ADDENDUM REPORT

Continuation of the Sooke Basin Creosote Evaluation Study (Goyette and Brooks, 1998)

Year Four - Day 1360 & Day 1540



Prepared by:

D. Goyette Commercial Chemicals Division Environment Canada Pacific and Yukon Region K. M. Brooks Aquatic Environmental Sciences Port Townsend, WA 98368

Prepared for:

Creosote Evaluation Steering Committee Regional Program Report PR00-03

May 12, 2001

ABSTRACT

Govette and Brooks (1998) reported initial results for the Sooke Basin creosote evaluation study designed to evaluate the chemical (PAH) and biological affects of creosote treated and untreated wood structures constructed in sensitive marine environments. Sediment toxicity was found adjacent to both the treated and untreated structures. Statistically significant increases in sediment PAH concentrations were observed within 7.5 metres of the creosote treated structures, but significant biological effects were confined to a distance of 0.65 metres. Slight adverse effects were observed to a distance of 2.0 metres in laboratory bioassays, but not in the benthic infaunal community. Model predictions (Brooks, 1994), suggested that observed sediment PAH concentrations would increase another 18% before reaching their maximum at about 1000 days post-construction. Field observations and the results of extensive PAH analyses suggested that PAH were transported from the treated piling to sediments in a particulate form. These observations were confirmed in preliminary laboratory experiments. However, this Particulate Creosote Transport Theory has not been studied in a rigorous manner. Additional observations suggested that at least some of the PAH contamination originated as microliter size droplets released from the air exposed portion of the piling during periods of high ambient air temperatures. It was hypothesized that these microparticles migrated through the water column without dissolving and that they remained intact as they worked their way downward into the sediments, where they were subject to microbial degradation (Govette and Brooks, 1998).

This addendum report presents the results of two additional surveys conducted 1360 and 1540 days after construction. The purpose of these surveys was to evaluate the longer-term environmental response to the structures; to validate model predictions of a decline in sediment PAH; to explore the source of PAH on the piling; and to examine the particulate transport hypothesis. The results of this study demonstrated a significant decline in sediment PAH concentrations on both days. Observed concentrations were unlikely to be toxic at all stations outside the perimeter of the creosote treated BMP dolphin. Concentrations of PAH in mussels growing directly on the piling were very low. In fact they were lower than those observed in mussel tissues collected from the reference station.

The creosote treated piling had become heavily encrusted with a diverse community of epifauna by Day 1360. The untreated mechanical control dolphin was deteriorating under attack by *Limnoria* and *Bankia* and supported fewer epifauna. Biodeposits from these communities, and from the failing untreated wood, exceeded the assimilative capacity of the sediments. The increased biological demand resulted in anaerobic conditions and concentrations of sediment sulfide that were likely toxic to some taxa to a distance of at least 20 meters downcurrent. Accumulations of this biological debris in the canisters confounded that part of the study. At the end of four years, the most significant environmental response to these structures was the establishment of a diverse and abundant epifaunal community on the piling and the attraction of large numbers of Dungeness crabs, starfish, finfish and other megafauna to what had become an artificial reef. While not assessed in this study, biological debris from this community created high biological demand in sediments resulting in significant increases in sulfide concentrations that were undoubtedly inimical to many infaunal species.

Keywords: Creosote Evaluation Study, Year Four; creosote; polycyclic aromatic hydrocarbons; PAH, Sooke Basin, Vancouver Island, B.C.

ACKNOWLEDGEMENTS

The authors are greatly indebted to S. Christie, D. Gerelle, G. Hafey, K. Hearn, and L. Laverdure, at Foreshore Technologies Inc. for their commitment to this study in providing high quality field and diving support.

The authors would also like to thank the staff at Environment Canada's Pacific Environmental Sciences Centre for their diligence and dedication to a high standard of analytical quality in completing the analytical chemistry and toxicity evaluations

Members of the Creosote Evaluation Steering Committee deserve recognition for their support and guidance during this lengthy study. Committee members are T. Appleton (DFO-SCH), K. Hutton (DFO-Habitat), H. Walthert, (CITW), David Webb (US Creosote Council), Barry Munson (EC - Prairie and Northern Region), and L. Churchland (GBEI).

Funding for this portion of the study was provided by:

Environment Canada - Pacific and Yukon and Prairie and Northern Regions; Department of Fisheries and Oceans - Habitat and Enhancement and Small Craft Harbours Branches; Georgia Basin Environmental Initiative; Canadian Institute of Treated Wood; and U.S. Creosote Council

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
List of Tables	v
List of Figures	vi
1.0 INTRODUCTION	1
1.2. Purpose and Scope.	2
2.0 SOOKE BASIN STUDY AREA	3
3.0 MATERIALS AND METHODS	4
3.1. Sampling Sites	
3.2. Sampling Schedule	
3.3. Sediment Transect Sampling	
3.4. Evaluated Endpoints.	
3.5. Catchment Experiments	
3.6. Sediment Analysis.	
3.6.1. Sulfide, Total Volatile Solids and Particle Size Analyses.	
3.7. Tissue PAH Analysis.	
3.8. Amphipod Bioassays.	
3.8.1. Bioassay "End Point" PAH Analysis	
3.9. Acute Toxicity Test Using a Photoluminescent Bacterium (Microtox TM)	
3.10. Underwater Observations and Photography.	
4.0 RESULTS AND DISCUSSION	13
4.1. BMP0.5 Interlaboratory QA/QC Splits	
4.2. Sediment Transect PAH Concentrations on Day1360 and Day1540	
4.2.1. Mechanical (MC0.5) and Open Control (OC0.0) Sediment PAH Concentrations	
4.2.2. BMP Downcurrent Transect Surface Sediment PAH Concentrations on Days 1360 and 1540	
4.3. Canister Studies.	
4.4. Toxicity associated with Eevated Sediment Total Sulfide Concentrations	27
4.5. Mussel (<i>Mytilus edulis</i>) Tissue PAH Levels	
4.6. Sediment 10-Day Amphipod (<i>Eohaustorius washingtonianus</i>) and Microtox™ Bioassay Results - Day 1360 and Day 1540	
4.6.1. Amphipod Bioassays	
4.6.2. Effect of Pretreatment on Sample PAH Concentration.	
4.6.3. Microtox TM Bioassay	
4.7. Sediment Characteristics.	
4.8. Observations and Underwater Photography	
4.8.1. Creosote Sheens.	
4.8.2. BMP Pilings.	
4.8.3. Invertebrate Community Growing on Treated and Untreated Piling.	
4.8.4. Marine Borer Attack (<i>Limnoria</i> ; <i>Bankia</i>).	
4.8.5. Catchment Containers	
5.0 Summary and Conclusions.	41
References	
PLATES	
APPENDIX I	

List of Tables

Table 1.	List of Parameters Measured During Year Four (Day 1360 and Day 1540) - Sooke Basin Creosote Evaluation Study
Table 2.	Interim Pass/Fail Criteria for 10-day Amphipod Sediment Toxicity Testing (Lee et al., 1995)11
Table 3.	Interpretation Guidelines for Microtox [™] Photoluminescent Bacterium Toxicity Tests
Table 4.	Laboratory QA/QC Split Samples Sediment PAH Concentration (ng/g, dry wt.) - Station BMP0.5 - Axys Analytical and Pacific Environmental Sciences - Day 1360
Table 5.	Summary Table Showing the Field Transect Surface Sediment Low Molecular Weight (LPAH), High Molecular Weight (HPAH) and Total (TPAH) PAH Concentrations (µg/g, dry weight) on Days 384 (Year One), 1360 and 1540 and 10-Day Amphipod Bioassay "End Point" PAH Concentration on Day 1540 Sooke Basin Creosote Evaluation Study.
Table 6.	Comparison of the BMP0.5 Total Surface Sediment PAH Concentration (µg/g, dry wt.) on Day 384 and 1540 with Environment Canada's Interim Sediment Quality Guidelines, the U.S. Environmental Protection Agency and Washington State (WAC 173-204-320) Numerical Sediment Quality Standards for Individual PAH and the sum of Low and High Molecular Weight PAH
Table 7.	Determination of the Toxicity of Individual PAH Compounds and the Sum of Their Predicted Toxicities Using the Methodology of Swartz (1999)
Table 8.	PAH Concentrations in Material Collected From Catchment Containers Placed at the BMP and Mechanical Control Sites on Day 1360 and Recovered on Day 1540 - Sooke Basin Creosote Evaluation Study - Year Four.
Table 9.	Concentration of PAH (ng/g, wet weight) Observed in Mussels Grown in Cages (Day 0 through Day 384) With That Observed in Mussels Taken from the Surface of the Piling on Day 1540 - Sooke Basin Creosote Evaluation Study.
Table 10.	Amphipod (<i>Eohaustorius washingtonianus</i>) 10-day Sediment Bioassay Results Comparing Year One (Day 384) to Year Four (Days 384, 1360 and 1540) - Sooke Basin Creosote Evaluation Study29
Table 11.	Sediment Hydrogen Sulfide (μ M & mg/L), Total Volatile Solids (TVS/g), Field Transect Total PAH (μ g/g) Concentrations on Days 1360 and 1540 and Amphipod Bioassays "End Point" PAH Concentrations on Day 1540 - Sooke Basin Creosote Evaluation Study - Year Four.
	Acute Liquid Phase Microtox™ Result for Year Four (Day1540) - Sooke Basin Creosote Evaluation Study - 1999
Table 13.	Solid Phase Microtox TM Test Results for Year Four (Day1540) - Sooke Basin Creosote Evaluation Study
Table 14.	Sooke Basin Creosote Evaluation Study: Comparison of Liquid and Solid Phase (LC ₅₀) Sediment Microtox TM Tests - Days 0 to 1540
Table 15.	Table Showing Sediment Particle Size Distribution and Total Volatile Solids Content - Day 1360 and Day 1540 - Sooke Basin Creosote Evaluation Study.
Table 16.	Summary Table Showing Results From Day 1360 and Day 1540 Compared to Year One (Days 14, 180 and 384) - Sooke Basin Crossote Evaluation Study-

List of Figures

Figure 1.	Sooke Basin Study Area	3
Figure 2.	Test Site and Piling Layout - Sooke Basin Creosote Evaluation Study.	4
Figure 3.	Benthic Grab Used in the Sooke Basin Creosote Evaluation Study	5
Figure 4.	Modified Transect Sampling Positions at the BMP Site to Accommodate the Additional Sampling Program in Year Four - Sooke Basin Creosote Evaluation Study.	6
Figure 5.	Modified Quadrant Sampling Positions Used During the 1999 Year Four Sampling Program - Sooke Basin Creosote Evaluation Study	6
Figure 6.	Photograph showing Catchment Containers Before Installation in June, 1999	7
Figure 7.	Photograph Showing Bioassay Test Facilities on Day 1540.	10
Figure 8.	Surface Total PAH Concentration at the Mechanical Control (MC0.0) - Day 0 to Day 1540 ($\mu g/g$, dry weight) - Sooke Basin Creosote Evaluation Study.	14
Figure 9.	Total Surface Sediment PAH Concentration (µg/g, dry weight) at the Open Control (OC0.0) from Day 0 to Day 1540 - Sooke Basin Creosote Evaluation Study.	14
Figure 10.	Comparison of Surface Sediment Total PAH Concentration ($\mu g/g$, dry wt.) at the BMP treatment site Day 384 \underline{vs} . Day1360 and Day1540 - Sooke Basin Creosote Evaluation Study. Polynomial fits to the Day1360 and Day1540 data are provided.	17
Figure 11.	Comparison of Surface Sediment Total PAH Concentration ($\mu g/g$, dry wt.) at the BMP Treatment Site Day 384 \underline{vs} . Day1360 and Day1540 - Sooke Basin Creosote Evaluation Study	
Figure 12.	Sooke Basin Creosote Evaluation Study: Total PAH Concentrations (µg/g, dry weight) in Surface Sediments at the BMP Piling Downcurrent and Upstream Distance Intervals from Day 0 to Day 1540 (Year Four).	19
Figure 13.	Sediment Concentrations of Total Sulfide on the BMP Downcurrent Transect During Sampling on Days 1360 and 540 - Sooke Basin Creosote Evaluation Study	27
Figure 14.	Bioassay Test Jar Taken at the End of the Pre-aeration and Bioassay Period	32
Figure 15.	Comparison of Amphipod (<i>Eohaustorious washingtonianus</i>) Percent Survival Between Year One (Day384) and Year Four (Day1360 and Day1540) - Sooke Basin Creosote Evaluation Study	33
Figure 16.	Photographs Showing the Creosote Sheens Observed on Benthic Sediment Samples From the <i>BMP</i> Sit Sooke Basin Creosote Evaluation Study - June 1996.	

1.0 INTRODUCTION

Despite widespread use along the British Columbia coast, very little information was available on the actual impact of creosote treated wood on the aquatic environment. Conflicts arising over the use of treated wood in sensitive marine environments prompted the need for more scientific information describing the 'real world' effects without interference from other sources of contamination. The appearance of oily surface sheens that have occurred during construction and knowledge of the chemical and biological effects associated with polycyclic aromatic hydrocarbons present in creosote has often led to a perception that adverse environmental effects are taking place. However, there are no previous studies presenting a clear understanding of the actual behavior of creosote treated wood in the environment. Between 1995 and 1999, a study to assess the environment's response to creosote treated wood projects was jointly funded by Environment Canada (EC), Department of Fisheries and Oceans (DFO), Province of British Columbia Ministry of Environment, Lands and Parks (MELP), Canadian Institute of Treated Wood (CITW) and the US Creosote Council. This study was conducted in an undisturbed location in Sooke Basin, Vancouver Island. Initially, these studies were designed to evaluate the temporal and spatial chemical and biological changes associated with freshly installed creosote treated pilings over a one-year period. Information obtained from these studies has providing a scientific basis for developing policies and guidelines on the placement and use of creosote treated wood in sensitive marine habitats.

Marine borers can rapidly destroy untreated wood in marine environments. Two principal borers on the B.C. coast are molluscan teredos (e.g. *Bankia setacea*) and the isopod crustacean (*Limnoria sp.*), commonly known as gribbles (Quayle, 1992). Protection from these marine borers normally requires some form of preservative treatment or the choice of an alternate material resistant to borer attack (e.g., steel, concrete, or plastic). One of the most common methods of protection against marine borer attack is to impregnate wood with creosote, a practice that has been used for centuries.

The Sooke Basin study involved the installation of three dolphins constructed with six piling each. The Weathered Piling (WP) dolphin was constructed with eight-year-old pilings treated by conventional methods. The second dolphin was constructed with pilings treated using industry's latest Best Management Practices (BMP). These practices are designed to produce a cleaner and more environmentally sensitive product (CITW and WWPI, 1997). The third structure, referred to as the Mechanical Control (MC), was constructed of untreated Douglas fir piling. It was designed to evaluate the environment's response to the physical structure and to organic compounds released from untreated wood. Contaminants typically accumulate in bottom sediments over time sediments are often associated with the highest chemical concentrations and the most significant biological responses. These studies were conducted in an undisturbed location in Sooke Basin to minimize confounding sources of PAH. The minimal current speeds and rich invertebrate communities at the study site coupled with fine-grained sediments, which have a strong affinity for organic contaminants, made this a worst case study. The project was evaluated using the model of Brooks (1994) prior to construction. The model predicted significant accumulations of PAH that were expected to cause adverse effects in the invertebrate community in the immediate vicinity of the dolphins.

Field surveys in 1995 were conducted on days 0, 14, 180 and 384 after installing the pilings. Results from the first year of study were reported in detail by Goyette and Brooks (1998). This report was peer reviewed and is available from the Environment Canada on CD-ROM. The results of the first year's study indicated that PAH lost from creosote treated wood can create toxic conditions within 0.65 meters of high densities of piling installed in worst case environments. Adverse effects on infauna or in bioassays were not documented at distances beyond 2.0 meters from the structures. The authors concluded that adverse effects associated with the use of creosote treated wood in marine environments could be easily managed. The model of Brooks (1994) predicted that sediment PAH concentrations would increase another 18% above those observed on Day 384 and peak approximately 1,000 days following construction. To assess the longer-term environmental response to creosote treated piling, a decision was made to continue with the field studies for an additional three-year period. This provided an opportunity to determine if sediment PAH concentrations and toxicity would increase beyond year one according to predictions; to measure seasonal changes in chemical contamination and toxicity; and if possible, to determine where on the pilings most of the additional PAH migration was originating. These studies were considered important for confirming earlier conclusions that the environmental effects associated with creosote treated wood are manageable. This addendum report presents the results of additional field studies conducted in 1999 on Days 1360 and 1540, four years after piling installation.

1.2. Purpose and Scope.

Goyette and Brooks (1998) reported that 384 days following piling installation, the maximum predicted and observed total PAH concentrations were significantly elevated (5.5 μ g/g and 4.8 μ g/g, respectively) to a distance of 7.5 metres downcurrent from the BMP treated dolphin. Biologically significant increases in sediment PAH were not observed at further distances. Observed TPAH concentrations declined sharply between 7.5 and 10 metres, averaging 0.53 μ g/g (n=13) at 10 metres and beyond, below the Threshold Effects Level or TEL of 0.75 μ g/g, dry weight. Sediment PAH concentrations were similar at both the BMP and WP treatment sites. Model predictions (Brooks, 1994) of peak PAH concentrations in sediments were somewhat conservative from the environment's point of view because they predicted more sediment PAH than was actually observed at all distances from the dolphins.

It was also postulated by Goyette and Brooks (1998) that the primary sources of creosote contamination to the bottom sediments were from the initial surface sheen which formed during construction and more importantly, from creosote micro-droplets which settled directly onto the bottom sediments from the above water and intertidal portions of the piling exposed to solar heating. These minute droplets appeared to pass through the water without dissolving or breaking up. This hypothesis was supported by the appearance of small oily microsheens throughout the upper six cm of the sediment column and by the patchy nature of the PAH distribution in sediments. In addition, very low (<31 ng/L) concentrations of dissolved PAH were observed immediately adjacent to the dolphins 247 to 261 after construction (Goyette and Brooks, 1998). It was hypothesized that solar heating of the black creosote treated piling was likely bringing fresh creosote from the interior of the wood to the piling surface. Microdroplets of these exudates were likely dislodged by wave action and/or gravity. This phenomenon is thought to occur primarily during warm summer months from sections of the piling exposed

during low tides and not insulated by the encrusting epifaunal community. The losses would likely continue as long as a fresh supply of creosote remained within the wood or until the wood surface is sealed by the accumulation of tar-like material on the pilings' surface. The authors referred to this hypothesis as the *Particulate PAH Transport Hypothesis*. The specific goals of this phase of the creosote evaluation study were:

- a) to determine if the level of sediment PAH contamination had, in fact, increased further since Year One at the BMP site;
- b) to confirm whether or not adverse biological effects remained confined to the immediate footprint of the dolphins or did they extend further downcurrent;
- b) to validate model predictions for another 18% increase in concentration, three years post-construction;
- d) and to further investigate the *Particulate PAH Transport Hypothesis*.

Since very little difference was observed between data from the WP and the BMP pilings sites, the sediment sampling program focused on the BMP, MC and OC sites.

2.0 SOOKE BASIN STUDY AREA.

The test site is located in a relatively well-protected area near Pim Head on the southern shore of Sooke Basin (Figure 1). The study area is removed from intense human activity or other potential sources of PAH contamination. Test site characteristics were described in more detail by Goyette and Brooks (1998).

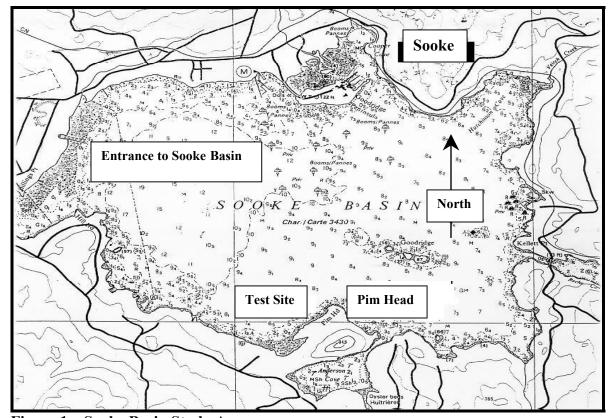


Figure 1. Sooke Basin Study Area

3.0 MATERIALS AND METHODS

Since creosote losses were thought to be exacerbated by solar heating, sampling was conducted before hot summer days on June 21 (Day 1360) and again on October 11, 1999 (Day 1540) following the period when most creosote was expected to be lost due to heating effects. Sets of six canisters each were installed at four water depths under the BMP dolphin to capture PAH migrating from the pilings during warm summer months. A similar set of canisters was installed at the MC dolphin as a control. Protocols were identical to those described by Goyette and Brooks (1998) with the exceptions discussed in the following sections.

3.1. Sampling Sites. Figure (2) shows the dolphin layout, which consisted of three treatment sites and an open control (OC) positioned along the 12.2 m depth contour, approximately 60 m from shore. Each structure was constructed with six Class A pilings tied together at the top to form a small dolphin. The WP was located at the southernmost end of the test site. The BMP dolphin was situated in the middle and the MC dolphin was located to the north. No structures were placed at the OC reference station, located 21 m north of the MC structure. The WP and BMP dolphins were separated by 70 m. All pilings were Class A, Douglas fir (*Pseudotsuga menziesii*), with an average diameter of ca. 30 cm. The dolphin's footprint varied between 2.4 and 4.1 m in diameter.

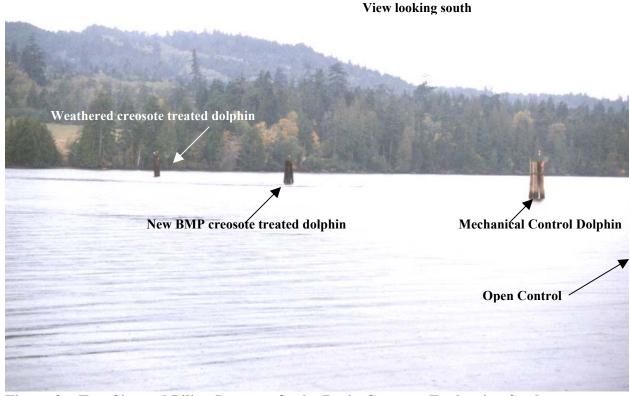


Figure 2. Test Site and Piling Layout - Sooke Basin Creosote Evaluation Study.

- **3.2.** Sampling Schedule. Field surveys were conducted during the weeks beginning June 21 (Day 1360) and October 11, 1999 (Day 1540). Sediment samples were collected in June from the BMP downcurrent transect at the 0.0, 0.5, 2.0, 5.0, 10 and 20 m stations. Additional samples were collected 0.5 metres downcurrent from the MC dolphin and at the OC site for PAH analysis and toxicity testing. The timing of these samples was selected to determine if there was any seasonal variation in sediment PAH concentrations or in the biological response determined by laboratory bioassay. Canisters were set out in June and recovered in October following the summer period when air temperatures were expected to exacerbate PAH losses from the BMP dolphin. A set of containers was also installed on the MC dolphin. The October 1999 sampling was scheduled to coincide with the last data collected in the previous study (October 1996).
- 3.3. <u>Sediment Transect Sampling</u>. The sediment-sampling program focused mainly on the BMP dolphin. Earlier surveys involved a combination of single and replicate samples taken at closely spaced intervals on both the upstream and downcurrent transects at the BMP treatment site. To keep costs down, sampling on Days 1360 and 1540 was confined to the 0.0, 0.5, 2.0, 5.0, 10 and 20 m stations on the downcurrent transect and at the MC and OC treatments. Triplicate samples were collected at each location, two from separate grabs (replicates # 1 & 2) and a third (#3) from a larger, composite sample collected from multiple grabs for amphipod and MicrotoxTM bioassays. Except for Station BMP 0.5, where all three replicates were analyzed, only replicates #1 and #3 were submitted for PAH analysis. Replicate #2 was archived for future reference. Analysis of composite Replicate #3 provided a direct evaluation of sediment PAH concentrations in the amphipod and MicrotoxTM tests.

Sampling procedures followed those used during all previous surveys as outlined by Goyette and Brooks (1998). Divers used the hand held benthic sampler (0.032 m²) described in Figure (3) to accurately retrieve samples from precise locations within the previously established grid. To accommodate this extra sampling program, two additional downcurrent transects, defined in Figure (4), were added to the seaward side of the BMP site. These transects remained within an extension of the dolphin's footprint



Figure 3. Benthic Grab Used in the Sooke Basin Creosote Evaluation Study.

on the downcurrent vector. This avoided resampling the same areas used during previous surveys. Each sampling point was divided into four quadrants as shown in Figure (5). This approach was necessary because all samples, including those for amphipod and Microtox bioassays, were collected from only from the top 2.0 cm of the sediment column. This required several grabs of virgin sediment at each station. Sediment samples from the BMP site were collected from the least contaminated (i.e. furthest distance from the dolphin) to the most contaminated stations using a single precleaned sampling fixture and utensils. Separate sets of

sampling equipment were used at the MC and OC sites. This approach also kept the divers from working over areas yet to be sampled.

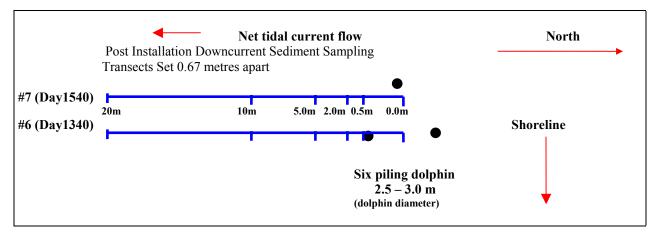


Figure 4. Modified Transect Sampling Positions at the BMP Site to Accommodate the Additional Sampling Program in Year Four - Sooke Basin Creosote Evaluation Study.

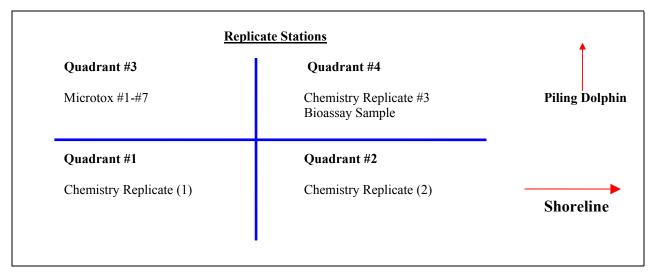


Figure 5. Modified Quadrant Sampling Positions Used During the 1999 Year Four Sampling Program - Sooke Basin Creosote Evaluation Study.

3.4. Evaluated Endpoints. Chemical analyses on Days 1360 and 1540 were restricted to 15 parental PAHs, excluding benzo(e)pyrene and the alkylated PAHs, which were included in previous surveys. Bioassays were conducted with *Eohaustorius washingtonianus*, because it appeared to be more sensitive than *Rhepoxynius abronius*, which had also been evaluated during the first year's work. Solid and liquid phase MicrotoxTM bioassays were conducted only on samples from Day 1540. A list of evaluated parameters is provided in Table (1). Analysis of PAH was also conducted on sediment from the catchment containers and from mussels growing directly on the BMP and MC piling on Day 1540.

Table 1. List of Parameters Measured During Year Four (Day 1360 and Day 1540) - Sooke Basin Creosote Evaluation Study.

Sediment PAH. PAH in surficial (0 - 2.0 cm) sediments. Low molecular weight compounds are presented in italics. Benzo(a)fluoranthene and benzo(b)fluoranthene are grouped as benzofluoranthenes

NaphthalenePyreneIdeno(123-cd)pyreneAcenaphthyleneBenz(a)anthraceneBenzo(ghi)peryleneAcenaphtheneChryseneDibenz(a,h)anthraceneFluoreneBenzo(a)fluorantheneBenzo(a)pyrene

Phenanthrene Fluoranthene

Anthracene Benzo(b)fluoranthene

Biological Endpoints

Amphipod bioassays – Mortality in *Eohaustorius washingtonianus*. MicrotoxTM assays (pore water and solid phase) (Day1540 only) Determination of tissue PAH concentrations in mussels (*Mytilus edulis edulis*) growing directly on the piling

Additional Tests

Sediment total sulfide concentrations (probe)

Bioassay PAH concentrations following aeration to reduce sulfide concentrations Bioassay sediment sulfide concentrations following aeration (probe and Hach Kit) Sediment Grain Size Distribution (sieve and pipette per Plumb, 1981) Sediment Total Volatile solids (percent of dry sediment weight) Catchment container dry weight content (g)

Underwater Observations and Photography

3.5. Catchment Experiments. Two-liter canisters (ice cream pails) were placed inside the BMP and MC dolphins on wooden platforms at selected depths in June 1999 (Figure 6) to capture PAH settling from various sections of the pilings. Two canisters in each set contained an oil absorbent cloth covered by a ½ inch wire mesh cone. Four additional canisters held 2 cm of commercial silica sand. Two of these were covered with wire mesh cones and two were left uncovered. Sets of canisters were placed: (1) above the high water mark; (2) immediately below Mean Lower Low Water



Figure 6. Photograph showing Catchment Containers Before Installation in June, 1999.

(-3' chart datum); (3) at mid-depth (-14' chart datum); and (4) near the bottom (-23' chart datum). One additional set of six canisters was installed at the MC dolphin (-3' chart datum) as a reference. Plastic sheeting was spread across the platforms to isolate each set of canisters. This isolation was designed to determine the vertical distribution of the sources of PAH. The canisters were retrieved in October 1999 for physicochemical analyses.

3.6. Sediment Analysis. PAH analysis was conducted by Environment Canada's Pacific Environment Sciences Center (PESC) using High Resolution Gas Chromatography/Low Resolution Mass Spectrometry (HRGC/LRMS). Analytical methods and QA/QC procedures followed standard procedures develop by PESC (vers2.0, January, 1997). Mass spectrometry data was acquired in Total Ion Mode (TIM) and selected ions quantified using multilevel Internal Standards.

Recovery efficiency was calculated and reported using five surrogate standards - Naphthalene-d8 (x=51%), Acenaphthene-d10 (x=70%), Phenanthrene-d10 (x=96%), Chrysene-d12 (x=84%) and Perylene-d12 (x=80%). Reported results are not recovery corrected in this report. Average PAH surrogate recoveries for sediments by PESC was about 76% (Std. Dev. 11%). The method detection limit (Median Detection Limit) for sediment is given as 0.02 μ g/g, dry weight. In contrast to Axys Analytical Ltd., which was responsible for all previous analyses, concentrations below quantification criteria (NDR) were not reported by PESC.

Since the chemical analysis for this segment of the study was being done by a different laboratory, a thoroughly homogenized split sediment sample from Station BMP 0.5 was collected on Day 1360 and submitted to both PESC and Axys Analytical Ltd., for PAH analysis.

3.6.1. Sulfide, Total Volatile Solids and Particle Size Analyses.

Sulfide analysis on the field samples was conducted by Aquatic Environmental Science (AES) using a sulfide probe method (EPA Standard Method 376.1/9030). Field samples were placed in plastic containers, fixed in the field with zinc acetate and taken directly to the Aquatic Environmental Sciences laboratory. Samples were then treated with a sulfide anti-oxidant buffer solution (SAOB) and analyzed using an OrionTM advanced portable ISE/pH/mV/ORP meter Model 290A with a Model 9616 BNC *Ionplus* Silver/Sulfide electrode. Analysis of the bioassay sediment and overlying water during the bioassay tests was conducted at PESC using sulfide probe methods similar to those used by AES and a Hach Kit method using color chart and sulfide effervescence.

Total Volatile Solids (TVS) were determined using Standard Method 2540.E. Samples were dried at 103 ± 2 °C in aluminum boats that had been pre-cleaned by combusting at 550°C for 30 minutes. Drying was continued until no further weight reduction was observed. The samples were then combusted at 550°C for 30 minutes or until no further weight loss was recorded. Total Volatile Solids were calculated as the percent difference between the dried and combusted weights. Quality assurance involved triplicate analysis on one of every 20 samples or on one sample per batch if fewer than 20 samples were analyzed. A maximum of 20 percent Relative Percent Difference was established as the Data Qualification Control Limit.

Sediment Grain Size (SGS) analyses were accomplished on surficial sediments (top two cm of the sediment column). Sediment samples were wet sieved on a 0.064 mm sieve. The fraction retained on the sieve was dried in an oven at $92 \pm 2^{\circ}$ C and processed using the dry sieve and pipette method of Plumb (1981). The sieves used for the analysis had mesh openings of 20, 0.89, 0.25 and 0.064 mm. Particles passing the 0.064 mm sieve were analyzed by sinking rates in a column of water (pipette analysis).

3.7. Tissue PAH Analysis.

Previous tests conducted to determine mussel tissue whole body PAH concentrations were done using caged mussels (*Mytilus edulis edulis*), which were being held for growth, survival and spawning success experiments. The *in-situ* bioassay mussels closest to the piling were suspended at a distance of approximately 15 cm. During the 1999 studies, mussels were collected directly from the pilings at the BMP and MC sites to see if differences existed between tissue levels of PAH in the caged mussels and those directly attached to the creosote treated pilings. Tissue analysis was conducted by PESC using High Resolution Gas Chromatography/Low Resolution Mass Spectrometry (HRGC/LRMS).

3.8. Amphipod Bioassays.

Bioassay tests on the amphipod, *Eohaustorius washingtonianus*, were performed at PESC's Toxicity Laboratory. *Eohaustorius washingtonianus* samples were field collected at Esquimalt Lagoon, Victoria, B.C., by Biologica Environmental Services. Samples were collected and delivered to the laboratory within five days of test initiation. Amphipods were acclimated to $15 \pm 1^{\circ}$ C in Esquimalt Lagoon sediment under continuous light and aeration. These conditions were maintained for about two days prior to test initiation.

Normally amphipod tests are initiated shortly after sediments are collected. In this case, bioassay samples were pre-aerated for 10 days prior to adding the amphipods to reduce or eliminate toxic effects associated with high sulfide concentrations. The Sooke Basin test site was initially chosen, in part, because of the lack of H_2S odor in the sediment. It was apparent during the June 1999 survey that the buildup and decomposition of fouling organisms and their waste falling from the pilings had caused a marked increase in sediment sulfides. This condition was exacerbated at the MC dolphin because of wood debris dislodged by boring organisms. Total sulfide measurements on field transect samples revealed that total sulfide concentrations in proximity to the pilings were as high as 7500 micromoles - well above the 97.5 μ mole 48-hr LC50 reported by Wang and Chapman (1999) for *Eohaustorius*.

Each bioassay sample was pre-aerated for 10 days prior to adding the amphipods. The overlying water was changed daily during this period. To avoid disturbing the actual test sample, a sixth replicate bioassay jar from each station was setup to periodically monitor sulfide using the Hach Kit method. Amphipods were introduced after the 10-day pre-aeration period and the standard static 10-day acute toxicity tests were performed according to procedures outlined in Environment Canada (1992a). The overlying water was not changed during the bioassay period to avoid placing undue stress on the animals. Tests were conducted on samples

from the 0.0, 0.5, 2.0, 10 and 20 m BMP downcurrent transect stations, the MC 0.5 m station and the OC. In addition, a separate bioassay jar containing material from inside the perimeter of the Mechanical Control dolphin (MC 0.0) was spiked with a Standard PAH solution at 1 µg/g of each PAH compound for a total of 15 µg/g. PAH measurements were taken at the end of the pre-aeration and 10-day bioassay periods to determine if aeration had affected individual PAH concentrations. Only a small sub-sample was taken at the end of the pre-aeration period for PAH analysis. The entire contents of the jar were thoroughly mixed at the end of the bioassay period

before sampling for PAH

analysis.

Each container was carefully filled with a fresh laboratory supply of sand-filtered seawater from Burrard Inlet, being careful not to disturb the sediment layer. Twenty randomly selected *E*. washingtonianus were added to each of five replicate jars per evaluated station. The bioassays were conducted in an environmental chamber at 15 + 1°C under continuous light (Figure 7). Water quality (temperature, pH, salinity and dissolved oxygen) was measured periodically throughout the tests.



Figure 7. Bioassay Test Facilities on Day 1540.

At the conclusion of the bioassays, the total number of emergent (dead and alive) amphipods on the sediment surface (or swimming in the water column) of each test container was recorded (% at surface). The sediments were wet-sieved through a 0.5 mm stainless steel screen, and total surviving, dead and missing amphipods were recorded (% survival).

In addition, 96-h LC₅₀ reference toxicant tests were run concurrently with each set using various concentrations of cadmium chloride in seawater to assess the acceptability of test conditions and the amphipods' sensitivity in reference to historical performance under the same conditions (including absence of substrate and darkness).

The OA/OC and toxicity criteria of Lee et al., 1995 (Table 2) were used to evaluate Sooke Basin sediments in amphipod bioassays. All data were tested for normality using the Shapiro and Wilk test and the homogeneity of variance was tested using Bartlett's test in the TOXSTAT statistical program (Gulley et al. 1989). If any of the treatments showed zero variance (i.e. identical survival rate in all replicates), that treatment was removed from the analysis since treatments with zero variance will always result in a rejection of the test for normality and homogeneity of variance (US EPA, 1994). If the data passed the tests for normality and homogeneity of variance, a two-sample one-tailed t-test with equal variance ($\alpha =$ 0.05) was used to determine whether test sediment survival was lower than reference sediment survival. If data failed the test for normality of homogeneity of variance, the data were transformed using an arcsine – square root transformation developed by Anscombe and described in Zar (1984) before being reanalyzed. If the transformed data passed tests for normality and homogeneity of variance, the two-sample, one-tailed *t*-test with equal variance was performed on the transformed data. If the transformed data still failed tests for homogeneity of variance, but passed the test for normality, a two-sample, one-tailed *t*-test with unequal variance was used on the transformed data to determine whether survival in each test and in the reference sediment was significantly lower from that in the control.

It should be noted that biological significance in laboratory tests does not necessarily reflect environmental significance as noted in Goyette and Brooks (1998), and that it is up to the researcher evaluating the study site to determine relevant toxic responses.

Table 2. Interim Pass/Fail Criteria for 10-day Amphipod Sediment Toxicity Testing (Lee et al., 1995).

Condition	Requirement
Reference sediment Available	 Control Sediment Survival ≥ 90% Reference Survival ≥ 80% or abandon reference comparison If % control survival - % reference survival ≥ 20% & statistically lower, abandon the reference comparison Test sediment toxic if: % reference survival - % test survival ≥ 20% and is statistically lower
Reference Sediment Unavailable Or Abandoned.	 Control Sediment Survival ≥ 90% Test sediment toxic if: % control survival - % test survival ≥ 30% and is statistically lower.

In order for a test to be considered valid, amphipod survival in the control sediment must be 90% or greater (Environment Canada, 1992a). The LC₅₀ values (and associated 95% confidence limits) for the positive reference toxicant tests were determined using the Environment Canada computer program based on Stephan (1977).

3.8.1. Bioassay "End Point" PAH Analysis

Pre-aerating the sediment for 10 days to reduce the sulfide concentration could potentially reduce the PAH concentration in bioassay samples. Normally, five replicate jars are used in bioassay tests to evaluate toxicity. In addition to the spiked sample from MC 0.0, a sixth replicate jar for each sampling station was setup solely to monitor the PAH concentration at the end of the bioassay period. Results were compared with concentrations found in field Replicate #3, which came from the same composite sample used for the bioassays. The entire contents of each jar were thoroughly mixed at the end of the bioassay period before sub-sampling for PAH analysis.

3.9. Acute Toxicity Test Using a Photoluminescent Bacterium (MicrotoxTM).

Seven (7) field sediment samples were collected at each station in labeled centrifuge tubes on Day 1540 for liquid and solid phase MicrotoxTM assays. A marine bioluminescent bacterium, *Vibrio fischeri*, was used to assess the toxicity of the test sediments using the MicrotoxTM test system. Procedures were outlined in detail by Goyette and Brooks (1998). Vials of freeze-dried *V. fischeri*, stored at $-20 \pm 2^{\circ}$ C, were reconstituted in 1.0 mL of distilled water and incubated at $5.5 \pm 1^{\circ}$ C for no less than 20 minutes prior to use in liquid and solid phase tests. Test results were based on measured light output in the presence of various levels of test substance in aqueous solutions, which were compared with light output of a control blank (*i.e.* bacterial cell suspension in diluent only). Light output is a product of the electron transport system and relates directly to the metabolic state of the bacteria (Schiewe *et al.*, 1985). The degree of light loss (degree of metabolic inhibition in the bacteria) indicates the degree of toxicity of the sample.

Each of the full 50 mL polystyrene tubes was centrifuged for 30 minutes at 4,000 rpm and 4°C to extract the pore water. The pore water was immediately decanted and tested within 24 hours for toxicity using liquid-phase testing procedures for screening and IC₅₀ determination outlined by Microbics Corporation (1992a) and Environment Canada (1992b). Natural seawater, adjusted with natural brine salts to match the salinity of the pore water samples, was used as control/dilution water during liquid-phase testing. Light emission readings were recorded after 5 and 15 minutes (also after 30 minutes for baseline and Day 14 samples) of incubation at 15.0 \pm 0.5°C in controls and test solutions.

After centrifugation, the sediment remaining in one of the tubes from each station was homogenized for solid phase testing, which was carried out according to methods outlined by Microbics Corporation (1992b). Bacteria were incubated for 20 minutes at ambient room temperature in a series of aqueous solutions of various concentrations made up of the sediment sample and a 3.5% solution of Reagent Grade NaCl crystals dissolved in de-ionized water. Following this incubation period of direct bacterium-particle interaction, the solutions were filtered and 500 μL of each filtrate was transferred to a corresponding glass cuvette within the incubation unit. After a further five minute incubation period at $15.0 \pm 0.5\,^{\circ}\text{C}$, light emission from each concentration was measured. A Microtox model 500 Toxicity Analyzer (Microbics Corporation) controlled by the appropriate Microtox software (versions 7.03 and 7.81) was used for all procedures.

A 50 to 100% inhibition of light production during the screening test (using a 100% concentration only) indicates that further testing using serial dilutions of the pore water may allow determination of an IC₅₀ value. The degree of light loss (*i.e.* degree of metabolic inhibition in the bacteria) indicates the degree of toxicity of the sample. A dose-response curve was determined by Microbics software (Version 7.81 for liquid-phase; Version 7.03 for solid-phase), on which the IC₅₀ was located. A 95% confidence range was also reported. The IC₅₀ is the inhibiting concentration of a sample causing a 50% decrease in the bacterial light output under defined conditions of exposure time and test temperature. Interpretation guidelines for these tests are given in Table (3).

Table 3. Interpretation Guidelines for Microtox[™] Photoluminescent Bacterium Toxicity Tests (Beckman, Inc. 1982; PSEP 1986).

Type of Test and Condition

	Solid-Phase 5	minute IC ₅₀	<u>Liquid-phase 15 minute IC₅₀</u>
	Wet Weight	Dry Weight	
Practically nontoxic:	>1.0%	>0.5%	>100%
Moderately toxic:	0.1 to 1.0%	0.1 to 0.5%	50 to 100%
Toxic:	< 0.1%	<0.1%	<50%

3.10. <u>Underwater Observations and Photography</u>.

Foreshore Technologies Inc. routinely recorded underwater observations including 35 mm still photographs and 8 mm digital and analog video during the first year of the study. This provided a record of the development of the epifaunal community on each structure and of megafauna inhabiting the bottom and water column. Diver observations were recorded on the videotape as audio input. The inshore piling of each dolphin was routinely videotaped from the surface to the mud line. These observations were continued in Year Four.

4.0 RESULTS AND DISCUSSION.

4.1. BMP 0.5 Interlaboratory QA/QC Splits.

All PAH analysis conducted in 1995-96 were accomplished by Axys Analytical Ltd., Sidney B.C. Analyses for the 1999 study were completed at Environment Canada's Pacific Environment Centre. This report compares 1995-96 results with 1999 results. Therefore, it was considered important to evaluate consistency between the two laboratories. A split sediment sample from Station BMP0.5 was collected on Day 1360 and submitted to both laboratories for comparison. The results are given in Table (4). A two-sample t-test, with separate variance estimates, was used to assess the differences between laboratories. The results from Axys for phenanthrene (1900 ng/g) and pyrene (1850 ng/g) were significantly higher ($\alpha = 0.05$) than reported by PESC (1290 ng/g for phenanthrene and 1190 ng/g for pyrene). The differences for other compounds and for LPAH, HPAH and TPAH were not significantly different. It should be noted that one replicate from Axys was significantly higher than either PESC replicate and the remaining three replicates from Axys were significantly lower than those from PESC. Some analytical variability was expected due to variations in recovery rates, the method of reporting detection limits and the patchy nature of PAH in sediments described by Goyette and Brooks (1998). These results were considered acceptable to allow comparisons between the results from the two laboratories.

4.2. Sediment Transect PAH Concentrations on Day 1360 and Day 1540.

PAH concentrations (LPAH, HPAH and TPAH) for each transect sample on Days 1360 and 1540 are compared with results from Day 384 in Table (5). Concentrations are given in μg/g dry weight. Raw data on the individual PAH compounds from each sampling station are given in Appendix I (a-f), including those collected at the end of the 10-day bioassay period. Sediment

concentrations of PAH at the end of the bioassay and a sample from Station MC 0.0 spiked with a PAH Standard equivalent to $1.0~\mu g$ of each PAH compound/g dry sediment are also included in Table (5). These results will be discussed in more detail in the bioassay section.

4.2.1. Mechanical (MC 0.5) and Open Control (OC 0.0) Sediment PAH Concentrations.

PAH concentrations at the MC (Figure 8) and OC (Figure 9) sites were consistent low (<0.20 µg/g) throughout the entire study.

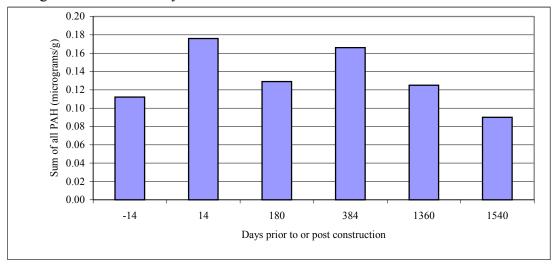


Figure 8. Surface Total PAH Concentration at the Mechanical Control (MC 0.0) - Day 0 to Day 1540 (μ g/g, dry weight) - Sooke Basin Creosote Evaluation Study.

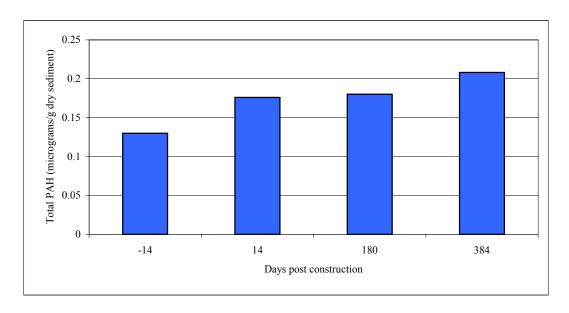


Figure 9. Total Surface Sediment PAH Concentration ($\mu g/g$, dry weight) at the Open Control (OC 0.0) from Day 0 to Day 1540 - Sooke Basin Creosote Evaluation Study.

Table 4. Laboratory QA/QC Split Samples for Station BMP 0.5 on Day 1360. Axys Analytical and Pacific Environmental Science Center. Individual PAH Compound Concentrations are given in ng/g, dry wt. Total PAH (TPAH) concentrations are in μ g/g dry weight.

	<u>A</u>	XYS Analyti	<u>cal</u>		Pacific Environmental Sciences Centre				
РАН	A	В	Mean	1	2	3	4	Mean (Uncorrected for recover rate)	Mean (Corrected for recovery rate)
Naphthalene	260	230	245	240	180	220	170	203	251
Acenaphthylene	10	10	10	40	20	20	20	25	31
Acenaphthene	540	510	525	520	450	490	430	473	503
Fluorene	370	420	395	410	330	340	300	345	367
Phenanthrene	1700	2100	1900	1520	1250	1250	1140	1290	1217
Anthracene	310	670	490	680	350	270	260	390	368
LPAH	3190	3940	3565	3410	2580	2590	2320	2726	2737
Fluoranthene	3300	2800	3050	4650	1840	2740	1930	2790	2632
Pyrene	2000	1700	1850	1510	990	1200	1060	1190	1123
Benz(a)anthracene	1300	1300	1300	1930	680	620	670	975	1121
Chrysene	1800	2200	2000	2730	1030	1040	910	1428	1641
Benzofluoranthenes	1400	1300	1350	2160	870	880	810	1180	1356
Benzo(a)pyrene	440	440	440	890	350	320	330	473	544
Dibenz(ah)anthracene	54	48	51	80	40	40	40	50	58
Indeno(1,2,3-cd)pyrene	160	170	165	310	150	130	140	183	210
Benzo(ghi)perylene	130	130	130	240	110	100	100	138	159
НРАН	10584	10088	10336	14500	6060	7070	5990	8407	8844
TPAH (μg/g)	13.77	14.02	13.90	17.91	8.64	9.66	8.31	11.13	11.58
Surrogate Recovery (%)								1	
Naphthalene d-8	52	130	86	89	70	90	76	81	79
Acenaphthylene d-10	60	100	80	95	86	92	102	54	93
Phenanthrene d-10	63	82	73	106			106	106	106
Pyrene d-10	72	92	82	90	86	82	89	87	87
Chrysene d-12	81	120	101						
Benzo(a)pyrene d-12	58	74	66						
Perylene d-12	49	63	56	90	87	82	89	87	87
Dibenz(ah)anthracene d-14	34	41	38						
Benzo(ghi)perylene d-12	49	54	52						

Table 5. Summary Table Showing the Field Transect Surface Sediment Low Molecular Weight (LPAH), High Molecular Weight (HPAH) and Total (TPAH) PAH Concentrations (μ g/g, dry weight) on Days 384, 1360 and 1540 and 10-Day Amphipod Bioassay "End Point" PAH Concentration on Day 1540. - Sooke Basin Creosote Evaluation Study.

Station	LPAH	HPAH	TPAH
384BMP0.0	6.6	23.3	29.9
1360BMP0.0	1.1	5.8	6.9
1540BMP0.0	1.1	5.7	6.8
Bioassay "End Point"	0.57	4.0	4.6
384BMP0.5	2.4	11.9	14.3
1360BMP0.5	1.0	4.3	5.3
1540BMP0.5	2.0	5.1	7.1
End of Day 1540 Bioassay	0.39	2.7	3.1
384BMP2.0	1.3	6.6	7.9
1360BMP2.0	0.54	2.3	2.8
1540BMP2.0	0.20	1.3	1.5
End of Day 1540 Bioassay	0.08	1.2	1.3
384BMP5.0	0.75	2.5	3.2
1360BMP5.0	0.007	0.047	0.05
1540BMP5.0	0.23	0.93	1.2
End of Day 1540 Bioassay	0.07	0.97	1.0
384BMP10	0.52	1.7	2.2
1360BMP10	0.51	0.92	1.4
1540BMP10	0.005	0.47	0.48
End of Day 1540 Bioassay	<0.02	0.44	0.46
384BMP20	0.12	0.38	0.50
1360BMP20	0.13	0.41	0.54
1540BMP20	0.02	0.19	0.21
End of Day 1540 Bioassay	0.02	0.23	0.25
384MC0.5	0.03	0.13	0.16
1360MC0.5	0.03	0.095	0.12
1540MC0.5	<0.02	0.09	0.11
End of Day 1540 Bioassay	<0.02	0.04	0.06
384OC0.0	0.058	0.16	0.22
End of Day 1540 Bioassay	<0.02	0.08	0.10
MC0.0 (#1) ¹			
Post aeration Spiked Sample	1.7	4.8	6.5
Post Bioassay Spiked Sample	4.7	12.7	17.4

Note: ¹MC 0.0 (#1) was spiked with a known PAH Standard at 1 µg of each PAH species/g dry sediment for a total of 15 µg PAH/g dry sediment.

4.2.2. BMP Downcurrent Transect Surficial Sediment (≤ 2.0 cm depth) PAH Concentrations on Days 1360 and 1540.

One striking aspect in the data from Year Four is the noticeable drop in sediment PAH concentrations at all BMP stations when compared with same station data from Day 384. Concentrations near the BMP dolphin were less than a third of what they were at the end of Year One. The model developed by Brooks (1994) predicted that sediment concentrations would peak approximately 1,000 days following construction at a TPAH value ~18% higher than observed on Day 384. Samples were not collected in year three and the exact timing and PAH concentration at the peak was not documented. Figure (10) is a three-dimensional, distance weighted least squares plot of sediment PAH concentrations observed within 5 meters of the BMP dolphin during this study. Few data points were obtained following Day 384, but the results suggest that sediment PAH at the BMP 0.5 m station peaked very close to the Day 384 value at about 600 days following construction.

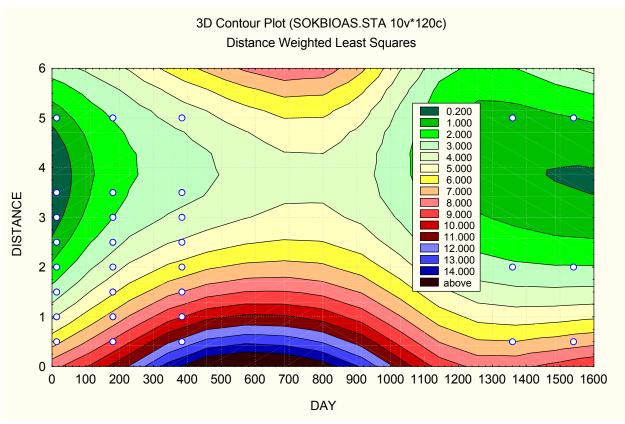


Figure 10. Comparison of Surface Sediment Total PAH Concentration ($\mu g/g$, dry wt.) at the BMP treatment site - Day 384 \underline{vs} . Day1360 and Day1540 - Sooke Basin Creosote Evaluation Study. Polynomial fits to the Day1360 and Day1540 data are provided.

There were no apparent seasonal differences in sediment PAH concentrations. Samples from inside the BMP dolphin (Station BMP 0.0) were essentially the same on Day 1360 (6.9 \pm 1.8 μ g/g) and Day 1540 (6.9 \pm 2.1 μ g/g). The range in sediment PAH concentrations under the dolphins on these days was 5.6 to 8.3 μ g TPAH/g, which was considerably lower than the 29.9 μ g/g measured in similar samples taken on Day 384 (Table 5). Concentrations at Station

BMP0.5, were also down, averaging $5.3 \pm 2.0 \,\mu\text{g/g}$ on Day 1360 and $7.1 \pm 3.2 \,\mu\text{g/g}$ on Day 1540, compared to 14.3 $\,\mu\text{g/g}$ measured in mixed samples on Day 384. Replicate samples from BMP 0.5 on Day 384 averaged 18.9 ± 10.2 . Seventy-five percent of the total sediment PAH concentration measured on Day 1540 in samples from BMP0.5 was made up of phenanthrene (12%), anthracene (12%), fluoranthene (25%), chrysene (16%) and benzo(a)anthracene (10%). Concentrations of PAH at both stations were well below predictions by Goyette and Brooks (1998). The remaining downcurrent sampling stations were also below levels previously recorded on Day 384. Figure (11) compares the total PAH concentrations along the BMP transect on Days 1360 and 1540 with Day 384. Figure (12) describes the temporal and spatial changes in total PAH concentration at the BMP site over the entire study period.

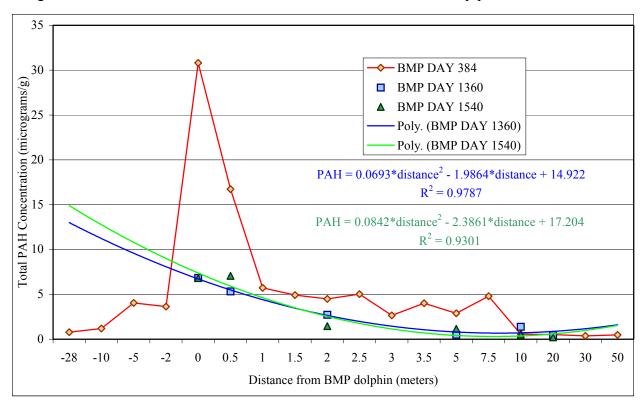


Figure 11. Comparison of Surface Sediment Total PAH Concentration ($\mu g/g$, dry wt.) at the BMP treatment site - Day 384 \underline{vs} . Day1360 and Day1540 - Sooke Basin Creosote Evaluation Study. Polynomial fits to the Day 1360 and 1540 data are provided.

Low molecular weight PAHs, such as naphthalene, degrade rapidly, while the higher molecular weight PAHs such as benz(a)anthracene and benzo(a)pyrene are more resistant to microbial attack. Herbes (1978) reported turnover times for naphthalene, anthracene and benz(a)anthracene of 13, 62 and 300 hours respectively. Mueller *et al.* (1991) found that natural microbial communities mineralized 94% of the low molecular weight PAH in 14 days but only 53% of the high molecular weight PAH was degraded during the same period. They also noted that the most rapid biodegradation of PAH occurred at the water/sediment interface.

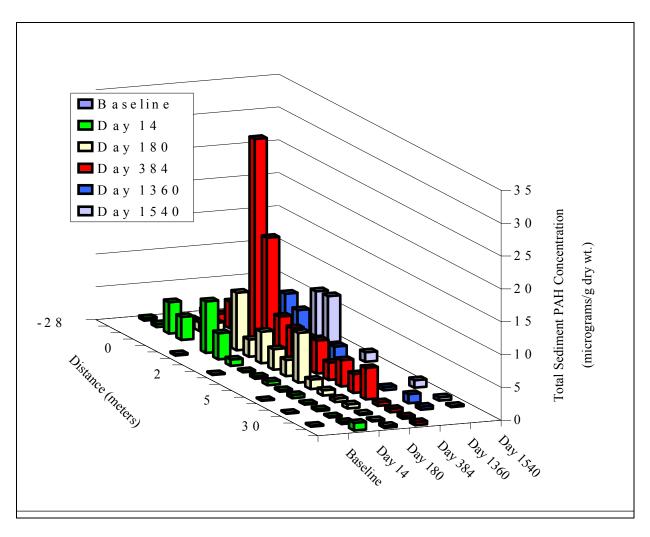


Figure 12. Sooke Basin Creosote Evaluation Study: Total PAH Concentrations ($\mu g/g$ dry weight) in Surface Sediments at the BMP Piling Downcurrent from the Baseline through Day1540.

Saylor and Sherrill (1981) and Cerniglia and Heitkamp (1991) summarized the available literature describing the half-life of PAH in aquatic environments. The results were highly variable and depended on PAH species together with a range of environmental and biological factors such as temperature, the presence of cometabolites, the nature of the microbial community and the availability of oxygen. A broad range of bacteria and fungi have been observed to rapidly degrade numerous low and high molecular weight PAH (Grifoll *et al.*, 1994; Stringfellow and Aitken, 1994; Cerniglia and Heitkamp, 1991). Bacterial communities in polluted areas metabolize PAH more quickly than those in unpolluted areas and low molecular weight PAH are metabolized more quickly than high molecular weight PAH in these environments (Herbes and Schwall, 1978). Naphthalene has a short half-life (hours to days), whereas the five-ringed benzo(a)pyrene has a long half-life (years under unfavorable conditions). However, Kanaly and Bartha (1999) demonstrated significant biodegradation of B(a)P in the presence of complex hydrocarbon mixtures. Crude oil, distillates of heating oil, jet fuel, and diesel fuel supported up to 60% reduction to carbon dioxide and water of 80 μg

B(a)P/g soil in 40 days. Millette *et al.* (1995) also demonstrated the interdependence and cometabolism of mixtures of creosote derived PAH following an initial lag time of 5 to 7 days during which the natural microbial community was selected for those phenotypes capable of efficiently metabolizing PAH. In their study, 60 to 75% of the phenanthrene was mineralized within 30 days. These studies suggest that in the presence of complex cometabolites, phenanthrene, which comprises approximately 19% of new creosote oil, may be rapidly degraded once it migrates from the wood. There are a number of possible reasons for the observed decline in surficial sediment PAH concentrations between Year One and Year Four.

- a) Differences in analytical results from the Axys and PESC laboratories. Although the analytical methods were similar, some difference between labs might be expected due to variations in recovery rates and reported detection limits. However, results from split samples taken in June 1999 and analyzed by both labs, indicated significant differences for only two of the 15 PAH compounds tested. The differences in LPAH, HPAH and TPAH were not significant at $\alpha = 0.05$. It is unlikely that the large observed differences between Days 384 and 1360 or 1540 could be attributed to the laboratories.
- b) Catchment experiments. The platforms and plastic sheeting installed during canister experiments may have intercepted PAH falling from the pilings. This may have been a factor at the base of the dolphin, but it could not explain the drop in PAH concentrations at the 0.5 m and further downcurrent stations. It is worth noting that the sheeting was only installed for a relatively short period of time and that the PAH levels in fine mud collected during the canister experiments ranged between 4.3 μ g/g and 8.7 μ g/g, very similar to the levels found in transect sediments at the base of BMP dolphin.
- c) Increased degradation through microbial cometabolism. The following is provided as a brief review of the literature describing microbial degradation. The volume of information provided should not necessarily imply relative importance over the other factors. The ultimate fate of PAHs that accumulate in sediments is believed to be biotransformation and degradation by bacteria, fungi and algae (EPA, 1980; Borthwick and Patrick, 1982; Cerniglia, 1984; Boldrin *et al.*, 1993). Borthwick and Patrick (1982) estimated the chemical and biological half-life of the dissolved components of marine grade creosote at less than one week in laboratory experiments. More recently, Bestari *et al.* (1998a, 1998b) observed an exponential decline in creosote derived PAH released into microcosms. The concentration of PAH in these microcosm studies reached background levels by the end of their 84-day study.

Numerous studies have examined the degradation of creosote in laboratory and natural environments. Bogan and Lamar (1995) showed that white rot basidiomycetes are able to degrade a broad spectrum of intermediate and heavier creosote-derived PAH. Mueller *et al.* (1989) provided an excellent review of bioremediation technologies designed to remove PAH, including the high molecular weight compounds, from creosote-contaminated sites. Ingram (1982) observed that the concentration of creosote in leaching vats increased to greater than 700 µg/L in the first 72 hours and then decreased to less than 34 µg/L at the end of 20 days. He attributed that decrease to bacterial metabolism of the PAH migrating from the creosote treated piling assessed in his study. Similarly, Tagatz *et al.* (1983) noted that creosote concentrations

decreased by 42% over an eight-week period in sediments artificially contaminated in mesocosm studies. They also attributed the decrease to microbial metabolism.

Brooks (1994) concluded that the half-life of the suite of low molecular weight PAH associated with creosote treated wood was approximately 30.5 days at 20°C, while the suite of high molecular weight compounds degraded more slowly with a half-life of 242.3 days at 20°C in aerobic conditions (reduction oxidation potential discontinuity >4.0 cm). The half-life of the mixture of all PAH lost from creosote treated wood was predicted to be 214.8 days at 20°C. The TPAH concentration measured at the 0.5-meter downcurrent station in the Sooke Basin study on Day 384 was 14.3 μ g/g dry sediment. The concentration of TPAH at this station was 5.3 $\pm \mu$ g/g on Day 1360 and 7.1 \pm 3.2 μ g/g on Day 1540. This represents a reduction of sediment PAH to between 37% and 50% of the maximum observed on Day 384. The half-life of creosote derived PAH in sediment cannot be inferred from these data because it is likely that small amounts PAH continued to be lost from the dolphins.

Sediment concentrations of volatile solids were significantly increased by biodeposits from the epifaunal community that took up residence on creosote treated piling. While it is known that the addition of complex organic carbon enhances PAH catabolism – it has also been demonstrated that microbial degradation of high molecular weight compounds is slowed under anaerobic conditions. Therefore, while the added organic carbon may have enhanced the metabolism of sediment PAH as discussed above, these same organic deposits created anaerobic conditions in sediments around all of the dolphins – perhaps inhibiting PAH metabolism. How these antagonistic factors affect degradation rates in open aquatic environments is unknown. However, the results of this study suggest that PAH degradation proceeded more quickly than was expected. The following hypotheses are presented as possible reasons for the observed decreases in sediment PAH concentrations.

- d) **Burial of PAH contaminated sediment from piling debris**. It is possible that debris falling from the pilings had partially buried the PAH contaminated sediment. Over the last three years, a substantial amount of shell and other debris had built-up at the base of the dolphins. The amount of material falling from the pilings due to animal grazing would be more than adequate to bury previously contaminated sediment and dilute recent deposits. As much as 413 g of debris was collected by the mid-piling canister at the BMP site within a relatively short four-month period. If this deposition rate remained constant throughout the year, it would represent an annual biodeposit of 45 kg/m²-y. At the MC dolphin, over 938 g of biodeposits were collected by canisters placed at the same depth. This represented about 104 kg of biological debris/m²-y. These deposition rates would likely have a significant impact on sediment PAH concentrations around the base of the dolphins. However, this would not explain the decreased concentrations observed at those stations located further downcurrent.
- e) Lower PAH migration rates from the upper and above water portions of the piling. As shown in Plates (1) through (4), BMP piling surfaces that were exposed at low tide became covered with a tar-like residue similar to the weathered tar found in asphalt paving. This material appeared insoluble and it may have acted to anneal the piling, reducing further losses. This hypothesis was not investigated in this study, but it deserves further evaluation.
- f) **Interception of PAH by epifauna growing on the pilings**. As shown in Plate (5), a substantial community of mussels, anemones and other invertebrates developed on

the pilings over the four-year study – particularly after Day 384. This thick layer of growth may have been sufficient to provide a physical barrier to the migration of creosote. This fouling community included an interior mass of detrital waste that was high in organic carbon. Polycyclic aromatic hydrocarbons migrating from the treated wood were likely bound in this organic matrix where they were rapidly cometabolized (see discussion above) with the detritus by a flourishing microbial community.

Many of these factors likely contributed to the reduction in sediment PAH concentrations following Year One. Which factor(s) were dominant was not specifically investigated. The relative roles of burial, microbial degradation and lower creosote leaching rates are definitely worthy of further research and would provide valuable information on the long term effects of creosote treated wood in aquatic environments.

Year Four results indicated that sediment PAH concentrations peaked earlier and declined more quickly than predicted. Model predictions did not take into account possible enhanced microbial catabolism of PAH associated with biodeposits at the base of the pilings, nor the extent to which piling growth might restrict creosote migration. Both factors appear important in evaluating the environmental response to creosote treated wood.

Over the past few years, Environment Canada (1995) and other agencies in Canada and the US have developed a set of numerical benchmarks for evaluating the biological affects associated with PAH compounds. These are intended to provide guidance for the regulatory framework dealing with PAH contaminated sediments. In some cases, benchmarks are intended as screening tools for further investigation. Washington State's Sediment Quality Standards (WAC 173-204-320) provide a legal basis for regulatory action. Results from Day 384 and Day 1540 at Station BMP 0.5 are compared with Environment Canada's Interim Sediment Quality Guidelines (ISQGs), the draft US EPA standards, and Washington State Sediment Quality Standards in Table (6). The ISQG TEL values represent the Threshold Effects Level and the PEL is the Probable Effects Level. Both of these benchmarks are based on a large field and laboratory derived chemical and biological database developed in Canada and the US. Sooke Basin concentrations that exceeded the PEL values are shaded in Table (6).

Polycyclic aromatic hydrocarbons bind tightly to organic matter in aquatic environments. This reduces their bioavailability and therefore their toxicity. This is reflected in many sediment quality criteria. Biodeposits from epifauna growing on the piling more than doubled the organic carbon content of near field sediments from 1.04% on Day 384 to 2.37% on Days 1360 and 1540. In addition to increasing the rates of microbial catabolism, this organic carbon may have also reduced the bioavailability of PAH to eukaryotes. Swartz (1999) published Consensus Sediment Quality Benchmarks for mixtures of PAH that included consideration of environmental levels of organic carbon. The results of assessing sediment toxicity on Day 1540 at the BMP dolphin are provided in Table (7). None of the observed PAH concentrations exceeded the mean of the two Swartz (1999) benchmarks – a value above which a toxic response could be expected. Only chrysene exceeded the threshold effects level. The point is that the increased concentration of organic carbon in near field sediments had likely rendered the remaining PAH non toxic and no adverse biological affects were anticipated in association with sediment PAH— even under the footprint of the six piling BMP dolphin.

Table 6. Comparison of the BMP 0.5 Sediment TPAH Concentration (μg/g, dry wt.) on Days 384, 1360 and 1540 with Environment Canada's Interim Sediment Quality Guidelines, the U.S. Environmental Protection Agency and Washington State (WAC 173-204-320) Numerical Sediment Quality Standards for Individual PAH and the sum of Low and High Molecular Weight PAH. Washington State and U.S. EPA standards are in μg/g organic carbon at the observed mean Total Organic Carbon content of 1.04%. Values exceeding the individual Probable Effects Level (PEL) are shaded.

PAH Compound	Environment Canada's Interim Sediment Quality Guidelines (ISQG) in µg/g		Proposed EPA Standard	Washington Standard	days after I	0 and 1540 stallation at lowncurrent	
	(TEL)	(PEL)			Day 384	Day 1360	Day 1540
Naphthalene	0.03	0.39		1.03	0.029	0.203	0.020
Acenaphthylene	0.01	0.13		0.67	0.032	0.025	0.027
Acenaphthene	0.01	0.09	2.39	0.17	0.165	0.473	0.06
Fluorene	0.02	0.144		0.24	0.300	0.345	0.20
Phenanthrene	0.09	0.54	2.50	1.04	1.300	1.290	0.87
Anthracene	0.05	0.24		2.29	0.615	0.390	0.83
Total LPAH	0.20	1.55		3.85	2.441	2.725	2.0
Fluoranthene	0.11	1.49	3.12	1.66	3.550	2.790	1.7
Pyrene	0.15	1.40		10.40	1.600	1.190	0.28
Benz(a)anthracene	0.08	0.69		1.14	1.500	0.975	0.71
Chrysene	0.11	0.846		1.14	2.350	1.428	1.1
Benzofluoranthenes				2.39	1.550	1.180	0.69
Benzo(a)pyrene	0.09	0.76		1.03	0.785	0.473	0.28
Dibenz(ah)anthracene	0.01	0.14		0.12	0.059	0.050	0.05
Ideno(1,2,3-cd)pyrene				0.35	0.275	0.183	0.12
Benzo(ghi)perylene				0.32	0.190	0.138	0.09
Total HPAH	0.55	5.33		9.98	11.859	8.405	5.1
Total PAH	0.75	6.88		13.831	14.300	11.100	7.1

Goyette and Brooks (1998) found that 384 days following construction, the accumulation of sedimented PAHs was restricted to distances \leq 7.5 metres (4.8 µg/g, TPAH) from the BMP dolphin. Levels of PAH outside 7.5 metres were generally low (0.4 - 2.3 µg/g) and well below regulatory sediment quality criteria or levels at which biological responses are expected (Johnson, *et al.* 1994). After four years, the accumulated PAHs had receded to a distance of 0.5 metres from the BMP dolphin at an average TPAH concentration of 7.1 µg/g. Beyond that point, total PAH concentrations were generally \leq 2.0 µg/g. Based on the preceding discussion, toxic responses to sediment PAH were not anticipated on either Day 1360 or Day 1540.

Table 7. Determination of the Toxicity of Individual PAH Compounds and the Sum of Their Predicted Toxicities Using the Methodology of Swartz (1999). The TEL, LC_{50} and the mean of these two values are corrected to the observed organic carbon value of 2.37%. This data is from the area inside the footprint of the BMP dolphin on Day 1540.

Compound	Sum PAH TEL	Sum PAH LC50	Mean	Observed	TU
Naphthalene	0.308	1.683	0.995	0.010	0.010
Acenaphthylene	0.071	0.356	0.213	0.035	0.164
Acenaphthene	0.095	0.545	0.320	0.055	0.172
Fluorene	0.403	2.133	1.268	0.105	0.083
Phenanthrene	0.687	3.674	2.180	0.540	0.248
Anthracene	0.498	2.702	1.600	0.405	0.253
Fluoranthene	1.635	8.793	5.214	1.480	0.284
Pyrene	2.133	11.400	6.766	0.820	0.121
Benz(a)anthracene	0.498	2.631	1.564	0.700	0.448
Chrysene	0.735	4.005	2.370	1.325	0.559
Benzo(b)fluoranthene	0.782	4.266	2.524	0.780	0.309
Benzo(k)fluoranthene	0.687	3.674	2.180	0.300	0.138
Benzo(a)pyrene	0.782	4.242	2.512	0.300	0.119
Dibenzo(ah)anthracene	0.237	3.318	1.778	0.045	0.045
Benzo(ghi)perylene					
Sum Toxic Units for LPAH	2.062	11.092	6.577	1.150	0.175
Sum Toxic Units for HPAH	7.489	42.328	24.909	5.705	0.229
Sum Toxic Units for TPAH	9.551	53.420	31.485	6.900	0.169

4.3. Canister Studies.

Field observations during the first year suggested that the bulk of the creosote contamination in sediments consisted of minute creosote droplets, which fell directly from the pilings. The major source appeared to be the above water and intertidal sections of the dolphins, which were heated by the sun. The appearance of a tarry residue on the upper surface of the BMP pilings suggested that the creosote, being black in color, was absorbing heat from the sun. Temperatures, at times, could be high enough to cause the creosote to melt and trickle down into the water or to be picked up by the incoming tide. The purpose of the canister studies was to determine if the air exposed portion of the pilings was the major source of creosote contamination.

Results from the canister studies are provided in Table (8). Unfortunately, the tight configuration of the dolphin did not allow placement of the above water containers high enough to avoid wave action during heavy weather. Consequently, most of the contents had been washed out by the time they were retrieved. In addition, shell debris and decaying biological matter falling into the lower containers that were not covered by wire mesh prevented the creosote droplets from reaching the silica sand. The small amount of sand recovered from these containers in October held only $1.2~\mu g$ PAH/g dry sand and the results were considered inconclusive. Sand from the MLLW container exposed to the above water and intertidal areas

of the piling had a TPAH concentration of 32 μ g/g. The most reliable data came from the containers with oil absorbent cloth covered by wire mesh. These had collected enough fine mud for PAH analysis without the shell debris. Total PAH concentrations in the mud samples were 7.7 μ g/g at MLLW; 8.7 μ g/g at mid-depth; and 4.3 μ g/g in the bottom container. The results of this canister study did not provide a clear indication of what specific section of the piling, if any, was contributing the most PAH. Either all areas of the pilings were contributing equally, or attempts to isolate each section from the others were not successful. It is worth noting that the PAH concentration in the mud from each of the containers was very similar to that found in sediments at the base of the BMP dolphin (BMP 0.0 and BMP 0.5) on Days 1360 and 1540. Previous canister studies using Kaolin clay placed at MLLW and 0.6 m above the bottom at the BMP site produced PAH concentrations ranging between 28 μ g/g and 51 μ g/g (Goyette and Brooks, 1998).

Dry weight measurement of the debris collected by the canisters gave an indication of the amount of material falling from the pilings. The bottom canister at BMP 0.0 contained 156 g and the middle container 413 g. The middle container on the Mechanical Control piling contained considerably more at 939 g. This was likely because the untreated piling were quickly deteriorating under attack by *Limnoria* and *Bankia*. This deterioration caused failure of the surface layers of wood resulting in the sedimentation of attached fouling communities and woody debris.

Table 8. PAH Concentrations in Material Collected From Catchment Containers Placed at the BMP and MC Sites on Day 1360 and Recovered on Day 1540 - Sooke Basin Creosote Evaluation Study - Year Four. All values, except for TPAH are provided in ng PAH/g dry sediment. Given PAH concentrations have not been recovery corrected.

Treatment site		BMP Dolphin						
Tray Location Chart datum Substrate	Control sand	Above water	MLLW (#2) -3 ft. sand	MLLW (#1) -3 ft mud	Mid-depth -13 ft mud	Bottom -23 ft mud	Control MLLW (#1) -3 ft sand	
РАН	Control samu	Sunce	Sund	uu	uu	uu	Sund	
Naph.	<10	<20	<20	20	<20	<20	<20	
Aceny.	<10	<20	50	20	20	<20	<20	
Acen.	<10	<20	1460	650	420	250	<20	
Fluor.	<10	<20	1530	400	390	210	<20	
Phen.	<10	220	8470	1640	1980	970	<20	
Anth.	<10	<20	690	140	240	110	<20	
LPAH	<10	220	12200	2870	3050	1540	<20	
Fluoranth.	<10	460	8570	2240	2160	1140	<20	
Pyrene	<10	270	5470	1300	1350	630	<20	
B(a)Anth.	<10	30	1640	330	530	250	<20	
Chrysene	<10	100	1490	310	580	300	<20	
B(FI)	<10	90	1530	360	550	270	<20	
B(a)Pyr.	<10	30	580	130	210	100	<20	
Dibenz(ah)Anth.	<10	<20	70	<20	<20	<20	<20	
Indeno.	<10	<20	250	80	120	70	<20	
B(ghi)perylene	<10	<20	160	50	80	40		
НРАН	<10	980	19760	4800	5580	2800	<20	
TPAH (ng/g)	<10	1200	31960	7670	8630	4340	<20	
TPAH (μg/g)	<0.01	1.200	31.960	7.670	8.630	4.340	<0.020	
Surr. Recovery (%)								
Naph d-8		65	68	100	72	68	62	
Acen d-10		71	82	118	82	82	76	
Phen d-10		87	96	99	85	92	95	
Cry d-12		88	84	81	74	80	92	
Perylene d-12		90	93	89	76	83	95	

4.4. Toxicity Associated with Elevated Sediment Total Sulfide Concentrations.

Plate (5) depicts the diverse and abundant epifaunal community that established itself on the BMP piling by Day 1540. Grazing by starfish (Plate 6) and crabs resulted in significant biodeposits on the benthos (see Plate 11). The biological oxygen demand created by the microbial catabolism of this material exceeded the assimilative capacity of the sediments resulting in anaerobic conditions and elevated concentrations of sulfide. Sulfide concentrations observed on Days 1360 and 1540 at the BMP dolphin are summarized in Figure (13).

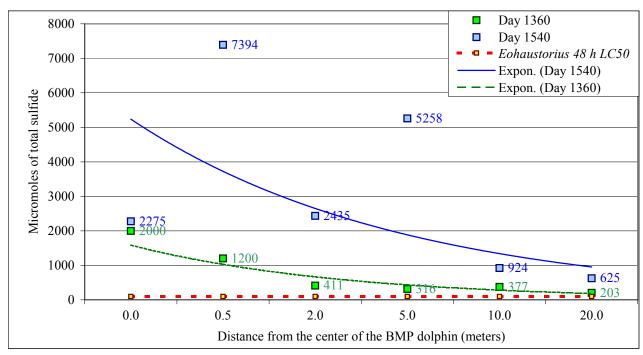


Figure 13. Sediment Concentrations of Total Sulfide on the BMP Dolphin Downcurrent Transect During Sampling on Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study. An exponential trendline is provided for the sulfide data on each day.

Wang and Chapman (1999) reviewed the toxicity of sediment total sulfide, which is taxa dependent. Sulfide concentrations causing mortality in 50 percent of test organisms (LC₅₀) ranged from 5.9 μmoles sulfide for the amphipod *Anisogammarus* to >1467 μmoles for *Mytilus edulis*. The organic carbon tolerant polychaete *Capitella capitata* has a lowest observed three-hour effects concentration (3 hr-LOEC) of greater than 470 μmoles sulfide. *Eohaustorius*, the amphipod genus used in amphipod toxicity tests for the Sooke Basin study has a 48-h LC₅₀ of 97 μmoles sulfide. Figure (13) includes this LC₅₀ as a benchmark. Figure (13) demonstrates that biodeposits associated with the epifaunal community resulted in sediment sulfide toxicity to at least 20 m downcurrent. The concentration of sulfides at the reference station (OC), where there were no structures, was not toxic at 36 micromoles. Similarly high sulfide concentrations were observed at the MC dolphin. These conditions were expected to significantly complicate sediment bioassays on these days.

4.5. Mussel (Mytilus edulis) Tissue PAH Levels.

During the first year of the study, mussel cages were placed on the BMP pilings and at distances of 5 and 10 m downcurrent from the dolphins for growth, survival and spawning success experiments. Samples were also taken at selected exposure intervals for whole body tissue PAH analysis. The 0.5 m cages were suspended by a metal bracket approximately 15 cm from the piling. They were not in direct contact with the piling. On Day 1540, additional samples consisting of two composites of 20 mussels growing directly on the piling were collected from the BMP and MC dolphins to determine if direct contact with the treated wood affected tissue PAH concentrations. Table (9) compares tissue PAH concentration observed on each of the sampling days at Sooke Basin. Open Control samples were obtained from caged mussels. The higher detection limits are associated with the change in analytical laboratories.

Prior to being placed in the water at Sooke Basin, mussel tissue PAH concentrations were measured at 16.15 ± 2.19 ng/g. The levels increased an average of 328 percent on Day 14 with the most significant increases observed in close proximity (within 15 cm) to the BMP and WP dolphins. Following this initial increase, PAH concentrations returned to normal by Day 185 and were lower than baseline levels by Day 384. On Day 1540, tissue concentrations in mussels attached directly to the pilings were all below the method detection limit of 20 ng/g. Only one replicate sample from the BMP site showed evidence of phenanthrene (9.0 ng/g), fluoranthene (10 ng/g) and pyrene (6 ng/g). However, all of these values were below the Method Detection Limit.

Table 9. Concentration of TPAH (ng/g wet weight) Observed in Mussels Grown in Cages (Day 0 through Day 384) With That Observed in Mussels Taken from the Surface of the Piling on Day 1540 - Sooke Basin Creosote Evaluation Study. Treatment designations correspond to the location of the mussel cages with respect to the sample stations. In other words, BMP2.0 represents caged mussels anchored 2.0 meters downcurrent from the BMP dolphin. WP = Weathered Piling; MC = Mechanical Control (untreated piling) and OC = Open Control with no structures.

Treatment	Baseline TPAH (ng/g)	Day 14 TPAH (ng/g)	Day 185 TPAH (ng/g)	Day 384 TPAH (ng/g)	Day 1540 (TPAH (ng/g)
BMP (0.5)	16.15 ± 2.19	68.07 <u>+</u> 9.14	19.73 ± 0.32	8.29 ± 0.85	<20
BMP (2.0)		47.10 ± 3.80	32.39 <u>+</u> 21.43	8.73 <u>+</u> 1.13	
BMP (10.0)		47.04 <u>+</u> 7.26	15.39 ± 0.48	15.53 ± 0.78	
WP (0.5)		58.40 ± 14.71	21.15 ± 2.46	15.16 ± 1.23	
MC0.5					<20
OC0.0		44.12 <u>+</u> 8.09	19.61 <u>+</u> 2.20	11.12 <u>+</u> 1.16	

4.6. <u>Sediment 10-Day Amphipod (Eohaustorius washingtonianus)</u> and MicrotoxTM Bioassay Results – Days 1360 and 1540.

4.6.1. Amphipod Bioassays

Static 10-day sediment bioassays using the amphipod *Eohaustorius washingtonianus* were performed on Days 1360 and 1540 in sediments from the 0.0, 0.5, 2.0, 5.0, 10 and 20 m downcurrent BMP stations and from M C0.5 and OC 0.0. *Eohaustorius* prefers to remain in the sediment and therefore was chosen over *Rhepoxynius abronius* for the single species bioassay tests during this portion of the study. Results from the amphipod tests are compared with Day 384 bioassays in Table (10). Tests were performed on sediment from the top 2 cm only. Previous data had shown that PAH concentrations decreased steadily from the surface of the sediment column to a depth of 6 - 8 cm and declined rapidly below that depth. Sediment bioassays based on the top 2 cm of the sediment column, where the highest PAH concentrations were found, were considered more representative of potential infaunal exposure than bioassays using the entire contents of the 10 cm deep grab (Goyette and Brooks, 1998).

Table 10. Amphipod (*Eohaustorius washingtonianus*) 10-day Sediment Bioassay Results Comparing Year One (Day384) to Year Four (Days 384, 1360 and 1540) - Sooke Basin Creosote Evaluation Study. Toxic samples (Lee *et al.* 1995) are shaded.

Treatment			R	eplicates	3		Mean		
		1	2	3	4	5			
Esquimalt Lagoon - Day 1360 & 1540 (Control)	% survival	90	90	95	95	100	94	4.2	
,	% at surface	0	0	0	0	0	0	0.0	
384BMP0.0	% survival	20	30	40			30	10	
	% at surface	0	0	0			0	0.0	
1360BMP0.0	% survival	0	0	0	55	0	11.0	24.6	
	% at surface	2	4	5	2	4	3.4	1.3	
1540BMP0.0	% survival	45	70	90	80	80	73	17.2	
	% at surface	0	5	0	15	20	8.0	9.1	
384BMP0.5	% survival	40	10	30	30	70	36	22	
	% at surface	30	0	0	0	0	6.0	13.4	
1360BMP0.5	% survival	40	65	30	60	55	50	14.6	
	% at surface	15	35	20	5	5	16	12.4	
1540BMP0.5	% survival	75	35	45	75	45	55	18.7	
	% at surface	25	35	15	10	20	21	9.6	
384BMP2.0	% survival	60	20	60	70	20	46	24.1	
	% at surface	50	0	0	10	0	12	21.7	
1360BMP2.0	% survival	80	60	65	70	75	70	7.9	
	% at surface	0	0	5	5	0	2.0	2.7	

Table 10 (cont'd). Amphipod (*Eohaustorius washingtonianus*) 10-day Sediment Bioassay Results Comparing Year One (Day384) to Year Four (Days 384, 1360 and 1540) - Sooke Basin Creosote Evaluation Study. Toxic values are outlined and shaded in bold. Marginal toxicity has been lightly outlined and shaded.

Treatment		Replicates					Mean	sd
		1	2	3	4	5		
1540BMP2.0								
	% survival	35	60	85	95	70	69	23.3
	% at surface	15	10	40	0	5	14	15.6
384BMP5.0								
	% survival	90	60	90	90		83	15
	% at surface	20	0	0	10		7.5	9.6
1360BMP5.0								
	% survival	80	80	100	75	65	80	12.7
	% at surface	0	5	5	20	0	6.0	8.2
1540BMP5.0								
	% survival	75	55	95	90	85	80	15.8
	% at surface	5	0	0	5	0	2.0	2.7
1360BMP10								
10000011111 10	% survival	30	0	90	85	100	92	7.6
	% at surface	0	0	10	5	0	3.0	4.5
1540BMP10	% survival	65	45	90	75	90	73	18.9
	% at surface	0	10	5	0	0	3.0	4.5
1360BMP20								
1000001111 20	% survival	90	80	75	70	95	82	10.4
	% at surface	5	5	15	5	5	7.0	4.5
1540BMP20								
10-10 Dilli 20	% survival	100	100	70	65	100	87	17.9
	% at surface	0	0	0	0	0	0	0.0
384MC0.5								
	% survival	40	50	80	70	40	56	18.2
	% at surface	20	0	0	40	20	16	16.7
1360MC0.5								
	% survival	65	15	80	50	55	53	24.1
	% at surface	15	0	15	10	15	11	6.5
1540MC0.5								
	% survival	95	80	100	90	85	90	7.9
	% at surface	0	0	5	0	0	1.0	2.2

Table 10 (cont'd). Amphipod (*Eohaustorius washingtonianus*) 10-day Sediment Bioassay Results Comparing Year One (Day384) to Year Four (Days 384, 1360 and 1540) - Sooke Basin Creosote Evaluation Study. Toxic values are outlined and shaded in bold. Marginal toxicity has been lightly outlined and shaded.

Treatment				Replicates	S		Mean	sd
		1	2	2 3	4	5		
384OC0.0								
	% survival	90	90	90	90	90	90	0.0
	% at surface	10	0	0	0	0	2.0	4.5
1360OC0.0								
	% survival	80	90	80	90	90	86	5.5
	% at surface	15	15	0	5	0	7.0	7.6
1540OC0.0								
	% survival	90	100	95	80	100	93	8.4
	% at surface	0	0	0	0	0	0	0.0

Samples on Day 384 showing a statistically significant acute toxicity response as defined by Lee et al. (1995) are shaded in Table (10). These designations were made by PESC in relation to responses measured in Esquimalt Lagoon reference sediments. Survival at the Sooke Basin reference station (OC) was also significantly less than observed in Esquimalt Lagoon reference sediments. On Day 384, amphipod survival in BMP 0.5 and MC0.5 sediments was significantly less than at the OC. This implied a negative effect at both sites. It was hypothesized that toxicity at the MC dolphin was associated with naturally occurring wood extracts while toxicity at the BMP site was likely due to sedimented PAH lost from the treated wood. No adverse effects were apparent at 2.0 and 5.0 meters downcurrent from the BMP site on Day 384.

Despite the drop in PAH concentration, amphipod survival in sediment from inside the BMP dolphin perimeter (BMP 0.0) remained low at 11% on Day 1360 (Table 10). At the same time, although still considered toxic, survival at BMP 0.5 improved slightly to 50% from the 36% observed on Day 384. Reduced survival was observed at BMP 2.0, but the decrease did not meet the criteria of Lee *et al.* (1995) to be designated toxic. Acute toxicity was not observed at any station beyond two metres. As previously discussed, the observed toxicity was likely associated with high sulfide concentrations, which exceeded the 48 h LC₅₀ for the test organism. In October 1999 (Day 1540), survival at BMP 0.0 improved considerably to 73%. The BMP 0.5 samples remained about the same at 55%. Survival in samples from the MC dolphin on Day 1360 was also poor at 53%. In both cases, toxicity was most likely associated with sediment sulfide, which was not adequately removed by the 10-day pre-aeration procedure.

It was apparent on Day 1360 that sediment sulfide concentrations at both the BMP and MC sites had increased considerably over the past three years. Samples taken in June from the BMP and MC site had sulfide concentrations ranging between 1871 and 2120 μmoles compared to 37.4 μmoles at the OC (Table 9). On Day 1540, concentrations at the BMP site increased to 7828 μmoles, which is toxic to MicrotoxTM. Steps were taken to lower the sulfide concentrations in Day 1540 samples by aerating the sediment for 10 days prior to introducing the amphipods.

The overlying water was also replaced daily during the aeration period by pouring off the contents from each jar. Although no attempt was made to manually stir the sediment at the bottom of the test jars, this procedure was sufficient to partition the sediment into two distinct layers. A layer of coarse material accumulated on the bottom with a fine layer of oxidized sediment, where *Eohaustorius washingtonianus* prefer to spend most of their time, on top (Figure 14).

While not completely removing all sulfides, this procedure did succeed in lowering the concentrations. Sulfides were measured at the end of aeration at 102.9 μ moles in BMP 0.0 sediment and 21.8 μ moles in BMP 20 sediment. End point measurements of the overlying bioassay water with a Hach Kit varied between 156 and 218 μ moles in the BMP 0.0 and BMP 0.5 tests. The sulfide concentrations at BMP 0.0 and BMP 0.5

remained higher than the LC₅₀ (97 μ moles) for *Eohaustorius*. No sulfide was detected in the samples from the BMP, MC or OC sites. Probe analysis taken at the same time gave lower results of 40.5 μ moles at BMP 0.0 and 31.2 μ moles BMP 0.5. A strong



Figure 14. Bioassay Test Jar Taken at the End of the Pre-aeration and Bioassay Period. Note the layer of fine oxidized sediment on the surface

hydrogen sulfide odor was still present in the BMP 0.0 and BMP 0.5 samples at the end of the bioassay tests but not in any of the other samples. Results are provided in Table (11) and Summary Table (16) at the end of this report.

Table 11. Sediment sulfide (μ M & mg/L), Total Volatile Solids (TVS/g) and Field Transect Total PAH (μ g/g) Concentrations on Days 1360 and 1540 and Amphipod Bioassays "End Point" PAH Concentrations on Day 1540 - Sooke Basin Creosote Evaluation Study - Year 4

Station	Exposure Period	H₂S Conc. (µM)	H₂S Conc. (mg/L)	TVS/g	Field TPAH µg/g	End Pt. TPAH μg/g
BMP0.0	Day1360	2000	68.0	0.0301		
	Day1540	2275	77.3	0.0395	6.9	4.6
BMP0.5	Day1360	1200	41.0	0.0369		
	Day1540	7394	251	0.0475	7.1	3.1
BMP2.0	Day1360	411	14.0	0.0352		
	Day1540	2435	83.0	0.0327	1.5	1.3
BMP5.0	Day1360	316	10.7	0.0270		
	Day1540	5258	179	0.0499	1.2	1.0
BMP10	Day1360	377	12.8	0.0485		
	Day1540	924	31.4	0.0385	0.6	0.4
BMP20	Day1360	203	6.9	0.0255		
	Day1540	625	21.2	0.0399	0.2	0.2

Table 11 (continued). Sediment sulfide (μM), Total Volatile Solids (TVS), Field Transect TPAH ($\mu g/g$), on Days 1360 and 1540 and Amphipod Bioassay "End Point" PAH Concentrations on Day 1540 - Sooke Basin Creosote Evaluation Study - Year 4

Station	Exposure Period	Sulfide (µM)	TVS (Proportion)	Field TPAH (µg/g)	End Pt. TPAH (μg/g)
MC0.5	Day1360	1790	0.0835		
	Day1540	1068	0.0791	0.09	0.04
OC0.0	Day1360				
	Day1540	36	0.0252		0.08
MC0.0 (spiked)	bioassay end point				17.5

Pre-aerating the bioassay samples on Day 1540 markedly improved amphipod survival. Survival in the Mechanical Control samples increased from the 53% on Day 1360 to 90% on Day 1540. This suggested that the toxicity on Day 1360 was due to sulfide. Survival in the BMP 0.0 samples also increased to 73% from the earlier 11%. No statistically significant changes in survival ($\alpha = 0.05$) were observed in the BMP 0.5 and BMP 2.0 samples, which remained between 70 and 80%. Survival in the OC samples was consistent at 86 and 93% on Days 1360 and 1540 respectively. Figure (15) compares the percent survival rates on Days 1360 and 1540 to Day 384. Sediment total sulfide concentrations on Day 1360 and Day 1540 are provided together with the 97 µmole sulfide LC₅₀ for *Eohaustorius washingtonianus*.

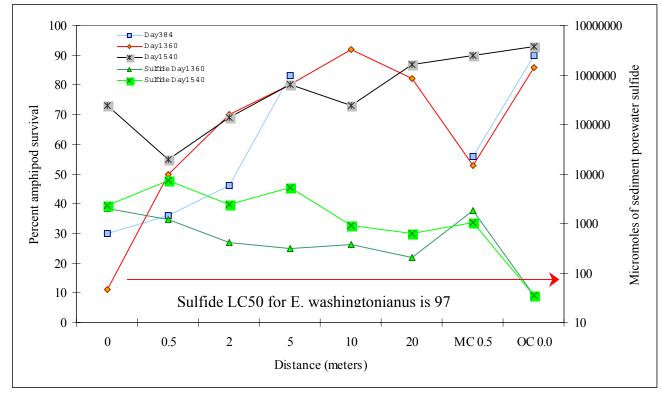


Figure 15. Comparison of Amphipod (*E. wash*) Percent Survival Between Year One (Day384) and Year Four (Days 1360 and 1540) - Sooke Basin Creosote Evaluation Study.

The fact that toxic concentrations of sulfide could still be detected in sediment from BMP 0.5 at the end of the bioassay tests suggests that the lack of improvement in amphipod survival was mainly due to the residual sulfide rather than the low concentration of PAHs present. Sediment PAH concentrations on Day 1540 were close to Long and Morgan's (1990) Effects Range Low (ER-L) of 4.02 µg TPAH/g dry sediment and were unlikely to be acutely toxic.

4.6.2. Effect of Aeration on PAH Concentrations. Aeration of the bioassay sediment could have affected sediment PAH concentrations. To test for changes, a sixth bioassay jar from each site and an additional jar containing sediment from Station MC 0.0, spiked with a Standard PAH solution equal to $15 \mu g/g$ total PAH ($1.0 \mu g$ PAH/g for each of the 15 measured PAH compounds) were run concurrently with the bioassays. The spiked sample was analyzed for PAHs at the end of the pre-aeration period and again at the end of the bioassays tests. Transect samples were analyzed for "End Point" PAH concentration at the end of the bioassay. Results are provided Table (5) and summarized in Table (13) at the end of this report. Initial PAH concentrations (prior to aeration and bioassays) can be estimated from Replicate #3, which came from the same homogenized sample as the bioassay sediments.

The expected TPAH concentration in the spiked MC 0.0 sample should have been 15 μ g/g plus the original sediment PAH concentration. The TPAH concentration after the 10-day pre-aeration period was 6.5 μ g/g, considerably below what was expected. The TPAH concentration in the same sample at the end of the bioassay was 17.5 μ g/g, close to the expected value. The initial lower result was thought to be due to the analysis of a small subsample at the end of the pre-aeration period. The sample taken at the end of the bioassay period was collected after thoroughly mixing the entire contents of the bioassay jar. Since the latter result corresponds to what would be expected, based on the 15 μ g PAH/g spike, the latter result is considered most valid. "End Point" PAH concentrations in the BMP 0.0 and BMP 0.5 transect samples were somewhat lower after the bioassay at 4.6 μ g/g and 3.1 μ g/g respectively, down from the 6.9 μ g/g and 7.1 μ g/g measured in the field samples (Table 13). Significant changes in PAH concentration were not observed in the other bioassay samples. Based on the weight of evidence, it was concluded that pre-aeration and aeration during the bioassay did not significantly affect PAH concentrations.

4.6.3. MicrotoxTM Bioassay.

Results of the liquid and solid phase sediment MicrotoxTM bioassay tests completed by PESC are presented in Table (12). MicrotoxTM tests were conducted only on samples from Day 1540. Tests were performed on raw field samples and no attempts were made to lower the sulfide concentrations through pre-aeration. Separate tests were performed on a standard sodium sulfide solution. The liquid phase 5 and 10 min. IC₅₀ results were equivalent to 21% and 18% of the sulfide concentrations observed in field samples (Table 12).

Solid phase tests were conducted on sediment from one of the seven centrifuge tubes. Liquid phase tests were first screened at 100% concentration to determine if a positive response occurred (i.e. >50% decrease in light output) at either the 5 or 15-minute exposure intervals. If the screening test was positive, then further tests were done with serial dilutions to determine the IC_{50} value at the 95% confidence interval. IC_{50} values were derived after 5 and 15-minute

exposure periods. The exposure interval for solid phase testing was 25 minutes. Toxicity was then determined by the degree of light loss. Interpretation guidelines developed by Microbics Corporation (1992a) are provided in Table (3).

On Day 384, screening tests produced a greater than 50% decrease in light production for samples taken at all Sooke Basin stations including the OC and MC sites when compared to the laboratory controls. Further tests showed Toxic responses at stations BMP 0.0, BMP 2.0, WP 0.5 and MC 0.5. Stations BMP 0.5, BMP 5.0, WP 2.0 and OC 0.0 were Moderately Toxic. However, when the results were normalized to light output at the MC station, none of the stations at either the BMP or WP sites were acutely toxic (Goyette and Brooks, 1998). The BMP 0.0 and the WP 0.5 stations were Toxic with light inhibitions of 30% and 18% respectively. It was not possible to compare treatment sites with the OC because the smallest dilution was 50% and the IC $_{50}$ values reported at the OC, BMP 5.0 and WP 2.0 sites were reported only as >50%. With the exception of the OC all liquid phase results were toxic with IC $_{50}$ values ranging from 3.6% at Station BMP 10 to 41.4% at MC 0.5. Since no attempt was made to remove sulfides prior to conducting the MicrotoxTM tests, it is likely that much of the toxicity was attributable to high sulfide content.

Solid phase IC₅₀ results from samples at Stations BMP 0.0 to BMP 5.0 and MC 0.5 were all toxic. Stations BMP 10 and BMP 20 were Moderately Toxic. Results from the Roberts Bank (Laboratory Control) and the Sooke Basin Open Control samples were essentially the same. Table (14) summarizes the Microtox liquid and solid phase results and sediment PAH concentrations from Baseline (B) through Day 1540. Toxic responses prior to Day 1360 were likely due to elevated sediment PAH concentrations since no sulfide odor was detected during that time. From Day 1360 on, toxic responses were more likely due to high sulfide concentrations.

Table 12. Acute Liquid Phase Microtox[™] Result for Year Four (Day1540) - Sooke Basin Creosote Evaluation Study - 1999. 95% confidence intervals (CI) are provided for the 5 and 15-minute tests.

	15 Min. 100%	5 Minute		1.	5 Minute
Site	Screening Test Percent light	IC50	95% C.I.	IC50	95% C.I.
1540 BMP 0.0	99.0	15.4%	13.0 - 18.1	8.7%	7.9 - 9.5
1540 BMP 0.5	99.9	7.5%	6.7 - 8.4	4.5%	4.2 - 4.6
1540 BMP 2.0	99.5	9.1%	8.6 - 9.6	5.3%	5.2 - 5.4
1540 BMP 5.0	98.8	7.3%	6.2 - 8.6	4.2%	3.7 - 4.8
1540 BMP 10	97.9	5.7%	4.7 - 6.8	3.6%	3.0 - 4.4
1540 BMP 20	92.6	9.1%	7.6 - 10.9	6.7%	5.4 - 8.4
1540 MC 0.5	70.9	85.3%	40.1 - 181.5	41.4%	36.3 - 47.3
1540 OC Sulfide	42.2	Not Acutely Toxic 21%		Not A	cutely Toxic

35

Table 13. Solid Phase MicrotoxTM Test Results for Year Four (Day1540) - Sooke Basin Creosote Evaluation Study - 1999. None of the results were Toxic. Marginally toxic results (Microbics Corporation, 1992a) are shaded

	5 N	linute	15 N	Minute
Day and Station	IC50 (wet)	95% C.I.	IC50 (dry)	95% C.I.
1540 BMP 0.0	0.19%	0.15 - 0.25	0.13%	0.10 - 0.16
1540 BMP 0.5	0.20%	0.19 - 0.21	0.13%	0.12 - 0.13
1540 BMP 2.0	0.16%	0.16 - 0.17	0.12%	0.11 - 0.12
1540 BMP 5.0	0.17%	0.16 - 0.19	0.12%	0.10 - 0.13
1540 BMP 10	0.45%	0.41 - 0.49	0.31%	0.28 - 0.34
1540 BMP 20	0.43%	0.37 - 0.50	0.29%	0.25 - 0.34
1540 MC 0.5	0.23%	0.18 - 0.28	0.14%	0.12 - 0.18
1540 OC 0.0	1.3%	1.1 – 1.5	0.9%	0.79 - 1.1
Roberts Bank	0.86%	0.80 - 0.93	0.85%	0.79 - 0.92

4.7. Sediment Characteristics.

In addition to the elevated sulfide concentrations, other sediment characteristics also changed following piling installation. By Day 1360, a significant amount of shell debris had built up at the base of the BMP and MC dolphins, altering the particle size distribution. The amount of shell (gravel) decreased exponentially out to 10 m downcurrent from the BMP pilings (Table 15). Total volatile solids (TVS) were also elevated at both sites except for the 5.0 and 20 m BMP stations (Table 13). Assuming that TOC is about 60% of TVS (Brooks, unpublished 1997), TOC concentrations ranged from ca. 1.8% to 5.0% at the BMP site. These values are two to five times those measured at the beginning of these studies.

4.8. Observations and Underwater Photography

4.8.1. Creosote Sheens

As pointed out earlier and described in detail by Goyette and Brooks (1998), contamination of the bottom sediments appeared to be related to the presence of minute creosote droplets coming from the pilings. Preliminary laboratory studies indicated that creosote microdroplets tend to remain intact while they work their way downward into ground shell or quartz sediments. They resisted attempts to disperse them by stirring. The small sheens observed in Figure (16) occurred when Sooke Basin sediment samples were exposed to the air. It also appeared that these droplets had an affinity for rocks and shells buried in the sediments. Small hydrocarbon sheens were observed in samples collected on Day 1360 and Day 1540 and in the catchment's containers. However, the number of sheens was qualitatively fewer than observed in previous surveys.

Table 14. Sooke Basin Creosote Evaluation Study: Comparison of Liquid and Solid Phase (LC_{50}) Sediment MicrotoxTM Tests - Baseline to Day1540.

Surface Sediment PAH Concentration	Station/Exposure Period	Liquid Phase 100% Screen (%)	Liquid Phase (IC ₅₀ at 95% CI) (%)	Liquid Phase (IC ₅₀ at 95% CI) (%)	Solid Phase
TPAH (μg/g)	Station	15 min.	5 min.	15 min.	LC ₅₀
Open Control		Exposure	Exposure	Exposure	(%)
-	D 10000				0 =0 (0 0= 0 0=)
0.13	B ¹ OC0.0	No decrease			0.79 (0.65-0.97)
0.18	14OC0.0	No decrease			0.94 (0.80-1.1)
0.18	180OC0.0	No decrease			1.5 (1.4-1.8)
	270OC0.0	8.6	. 50	. 50	3.1 (1.5-6.5)
0.73	384OC0.0	>50	>50	>50	1.8 (1.5-2.0)
 Mechanical Control	1540OC0.0	42.2	Nt	nt	0.93
0.11	BMC0.5	No decrease			0.80 (0.75-0.85)
0.11	14MC0.5	No decrease			0.57 (0.47-0.70)
0.13	180MC0.5	20	not performed	n/p	1.0 (0.94-1.1)
0.13	TOUNICU.S	20	(n/p)	11/10	1.0 (0.34-1.1)
0.2	384MC0.5	>50	43.0 (27.4 - 67.4)	35.7 (24.4 - 52.2)	1.2 (1.0-1.4)
	535MC0.5	99.6	16.4 (12.6 - 21.3)	12.8 (9.3 - 17.5)	0.53 (0.49-0.59)
0.09	1540MC0.5	70.9	85.3 (40.1 - 181.5)	41.2 (36.3 - 47.3)	0.14 (0.12 - 0.18)
BMP Pilings					
29.9	384BMP0.0	>50	27.8 (23.9 - 32.3)	25.1 (21.6 - 29.0)	0.72 (0.7-0.74)
	535BMP0.0	82.0	34.6 (29.1 - 41.1)	38.9 (30.1 - 50.4)	0.54 (0.50-0.59)
6.9	1540BMP0.0	99.0	15.4 (13.0 - 18.1)	8.7 (7.9 - 9.5)	0.13 (0.1 - 0.16)
0.17	BBMP0.5	No decrease			0.49 (0.45-0.52)
7.8	14BMP0.5	No decrease			0.79 (0.69-0.90)
8.8	180BMP0.5	No decrease			1.6 (0.96-2.7)
54.4	270BMP0.5	n/p	n/p	n/p	4.4 (1.6-10)
18.9	384BMP0.5	>50	>50	67.2 (41.0-110.4)	1.0 (0.92-1.2)
	535BMP0.5	91.4	24.7 (21.9 - 27.9)	23.2 (20.2 - 26.7)	0.34 (0.31-0.38)
7.1	1540BMP0.5	99.9	7.5 (6.7 - 8.4)	4.5 (4.3 - 4.7)	0.13 (0.12 – 0.13)
0.18	14BMP2.0	No decrease			0.49 (0.39-0.63)
3.1	180BMP2.0	No decrease			1.0 (0.99-0.74-1.3)
8.2	384BMP2.0	>50	>50	47.2 (26.1 - 85.4)	0.77 (0.7-0.82)
	535BMP2.0	3.0	n/p	n/p	0.40 (0.37-0.43)
1.5	1540BMP2.0	99.5	9.1 (8.6 - 9.6)	5.3 (5.2 - 5.4)	0.12 (0.11 – 0.12)
0.49	14BMP5.0	No decrease			0.53 (0.52-0.55)
0.81	180BMP5.0	No decrease			0.79 (0.68-0.92)
2.9	384BMP5.0	>50	>50	>50	0.98 (0.97-0.99)
	535BMP5.0	78.8	>50	>50	0.83 (0.82-0.84)
1.2	1540BMP5.0	98.8	7.3 (6.2 - 8.7)	4.2 (3.7 - 4.8)	0.12 (0.10 – 0.13)
0.29	14BMP10	No decrease			0.75 (0.70-0.81)
0.62	180BMP10	n/p			
0.6	1540BMP10	97.6	5.7 (4.7 - 6.8)	3.6 (3.0 - 4.4)	0.31 (0.28 – 0.34)
0.2 The "B" preceding the tro	1540BMP20 eatment identifier signif	92.6 ies samples collecte	9.2 (7.7 - 10.9)	6.7 (5.4 - 8.4) survey – before the do	0.29 (0.25 – 0.34) Olphins were constructed.

Table 15. Table Showing Sediment Particle Size Distribution and Total Volatile Solids Content - Day 1360 and Day 1540 - Sooke Basin Creosote Evaluation Study.

		Particle	Size		
Site	Gravel	Total Sand	Silt	Clay	Total Volatile Solids
	%	%	%	%	TVS/g
1360BMP0.0	24.2	54.5	13.0	8.4	0.030
1540BMP0.0					0.040
1360BMP0.5	21.2	52.5	17.1	9.2	0.037
1540BMP0.5					0.048
1360BMP2.0	8.0	61.8	19.6	10.6	0.035
1540BMP2.0					0.033
1360BMP5.0					0.027
1540BMP5.0					0.050
1360BMP10	3.6	58.9	26.1	11.3	0.048
1540BMP10					0.039
1360BMP20	0.45	73.9	16.6	9.1	0.026
1540BMP20					0.040
1360MC0.5	35.4	35.2	18.3	11.1	0.084
1540MC0.5					0.079
1360OC0.0					
1540OC0.0					0.025

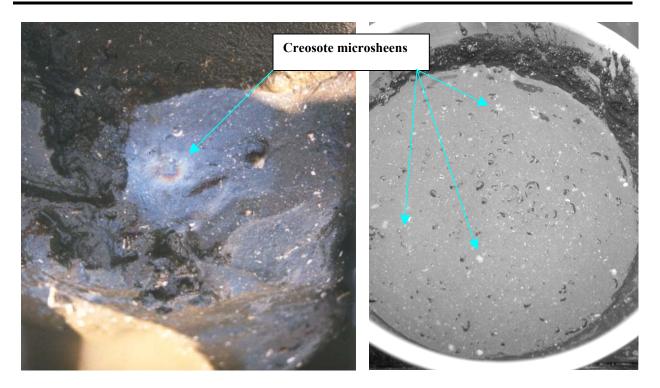


Figure 16. Creosote Sheens Observed on Benthic Sediment Samples From the BMP Site - Sooke Basin Creosote Evaluation Study - June 1996.

4.8.2. BMP Pilings.

The appearance of freshly treated BMP pilings is very similar to what could best be described as a blackboard, smooth and black in color, with no visible surface residue. One could touch the wood without getting creosote on the hands. Over time, the exposed portion of the BMP piling down to the mussel growth line began to appear like "conventionally" treated wood. A tar like residue developed on the surfaces of the wood located above water and exposed during low tides. Plates (1) and (2) were taken on Day 1540 showing the above water and intertidal sections of the BMP pilings. Plates (3) and (4) are close-up views of the creosote residue on the piling surface. This thick tarry residue had developed on the upper surface of the piling and in some cases, heat from the sun had caused the creosote to exude from the wood and trickle down the piling, forming a drop at the end as it cooled. This would explain the small sheens that periodically appeared on the surface of the water around the treated dolphins and further supports the Particulate PAH Transport Hypothesis put forth by Goyette and Brooks (1998).

4.8.3. Invertebrate Community Growing on Treated and Untreated Piling.

All of the pilings on Day 14 were relatively 'clean' with little or no evidence of new marine growth. By Day 180, a light film of brown filamentous algae had developed on the BMP piling. This film became progressively thicker near the water's surface. A marine community had started to establish itself on the pilings by Day 384. The amount of growth appeared to be slightly greater on the MC pilings than at the BMP site, particularly with respect to the number of mussels. By Day 1540, a diverse and abundant biological community covered the entire length of the BMP pilings and any object left in place during piling installation, such as transect lines, marker pins, and mussel cage brackets. Both the BMP and MC dolphins were covered with an abundance of mussels, barnacles, numerous starfish (up to 15-20 individuals in any given section), plumose sea anemones, calcareous tube worms, hermit crabs, coonstripe shrimp, tunicates, marine snails, sea cucumbers, sponges, filamentous algae and other marine organisms. Large plumose anemones were attached to the inside of the catchment containers, which had been installed only four months previously. Whether they had grown there from juveniles or somehow found another way into the containers as adults is unknown.

A number of organisms normally seen on the mud bottom had also move up onto the pilings (e.g. large sea cucumbers - *Parastichopus*, juvenile Dungeness crabs, starfish, sculpins, coonstripe shrimp, etc.) An example of the marine growth at the lower end of the mussel zone on the BMP pilings is shown in Plate (5). Plate (6) shows numbers of anemones (a), starfish (b) and shrimp (c). Plate (7) depicts examples of various fish species (perch and needlefish), which had taken up residence since the dolphins were installed. Colonies of sea anemones had also established themselves on the sampling transect lines and marker pins left in place since piling installation, four years previously (Plate 8). Literally hundreds of juvenile Dungeness crabs migrated to the base of the *BMP* dolphins during diving operations on Day 1540 to feed on mussels and other debris, which had been stirred up by the divers (Plate 9). Much of the marine growth seen on Day 1540 had established itself since the last survey in Year One (Day 384). The amount of growth on the pilings was thick enough to severely restrict creosote migration out from the piling surface.

Contrary to previous observations, the amount of marine growth on the BMP pilings was noticeably greater than the MC pilings. The reason for this appeared to be associated with the marine borer attack taking place on the untreated wood. The wooden foundation on which many of the marine organisms were attached was constantly being undermined by *Limnoria* and *Bankia*.

4.8.4. Marine Borer Attack (Limnoria; Bankia).

The impact of borer activity on untreated wood left in the marine environment was clearly visible by Year Four, indicating the need for some form of wood preservation. The surface of the MC pilings was riddled with small burrows from the isopod *Limnoria*, commonly known as 'gribbles' (Plate 10). These organisms live close to the surface of the wood, going progressively deeper as the wood breaks off. It was estimated that as much as 2.0 to 4.0 cm of wood had been lost from the piling since first installed, most of which occurred during the last three years. The untreated pilings had also been attacked by Teredos and numerous ~2.0 cm diameter tunnels were exposed by *Limnoria* (Plates 11 a & b). These are not normally visible on the surface of the wood. While the piling still supporting a reasonable epifaunal community, the *Limnoria* left large patches of bare unstable wood, which was constantly being eroded away, taking the attached organisms with it. Mussels on the MC dolphin were noticeably smaller and younger than those on the BMP pilings. This was likely because larger mussels were constantly falling off with the unstable wood. New mussel recruits recolonized the exposed wood surface to begin the cycle anew. Overall, the amount of growth on the MC dolphin was noticeably less than on the BMP dolphin. The impact from this biological fallout could be seen at the base of the MC piling where a substantial build up of shell, wood and other debris had occurred. Consequently, sediments around the base of the MC dolphin had turned anoxic, producing toxic concentrations of sulfide. The rate of deposition was much greater at the untreated MC site than the BMP site, largely due to the marine borer attack. Deposition at the BMP site largely resulted from the numerous starfish, which were feeding on the mussels and barnacles attached to the piling.

4.8.5. Catchment Containers

Plate (12) shows the canisters on the MC dolphin shortly after installation in June 1999. Plates (11) to (15) depict the canisters and accumulated debris four months after installation. The uncovered eight inch deep container shown in Plate (15) was completely filled with shell debris and decaying biological matter. Assuming a constant rate of deposition throughout the year, these deposits represented $\sim 100 \text{ kg/m}^2$ -y at the MC dolphin and 45 kg/m²-y at the BMP dolphin.

5.0 Summary and Conclusions.

The results of this four year study to evaluate the physicochemical and biological response to freshly installed creosote treated pilings installed in a worst case environment are summarized below:

- ♦ Water column concentrations of PAH remained close to background concentrations throughout the study. Biologically insignificant increases in mussel tissue concentrations of PAH were observed during the first two weeks of the study. By Day 185, mussel tissue concentrations declined to those observed at the reference station. Mussels growing directly on the heavily fouled BMP treated piling did not contain elevated tissue concentrations of PAH at the end of the study.
- ♦ After the 1996 studies, Goyette and Brooks (1998) concluded that maximum predicted and observed total sediment PAH concentrations were significantly elevated (5.5 μg/g and 4.8 μg/g, respectively) to a distance of 7.5m downstream from the BMP treated dolphin, but not at 10 m and beyond. Observed PAH concentrations declined sharply between 7.5 and 10 m, averaging 0.53 μg/g (n=13), well below the Threshold Effects Level (TEL) of 0.75 μg/g dry weight. Statistically insignificant decreases in the abundance of PAH sensitive species (see Goyette and Brooks, 1998 for their identification) were found at distances less than 0.65 m from the perimeter of the creosote treated structures. Slight adverse effects were observed at 2.0 m downcurrent in laboratory sediment bioassays but not in the infaunal community. No significant effects were observed on mussel growth, survival, or spawning success. Sediment concentrations of PAH at the BMP dolphin peaked sometime between Day 384 and Day 1360 and then declined.
- ♦ Multi-tiered toxicity testing based on 10-day amphipod bioassays, liquid and solid phase MicrotoxTM tests and echinoderm sperm tests demonstrated toxicity at distances ≤ 0.5 metres from the BMP dolphin at 384 days post construction. Equivocal evidence of toxicity was observed in bioassays downstream to a distance of 2.0 metres but not beyond. The PAH compounds, fluoranthene and phenanthrene appeared to be the major contributors to sediment toxicity. It was postulated that the primary mode of contamination occurred as microliter size particles of creosote present in sediments to depths of 6 cm and at the air water interface. It was hypothesized that these microparticles were generated during hot summer weather.
- Continuing the studies into 1999 revealed that by the fourth year, sediment PAH at the BMP treatment site had declined considerably to about one-third of what it was on Day 384. Surface sediment concentrations inside the footprint of the BMP dolphin on Day 1540 averaged 6.9 μ g/g compared to 29.9 μ g/g observed on Day 384. Significant increases in sediment PAH had receded to a distance of 0.5m downstream from the BMP treated structure. Slightly elevated PAH levels (1.5 μ g/g) were observed to a distance of 2.0 m downstream. Results from Day 1360 and Day 1540 compared to all previous results are summarized in Table (16).
- ♦ Adverse effects on the benthic environment associated with creosote derived PAH were not apparent on Day 1360 or Day 1540 because PAH concentrations had significantly declined and sediment TOC concentrations had significantly increased reducing the bioavailability of PAH. By Days 1360 and 1540, adverse effects were associated with high sediment sulfide

concentrations at both the treated and untreated dolphins created by biodeposits from the invertebrate community that had taken up residence on all of the structures and by the deteriorating untreated piling at the MC site. These biodeposits were greater under the untreated dolphin – presumably because the wood was being eroded by *Limnoria sp*. The failing woody debris carried the sessile invertebrates with it to the benthos. Assuming the measured deposition rates were constant throughout the year, deposition at the untreated MC dolphin was equivalent to 100 kg/m^2 -y. This was more than twice the 45 kg/m^2 -y rate observed at the BMP dolphin.

- ♦ Amphipod survival in sediments, initially containing >2000 μmoles of total sulfide, markedly improved when bioassay samples were pre-aerated to reduce or remove the sulfide. Survival at the MC site increased from 53% in un-aerated samples taken on Day 1360 to 90% in pre-aerated sediments collected on Day 1540. Survival in samples from the BMP site improved only slightly with pre-aeration and still remained toxic with unacceptably high concentrations of sediment sulfide which exceeded the LC₅₀ for the test amphipod − even after aeration for 10 days. Similarly treated samples spiked with a Standard PAH solution suggested that aeration did not decrease the PAH concentrations.
- ♦ The decline in sediment PAH concentration downcurrent from the BMP structure is thought to have been due to decreased movement of PAH through the epifaunal biomass and to enhanced microbial cometabolism in the complex organic mixture of biodeposits and PAH. Biodeposits from the epifaunal community may have also diluted sediment PAH concentrations.
- ♦ The presence of the piling structures, both treated and untreated, led to the development of an abundant and diverse marine community comprised of various species of fish, invertebrates and algae one that was not present prior to construction. The benthic infaunal community structure, while not enumerated on the last two sampling days, was expected to develop into one with a species composition tolerant of low oxygen levels and elevated sulfide concentrations.
- The risk assessment model of Brooks (1994) has proven conservative in that it has consistently predicted higher environmental concentrations of PAH than have been observed in this study. Sediment concentrations of PAH appeared to peak earlier than predicted by the model – likely because of reduced migration of PAH through the dense community of fouling organisms, burial by debris from the dolphins, and partly due to the enhanced microbial cometabolism of PAH in the organically enriched sediments. It is also possible that the tarry residue that formed on the air-exposed surface of the piling may have functioned to partially anneal the surface, somewhat restricting further migration of creosote from the interior of the piling. Sediment concentrations of PAH had decreased to concentrations less than those associated with toxicity by the end of the study. It appeared that the longest lasting effect of these structures was the establishment of a luxurious epifaunal community that was not previously present. Biodeposits from this community created anaerobic conditions in nearfield sediments, resulting in toxic concentrations of sulfide. These anaerobic conditions were apparent at both the MC site constructed of untreated Douglas fir piling and at the creosote treated BMP site. Rapid deterioration of the untreated piling resulted in a reduced fouling community because the attached organisms were constantly sloughing off and falling to the bottom. It appeared that these effects would remain until the untreated piling completely deteriorates, or until the creosote treated piling are removed.

Table 16. Summary Table Showing Results From Day 1360 and Day 1540 Compared to Year One (Days 14, 180 and 384) - Sooke Basin Creosote Evaluation Study - 1999.

Station/ Transect Exposure PAH Period	PAH End Point PAH		Sediment Bioassay End Point H ₂ S Conc.	Water End Point Sulfide (Hach Kit)	Water End Point Sulfide (Probe)	Bioassay End Point Sulfide Odour	E. wash.	E. wash.	Microtox Liquid Phase	Microtox Liquid Phase	
	μg/g	μg/g	mg/L	mg/L	mg/L	mg/L	Odour	% survival	% at surface	%	15min IC ₅₀ dry wt.
BMP0.0 14											
384	29.9							30 ± 10	0 ± 0.0	25.1	0.72
1360	6.9 ± 1.8		68.0					11 ± 24.6	3.4 ± 1.3		
1540	6.9 ± 2.1	4.6	77.3	3.3	5.0	1.3	strong	73 ± 17.2	8.0 ± 9.1	8.7	0.13
BMP0.5 14	7.8 ± 4.4										
180	8.8 ± 1.6										
384	18.3 ± 9.8										
1360	5.3 ± 2.0		41					50 ± 14.6	16 ± 12.4		
1540	7.1 ± 3.2	3.1	251	2.6	7.0	1.0	strong	55 ± 18.7	21 ± 9.6	4.5	0.13
BMP2.0 14	4.5										
180	3.1										
384	7.9							46 ± 24	12 ± 21.7	47.2	0.77
1360	2.8 ± 0.4		14					70 ± 7.9	2.0 ± 2.7		
1540	1.5 ± 0.1	1.3	83	1.5	0	< 0.05	slight	69 ± 23.3	14 ± 15.6	5.3	0.12
BMP5.0 14	2.9 ± 0.8										
180	0.8 ± 0.4										
384	2.8 ± 0.8							83 ± 15	7.5 ± 9.6	<50	0.98
1360	0.5 ± 0.3		10.7					80 ± 12.7	6.0 ± 8.2		
1540	1.2 ± 0.1	1.0	179	1.4	0	< 0.05	slight	80 ± 15.8	2.0 ± 2.7	4.2	0.12

Table 16 (continued). Summary Table Showing Results From Day1360 and Day 1540 Compared to Year One (Days 14, 180 and 384) - Sooke Basin Creosote Evaluation Study - 1999.

Station/ Exposure Period	Transect PAH	Bioassay End Point PAH	Sediment Field H ₂ S Conc.	Sediment Bioassay End Point H ₂ S Conc.	Water End Point Sulfide (Hach Kit)	Water End Point Sulfide (Probe)	Bioassay End Point Sulfide Odour	E. wash.	E. wash.	Microtox Liquid Phase	Microtox Liquid Phase
	μg/g	μg/g	mg/L	mg/L	mg/L	mg/L		% survival	% at surface	%	15min IC ₅₀ dry wt.
BMP10 14	0.6 ± 0.1										
180	0.6 ± 0.3										
384	0.6 ± 0.1										
1360	1.4 ± 0.6		12.8					92 ± 7.6	3.0 ± 4.5		
1540	0.6 ± 0.2	0.4	31.4	1.1	0	1.1	none	73 ± 18.9	3.0 ± 4.5	3.6	0.31
BMP20 14	0.18										
180	0.22										
384	0.5										
1360	0.5 ± 0.2		6.9								
1540	0.2 ± 0.0	0.2	21.2	0.7	0	< 0.05	none	87 ± 17.9	0.0 ± 0.0	6.7	0.29
MC0.5 14	0.2 ± 0.05										
180	0.1										
384	0.16							56 ± 18	16 ± 16.7	35.7	1.2
1360	0.13 ± 0.02		60.9					53 ± 24.1	11 ± 6.5		
1540	0.09 ± 0.0	0.04	36.3	0.7	0	< 0.05	none	90 ± 7.9	1.0 ± 2.2	41.4	0.14
OC0.0 14	0.18 ± 0.02										
180	0.18 ± 0.04										
384	0.2 ± 0.05							90 ± 0.0	2.0 ± 4.5	>50	1.8
1360								86 ± 5.5	7.0 ± 7.6		
1540		0.08	1.2	0.8	0	0.2	none	93 ± 8.4	0 ± 0.0	non-toxic	0.93
MC0.0 (#1)											
Post Aeration	6.5										
Post Bioassay	17.5										

References

- Beckman, Inc. 1982. *Microtox System Operating Manual*. Beckman Instruments, Microbics Operations, Carlsbad, CA.
- Bestari, K.T.J., R.D. Robinson, K.R. Solomon, T.S. Steel, K.E. Day and P.K. Sibley. 1998a. Distribution and Composition of Polycyclic Aromatic Hydrocarbons within Experimental Microcosms Treated with Creosote-Impregnated Douglas Fir Pilings. Environmental Toxicology and Chemistry 17(12) 2369 2377.
- Bestari, K.T., R.D. Robinson, K.R. Solomon, T.S. Steele, K.E. Day and P.K. Sibley. 1998b. Distribution and Composition of Polycyclic Aromatic Hydrocarbons Within Experimental Microcosms Treated with Liquid Creosote. Environmental Toxicology and Chemistry 17(12) 2359 2368.
- Bogan, B.W. and R.T. Lamar. 1995. One-Electron Oxidation in the Degradation of Creosote Polycyclic Aromatic Hydrocarbons by *Phanerochaete chrysosporium*. Applied and Environmental Microbiology. Vol. 61(7). Pp. 2631-2635.
- Borthwick, P.W. and J.M. Patrick. 1982. Use of Aquatic Toxicology and Quantitative Chemistry to Estimate Environmental Deactivation of Marine-Grade Creosote in Seawater. Environmental Toxicology and Chemistry. Vol. 1, pp. 281-288.
- Boldrin, B., A. Tiehm and C. Fritasche. 1993. Degradation of phenanthrene, fluorene, fluoranthene and pyrene by a *Mycobacterium sp.* Applied Environmental Microbiology Vol. 59. pp. 1927-1930.
- Brooks, K.M. 1994. Literature Review, Computer Model and Assessment of the Potential Environmental Risks Associated with Creosote Treated Wood Products Used in Aquatic Environments. Prepared for the Western Wood Preservers Institute, 601 Main Street, Suite 401, Vancouver, WA 98660. 65 pages, plus Appendices.
- Brooks, K.M. Unpublished 1997. Recommendations to the British Columbia Ministry of Environment regarding establishment of surrogate physicochemical standards in lieu of infaunal assessments at salmon farms.
- Cerniglia, C.E. 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Advances in Applied Microbiology Vol. 30. pp. 31-71.
- Cerniglia, C.E. and M.A. Heitkamp. 1991. Chapter 2, Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAH) in the Aquatic Environment.
- CITW and WWPI. 1997. Best Management Practices for the Use of Treated Wood in Aquatic Environments. Canadian Institute of Treated Wood and Western Wood Preservers Institute. Canadian version. January 1997. 33pp.

- Environment Canada. 1992a. Biological Test Method: Acute test for sediment toxicity using marine or estuarine amphipods. Environment Canada, Conservation and Protection. EPS 1/RM/26.
- Environment Canada. 1992b. Biological Test Method: Toxicity testing using luminescent bacteria. Environment Canada, Conservation and Protection. EPS 1/RM/24.
- Environment Canada. 1995. Standard Operating Procedure. Mutatox Genotoxicity Testing Using Luminescent Bacteria (*Vibrio fischeri*, Strain M169). Aquatic Toxicology Section, Pacific Environmental Science Centre.
- Environment Canada. 1995. Canadian Interim Sediment Quality Guidelines. Prepared by Guidelines Division, Evaluation and Interpretation Branch, Ecosystem Conservation Directorate, Environment Canada, Ottawa, Ont.
- EPA. 1980. Ambient water quality criteria for polynuclear aromatic hydrocarbons. U.S. Environ. Protection Agency. Rep. 440/5-80-069. 193 pp.
- Goyette, D. and K. Brooks. 1998. Creosote Evaluation Study Phase II: Sooke Basin Study Baseline to 535 Days Post Construction. 1995 1996. Prepared for the Creosote Evaluation Steering Committee. Environment Canada Regional Program Report PR98-04. Available in CD-ROM.
- Grifoll, M., S.A. Selifonov and P.J. Chapman. 1994. Evidence for a Novel Pathway in the Degradation of Fluorene by *Pseudomonas* sp. Strain F274. Applied and Environmental Microbiology. Vol. 60(7). Pp. 2438-2449.
- Gulley, D.D., A.M. Boelter and H.L. Bergman. 1989. TOXSTAT Release 3.2. University of Wyoming.
- Herbes, S.E. and L.R. Schwall. 1978. Microbial transformation of polycyclic aromatic hydrocarbons in pristine and petroleum contaminated sediments. Applied Environmental Microbiology. Vol. 35, pp. 306-316.
- Ingram, L.L., G.D. McGinnis, L.R. Gjovik and G. Roberson. 1982. Migration of Creosote and its Components from Treated Piling Sections in a Marine Environment. Journal of the American Wood-Preservers' Association. Pp. 1-8.
- Johnson, L., M. Myers, D. Goyette and R. Addison. 1994. Toxic Chemicals and Fish Health in Puget Sound and the Strait of Georgia. Proceedings of the BC/Washington Symposium on the Marine Environment, January 13 & 14, 1994. Edited by R.C.H. Wilson, R.J. Beamish, Fran Aitkens and J. Bell. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 1948.

- Kanaly, R.A. and R. Bartha. 1999. Cometabolic mineralization of benzo(a)pyrene caused by hydrocarbon additions to soil. Environmental Toxicology and Chemistry, Vol. 18(10). Pp. 2186-2190.
- Lee, D.L., S.G. Yee, M. Fennell and D.L. Sullivan. 1995. Biological Assessment of Three Ocean Disposal Sites in Southern British Columbia. Environment Canada, Pacific and Yukon Region. Regional Report: 95-07.
- Long, E.R., and L.G. Morgan. 1990. The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Rockville, MD
- Microbics Corporation. 1992a. Microtox™ Manual. A Toxicity Testing Handbook. Carlsbad, CA. Volume 1, Getting Started; Volume 2, Detailed Protocols; Volume 4, Data Quality and Applying Results.
- Microbics Corporation. 1992b. Microtox™ Update Manual. Carlsbad, CA.
- Millette, D., J.F. Barker, Y. Comeau, B.J. Butler, E.O. Frind, B. Clement and R. Samson. 1995. Substrate interaction during aerobic biodegradation of creosote-related compounds: A factorial batch experiment. Environmental Science and Technology. Vol. 29(8). Pp. 1944-1952.
- Mueller, J.G., P.J. Chapman and P. H. Pritchard. 1989. Creosote-contaminated sites Their potential for bioremediation. Environmental Science and Technology Vol. 23(10). Pp. 1197-1201.
- Mueller, J.G., D.P. Middaugh, S.E. Lantz and P.J. Chapman. 1991. Biodegradation of creosote and pentachlorophenol in contaminated groundwater: Chemical and biological assessment. Applied Environmental Microbiology. Vol. 57(5). Pp. 1277-1285.
- Plumb, R. H. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers, Vicksburg, MS.
- PSEP. 1986. Puget Sound protocols. Prepared for U.S. Environmental Protection Agency, Region 10, Seattle, WA.
- Quayle, D.B. 1992. Marine Borers in British Columbia. Canadian Special Publication of Fisheries and Aquatic Sciences 115. 55pp.

- Sayler, G.S. and T.W. Sherrill. 1981. Bacterial degradation of coal conversion by-products (polycyclic aromatic hydrocarbons) in aquatic environments. Final Report, Office of Water Research and Technology, U.S. Department of Interior matching grant program project B-040 TENN. Department of Microbiology and the Graduate Program in Ecology, University of Tennessee, Knoxville, Tennessee 37996. 80 pp.
- Schiewe, M.H., E.G. Hawk, D.I. Actor and M.M. Krahn. 1985. Use of bacterial bioluminescence assay to assess toxicity of contaminated marine sediments. Canadian Journal of Fisheries and Aquatic Sciences. Vol. 42, pp. 1244-1248.
- Stephan, E.E. 1977. Methods for calculating an LC₅₀. <u>In</u> F.L. Mayer and J.L. Hamelink (eds). Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials. Philadelphia, PA. ASTM STP 634.
- Stringfellow, W.T. and M.D. Aitken. 1994. Comparative physiology of phenanthrene degradation by two dissimilar pseudomonads isolated from a creosote-contaminated soil. Canadian Journal of Microbiology. Vol. 50(6). Pp. 432-438.
- Swartz, R.C. 1999. Consensus Sediment Quality Guidelines for Polycyclic Aromatic Hydrocarbon Mixtures. Environmental Toxicology and Chemistry, Vol. 18, No. 4. Pp. 780 787.
- Tagatz, M.E., G.R. :laia, C.H. Deans and E.M. Lores. 1983. Toxicity of creosote-contaminated sediment to field and laboratory colonized estuarine benthic communities. Environmental Toxicology and Chemistry. Vol. (2), pp. 441-450.
- U.S. Environmental Protection Agency (US EPA). 1994. Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods. Office of Research and Development. US EPA, Narragansett, Rhode Island. EPA 600/R-94/025.
- Wang, F. and P.M. Chapman. 1999. Biological implications of sulfide in sediment A review focusing on sediment toxicity. Environmental Toxicology and Chemistry. Vol. 18, No. 11, pp. 2526-2632.
- Zar. J.H. 1984. Biostatistical Analysis. 2nd edition. Prentice Hall: Englewood, N.J.

ADDENDUM REPORT

Continuation of the Sooke Basin Creosote Evaluation Study (Goyette and Brooks, 1998)

Year Four - Day 1360 and Day 1540

Part II. Plates

Prepared by:

D. Goyette Commercial Chemicals Division Environment Canada Pacific and Yukon Region

and

K.M. Brooks Aquatic Environmental Sciences Port Townsend, WA 98368

Prepared for:

Creosote Evaluation Steering Committee

Regional Program Report PR00-03

May 2001



<u>Plate 1. Photograph of BMP Piling Dolphin</u> In October 1999 (Year Four)

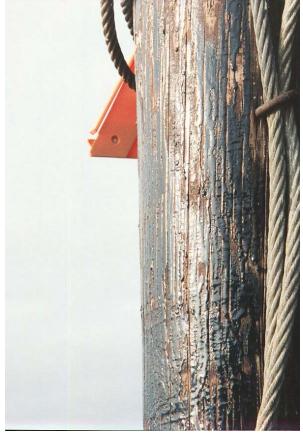


Plate 2. Creosote Residue on the surface of the BMP piling in October 1999 (Year Four). Note where exposure to solar heating has Brought creosote to the surface of the piling.



Plate 3. Close up view of the BMP piling surface taken at +10' Chart Datum showing the effects of solar heating four years after construction – October 1999.



Plate 4. Magnified view of the BMP piling surface taken at +8' Chart Datum four years after construction – October 1999



Plate 5. Marine growth on the BMP piling near the lowr end of the mussel (*Mytilus edulis trossulus*) zone (-14' Chart Datum) in October 1999, four years following construction.

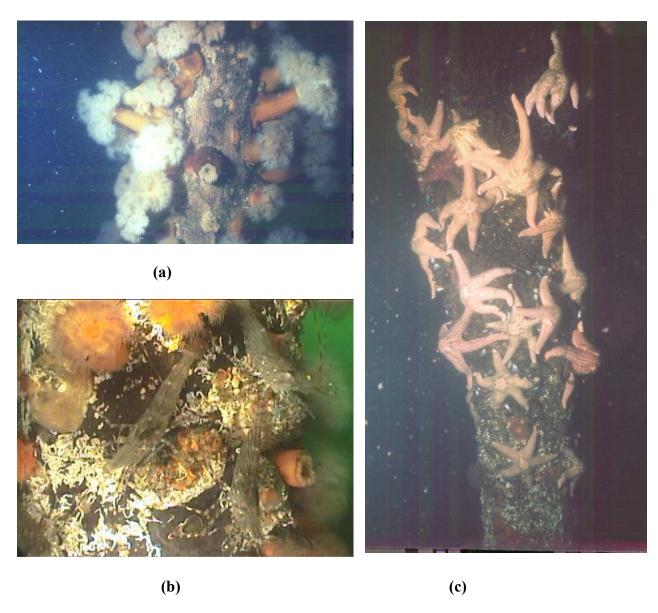


Plate 6. Examples of the epifaunal community resident on (a) the untreated Mechanical Control; (b) the BMP piling at mid depth; and (c) the BMP piling near the mud line.



Plate 7. Fish (*Cytomaster aggregata*) attracted to the untreated Mechanical Control dolphin in October 1999.

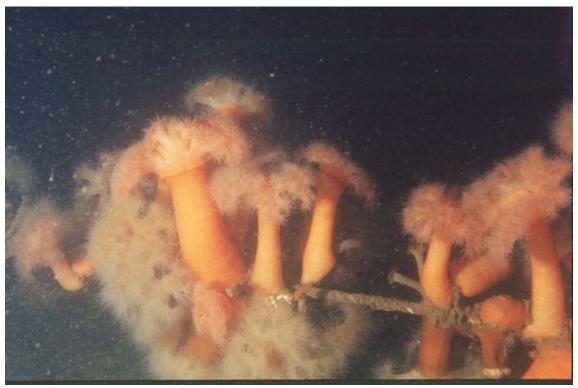


Plate 8. Plumose anemones (*Metridium senile*) growing on a transect line at the BMP piling dolphin in October 1995.





Plate 9. Juvenile Dungeness crab (*Cancer magister*) foraging around the BMP dolphin on Day 1540 (October 1999).

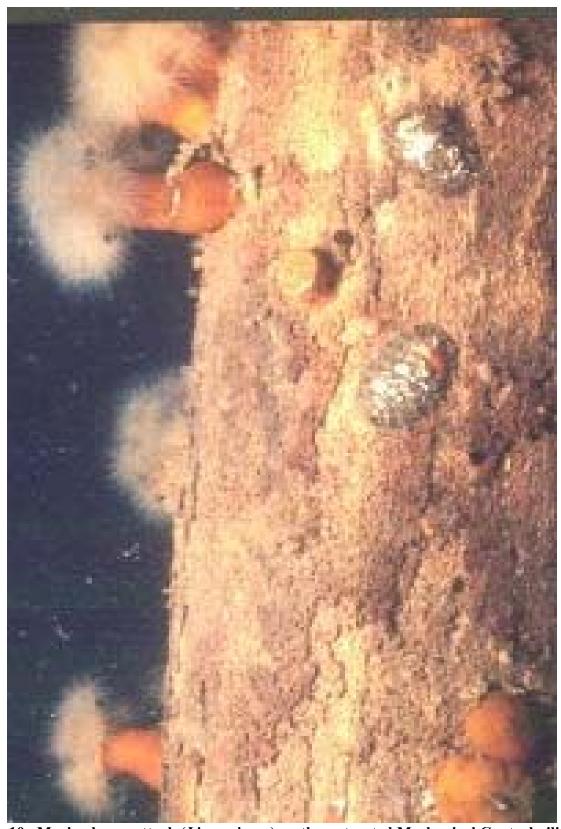


Plate 10. Marine borer attack (*Limnoria sp.*) on the untreated Mechanical Control piling recorded in October 1999, four years following construction.

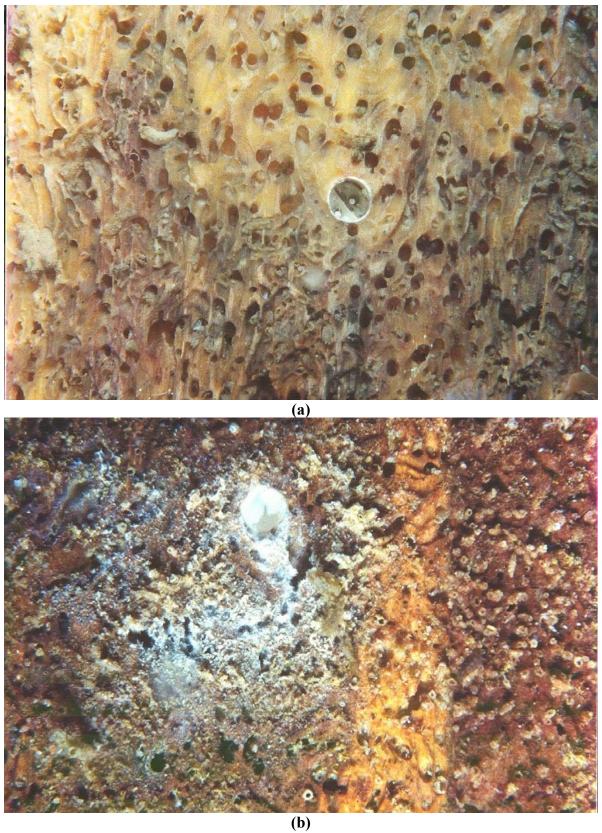


Plate 11. Close up view of the surface of the untreated Mechanical Control pile showing damage created by a) *Limnoria* and b) Toredo (*Bankia sp.*) four years after construction.



Plate 12. Typical canisters and platforms installed on Day 1360 at the BMP and MC dolphins (June 1999).



Plate 13. Canisters on the -13' (Chart Datum) platform at the BMP dolphin on Day 1540 (October 1999), four months after installation.



Plate 14. Debris collect by the Mechanical Control canisters after four months of exposure (June to October 1999)



Plate 15. Contents of the Mechanical Control canister after four months of exposure. Note the amount of debris collected by the 8 inch deep canister and the differences from those at the BMP site (Plate 13). Assuming a constant rate of deposition, the rate at the MC site equaled approximately 104 kg/m²-y.

ADDENDUM REPORT

Continuation of the Sooke Basin Creosote Evaluation Study (Goyette and Brooks, 1998)

Year Four - Day 1360 and Day 1540

Part III. Appendices

Prepared by:

D. Goyette Commercial Chemicals Division Environment Canada Pacific and Yukon Region

and

K.M. Brooks Aquatic Environmental Sciences Port Townsend, WA 98368

Prepared for:

Creosote Evaluation Steering Committee

Regional Program Report PR00-03

May 2001

Appendix Ia. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station BMP0.0.

Exposure	384BMP0.00		1360E	BMP0.0			1540	BMP0.0		
Period/Distance (m) Replicate #	mixed	1	2	Mean	Std. Dev.	1	3	Mean	Std. Dev.	Bioassay End Pt.
РАП										ī
Naph.	43	20	<20	20		<10	<10	<10		<20
Aceny.	46	30	20	25	7.1	30	40	35	7.1	30
Acen	350	90	50	70	28	50	60	55	7.1	20
Fluor.	740	110	70	90	28	80	130	105	35	50
Phen.	3600	590	380	485	148	310	770	540	325	190
Anth.	1800	450	300	375	106	420	390	405	21	280
LPAH	6579	1290	820	1065	332	890	1390	1140	354	570
Fluoranth.	6700	1570	1050	1310	368	1010	1950	1480	665	1010
Pyrene	3400	780	520	650	184	520	1120	820	424	500
B(a)Anth.	3100	970	690	830	198	610	790	700	127	40
Chrysene	4600	1680	1210	1445	332	1270	1380	1325	78	1210
B(FI)	2900	980	740	860	170	620	940	780	226	740
B(a)Pyr.	1600	400	290	345	78	240	360	300	85	290
Dibenz(ah)Anth.	120	70	60	65	7.1	40	50	45	7.1	40
Indeno.	530	180	130	155	35	100	160	130	42	130
B(ghi)perylene	370	140	110	125	21	80	120	100	28	80
НРАН	23320	6770	4800	5785	1393	4490	6870	5680	1683	4040
TPAH (ng/g) TPAH (μg/g)	<mark>29899</mark> 29.90	8060 8.06	5620 5.62	6850 6.85	1725 1.72	5380 5.38	8260 8.26	6820 6.82	2036 2.04	4610 4.61
Surr. Recovery (%)				<u> </u>						
Naph d-8	75	52	56	54		54	51	53		64
Acen d-10	82	63	69	66		78	74	76		73
Phen d-10	88	77	82	80		93	89	91		81
Cry d-12	78	71	74	73		83	80	82		79
Perylene d-12	90	74	78	76		89	89	89		78

Appendix Ib. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station BMP0.5.

Exposure Period/Distance (m)	Day 384			1360BMP0.5					`154	10BMP0.5		
Replicate #	mixed	1	2	3	Mean	Std. Dev.	1	2	3	Mean	Std. Dev.	Bioassay End Pt.
РАН												
Naph.	29	<20	<20	30	30		10	20	30	20	10	<20
Aceny.	32	<20	<20	<20	<20		30	30	20	27	5.8	20
Acen.	165	160	70	120	117	45	40	50	90	60	26	30
Fluor.	300	200	70	90	120	70	60	440	110	203	206	40
Phen.	1300	1000	320	410	577	369	360	1720	540	873	739	140
Anth.	615	310	130	210	217	90	400	1840	250	830	878	160
LPAH	2441	1670	590	860	1040	562	900	4100	1040	2013	1808	390
Fluoranth.	3550	1790	1000	1250	1347	404	1670	2360	1200	1743	583	640
Pyrene	1600	970	550	650	723	219	350	320	170	280	96	330
B(a)Anth.	1500	650	290	570	503	189	810	920	400	710	274	350
Chrysene	2350	1060	410	810	760	328	1170	1430	700	1100	370	620
B(FI)	1550	650	310	650	537	196	840	790	440	690	218	420
B(a)Pyr.	785	260	110	260	210	87	350	320	170	280	96	170
Dibenz(ah)Anth.	59	50	<20	40	45	7.1	50	50	40	47	5.8	30
Indeno.	275	130	80	110	107	25	150	120	80	117	35	80
B(ghi)perylene	190	90	50	100	80	26	110	90	60	87	25	50
НРАН	11859	5650	2800	4440	4297	1430	5500	5400	3260	5053	1617	2690
TPAH (ng/g) TPAH (μg/g)	14300 14.30	7320 7.32	3390 3.39	5300 5.30	5337 5.34	<mark>1965</mark> 1.96	6400 6.40	10500 10.50	4300 4.30	7067 7.07	3153 3.15	3080 3.08
Surr. Recovery (%)												
Naph d-8	75	62	60	61	61		55	60	64	60		60
Acen d-10	82	72	69	70	70		75	74	80	76		71
Phen d-10	88	85	79	78	81		90	84	92	89		79
Cry d-12	78	76	70	68	71		81	74	80	78		77
Perylene d-12	90	76	72	70	73		90	80	88	86		78

Appendix Ic. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station BMP2.0.

Exposure Period/Distance (m)	Day 384			1360	BMP2.0					1540BMI	P2.0	
Replicate #	Mixed	1a	1b	2 a	2 b	Mean	Std.Dev.	1	3	Mean	Std. Dev.	Bioassay End Pt.
РАН												Liid i t.
Naph.	18	<20	<20	<20	<20	<20		<10	<10	<10		<20
Aceny.	21	<20	<20	<20	<20	<20		<10	<10	<10		<20
Acen.	110	30	50	40	40	40	8.2	30	10	20	14	<20
Fluor.	180	70	90	50	50	65	19	30	20	25	7.1	<20
Phen.	620	320	440	230	230	305	99	120	80	100	28	40
Anth.	340	130	130	150	100	128	21	60	50	55	7.1	40
LPAH	1289	550	710	470	420	538	127	240	160	200	57	80
Fluoranth.	1800	630	880	740	670	730	110	410	390	400	14	360
Pyrene	710	360	500	400	360	405	66	250	230	240	14	180
B(a)Anth.	950	190	270	310	240	253	51	150	130	140	14	140
Chrysene	1400	210	410	480	360	365	114	170	160	165	7.1	170
B(FI)	970	200	270	350	250	268	62	180	160	170	14	170
B(a)Pyr.	480	80	100	130	90	100	22	70	60	65	7.1	60
Dibenz(ah)Anth.	37	<20	<20	<20	<20	<20		<10	<10	<10		20
Indeno.	170	50	40	50	30	43	9.6	50	40	45	7.1	60
B(ghi)perylene	120	40	30	40	30	35	5.8	30	30	30	0.0	30
НРАН	6637	1760	2500	2500	2030	2199	366	1310	1200	1255	78	1190
TPAH (ng/g) TPAH (μg/g)	7926 7.93	2310 2.31	3210 3.21	2970 2.97	2450 2.45	2737 2.74	425 0.42	1550 1.55	1360 1.36	1455 1.46	134 0.13	1270 1.27
Surr. Recovery (%)						1						<u> </u>
Naph d-8	79	52	69	63	67	63		59	61	60		76
Acen d-10	80	68	87	74	82	78		78	76	77		83
Phen d-10	85	78	96	81	94	87		90	92	91		95
Cry d-12	77	69	103	84	99	89		79	83	81		77
Perylene d-12	89	70	92	74	84	80		87	90	89		77

Appendix Id. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station BMP5.0.

Exposure Period/Distance (m)	Day384	1360BMP5.						1540BMP5	5.0	
Replicate #	mixed	0 1	2	Mean	Std. Dev.	1	3	Mean	Std. Dev.	Bioassay End Pt.
PAH										
Naph.	20	<20	<20	<20		<20	<20	<20		<20
Aceny.	4.2	<20	<20	<20	<20	<20	<20	<20		<20
Acen.	99	<20	<20	<20	<20	40	<20	40		<20
Fluor.	100	<20	<20	<20		40	30	35	7.1	<20
Phen.	380	40	50	45	7.1	150	100	125	35	40
Anth.	150	20	20	20	0.0	50	50	50	0.0	30
LPAH	753	60	70	65	7.1	280	180	230	71	70
Fluoranth.	860	170	90	130	57	320	250	285	49	280
Pyrene	520	110	60	85	35	210	160	185	35	190
B(a)Anth.	300	90	30	60	42	110	100	105	7.1	90
Chrysene	380	80	30	55	35	130	100	115	21	100
B(FI)	250	90	50	70	28	150	110	130	28	120
B(a)Pýr.	120	40	40			50	40	45	7.1	40
Dibenz(ah)Anth.	8.3	<20	<20	<20		<20	<20	<20		20
Indeno.	39	40	<20	40		40	40	40	0.0	40
B(ghi)perylene	31	30	<20	30		30	20	25	7.1	20
НРАН	2508	650	300	470	276	1040	820	930	156	900
TPAH (ng/g) TPAH (μg/g)	3262 3.26	710 0.71	370 0.37	535 0.54	<mark>269</mark> 0.27	1320 1.32	1000 1.00	1160 1.16	226 0.23	<mark>970</mark> 0.97
Surr. Recovery (%)		1		I						
Naph d-8	73	53	62	58		72	64	68		64
Acen d-10	74	68	73	75		82	77	80		76
Phen d-10	71	75	86	81		89	83	86		75
Cry d-12	59	70	83	77		84	73	79		77
Perylene d-12	63	71	81	76		84	72	78		78

Appendix Ie. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station BMP10.

14 4.5 78	<20 <20 <30	20 <20	Mean 20 <20	Std. Dev.	<20	3 <20	Mean	Std. Dev.	Bioassay End Pt.
4.5 78	<20				<20	<20	<20		<20
4.5 78	<20				<20	<20	<20		~20
78		<20	~20						~20
	30		~20		<20	<20	<20		<20
79	30	110	70	57	<20	<20	<20		<20
. •	20	100	60	57	<20	<20	<20		<20
250	160	500	330	240	30	50	40	14	<20
89	30	50	40	14.1	<20	20	20		<20
515	240	780	510	382	30	70	60	28	<20
480	290	460	375	120	90	210	150	55	120
210	200	290	245	64	80	160	120	57	90
220	70	90	80	14.1	30	70	50	28	40
310	80	80	80	0.0	30	60	45	21	70
250	90	90	90	0.0	80	40	60	28	70
120	30	30	30	0.0	20	<20	20		20
8.7	<20	<20	<20		<20	<20	<20		<20
39	30	<20	30		<20	40	40		30
32	<20	<20	<20		<20	30	30		<20
670	790	1040	915	177	330	610	515	198	440
184	1030	1820	1425	559	360	680	520	226	440 0.44
5 4 2 2 3 2 1 3 3 4 4 4 4 4 7 7	880 810 820 810 850 220 3.7 39 332	240 280 290 200 200 200 200 200 200 200 200 20	240 780 280 290 460 200 290 20 70 90 20 70 90 20 80 80 250 90 90 20 30 30 3.7 <20	240 780 510 880 290 460 375 210 200 290 245 220 70 90 80 80 80 80 80 850 90 90 90 20 30 30 30 3.7 <20	315 240 780 510 382 380 290 460 375 120 310 200 290 245 64 320 70 90 80 14.1 310 80 80 0.0 350 90 90 90 0.0 30 30 30 0.0 37 <20	315 240 780 510 382 30 380 290 460 375 120 90 310 200 290 245 64 80 320 70 90 80 14.1 30 310 80 80 80 0.0 30 350 90 90 90 0.0 80 20 30 30 30 0.0 20 3.7 <20	315 240 780 510 382 30 70 880 290 460 375 120 90 210 810 200 290 245 64 80 160 820 70 90 80 14.1 30 70 810 80 80 90 30 60 850 90 90 90 90 80 40 20 30 30 30 0.0 20 <20	315 240 780 510 382 30 70 60 880 290 460 375 120 90 210 150 810 200 290 245 64 80 160 120 820 70 90 80 14.1 30 70 50 810 80 80 0.0 30 60 45 850 90 90 90 0.0 80 40 60 20 30 30 30 0.0 20 <20	315 240 780 510 382 30 70 60 28 880 290 460 375 120 90 210 150 55 810 200 290 245 64 80 160 120 57 820 70 90 80 14.1 30 70 50 28 810 80 80 0.0 30 60 45 21 850 90 90 90 0.0 80 40 60 28 20 30 30 30 0.0 20 <20

Appendix If. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station BMP20.

Exposure Period/Distance (m)	Day384		1360BN	/IP20		1540BMP20					
Replicate #	mixed	1	3	Mean	Std. Dev.	1	3	Mean	Std. Dev.	Bioassay End Pt.	
РАН											
Naph.	16	<20	<20	<20		<20	<20	<20		<20	
Aceny.	2.5	<20	<20	<20		<20	<20	<20		<20	
Acen.	18	<20	<20	<20		<20	<20	<20		<20	
Fluor.	22	<20	20	20		<20	<20	<20		<20	
Phen.	51	<20	110	110		20	20	20	0.0	20	
Anth.	14	<20				<20	<20	<20		<20	
LPAH	124	<20	130	130		20	20	20	0.0	20	
Fluoranth.	120	40	180	110	99	60	60	60	0.0	70	
Pyrene	79	30	130	80	71	50	40	45	7.1	60	
B(a)Anth.	34	30	50	40	14.1	20	20	20	0.0	20	
Chrysene	45	<20	40	40		20	20	20	0.0	30	
B(FI)	51	50	80	65	21	50	40	45	7.1	50	
B(a)Pyr.	23	30	<20	30		<20	<20	<20		<20	
Dibenz(ah)Anth.	1.5	50	30	40	14.1	<20	<20	<20		<20	
Indeno.	14	40	<20	40		<20	<20	<20		<20	
B(ghi)perylene	11	30	<20	30		<20	<20	<20		<20	
НРАН	379	300	510	475	148	200	180	190	14	230	
<mark>TPAH (ng/g)</mark> TPAH (μg/g)	502 0.50	300 0.30	640 0.64	605 0.60	240 0.24	220 0.22	200 0.20	210 0.21	14 0.01	230 0.23	
Surr. Recovery (%)											
Naph d-8	72	78	75	77		57	64	61		71	
Acen d-10	74	85	81	83		70	76	73		82	
Phen d-10	75	88	84	86		81	83	82		90	
Cry d-12	67	81	76	79		75	71	73		90	
Perylene d-12	72	80	69	75		72	66	69		67	

Appendix Ig. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Station MC0.5.

Exposure Period/Distance (m)	Day384		1360	MC0.5		1540MC0.5					
Replicate #	mixed	1	3	Mean	Std. Dev.	1	3	Mean	Std. Dev.	Bioassay End Pt.	
PAH											
Naph.	NDR(4.5)	<20	<20	<20		<20	<20	<20		<20	
Aceny.	NDR(1.0)	<20	<20	<20		<20	<20	<20		<20	
Acen.	2.1	<20	<20	<20		<20	<20	<20		<20	
Fluor.	3.7	<20	<20	<20		<20	<20	<20		<20	
Phen.	22	<20	<20	<20		<20	<20	<20		<20	
Anth.	4.2	30	30	30	0.0	<20	<20	<20		<20	
LPAH	32	30	30	30	0.0	<20		<20		<20	
Fluoranth.	39	30	30	30	0.0	30	30	30	0.0	20	
Pyrene	27	20	20	20	0.0	20	20	20	0.0	20	
B(a)Anth.	10	<20	<20	<20		<20	<20	<20		<20	
Chrysene	14	<20	<20	<20		<20	<20	<20		<20	
B(FI)	18	60	30	45	21	40	40	40	0.0	20	
B(a)Pyr.	6.8	<20	<20	<20		<20	<20	<20		<20	
Dibenz(ah)Anth.	NDR(1.0)	<20	<20	<20		<20	<20	<20		<20	
Indeno.	6.7	<20	<20	<20		<20	<20	<20		<20	
B(ghi)perylene	6.1	<20	<20	<20		<20	<20	<20		<20	
НРАН	128	110	80	95	21	90	90	90		60	
TPAH (ng/g) TPAH (μg/g)	<mark>160</mark> 0.16	<mark>140</mark> 0.14	110 0.11	125 0.12	<mark>21</mark> 0.02	<mark>90</mark> 0.09	90 0.09	90 0.09		60 0.06	
Surr. Recovery (%)				I						1	
Naph d-8	73	63	60	62		76	78	77		66	
Acen d-10	73	76	75	76		84	82	83		76	
Phen d-10	68	82	82	82		87	84	86		81	
Cry d-12	68	70	73	72		76	78	77		78	
Perylene d-12	65	72	76	74		76	72	74		83	

Appendix Ih. Comparison of the BMP Downcurrent Transect Sediment PAH Concentrations (ng/g, dry wt.) on Day384 (Year1) with Days 1360 and 1540 - Sooke Basin Creosote Evaluation Study - Stations OC0.0 & MC0.0 (#1).

Exposure	Day384	1540OC0.0	MC0.0 #1			
Period/Distance (m) Replicate # PAH	mixed	Bioassay End Pt.	Post Aeration	Post Bioassay		
Naph.	8.8	<20	40	160		
Aceny.	2.2	<20	260	780		
Acen.	6.8	<20	100	410		
Fluor.	9.8	<20	340	910		
Phen.	24	<20	480	1230		
Anth.	5.9	<20	460	1260		
LPAH	58	<20	1680	4750		
Fluoranth.	43	20	550	1510		
Pyrene	33	20	570	1460		
B(a)Anth.	13	<20	500	1380		
Chrysene	18	20	480	1330		
B(FI)	21	20	910	2540		
B(a)Pýr.	19	<20	500	1220		
Dibenz(ah)Anth.	1.1	<20	420	1070		
Indeno.	8.3	<20	450	1140		
B(ghi)perylene	7.6	<20	430	1090		
НРАН	164	80	4810	12740		
TPAH (ng/g)	222	80	6490	17490		
TPAH (μg/g)	0.22	0.08	6.49	17.49		
Surr. Recovery (%)						
Naph d-8	63	66	52	61		
Acen d-10	66	76	63	73		
Phen d-10	81	81	74	79		
Cry d-12	80	78	72	76		
Perylene d-12	91	83	74	74		

Note: MC0.0 (#1) sample (post-aeration and post-bioassay) was spiked with a known PAH Standard at 1 µg/g per PAH compound.