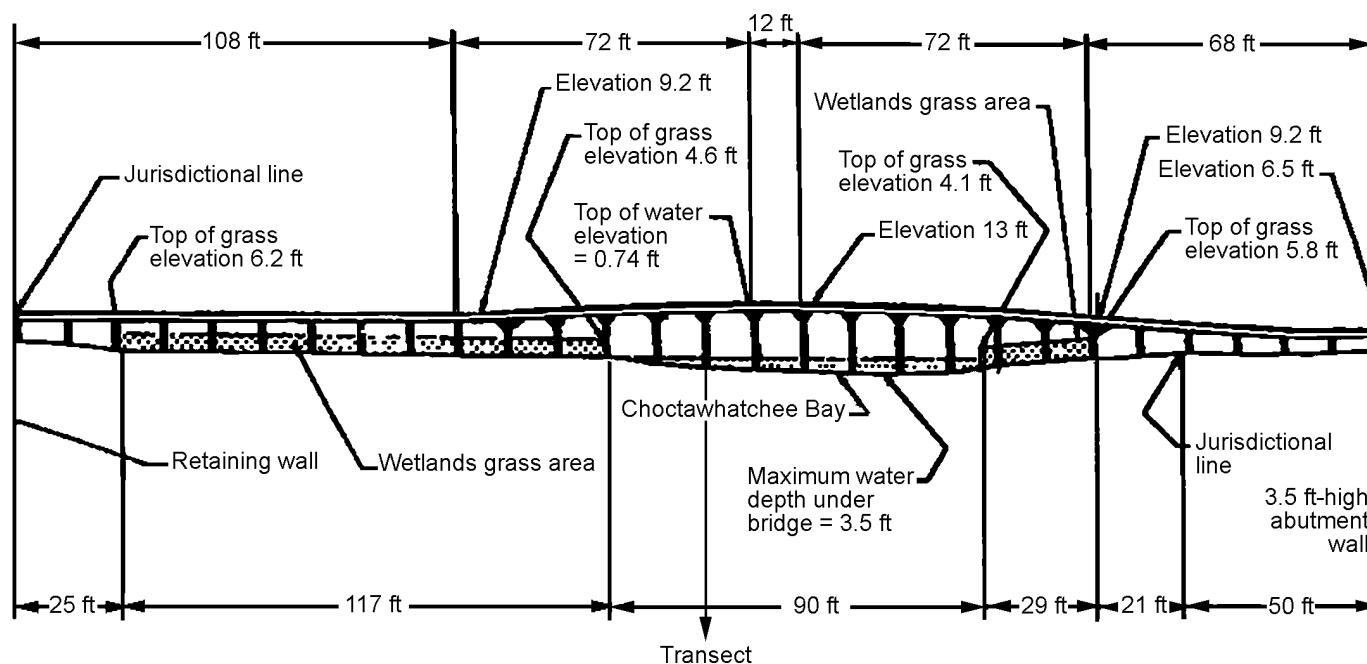


Figure 37—Elevation drawing of the Horseshoe Bayou Bridge in Sandestin Florida. The sampling transect is shown.

Table 40—Sandestin, Florida, rainfall

Month	Rainfall (mm (in.))	
	Average	1998
January	113.5 (4.47)	255.0 (10.04)
February	124.4 (4.90)	245.1 (9.65)
March	143.8 (5.66)	203.5 (8.01)

preceding 2 weeks. Rainfall was documented because most of the structure is located above water. The fact that significant rainfall occurred during construction and immediately prior to evaluating this bridge is important because it exacerbates the potential for metal loss from the overhead structure.

Figure 38 shows that portion of the bridge crossing Choctawhatchee Bay. Figure 39 is a copy of the elevation plan for this bridge with the location of the sampling transect annotated. Water depths in Horseshoe Bayou under the bridge varied between 0.0 and 104 cm. The salinity was 25.5 ppt and the water temperature 15.8°C. The pH of the water was 6.9. Currents were measured using a drogoue. They were less than 1.0 cm/s during slack tide and increased to 2.5 cm/s 3 h following slack. The water depths given in Table 41 were measured during low slack tide. All suspended solids in the water were organic (volatile).

#### ***Dissolved Copper, Chromium, and Arsenic***

Figure 39 summarizes the concentrations of dissolved metals in the water column as a function of distance from the Horseshoe Bayou Bridge. The U.S. EPA chronic marine water quality criteria are 2.5 µg copper/L, 36 µg arsenic/L, and 50 µg chromium(VI)/L.

All observed water column metal concentrations were close to background, and none approached its respective criterion. These results suggest that there were no adverse biological effects associated with dissolved metals lost from this newly constructed bridge treated with CCA-C. Differences in the concentration of dissolved metals were not significant between any two stations. Metal concentrations were slightly higher further from the bridge.

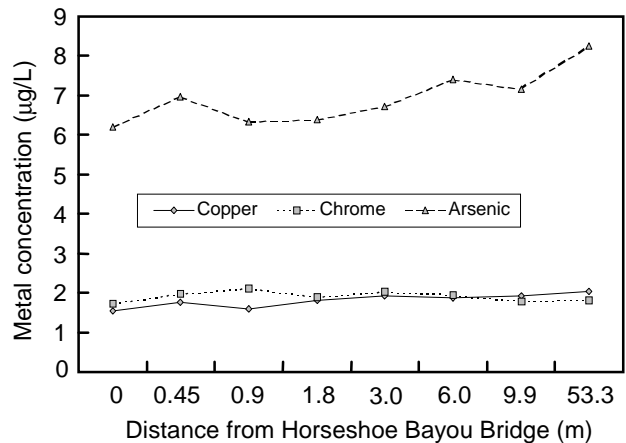
#### ***Sedimented Copper, Chromium, and Arsenic***

During construction of this bridge, 1,568 holes, each 19 mm (0.75 in.) in diameter and 0.35 m (14 in.) long, were drilled in the treated wood to secure structural members with galvanized bolts. Shavings from these borings were not cleaned up, and they blew into the estuary, forming mats in the grass (Fig. 40). No effort was made to avoid these shavings during sampling. Sediment physicochemical characteristics observed on March 22, 1998, are summarized in Table 42. Sediment concentrations of copper, chromium, and arsenic are higher within 3 m (10 ft) of the bridge. The distribution





**Figure 38—The Horseshoe Bayou Bridge crossing Choctawhatchee Bay in Sandestin, Florida. The sampling transect is noted.**



**Figure 39—Dissolved copper, chromium, and arsenic observed in salt water as a function of distance from the Horseshoe Bayou Bridge in Sandestin, Florida.**

**Table 41—Physicochemical characteristics observed on March 22, 1998, in the water column at Horseshoe Bayou Bridge, Choctawhatchee Bay<sup>a</sup>**

Station	Depth (cm)	pH	Cu (µg/L)	Cr (µg/L)	As (µg/L)	TSS (mg/L)	TVS (mg/L)	Salinity (ppt)	(°C)
-52.5 m (-175 ft) control	30	8.1	2.03 ± 0.35	1.80 ± 0.25	8.27 ± 2.10	3.6	3.6	25.0	15.8
0.0 under bridge	40	8.1	1.55 ± 0.10	1.72 ± 0.35	6.19 ± 0.43			25.0	15.8
0.45 m (1.5 ft) downstream	40	8.1	1.75	1.98	6.97	10.0	10.0	25.0	15.8
0.9 m (3.0 ft) downstream	30	8.1	1.59	2.10	6.34			25.0	15.8
1.8 m (6.0 ft) downstream	30	8.1	1.80	1.88	6.38	5.2	5.2	25.1	15.8
3 m (10 ft) downstream	30	8.1	1.92	2.02	6.71			25.1	15.8
6 m (20 ft) downstream	30	8.1	1.86	1.95	7.41			25.0	15.8
9.9 m (33 ft) downstream	30	8.1	1.92	1.78	7.16	4.4	4.4	25.0	15.8

<sup>a</sup>The control and samples taken under the bridge are the mean of three replicates. Where appropriate, values include 95% confidence limits on the mean.

of these metals was very patchy, and high values were associated with variance to mean ratios that significantly exceed one. The ratio of CuO:CrO<sub>3</sub>:As<sub>2</sub>O<sub>5</sub> in CCA is 18.5:47.4:34 by weight (AWPA 1996). In all except results from the sample collected 0.9 m (3 ft) from the bridge, the ratio of metals in these sediment samples approximated that of CCA-C. The presence of the wood chips, coupled with the patchy distribution and the ratio of metals, suggests that the observed metals were in fact associated with the debris and were not the result of loss from the preserved wood followed by sorption and sedimentation. These metals remain fixed in the wood debris and were not generally bioavailable. However, this debris represents an “unnecessary environmental risk.” Sediment concentrations of copper, chromium, and arsenic are summarized in Figure 41.

Sediment concentrations of metals observed in the vicinity of the Horseshoe Bay Bridge are summarized in Table 43 along with sediment benchmarks from Table 38. The maximum sediment concentration of copper did not exceed the TEL. However, the maximum concentrations of chromium and arsenic did exceed their respective TEL, and it was possible that adverse effects might be observed in the biota if this metal was bioavailable. However, from a realistic point of view, mean metal concentrations at all stations were less than or equal to the (TEL + PEL)/2 benchmark used in this assessment, and adverse effects were considered unlikely. This is especially true in light of the preceding discussion regarding the nature of the nearfield metals and the likelihood that they were fixed in the shavings and not bioavailable.

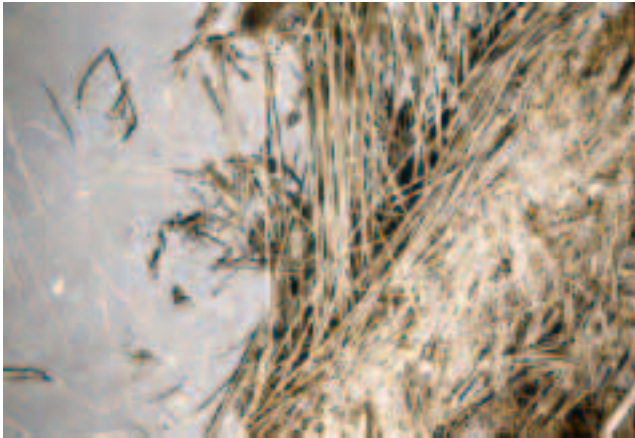


Figure 40—CCA-treated wood shavings deposited in Choctawhatchee Bay as a result of in situ boring of the bridge structure during construction.

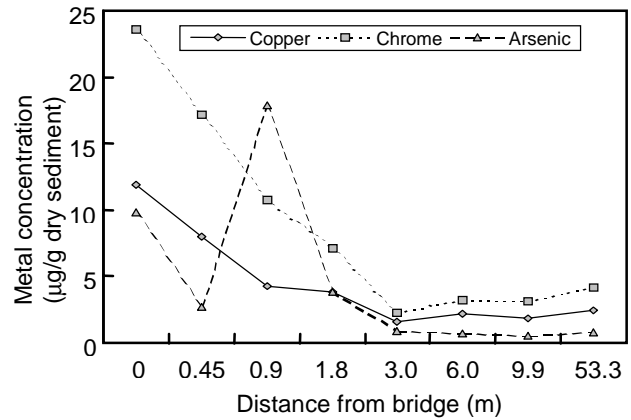


Figure 41—Sediment concentrations of copper, chromium, and arsenic observed in the vicinity of Horseshoe Bayou Bridge in Sandestin, Florida.

Table 42—Physicochemical characteristics observed on March 22, 1998, in sediments at Horseshoe Bayou Bridge, Choctawhatchee Bay<sup>a</sup>

Station	Depth (cm)	Cu (mg/kg)	Cr (mg/kg)	As (mg/kg)	TVS (%)	Sand (%)	Silt and clay (%)
-52.5 m (-175 ft) control	30	2.43 ± 0.43	4.13 ± 0.82	0.80 ± 0.11	1.34 ± 0.28	84.38 ± 4.21	14.27 ± 2.12
0.0 under bridge	40	11.87 ± 13.01	23.57 ± 30.16	9.80 ± 14.90	2.95 ± 1.33	79.47 ± 4.63	20.19 ± 4.43
0.45 m (1.5 ft) downstream	40	7.95 ± 11.60	17.20 ± 26.40	2.65 ± 3.60	0.23	91.76	8.24
0.9 m (3.0 ft) downstream	30	4.25 ± 0.72	10.80 ± 0.64	17.90 ± 20.96	5.12	88.35	11.14
1.8 m (6.0 ft) downstream	30	3.80 ± 3.84	6.93 ± 7.84	3.80 ± 5.12	1.19	87.75	12.17
3 m (10 ft) downstream	30	1.55 ± 0.72	2.30 ± 1.44	0.85 ± 0.08	0.43	90.44	9.56
6 m (20 ft) downstream	30	2.15 ± 0.24	3.20 ± 0.48	0.70 ± 0.00	0.81	86.33	13.30
9.9 m (33 ft) downstream	30	1.80 ± 0.16	3.15 ± 0.40	0.55 ± 0.08	0.99	87.65	12.35

<sup>a</sup>The control and samples taken under the bridge are the mean of three replicates. Where appropriate, values include 95% confidence limits on the mean.

### Biological Response—Infauna

A total of 1,537 invertebrates representing 46 taxa were identified in the 18 samples covering 0.0309 m<sup>2</sup> each. Taxa representing a minimum of 1% of the total (15 individuals) were considered dominant. Table 44 describes the dominant taxa observed in these samples.

Dominant taxa represent 89% of the total invertebrate abundance in these samples. The invertebrate community is dominated by polychaetes, particularly deposit feeders that might be sensitive to sedimented metals that are bioavailable. The number of taxa, total species abundance, and dominant species abundance are displayed in Figure 42 as a function of distance upstream and downstream from the Horseshoe

**Table 43—Mean and maximum concentrations of copper, chromium, and arsenic observed in sediments adjacent to the newly constructed Horseshoe Bayou Bridge in Sandestin, Florida, compared with TEL and PEL**

Metal	Maximum value (mg/kg)	Maximum mean (mg/kg)	PEL	TEL	(TEL + PEL)/2
Copper	25.1	11.87	100.00	28.00	64.00
Chromium	54.3	23.57	120.00	36.00	78.00
Arsenic	31.0	17.90	48.00	11.00	30.00

Bayou Bridge. Sediment values of arsenic were included because this was the only metal that exceeded its respective benchmark, albeit by a small amount (31/30).

There was a shallow cline in the number of taxa observed in the vicinity of the Horseshoe Bayou Bridge. More taxa were found under the bridge than were found at further distances. This cline was investigated using linear regression analysis on  $\log(N + 1)$  transformed count data; log transformed continuous data; and arcsine(square root(proportion)) transformed proportional (TVS and fines) data. The number of taxa was determined only by the constant term and the depth of the reduction–oxidation potential discontinuity in a linear

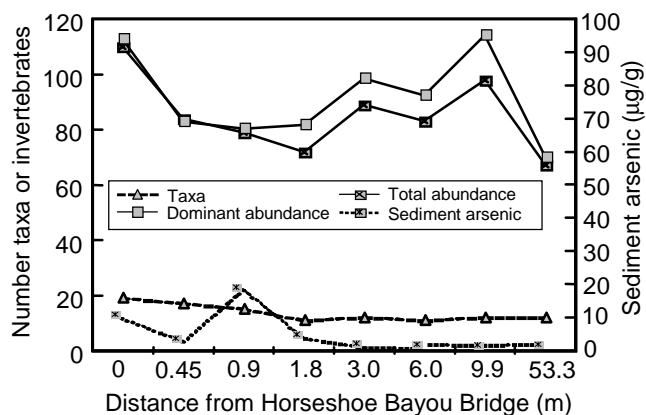
regression. The null hypothesis that the constant term and coefficient on RPD were zero was rejected at  $\alpha = 0.05$ , but the regression explained only 24% of the variation in the database. Total abundance and dominant taxa abundance were not significantly different ( $\alpha = 0.05$ ) between the three replicates collected under the bridge and those collected at the reference site.

The relationships between Shannon’s and Pielou’s Indexes and sediment physicochemical parameters are explored in Figure 43. These results are consistent with the hypothesis that the observed metals were associated with wood fibers (drill shavings) from bridge construction. This statement is supported by the exceptionally high value of TVS at Station 0.9 m (3 ft), where the high metal content was also observed. The proportion sediment silt and clay (FINES) was not elevated at this station, suggesting that the increase was not associated with the accumulation of detritus. The metals bound in the wood fibers would not be biologically available, and that explains the lack of significant negative correlation between biological endpoints and sediment metal concentrations. Shannon’s and Pielou’s Indexes were highest under the bridge and out to 0.9 m (3 ft) from the perimeter in the area where the highest concentrations of metals were also observed. This would be an unlikely finding if the metals were biologically available and of sufficiently high concentration to create adverse effects.

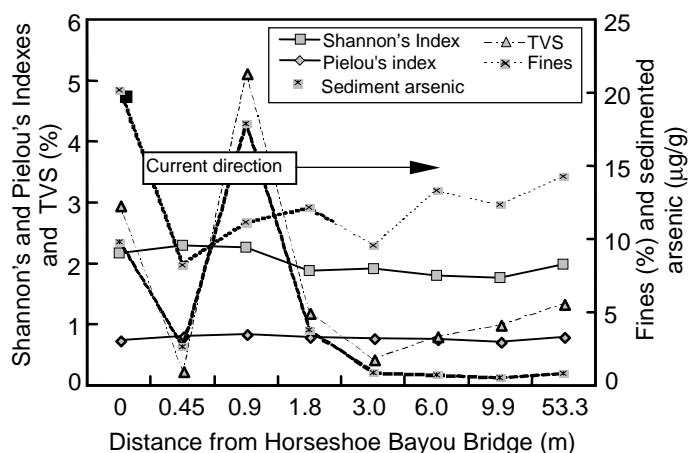
**Table 44—Dominant invertebrate taxa observed in sediment samples collected in the vicinity of Horseshoe Bayou Bridge in Sandestin, Florida<sup>a</sup>**

Dominant taxa	Code	Number of samples	Total abundance	Trophic group
Arthropods				
<i>Hargeria rapax</i>	AHARGPAX	15	51	
<i>Grandidierella bonnieroides</i>	AGRANDIB	12	59	
Mollusks				
<i>Neritina usnea</i>	MGNERITI	10	44	Vegetarian
<i>Tagelus plebeius</i>	MBTAGPLE	9	19	
<i>Eschadium recurvum</i>	MISCHREC	2	22	
Polychaetes				
<i>Neanthes succinea</i>	PNEASUC	17	119	Omnivore
<i>Neanthes micromma</i>	PNEAMIC	17	393	Omnivore
<i>Capitella capitata</i>	PCAPCAP	12	38	Detritivore
<i>Mediomastus ambiseta</i>	PMEDAMB	15	161	Detritivore
<i>Ampharete americana</i>	PAMPHAME	18	369	Detritivore
<i>Leitoscoloplos robustus</i>	PLEITORO	9	22	Detritivore
<i>Leitoscoloplos fragilis</i>	PLEITOFR	15	72	Detritivore
Abundance of dominant taxa			1,369	
Total abundance of all taxa			1,537	

<sup>a</sup>Eighteen samples were collected, each covering 0.0309 m<sup>2</sup>.



**Figure 42—The number of taxa, the abundance of all taxa, and only dominant taxa compared with sediment concentrations of arsenic observed at the Horseshoe Bayou CCA-C-treated bridge in Sandestin, Florida.**



**Figure 43—Biological endpoints (Shannon and Pielou Indexes) compared with sediment arsenic, total volatile solids (TVS), and percentage of silt and clay (FINES) as a function of distance from the recently constructed Horseshoe Bayou Bridge in Sandestin, Florida.**

A strong acid sample digestion (EPA 3000 Series) procedure was used in the analysis of metals. This procedure releases copper, chromium, and arsenic from wood fibers.

There was an observable decrease in the abundance of invertebrates in sediments located at intermediate distances from the perimeter of the bridge (stations 0.45, 0.9, 1.8 m). To investigate this further, transformed data were subjected to correlation analysis with all biological endpoints along one vector and sediment physicochemical parameters along the other. The resulting matrix is presented in Table 45. The number of taxa and Shannon's Index are negatively correlated with the depth of the RPD. This was unexpected because the deeper the RPD, the more "room" there is in the sediment column supporting aerobic metabolism. However,

the minimum depth of the RPD in these sediments was 1.0 cm, which in the author's experience is sufficient to support a healthy invertebrate community. The only significant correlation between sediment metals and biological response was the positive correlation between the abundance of *Capitella capitata* and sediment concentrations of arsenic.

The relationship between sediment physicochemical characteristics and biological response was summarized using the varimax normalized principal factors (MINRES) analysis (Table 46). Loadings >0.60 are in bold type. The first two factors explained 45.4% of the variation. The next two factors explained an additional 21.7%, giving a total of 67.2% for the four factors considered in this analysis.

Factor 1 is defined by physicochemical variables measuring the concentration of sedimented metals and TVS in the positive direction. Sediments in this area were reasonably homogeneous. The strong correlation between sediment metal levels and TVS was probably associated with treated wood shavings lost during drilling numerous holes in the structure during construction. These metals would not be biologically available and therefore would have little effect on the infaunal community. Sediments under the bridge contained more fines and less oxygen (the RPD was not as deep). However, these loadings were not significant.

Factor 2 is defined primarily by biological variables. The correlations described by these loadings suggest that the abundance of *Neanthes micromma*, *Mediomastus ambiseta*, and *Ampharete americana* were significant factors in determining the total taxa abundance and dominant species abundance. The arthropod *Hargeria rapax* and the number of taxa were also moderately positively correlated with the significant factors. Note that these orthogonal factors are independent and that sediment concentrations of metals (copper, chromium, and arsenic) have very low loadings on factor 2 and therefore have little influence on these biological metrics.

Factors 3 and 4 suggest interesting ecological relationships, but metals are not significantly loaded on either of these factors, suggesting that the relationships are independent of sediment concentrations of copper, chromium and arsenic, at least at the levels observed in this study.

The reason for the independence of the biological variables expressed in factors 2, 3, and 4 from the physicochemical variables described in factor 1 is that the metal levels are low and much of the metal observed is likely fixed in the wood shavings and not bioavailable.

Figure 44 displays the relationship between the variables and factors 1 and 2. The distance between Shannon's Index and measures of abundance (LABUND and LDABUND) suggests that increases in abundance were primarily dominated by a few species—particularly the polychaetes *Neanthes*

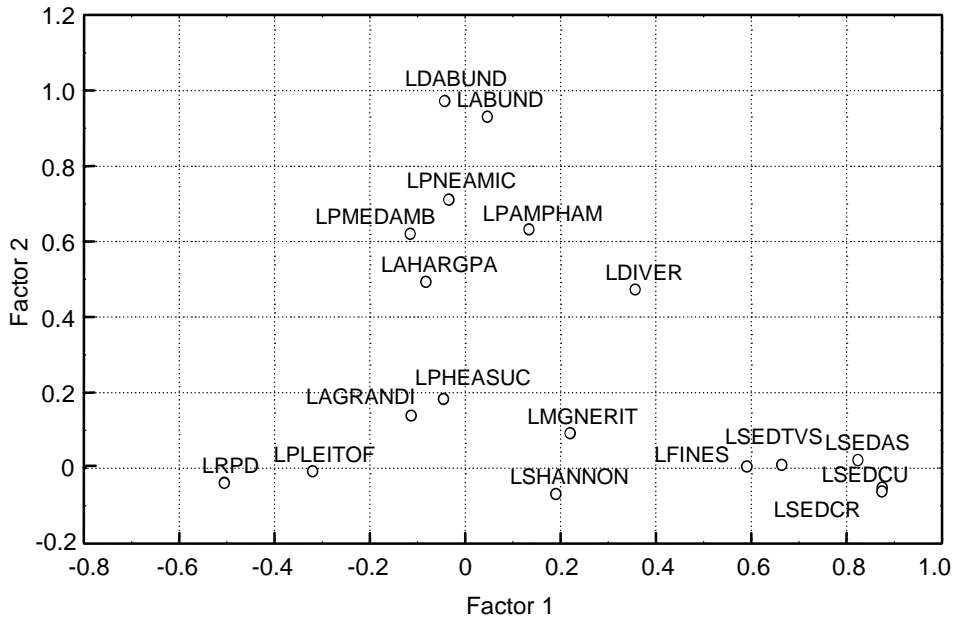
**Table 45—Matrix of Pearson correlation coefficients comparing biological endpoints, including individual dominant taxa and all metrics with sediment metal concentrations, depth of the aerobic layer (RPD), total volatile solids (TVS), and percentage silt and clay at the Horseshoe Bayou Bridge in Sandestin, Florida**

	RPD	Copper	Chromium	Arsenic	TVS	Percentage silt and clay
<i>Hargeria rapax</i>	-0.03	-0.03	-0.03	0.07	-0.13	-0.27
<i>Grandidierella bonnieroides</i>	0.39	-0.16	-0.19	-0.37	-0.09	0.37
<i>Neritina usnea</i>	-0.44	0.35	0.36	0.46	0.03	-0.35
<i>Tagelus plebeius</i>	0.02	0.09	0.06	-0.09	-0.18	0.10
<i>Ischadium recurvum</i>	-0.42	0.12	0.17	0.41	0.35	0.06
<i>Neanthes succinea</i>	-0.24	0.07	0.06	0.13	-0.11	-0.34
<i>Neanthes micromma</i>	0.04	-0.03	-0.04	0.02	-0.14	-0.13
<i>Capitella capitata</i>	-0.46	0.40	0.36	0.47	0.09	-0.01
<i>Mediomastus ambiseta</i>	-0.03	-0.28	-0.27	-0.10	0.15	0.03
<i>Ampharete americana</i>	-0.28	-0.04	-0.05	0.08	0.23	0.16
<i>Leitoscoloplos robustus</i>	0.40	-0.28	-0.30	-0.37	-0.38	-0.29
<i>Leitoscoloplos fragilis</i>	0.33	-0.37	-0.31	-0.26	0.02	-0.21
Total abundance	-0.06	0.07	0.07	0.09	-0.01	-0.05
Number taxa	-0.51	0.39	0.35	0.30	0.21	0.11
Shannon's index	-0.49	0.25	0.25	0.31	0.16	-0.15
Pielou's index	-0.17	-0.02	0.01	0.15	0.00	-0.32
Dominant abundance	0.03	-0.03	-0.03	0.02	-0.05	-0.07

**Table 46—Summary of loadings on four factors associated with a varimax normalized principal factors (MINRES) analysis of physicochemical and biological variables measured in sediments adjacent to Horseshoe Bayou Bridge in Sandestin, Florida<sup>a</sup>**

	Factor 1	Factor 2	Factor 3	Factor 4
<i>Hargeria rapax</i> (AHARGPAX)	-0.08	0.49	0.02	0.40
<i>Grandidierella bonnieroides</i> (AGRANDIB)	-0.11	0.14	<b>-0.94</b>	0.01
<i>Neritina usnea</i> (MGNERITI)	0.22	0.09	<b>0.62</b>	0.55
<i>Neanthes succinea</i> (PHEASUC)	-0.05	0.18	<b>0.70</b>	0.25
<i>Neanthes micromma</i> (LPNEAMIC)	-0.03	<b>0.71</b>	0.24	-0.25
<i>Mediomastus ambiseta</i> (LPMEDAMB)	-0.12	<b>0.62</b>	0.08	-0.08
<i>Ampharete americana</i> (LPAMPHAME)	0.13	<b>0.63</b>	-0.20	0.01
<i>Leitoscoloplos fragilis</i> (LPLEITOFR)	-0.32	-0.01	-0.49	0.38
Total taxa abundance (LABUND)	0.05	<b>0.93</b>	0.00	0.30
Number of taxa (LDIVER)	0.36	0.47	0.10	<b>0.62</b>
Shannon's Index (LSHANNON)	0.19	-0.07	0.17	<b>0.95</b>
Depth of the RPD (LRPD)	-0.51	-0.04	-0.28	-0.26
Sediment copper concentration (LSEDCU)	<b>0.87</b>	-0.05	0.11	0.08
Sediment chromium concentration (LSEDCR)	<b>0.87</b>	-0.06	0.12	0.09
Sediment arsenic concentration (LSEDas)	<b>0.82</b>	0.01	0.25	0.14
Sediment total volatile solids (LSEDTVSt)	<b>0.66</b>	0.01	-0.12	0.02
Sediment silt and clay (LFINES)	0.59	0.00	-0.41	-0.25
Dominant species abundance (LDABUND)	-0.04	<b>0.97</b>	-0.01	0.16
Explained variation	3.62	3.64	2.51	2.32
Proportion of total variation	0.20	0.20	0.14	0.13

<sup>a</sup>Loadings in bold type are >0.60.



**Figure 44—Varimax normalized principal factors (MINRES) analysis of selected physicochemical and biological parameters, displaying the relationship between factors 1 and 2. Variable codes are given in Appendix B.**

*micromma*, *Ampharete americana*, and *Mediomastus ambiseta* and the arthropod *Hargeria rapax*.

**Laboratory Bioassays**

Water samples for *Menidia berylina* bioassays were collected within 15 cm of the piling at low slack tide using a portable peristaltic pump and precleaned, acid-washed platinumized silicone tubing. Water samples were filtered in the field through Corning Costar Membra-Fil filters (0.45 µm). The bioassay results are summarized in Table 47.

Survival was excellent in all tests, and there were no statistically significant differences in survival between any of the stations. These results are consistent with the low water

**Table 47—Summary results of Horseshoe Bayou water column bioassays using *Menidia berylina*<sup>a</sup>**

Replicate	Laboratory control	Surviving amphipods (%)			
		-52.5 m (-175 ft) control	+0.45 m (+1.5 ft)	+1.8 m (+6.0 ft)	+9.9 m (+33.0 ft)
1	90	90	80	80	90
2	82	100	90	90	70
3	100	80	90	90	100
4	100	100	90	100	100
Mean	93	92.5	87.5	90.0	90.0

<sup>a</sup>An initial amphipod count of 10 or 11 per replicate.

column concentrations of metals observed in the Horseshoe Bayou water.

**Summary of Biological Response**

Marine organisms are more susceptible to copper intoxication than are most freshwater taxa. This is reflected in the lower U.S. EPA dissolved copper standard in salt water (2.5 µg/L) than in freshwater (generally 5 to 20 µg/L). Therefore, this large structure, located in an area of very slow currents, represents a worst-case analysis. This evaluation was completed during construction of the bridge when metal loss rates from CCA-treated wood would be highest. Only background concentrations of copper, chromium, and arsenic were observed dissolved in the water column. This suggests that the wood was well treated and fixed prior to installation.

Moderately elevated levels of copper, chromium, and arsenic were observed in sediments adjacent to the bridge. The results of this analysis suggest the association with deposits of CCA-treated wood shavings lost from the bridge during drilling of 1,568 holes used to attach bracing and railings. The metals in these shavings were fixed to the wood fibers and likely had low bioavailability. However, this represents unnecessary risk and construction contracts should be conditioned to require immediate cleanup of this type of debris.

Adverse biological effects were not observed in infauna at the Horseshoe Bayou Bridge. Adverse effects would most likely have been observed during this study, conducted during construction, when metal loss rates were the highest. Principal factor's analysis indicated the biological endpoints



were independent of sediment or water column concentrations of copper, chromium, or arsenic. Shannon's Index and Pielou's Index were slightly higher within 0.9 m (3 ft) of the bridge compared with further distances, including the reference station. Similarly, the abundance and/or number of taxa observed at stations in the immediate vicinity of the bridge were as high as or higher than the same metrics observed at the reference station. Survival in laboratory bioassays was excellent at all stations, and statistically significant differences were not observed between any two stations.

Despite the loss of nearly 0.28 m<sup>3</sup> (10 ft<sup>3</sup>) of drill shavings to the local environment, the Horseshoe Bayou Bridge was having no effect on biological resources assessed in the evaluation.

### Results From Fountains CCA-Treated Bridge

The Fountains Bridge crosses a eutrophic freshwater marsh (Fig. 36). Figure 45 is a photograph of the bridge and local environment with the sampling transect indicated. Figure 46 depicts the environment adjacent to the bridge. This bridge was constructed 2 years prior to the evaluation. No water movement was detected in the vicinity of the Fountains Bridge, and it was anticipated what maximum sediment concentrations of copper, chromium, and arsenic associated with worst-case CCA-treated wood projects constructed in aquatic environments would be observed in sediments at this site.

### Rainfall in Sandestin, Florida

February and March of 1998 were wetter than normal in Sandestin, Florida. Table 40 describes rainfall patterns prior to the time of evaluating the new Horseshoe Bayou CCA-treated bridge. The weather was clear and sunny during this evaluation. However, 38 mm (1.5 in.) of rain fell February 16, 1998, and a total of 110 mm (4.23 in.) fell during the preceding 2 weeks. Rainfall was documented because most of the structure is located above water. The 2-year-old Fountains Bridge was expected to lose very little metal with long-term steady state loss rates of 0.114 µg/cm<sup>2</sup>-day for copper, 0.04 µg/cm<sup>2</sup>-day for arsenic, and 0.015 µg/cm<sup>2</sup>-day for chromium (Brooks 2000). These losses were less than 5% of predicted initial loss rates. Therefore, although the amount of rainfall recorded in the period immediately preceding this study was significant and greater than normal, the metals lost in association with the structure would likely not be detectable above background concentrations because of the age of the structure.

### Dissolved Copper, Chromium, and Arsenic

Table 48 summarizes physicochemical characteristics observed in the water column adjacent to the Fountains Bridge during March 20–23, 1998. Figure 47 summarizes the concentrations of total metals in the water column as a function of distance from the Fountains Bridge.



Figure 45—Fountains Bridge in Sandestin, Florida.



Figure 46—Marshlands where the invertebrate effects of the Fountains CCA-treated timber bridge were evaluated in Sandestin, Florida.

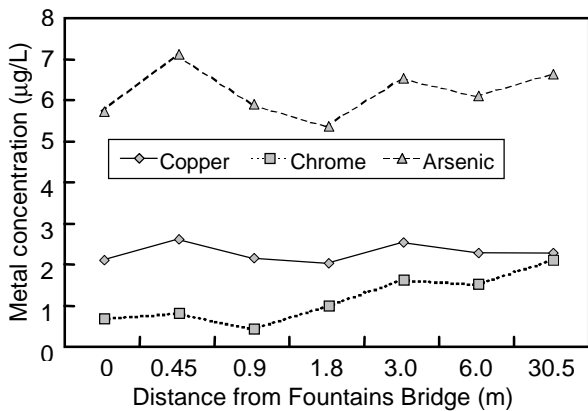
The marshland associated with the Fountains Bridge is eutrophic, with mats of vegetation and blue green algae. Alkalinity was low and hardness was moderate at 59.0 mg calcium carbonate/L. The low pH was not expected because of the amount of vegetation growing in the pond. However, increased metal loss would not be predicted from CCA-treated wood at the values observed (Cooper 1991). At the measured hardness, the U.S. EPA water quality criterion for copper is 6.49 µg Cu/L; chronic arsenic criterion is 190 µg As/L; chromium(VI) standard is 11.0 Cr(VI)/L, chromium(III) criterion is 134 µg Cr(III)/L. Cooper (unpublished data) noted that 95% of the chromium released from CCA-treated wood in leaching studies was in the chromium(III) valence state. In any case, increased levels of copper, chromium, and arsenic were not observed in the vicinity of the bridge and the background levels are well below chronic criteria.

These results suggest that there would be no adverse biological effects associated with dissolved metals lost from this 2-year-old bridge treated with CCA-C. Differences in the

**Table 48—Physicochemical characteristics observed in the water column at the Fountains Bridge in Sandestin, Florida on March 23, 1998<sup>a</sup>**

Station	Depth (cm)	Hardness (mg/L)	Alkalinity (mg/L)	pH	Copper (µg/L)	Chromium (µg/L)	Arsenic (µg/L)	TSS (mg/L)	TVS (mg/L)	Temp (°C)
0.0 under bridge	18.3	59.0 ± 21.5	17.1	6.40	2.11	0.68	5.73	1.52	1.52	15.8
0.45 m (1.5 ft) downstream	15.0	59.0 ± 21.5	17.1	6.41	2.62	0.80	7.12	1.52	1.52	15.8
0.9 m (3.0 ft) downstream	17.5	59.0 ± 21.5	17.1	6.35	2.15	0.44	5.91	1.52	1.52	15.8
1.8 m (6.0 ft) downstream	17.5	59.0 ± 21.5	17.1	6.20	2.03	1.00	5.37	1.52	1.52	15.8
3 m (10 ft) downstream	30.0	59.0 ± 21.5	17.1	6.25	2.53	1.61	6.52	1.52	1.52	15.8
6 m (20 ft) downstream	20.0	59.0 ± 21.5	17.1	6.25	2.29	1.52	6.11	1.52	1.52	15.8
9.9 m (33 ft) downstream	20.0	59.0 ± 21.5	17.1	6.29	1.40	1.37	7.48	1.52	1.52	15.8
30 m (100 ft) reference	18.3	59.0 ± 21.5	17.1	6.08	2.29	2.08	6.64	1.52	1.52	15.8

<sup>a</sup>The +30 m (+100 ft) reference station and samples taken under the bridge are the mean of three replicates. Where appropriate, values include 95% confidence limits on the mean.



**Figure 47—Dissolved copper, chromium, and arsenic observed in saltwater as a function of distance from the freshwater Fountains Bridge in Sandestin, Florida.**

concentration of dissolved copper and arsenic were not significantly different between any two stations, and maximum concentrations of arsenic were less than 20% of the current drinking water standard (50 µg/L). Chromium concentrations increased with distance from the bridge. Dissolved chromium was significantly higher at a distance of 30 m (100 ft) from the bridge compared with the 0.9-m (3.0-ft) station. Regression analysis indicated that the apparent slope observed in the chromium data was statistically significant. The null hypothesis that either the constant term or the coefficient on distance were zero was rejected at  $\alpha = 0.05$  ( $P_{Cr} = 0.004$  and  $P_{constant} = 0.000$ ). The resulting regression was

$$\text{Dissolved Chromium } (\mu\text{g/L}) = 0.090 + 0.001 \times \text{Distance}$$

### **Sedimented Copper, Chromium, and Arsenic**

Sediment physicochemical characteristics are summarized in Table 49, and sediment concentrations of copper, chromium, and arsenic are provided in Figure 48. Sediment levels of copper, chromium, and arsenic were slightly elevated to a distance between 3 and 10 m (10 and 20 ft) from the perimeter of the Fountains Bridge.

Sediment concentrations of metals observed in the vicinity of the Fountains Bridge are compared with sediment metal benchmarks in Table 50. All values, including the maxima observed in any sample, were less than the TEL, and no adverse biological effects could reasonably be expected.

### **Biological Response**

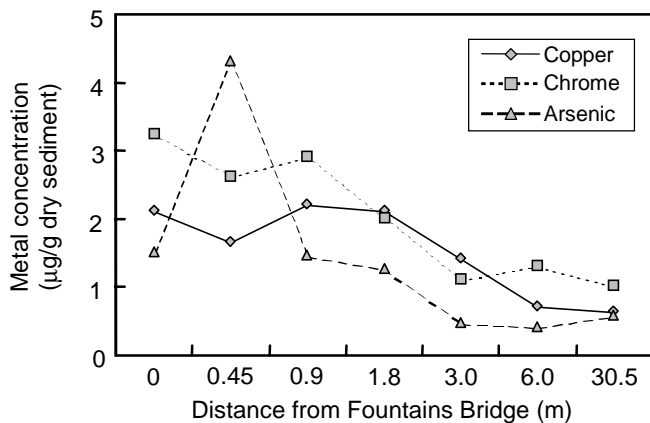
Sediments were heavily covered with vegetation. All vegetation and sediment to a depth of about 10 cm were removed from within a quadrat covering 0.03 m<sup>2</sup>. A total of 11,843 invertebrates representing 43 taxa were identified in the 18 samples. The invertebrate community was dominated by annelids (7,975 total). Taxa representing a minimum of 1% of the total, excluding annelids (11 taxa), were considered subdominant. Table 51 describes the dominant and subdominant taxa observed in these samples.

Dominant and subdominant taxa accounted for 98.2% of total abundance and included only 13 of the 43 taxa observed in 18 samples. Excluding annelids, the remaining 12 taxa represented 31% of the total abundance. All measures of diversity were based on the entire database. Abundance measures were analyzed using both total abundance and the abundance of only subdominant species.



**Table 49—Sediment physicochemical characteristics observed as a function of distance from the CCA-C-treated Fountains Bridge in Sandestin, Florida**

Station	Depth (cm)	Copper (mg/kg)	Chromium (mg/kg)	Arsenic (mg/kg)	TVS (%)	Sand (%)	Silt and clay (%)
0.0 under bridge	18.3	2.10 ± 1.08	3.23 ± 1.22	1.50 ± 0.74	1.13 ± 0.37	88.1 ± 5.4	8.3 ± 0.65
0.45 m (1.5 ft) downstream	15.0	1.65 ± 0.40	2.60 ± 0.80	4.30 ± 5.12	1.61	91.5	9.1
0.9 m (3.0 ft) downstream	17.5	2.20 ± 0.80	2.90 ± 1.12	1.45 ± 0.72	1.12	92.1	7.6
1.8 m (6.0 ft) downstream	17.5	2.10 ± 1.28	2.00 ± 0.48	1.25 ± 0.72	1.02	92.2	7.8
3 m (10 ft) downstream	30.0	1.40 ± 0.32	1.10 ± 0.16	0.45 ± 0.08	0.95	87.8	12.1
6 m (20 ft) downstream	20.0	0.70 ± 0.16	1.30 ± 0.48	0.40 ± 0.00	1.09	89.3	10.5
9.9 m (33 ft) downstream	20.0	1.20 ± 0.32	1.05 ± 0.56	0.63 ± 0.28	0.85	89.0	10.8
30 m (100 ft) reference	18.3	0.63 ± 0.46	1.00 ± 0.48	0.57 ± 0.17	1.42	90.6	9.3



**Figure 48—Sediment concentrations of copper, chromium, and arsenic observed in the vicinity of the Horseshoe Bayou Bridge in Sandestin, Florida.**

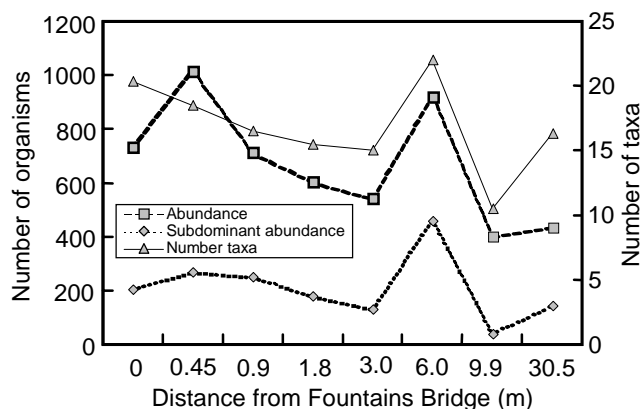
**Table 50—Mean and maximum concentrations of copper, chromium, and arsenic observed in sediments adjacent to the newly constructed Horseshoe Bayou Bridge in Sandestin, Florida, compared with TEL and PEL**

Metal	Maximum value (mg/kg)	Maximum mean (mg/kg)	TEL	TEL	TEL + PEL)/2
Copper	3.00	2.2 ± 1.28	100.00	28.00	64.00
Chromium	3.70	3.23 ± 1.22	120.00	36.00	78.00
Arsenic	7.50	4.30 ± 5.12	48.00	11.00	30.00

**Table 51—Dominant and subdominant invertebrate taxa observed in sediment samples collected in the vicinity of the Fountains Bridge in Sandestin, Florida<sup>a</sup>**

Dominant and subdominant Taxa	Code	Number of samples	Total abundance
Nematodes	NEMA	8	37
Annelida	ANNE	18	7,975
Mollusks			
Pseudosuccinea sp.	MGPSEU	11	54
Gyraulus sp.	MGGYS	15	779
Insecta			
Ephemeroptera			
<i>Caenis</i> sp.	AICAE	15	103
Diptera (Chironomoidea)			
<i>Bezzia</i> sp.	AICBEZ	18	154
Family Chironomidae	AICHR	16	152
<i>Larsia</i> sp.	AILRS	17	182
<i>Paramerina</i> sp.	AIPAAS	15	151
<i>Macropelopia</i> sp.	AIMACS	15	140
<i>Chironomus</i> sp.	AICHSS	18	1,426
<i>Paratendipes</i> sp.	AICPTS	18	395
<i>Zavrelimyia</i> sp.	AIZAV	17	85
Abundance of dominant and subdominant taxa			11,633
Abundance of subdominant taxa			3,658
Total abundance of all taxa			11,843

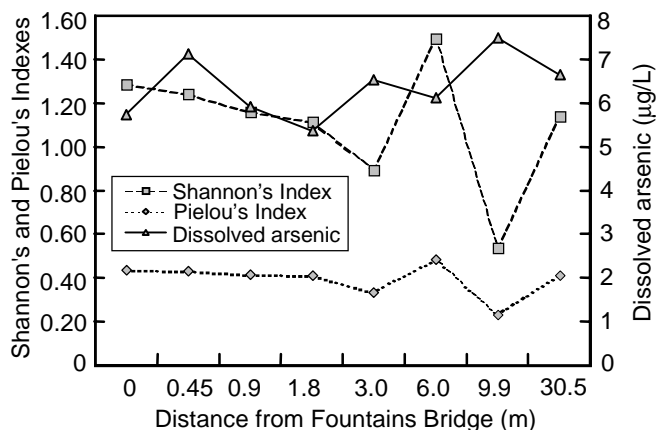
<sup>a</sup>A total of 18 samples were collected, each covering 0.030 m<sup>2</sup>.



**Figure 49—The number of taxa, the abundance of all taxa, and only dominant taxa compared with sediment concentrations of arsenic observed at the Horseshoe Bayou CCA-treated bridge in Sandestin, Florida.**

The number of taxa, total species abundance, and dominant species abundance are displayed in Figure 49 as a function of distance from the Fountains Bridge. There is an anomalous increase in all metrics at the 6-m (20-ft) station that does not correlate with any of the physicochemical variables. In addition, there is an apparent cline in all three metrics with decreasing values at increasing distances. Multiple regression indicated that none of these log transformed biological endpoints was significantly ( $\alpha = 0.05$ ) a function of distance (or any other independent physicochemical variables measured in this study).

Values of Shannon's and Pielou's Indexes are compared with observed concentrations of dissolved arsenic in Figure 50. Dissolved arsenic was chosen because all other significant metal endpoints were positively correlated with biological endpoints. The log transformed abundance of the chironomids *Macropelopia* sp. ( $p = -0.60$ ) and *Larsia* sp. ( $p = -0.68$ ) were negatively correlated with dissolved arsenic. Statistically significant clines were not observed in arsenic levels in this marsh, and high values in all biological endpoints were observed at the 0.45-m (1.5-ft) station, where arsenic levels were slightly elevated.



**Figure 50—Biological endpoints (Shannon and Pielou Indexes) compared with sediment arsenic, total volatile solids (TVS), and percentage of silt and clay (FINES) as a function of distance from the recently constructed Horseshoe Bayou Bridge in Sandestin, Florida.**

The results of a two-dimensional correlation analysis are provided in Table 52. All significant correlations ( $\alpha = 0.05$ ) between biological endpoints and sediment metal levels and dissolved copper or chromium were positive, indicating higher values of biological endpoints associated with increased metal concentrations. As previously noted, both dissolved and sedimented metal levels were low in the vicinity of the Fountains Bridge. It is likely that the biological differences observed were due to the stochastic nature of sampling patchy environments or covarying biological or physicochemical factors not evaluated in this study.

Table 52 suggests that there were reduced numbers of some taxa associated with increased fines (silt and clay), dissolved arsenic, and distance from the bridge. Several taxa were significantly more abundant at distances where sediments contained increased concentrations of copper and arsenic. These data were further explored using principle components analysis and principle factors (MINRES) analysis with varimax rotation. These analyses did not provide meaningful insight to this database. Physicochemical variables were not

**Table 52—Matrix of Pearson correlation coefficients comparing biological endpoints, including individual dominant taxa and all metrics with sediment metal concentrations, depth of the aerobic layer (RPD), total volatile solids (TVS), and percentage silt and clay (% FINES) at Fountains Bridge in Sandestin, Florida**

	AICHSS	AIMACS	ANNE	MGGYS	AICAE	AICBEZ	AICHR	AILRS	ABUND	DABUND	DIVERS	SHANNON	PIELOU
FINES	-0.595	-0.532	-0.336	-0.422	-0.744	0.101	-0.413	-0.752	-0.288	-0.263	-0.332	-0.445	-0.397
Distance arsenic	-0.440	-0.597	-0.261	-0.447	-0.364	0.135	-0.378	-0.683	-0.374	-0.505	-0.298	-0.618	-0.614
Distance chromium	-0.094	0.018	-0.277	-0.134	-0.024	-0.303	-0.073	0.088	-0.269	-0.203	-0.128	0.031	0.086
Distance	-0.111	-0.454	-0.595	-0.562	-0.484	-0.470	-0.487	-0.232	-0.535	-0.353	-0.262	-0.163	-0.114
Sediment copper	0.108	0.321	0.507	0.327	0.283	0.425	0.236	0.142	0.412	0.245	0.005	0.007	0.005
Sediment arsenic	0.348	0.471	0.549	0.379	0.473	0.322	0.221	0.305	0.422	0.244	0.158	0.101	0.068

**Table 53—Summary results of Fountains Bridge water column 96-h bioassays using the test cladoceran *Daphnia magna*<sup>a</sup>**

Replicate	Surviving animals (%)				
	Laboratory control	Upstream control	+0.45 m (+1.5 ft)	+1.8 m (+6.0 ft)	+9.9 m (+33.0 ft)
1	100	100	80	90	90
2	90	80	80	70	100
3	100	60	70	60	90
4	70	100	100	100	70
Mean	90.0	85.0	82.5	77.5	87.5

<sup>a</sup>Surviving animals with an initial count of 10 per replicate.

significantly differentiated from biological variables, consistent with the lack of clear relationships observed in the previous analysis.

### Bioassay Results

Bioassays were completed on water samples using the cladoceran *Daphnia magna*. Results are given in Table 53. The proportions of surviving test animals were arcsine(square root (proportion surviving)) transformed and *t*-tests were conducted to compare survival between treatment stations located at +0.45, +1.8, and +22.8 m (+1.5, +6.0, and +33.0 ft) from the bridge with the laboratory control and the local reference station located 30 m (100 ft) upstream from the bridge. The results are provided in Table 54.

Significant differences in cladoceran survival were not observed between any treatment station and either

the laboratory or the local control. These results are consistent with the low levels of metals found in this marsh and the lack of effects observed in the invertebrate community assessment.

### Summary of Physicochemical and Biological Evaluation

This CCA-treated timber bridge was constructed 2 years prior to the evaluation. It is a substantial bridge with a span of 30 m (100 ft). The Fountains Bridge crosses a freshwater marsh with no observable water movement, at least not during the 3 days of this evaluation. This bridge was chosen because it represents a worst case with respect to sedimented copper, chromium, and arsenic.

Water column concentrations of copper, chromium, and arsenic were below U.S. EPA water quality criteria, and there was no gradient indicating measurable loss from the bridge in the immediate past. Historical metal loss from the bridge was recorded in the sediments, with elevated concentrations of all three metals observed immediately adjacent to the structure and a clearly defined gradient that reached apparent background concentrations at some distance greater than 1.8 m (6 ft) but less than 3 m (10 ft) from the bridge perimeter. Maximum observed sediment levels for all three metals were low and less than their respective TELs.

Adverse biological effects were not expected in association with the observed metal levels and not one was observed. More taxa, in higher abundance, were found closer to the bridge than further away. However, the regression coefficient on distance was not statistically significant. Statistically significant differences in the *Daphnia magna* laboratory

**Table 54—Results of *t*-tests comparing survival of *Daphnia magna* at treatment stations located at distances of 0.45, 1.8, and 9.9 m (1.5, 6.0, and 33.0 ft) from the perimeter of the Fountains Bridge and laboratory (LAB) or local reference station (LB100)**

	Mean	Standard deviation	<i>N</i>	Difference	Standard deviation difference	<i>T</i>	<i>df</i>	<i>p</i>
LBLAB	1.35	0.28						
LB 0.45 m (1.5 ft)	1.19	0.26	4	0.150	0.52	0.58	3.00	0.60
LBLAB	1.35	0.28						
LB 1.8 m (6 ft)	1.17	0.31	4	0.170	0.53	0.64	3.00	0.57
LBLAB	1.35	0.28						
LB 9.9 m (33 ft)	1.27	0.24	4	0.080	0.31	0.52	3.00	0.64
LB100	1.28	0.34						
LB 0.45 m (1.5 ft)	1.19	0.26	4	0.090	0.25	0.71	3.00	0.53
LB100	1.28	0.34						
LB 1.8 m (6.0 ft)	1.17	0.31	4	0.109	0.15	1.44	3.00	0.24
LB100	1.28	0.34						
LB 9.9 m (33 ft)	1.26	0.24	4	0.019	0.51	0.07	3.00	0.95

bioassay were not observed at any distance from this bridge. In short, the bridge has created small increases in nearfield sediment concentrations of copper, chromium, and arsenic and no biological effect on the invertebrate community located in this marsh.

## Conclusions

Physicochemical and biological endpoints were evaluated at two creosote-treated bridges in Indiana, two penta-treated bridges in Oregon and Washington, and two CCA-treated bridges in Florida. These bridges were selected to present, as much as possible, worst-case projects with respect to preservative contamination of the water column and sediments. Preservative was lost from each bridge and could be detected at generally low levels in either the water column or in sediments.

Polycyclic aromatic hydrocarbons (PAHs) were detected in sediments immediately downstream from the two creosote-treated bridges. Higher sediment concentrations were observed adjacent to the new bridge (range = 1.24 to 5.31  $\mu\text{g}$  TPAH/g) than were observed at the 8-year-old bridge (0.98 to 2.56  $\mu\text{g}$  TPAH/g). Sediment levels of PAH exceeded the threshold effects level (TEL) for some compounds, and the concentration of phenanthrene was essentially equal to the sediment benchmark invoked for this assessment ((TEL + PEL)/2) at one station downstream from the newer bridge. Pipe Creek is located in an agricultural area and carries a heavy sediment load. The creek is shallow and slow moving, at least during the summer and late fall. The invertebrate community is a robust one dominated by annelids and chironomids. Adverse biological effects were not documented in the invertebrate community assessment or in laboratory bioassays on sediments from this bridge. The lack of biological response was attributed to the robust invertebrate community inhabiting the naturally stressful environment and to the low number of compounds that marginally exceeded the sediment benchmarks. Total PAH concentrations did not exceed appropriate benchmarks at either of these bridges.

Penta was not observed in the water column at either the Dairy Creek or West Fork Satsop River Bridges. Very low levels, less than the penta TEL, were observed in sediments under both bridges. No adverse biological effects were documented at either of these bridges, even though the healthy invertebrate communities included large numbers of pollutant-sensitive taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera.

The CCA-treated Horseshoe Bayou Bridge is a substantial HS20 bridge that crosses a sensitive marine environment. This bridge was chosen because of the volume of treated wood used and because marine organisms are very susceptible to copper intoxication. This susceptibility is seen in the low (2.5  $\mu\text{g}$  Cu/L) marine water quality standard. The evaluation was timed to coincide with the period during

construction in which maximum metal losses from the treated wood were anticipated. Marine water in the vicinity of the nearly complete bridge did not contain elevated levels of copper, chromium, or arsenic.

Elevated levels of copper, chromium, and arsenic were observed in association with sawdust and shavings lost from the Horseshoe Bayou Bridge during construction. The metals in these shavings were fixed in the wood fibers and not bioavailable. Adverse effects were not observed in either the indigenous infaunal community or in laboratory bioassays at this bridge.

The Fountains Bridge was 2 years old at the time of this evaluation. It crosses a eutrophic marsh with no observable water movement. It was anticipated that this bridge would represent a worst-case analysis with respect to sediment concentrations of metals lost from CCA-treated wood. As expected with a 2-year-old structure, increased levels of dissolved copper, chromium, and arsenic were not observed in the vicinity of this bridge. Increased concentrations of sediment copper, chromium, and arsenic were observed under this bridge. Metal levels declined with distance from the bridge and reached background concentrations between 1.8 and 3 m (6 and 10 ft) from the bridge's perimeter. Maximum metal concentrations were all well below their respective TELs, and adverse effects were not anticipated. The biological evaluation at this bridge did not reveal any adverse effects.

This study was designed to assess the environmental risks associated with timber bridges selected to represent worst-case conditions. In each case, preservative was lost from the bridges and could be detected in sediments but not in the water column. Sediment levels of contaminants were moderately high for the newer creosote-treated bridge and below TELs for all the other bridges. No adverse biological effects were observed in either the invertebrate community or in laboratory bioassays at any of these bridges.

These results suggest that there are minimal environmental risks associated with preservatives lost from timber bridges. These bridges were located in rural areas and carried few vehicles per day. Therefore, other sources of pollution, discussed in the introduction of this report, were thought to be minimal.

There is evidence that creosote can be lost from overhead bridge structures exposed to the sun during lengthy periods of hot weather. In addition, evidence of the deposition of coal tar in streams during road maintenance was obtained. Figure 51 depicts these losses at an unnamed bridge in Oregon. The lines of coal tar resulted from pouring tar onto the surface of cracks in the asphalt-covered roadway. Recall also the shavings observed under the newly constructed Horseshoe Bayou Bridge and the creosote oil dripping from the Narragansett Bay Bridge described in the introduction.



**Figure 51—Coal tar deposits in a small stream flowing under an unnamed bridge in Oregon. Coal tar was used to seal cracks in the asphalt covering on this bridge.**

All these problems represent an unnecessary environmental risk. These are risks that can be minimized, or even eliminated, through better construction and maintenance practices.

The conclusion to be reached is that timber bridges present little environmental risk but that best management practices for construction and maintenance should be developed and widely disseminated. These best management practices should apply to all bridges, regardless of the construction materials used.

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## Appendix A—Test America Reporting Limits for Polycyclic Aromatic Hydrocarbons (PAHs)

PNA 8310 Nonaqueous	Method reporting limit (mg/kg)	Special reporting limit (mg/kg)
Naphthalene	0.025	0.020
Acenaphthene	0.660	0.020
Acenaphthylene	0.660	0.020
Anthracene	0.660	0.020
Phenanthrene	0.660	0.020
Pyrene	0.180	0.020
Benzo(a)anthracene	0.0026	0.0026
Benzo(b)fluoranthene	0.0036	0.0036
Benzo(k)fluoranthene	0.0034	0.0034
Benzo(a)pyrene	0.0046	0.0046
Benzo(ghi)perylene	0.051	0.020
Chrysene	0.030	0.020
Dibenzo(a,h)anthracene	0.006	0.006
Fluoranthene	0.660	0.020
Fluorene	0.140	0.020
Ideno(1.2.3-cd)pyrene	0.0086	0.0086

## Appendix B—Freshwater Taxonomy Codes

CHYD	Class HYDROZOA	AISTEN	<i>Stenacron</i> species
NEMA	Phylum NEMATODA	AIBAET	Family BAETIDAE
ANNE	Phylum ANNELIDA	AIBAE	cf. <i>Baetis</i> species
MGJGS	<i>Juga</i> species	AIBAESS	<i>Baetis</i> sp.
MGLTS	<i>Leptotoxis</i> species	AICAL	<i>Callibaetis</i> species
MGPLS	<i>Pleurocera</i> species	AIAME	<i>Ameletus</i> species
MGSTL	Order STYLOMMATOPHORA	AICOE	Family COENAGRIONIDAE
MGFRS	<i>Ferrisia</i> species	AICOES	<i>Coenagrion</i> species
MGLXS	<i>Lanx</i> species	AIAS	cf. <i>Aeshna</i> species
MGLYS	cf. <i>Lymnaea</i> species	AIGOMS	<i>Gomphus</i> species
MGSTS	<i>Stagnicola</i> species	AILS	<i>Libellula</i> species
MGPSH	<i>Physa</i> species	AIPLE	Order PLECOPTERA
MGPSLS	<i>Physella</i> species	AINEM	Family NEMOURIDAE
MGGYS	<i>Gyraulus</i> species	AIAMS	<i>Amphinemura</i> species
MGHEL	cf. <i>Helisoma</i> species	AIOS	<i>Ostrocerca</i> species
MPPIS	<i>Pisidium</i> species	AIZAPS	<i>Zapada</i> species
MPSPH	<i>Sphaerium</i> species	AICAP	cf. <i>Capnia</i> species
AACAR	Order ACARINA	AIPEL	Family PELTOPERLIDAE
AARAN	Order ARANEAE	AICHL	Family CHLOROPERLIDAE
ACRUST	Subphylum CRUSTACEA	AISWE	<i>Sweltsa</i> species
ACDAPH	Family DAPHNIIDAE	AIPER	Family PERLODIDAE
ACDS	<i>Daphnia</i> Species	AISOS	<i>Isoperla</i> species
ACCYC	Suborder CYCLOPOIDA	AICALC	<i>Calineuria californica</i>
ACAMP	Order AMPHIPODA	AIHES	<i>Hesperoperla</i> species
ACHYS	<i>Hyalella</i> species	AICAED	Family CAECILIUSIDAE
ACCS	<i>Caecidotea</i> species	AIAEOL	Family AEOLOTHRIPIDAE
ACCAM	Family CAMBARIDAE	AIHET	Suborder HETEROPTERA
ACPAS	<i>Pacifastacus</i> species	AICOR	Family CORIXIDAE
ACOST	Class OSTRACODA	AISS	<i>Sigara</i> species
ADIPL	Class DIPLOPODA	AIGS	<i>Gerris</i> species
AIPA	<i>Podura aquatica</i> Linnaeus, 1758	AIMS	<i>Microvelia</i> species
AINEA	<i>Neanura</i> species	AITING	Family TINGIDAE [Terrestrial]
AIISO	Family ISOTOMIDAE	AIMIR	Family MIRIDAE [Terrestrial]
AIENT	Family ENTOMOBRYIDAE	AIHOM	Suborder HOMOPTERA
AIDIC	<i>Dicyrtoma</i> species	AICIC	Family CICAPELLIDAE
AICAE	<i>Caenis</i> species	AIMEMB	Family MEMBRACIDAE
AIEPHS	<i>Ephemerella</i> species	AIFLAT	Family FLATIDAE
AISERS	<i>Serratella</i> species	AIPSY	Family PSYLLIDAE
AIDRD	<i>Drunella doddsi</i>	AIAPH	Family APHIDIDAE
AIEPHA	<i>Ephemera</i> species	AIORTD	Family ORTHEZIIDAE
AIPAR	<i>Paraleptophlebia</i> species	AIMEG	Order MEGALOPTERA
AIHEP	Family HEPTAGENIIDAE	AISL	<i>Sialis</i> species (larva)
AICYN	<i>Cynigma</i> species	AISSA	<i>Sialis</i> species (adult fragment)
AIEPES	<i>Epeorus</i> species	AINEU	Order NEUROPTERA



## Appendix B—Freshwater Taxonomy Codes—con.

AICHEPS	<i>cf. Heptagenia</i> species	AIHEM	Family HEMEROBIIDAE [Terrestrial]
AIIRONS	<i>Ironodes</i> species	AICHRD	Family CHRYSOPIDAE [Terrestrial]
AIRHIS	<i>Rhithrogena</i> species	AICOL	Order COLEOPTERA
AIHSL	<i>Haliphus</i> species (larva)	AIHYHS	<i>cf. Hydropsyche</i> species
AIPELS	<i>Peltodytes</i> species	AIBS	<i>Banksiola</i> species
AIHYDR	Subfamily HYDROPORINAE	AIBRAD	Family BRACHYCENTRIDAE
AINEO	<i>Neobidessus</i> species (adult)	AIMIS	<i>Micrasema</i> species
AIHYSA	<i>Hydroporus</i> species (adult)	AILPS	<i>Lepidostoma</i> species
AIORES	<i>Oreodytes</i> species (larva)	AILIM	Family LIMNEPHILIDAE
AILSA	<i>Laccomis</i> species (adult)	AICHYS	<i>cf. Chyranda</i> species
AICOLY	Subfamily COLYMBETINAE	AIHAS	<i>Halesochila</i> species
AIASL	<i>Agabus</i> species (larva)	AIHYXS	<i>Hydatophylax</i> species
AIASA	<i>Agabus</i> species (adult)	AILES	<i>Lenarchus</i> species
AIRS	<i>Rhantus</i> species	AILIS	<i>cf. Limnephilus</i> species
AIGSL	<i>Gyrinus</i> species (larva)	AIPSS	<i>Psychoglypha</i> species
AIHEL5	<i>Helophorus</i> species	AIUENO	Family UENOIDAE
AIHYDL	Family HYDROPHILIDAE	AILEP	Order LEPIDOPTERA
AIBERS	<i>Berosus</i> species	AIGEO	Family GEOMETRIDAE
AITROPS	<i>Tropisternus</i> species	AIDIP	Order DIPTERA
AIES	<i>Enochrus</i> species	AITIP	Family TIPULIDAE
AIPASS	<i>Paracymus</i> species	AILIMO	Subfamily LIMONIINAE
AISTA	Family STAPHYLINIDAE	AIANTS	<i>Antocha</i> species
AIPTI	Family PTILIIDAE	AICRLS	<i>Cryptolabis</i> species
AIECT	<i>cf. Ectopria</i> species	AIDIS	<i>Dicranota</i> species
AICYPHS	<i>Cyphon</i> species	AIERS	<i>cf. Erioptera</i> species
AIELM	Family ELMIDAE	AIHES	<i>cf. Hesperoconopa</i> species
AILARA	<i>Lara</i> species	AIPEOS	<i>Pedicia</i> species
AIDUBS	<i>Dubiraphia</i> species	AIRHXS	<i>cf. Rhabdomastix</i> species
AIHETS	<i>Heterlimnius</i> species	AITPS	<i>cf. Tipula</i> species (adult)
AINS	<i>Narpus</i> species	AITIPS	<i>Tipula</i> species
AIOPT	<i>Optioservus</i> species	ACEC	Family CECIDOMYIIDAE
AISTNS	<i>Stenelmis</i> species	ASCI	Family SCIARIDAE
AIBUP	Family BUPRESTIDAE [Terrestrial]	APSY	Family PSYCHODIDAE
AICOC	Family COCCINELLIDAE [Terrestrial]	AMS	<i>Maruina</i> species (larva)
AILATD	Family LATHRIDIIDAE	ACPERS	<i>cf. Pericoma</i> species
AICHMD	Family CHRYSOMELIDAE	APSYS	<i>Psychoda</i> species
AIALTN	Subfamily ALTICINAE	AANI	Family ANISOPODIDAE
AIDS	<i>cf. Donacia</i> species	AIBTS	<i>Bittacomorpha</i> species
AICUR	Family CURCULIONIDAE	AIPTY	<i>Ptychoptera</i> species
AISCO	Family SCOLYTIDAE [Terrestrial]	AIDSA	<i>Dixella</i> species (adult)
AITRI	Order TRICHOPTERA	AIDSL	<i>Dixella</i> species (larva)
AIRHAS	<i>Rhyacophila</i> species	AICRT	Subfamily CERATOPOGONINAE
AIGLO	Family GLOSSOSOMATIDAE	AICBEZ	<i>cf. Bezzia</i> species
AIGLOS	<i>Glossosoma</i> species	AIPBEZ	<i>Probezzia</i> species

## Appendix B—Freshwater Taxonomy Codes—con.

AIHYDP	Family HYDROPTILIDAE	AIFOR	Subfamily FORCIPOMYIINAE
AIHYDS	<i>Hydroptila</i> species (larva)	AIATS	<i>Atrichopogon</i> species
AIOSL	<i>Oxyethira</i> species (larva)	ASIM	Family SIMULIIDAE
AIPOL	Family POLYCENTROPODIDAE	ASS	<i>Simulium</i> species (larva OR pupa)
AIPOSS	<i>Polycentropus</i> species	AICHR	Family CHIRONOMIDAE
AIHYL	Family HYDROPSYCHIDAE	AITNY	Subfamily TANYPODINAE
AIGUTS	<i>Gittipelopia</i> species	ACHS	cf. <i>Chrysops</i> species
AILRS	<i>Larsia</i> species	AGLS	cf. <i>Glutops</i> species
AIPAAS	<i>Paramerina</i> species	ASTRA	Family STRATIOMYIDAE
AICTHS	cf. <i>Thienemannimyia</i> species	ASTS	<i>Stratiomys</i> species (larva)
AIPRS	<i>Procladius</i> species	AEMP	Family EMPIDIDAE
AIPSS	<i>Psectrotanypus</i> species	ADOLI	Family DOLICHOPODIDAE
AICHSS	<i>Chironomus</i> species	ALONS	<i>Lonchoptera</i> species
AIDTS	<i>Dicrotendipes</i> species	ASYRD	Family SYRPHIDAE
AIGLS	<i>Glyptotendipes</i> species	AEPHY	Family EPHYDRIDAE
AIPPS	<i>Polypedilum</i> species	AMUS	Family MUSCIDAE
AITBS	<i>Tribelos</i> species	ATAC	Family TACHINIDAE
AIMPS	<i>Micropsectra</i> species	AHYM	Order HYMENOPTERA
AISTES	<i>Stempellinella</i> species	ABRAC	Family BRACONIDAE
AITYS	<i>Tanytarsus</i> species	AICH	Family ICHNEUMONIDAE
AIORTN	Subfamily ORTHOCLADIINAE	AICHLA	Superfamily CHALCIDOIDEA
AEUKS	<i>Eukiefferiella</i> species	AIENC	Family ENCYRTIDAE [Terrestrial]
APCLS	cf. <i>Parorthocladius</i> species	AIPLAT	Family PLATYGASTRIDAE [Terrestrial]
APSTS	<i>Psectrocladius</i> species	AFORM	Family FORMICIDAE [Terrestrial]
ARHS	<i>Rheocricotopus</i> species	AHAL	Family HALICTIDAE [Terrestrial]
ARHSS	<i>Rheosmittia</i> species	CPIS	Superclass PISCES
ASYNS	<i>Synorthocladius</i> species	CAUR	Order URODELA
APRS	cf. <i>Parochlus</i> species	CAAN	Order ANURA

## Appendix C—Marine Taxonomy Codes

Arthropods	Arthrop	Polychaetes	
<i>Hargeria rapax</i>	Ahargpax	<i>Neanthes succinea</i>	Pneasuc
<i>Rithropanopeus harrisi</i>	Arighhar	<i>Neanthes micromma</i>	Pneamic
<i>Edotea montosa</i>	Aedotmon	<i>Streblospio benedicti</i>	Pstrembe
<i>Gammarus mucronatus</i>	Agammucr	<i>Capitella capitata</i>	Pcapcap
<i>Corophium louisianum</i>	Acoroloui	<i>Mediomastus ambiseta</i>	Pmedamb
<i>Melita sp.</i>	Amelita	<i>Polydora socialis</i>	Ppolysoc
<i>Grandidierella bonnieroides</i>	Agrandib	<i>Ampharete americana</i>	Pamphame
<i>Palaemonetes pugio</i>	Apalaepu	<i>Eteone heteropoda</i>	Peteohet
<i>Callinectes cf. similis</i>	Acallisi	<i>Leitoscoloplos robustus</i>	Pleitoro
Unidentifiable arthropod	Aunident	<i>Leitoscoloplos fragilis</i>	Pleitofr
Chironomid larvae	Achirono	<i>Parandalia americana</i>	Pparanam
<i>Erichsonella attenuata</i>	Aerichat	<i>Unidentifiable polychaete</i>	Punident
<i>Meilita cf. nitida</i>	Amelinit	<i>Diopatra cuprrea</i>	Pdiopcup
		<i>Pecternaria gouldi</i>	Ppecteng
		<i>Owenia sp.</i>	Powenia
		<i>Megalona sp.</i>	Pmegalon
		<i>Aricidea fragilis</i>	Paricfrag
Mollusks		Others	
Gastropods		<i>Nemertea</i>	Nemertea
<i>Neritina usnea</i>	MGNeriti	<i>Platyhelminthes</i>	Platyhel
<i>Vitrinella sp.</i>	MGVitrin		
<i>Texadina sphinetostoma</i>	MGTexadi		
Unidentifiable gastropod	Mguniden		
Bivalvia			
<i>Tellina probina</i>	MBTelpro		
<i>Tagelus Plebeius</i>	MBTagple		
<i>Amygdalum papyrium</i>	MBAmypap		
Unidentifiable bivalve	Mbuniden		
<i>Crassostrea virginica</i>	Mbcravir		
<i>Mulinia lateralis</i>	Mmulinla		
<i>Ischadium recurvum</i>	Mischrec		
<i>Geukensia demissa</i>	Mgeukdem		
<i>Telina versicolor</i>	Mtelinve		