1994

Comparative Toxicity of Creosote and Creosote Contaminated Sediments

Padma T. Venkatraman
College of William and Mary - Virginia Institute of Marine Science

COMPARATIVE TOXICITY OF CREOSOTE AND CREOSOTE CONTAMINATED SEDIMENTS

A THESIS

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

---

by

Padma T. Venkatraman

1994

APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements

for the degree of

Master of Arts

T. V. Padma

Padma T. Venkatraman

Approved, April 1994

Robert C. Hale

Robert C. Hale, Ph.D., Co-Chairman

Romuald N. Lipcius

Romuald N. Lipcius, Ph.D., Co-Chairman

Robert J. Huggett

Robert J. Huggett, Ph.D.

Morris H. Roberts, Jr.

Morris H. Roberts, Jr., Ph.D.

Jacques von Montfrans

Jacques von Montfrans, M. S.

DEDICATION

This thesis is dedicated to Dr. Bhaskar Raju and Dr. Balagopal Raju.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Sincere thanks to Dr. Robert Hale for his patience, guidance and constant support. I am very grateful to Dr. Romuald Lipcius for encouraging my independence. Dr. Roberts gave valuable advice on conducting the toxicity tests. Dr. Huggett provided insightful comments on the study. Thanks to Jacques van Montfrans for his enthusiasm and encouragement.

I am grateful to Mr. Randall Baileys and Kopper's Chemical Industries for providing the creosote sample used in this study. This study was partially funded by a VIMS minor research grant.

Dr. Pete De Lisle and Dr. Ruth Williams are sincerely thanked for giving me their time. Mary Ann Volgelbein and Tanja Crockett provided invaluable help with the mysids. Ellen Harvey and Robby Mothershead were always cheerful and willing to help with analytical instrumentation. Ms. Elizabeth Bush taught me analytical procedures. Special thanks to Doug Dixon for his help. Dr. Roger Mann allowed me to use his balance. Dr. Bob Diaz increased my understanding of categorical data analysis. The VIMS publication department personnel patiently helped with preparing the slides. I also appreciate the support of the Lab Rats of Room 303, members of the crustacean ecology group and the affection of my mother, friends and family.

LIST OF TABLES

LIST OF FIGURES

Page

Abstract

Creosote is a widely used wood preservative, containing a complex mixture of aromatic compounds (ACs). Many creosote contaminated sites exist around the world, including the Elizabeth River, a subestuary of the Chesapeake Bay.

Whole creosote, creosote-contaminated Elizabeth River sediment (ERS), and water soluble fractions (WSFs) generated from each, were chemically analyzed. Initial identifications were made using gas chromatography (GC) with a flame ionization detector (FID) and confirmed by mass spectrometry (MS). Creosote was diluted and analyzed directly using GC-FID. Sediments were freeze dried and soxhlet extracted with dichloromethane (DCM). The extract was purified by gel permeation chromatography, subjected to open column chromatography and analyzed by GC-FID. WSFs were extracted with DCM and analyzed by GC-FID. Comparison of creosote, sediment and their respective WSFs showed that the WSFs were enriched in low molecular weight ACs (three or fewer aromatic rings). Sediment contained high proportions of creosote derived hydrophobic compounds. These differences in composition mimic the fractionation of creosote in the environment, which has important implications in respect to its fate, bioavailability and toxicity.

The 48h acute toxicities of the two WSFs to the bay mysid, *Mysidopsis bahia*, were compared using a static renewal exposure method to determine whether compositional differences resulted in differences in toxicity. The 48 h LC-50 for the creosote WSF and ERS WSF, based on total identified AC concentrations, were estimated to be 180 ug/l and 700 ug/l respectively. This indicates that the creosote WSF was about four times as toxic as the ERS WSF. This may be due to qualitative differences, such as the presence of higher concentrations of heterocyclic compounds (e.g. quinolines), in the creosote WSF.

The long-term effects of sublethal exposure to the ERS WSF were determined using a seven day exposure of *Mysidopsis bahia* to five sublethal concentrations of ERS WSF. Three sublethal endpoints: frequency of molting, somatic growth (measured by dry weight) and attainment of sexual maturity (indicated by the proportion of egg-bearing females in each replicate) were monitored. The ERS WSF significantly decreased somatic growth and delayed attainment of sexual maturity. The EC-50 for sexual maturity data was 15 ug/l total identified AC concentration. Molting was the least sensitive endpoint measured and did not differ significantly between control and treatments.

COMPARATIVE TOXICITY OF CREOSOTE AND CREOSOTE CONTAMINATED SEDIMENTS

CHAPTER I

GENERAL INTRODUCTION

Toxicological studies often focus on the effects caused by a single compound. Contaminated environments, however, contain mixtures of chemicals. This can result in enhancement or reduction of toxicity due to synergistic or antagonistic interactions between compounds.

The fate of a mixture in the aquatic environment is a function of its chemical composition, the physical characteristics of the water body in which it is present, as well as atmospheric, hydrodynamic and biological processes. These factors act on the parent mixture and transport, transform, fractionate and degrade it (Bennett, 1990). Fractionation is a process by which the components of a mixture separate, based on their properties. Polar compounds may partition into the aqueous phase, as a function of their solubility. High molecular weight compounds may rapidly sorb to sediment and organic matter (Knezovich *et al.*, 1987). Low molecular weight components may undergo volatilization. Degradation of a mixture over time may occur due to weathering processes such as

photooxidation and microbial action. Fractionation and degradation affect the mixture's composition and distribution within the ecosystem. As a consequence, biological uptake and toxicity are affected.

Some mixtures are composed predominantly of one group of compounds all having the same basic structure (a chemical class). Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds made up of carbon and hydrogen atoms that contain two or more fused aromatic rings, with or without aliphatic substitution (e.g. acenaphthene, methyl phenanthrene). Some closely related polycyclic compounds may contain elements such as nitrogen (e.g. quinoline), oxygen (e.g. dibenzofuran), or sulphur (e.g. dibenzothiophene) in the ring. These compounds are termed heterocyclics (Fig 1). Both groups are collectively referred to in this document as aromatic compounds (ACs). In this document, ACs with fewer than three aromatic rings will be referred to as 'low molecular weight', those with three aromatic rings as 'intermediate molecular weight' and those with more than three aromatic rings as 'high molecular weight'.

ACs may be formed by natural processes such as pyrogenesis, diagenesis and microbial synthesis. Anthropogenic activities such as the manufacture of petroleum gas, synthetic alcohol, coke, acetylene,

Figure 1. Structures of some ACs found in creosote.

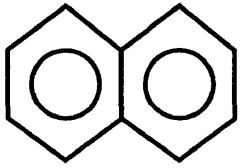
AROMATIC COMPOUNDS

Hydrocarbons

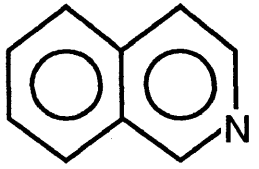
Heterocyclics

Low Molecular Weight

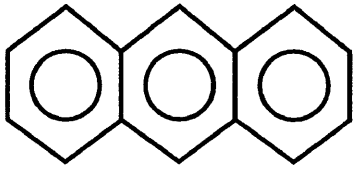
Naphthalene:



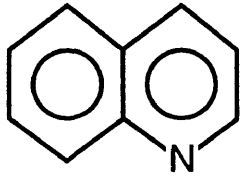
Isoquinoline:



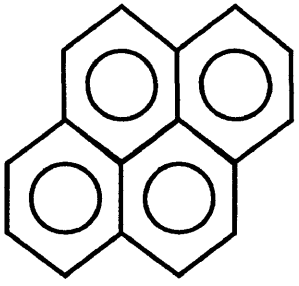
Anthracene:



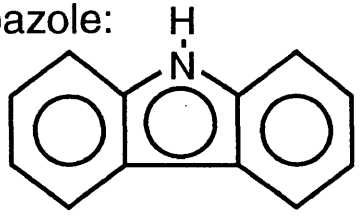
Quinoline:



Pyrene:



Carbazole:



High Molecular Weight

aluminum, organic solvents, coal tar and incomplete combustion of fossil fuels also release ACs (Neff, 1979, Enzminger and Ahlert, 1987).

ACs are the most abundant organic pollutant in the Chesapeake Bay (Helz and Huggett, 1987). Many ACs are suspected and a few are known to possess carcinogenic properties (O'Connor and Huggett, 1988).

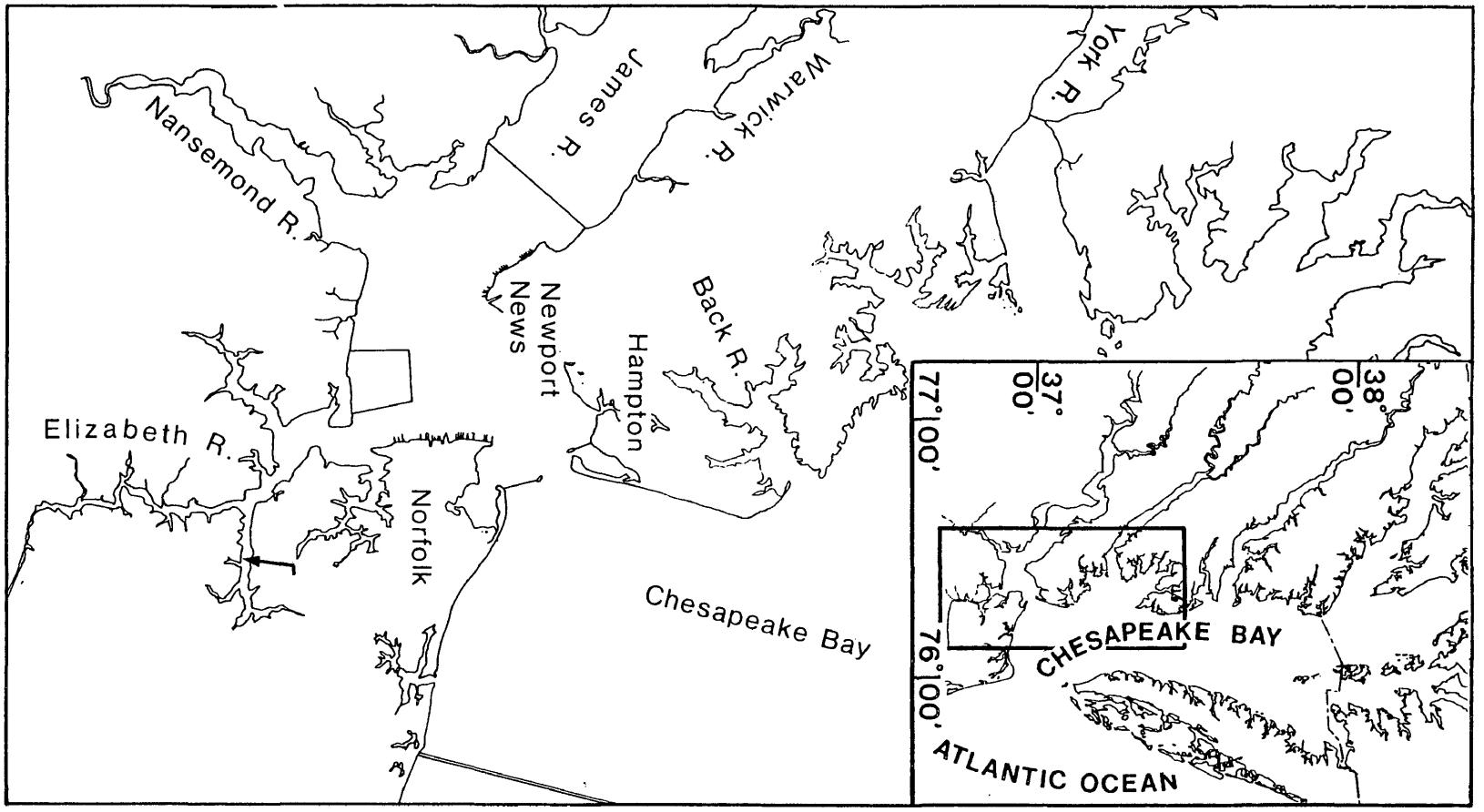
A variety of toxic effects of ACs on aquatic biota have been documented. ACs were lethal to *Daphnia magna* in laboratory tests (Munoz and Tarazona, 1993). Mortalities and lesions have been observed in the American oyster, *Crassostrea virginica*, upon exposure to ACs (Mahoney and Noyes, 1982). In the field, AC levels have been correlated with gross abnormalities in fish (Huggett *et al.*, 1992, Malins *et al.*, 1985).

Creosote is a complex mixture of ACs resulting from the distillation of coal tar. It a wood preservative that has been used widely since the seventeenth century (Mueller *et al.*, 1989 a). About 700 sites in the United States are known to be contaminated with creosote (Mueller *et al.* 1989 b). Process wastewater, leaching from dump sites, storage tank leaks and spills are major creosote inputs to the environment (Merril and Wade, 1985). After wood has been pressure treated, leaching is greatly reduced (Baileys and Webb, 1987).

Creosote has both short-term effects on biota (Borthwick and Patrick, 1982), as well as long-term effects, such as carcinogenesis (Black, 1982). The various compounds in creosote may differ in the type of toxic effect they produce. For instance, the genotoxic effects of creosote, as determined by the Ames *Salmonella* assay, reside in the high boiling point (>290°C) fraction of creosote (Nylund *et al.*, 1992). Comparison of toxicity of naphthalene and phenanthrene (two PAHs which commonly occur in creosote), showed that naphthalene had a greater lethality (Geiger and Buikema, 1982).

The toxicity of creosote-contaminated sediments from a number of different locations to biota has been documented (Malins and Roubal 1985; Tagatz *et al.*, 1983), including the Elizabeth River, a highly contaminated subestuary of the Chesapeake Bay (Roberts *et al.*, 1989). This river is situated in close proximity to three heavily populated and industrialized cities (Fig 2). Major sources of contamination are creosote plants, petroleum tank farms and wet and dry docks (Bieri *et al*, 1986). This river was the site of two major creosote spills in the 1960s. Huggett *et al.* (1992), measured sediment concentrations of creosote related AC as high as 15 g/kg (dry weight basis) at a contaminated site. A wood treatment facility on the

Figure 2. Map showing the location of the Elizabeth River.


Nansemond R.
James R.
Warwick R.
York R.
Newport News
Hampton
Back R.
Elizabeth R.
Norfolk
Chesapeake Bay
37° 00'
38° 00'
77° 00'
76° 00'
CHESAPEAKE BAY
ATLANTIC OCEAN

Southern Branch of the Elizabeth River, Atlantic Wood Industries, used creosote from the 1950s until 1992. This added significantly to existing pollution.

Sediment and water associated with the sediment of this river were lethal to *Leiostomus xanthurus* in laboratory experiments (Roberts *et al.*, 1989). External lesions, hepatic neoplasms, cataracts and finrot have been observed in several species of fish from this region (Hargis *et al.*, 1984; Volgelbein *et al.*, 1990). Depressions in biomass and abundance of several species of fish were observed in the most severely contaminated parts of the river (Huggett *et al.*, 1984). Fish taken from the Southern Branch of the river exhibited altered immunological function (Weeks and Wariner, 1984). The long-term effect of Elizabeth River sediment on biota is of concern since the sediment contains large quantities of high molecular weight AC that may act as carcinogens and mutagens.

Creosote contaminated sediments typically contain relatively greater percentages of ACs with low water solubilities than does whole creosote (Bieri *et al.*, 1986; Roberts *et al.*, 1989). A water soluble fraction (WSF) generated from whole creosote (simulating the environment which may be present shortly after a spill) would likely contain a larger concentration of soluble, low molecular weight ACs than one generated from

contaminated sediment. An important factor influencing the toxicity of ACs is their water solubility (Neff and Anderson, 1981). Since both water solubility, as well as short-term toxicity of ACs decrease with increasing molecular size (Neff 1979), it is reasonable to expect that the short-term lethal effect of contaminated sediment would be lower than that of fresh creosote. Previous observations on blue crab megalopae exposed to WSFs generated from whole creosote and creosote contaminated sediment indicated that the former had a much higher lethality (personal observations).

Benthic and epibenthic organisms in contact with the bottom are particularly susceptible to exposure from polluted sediments. The bay mysid, *Mysidopsis bahia*, is an epibenthic estuarine crustacean that tends to aggregate in high densities over sediments and in vegetated areas (Lussier *et al.*, 1988). The bay mysid plays a vital role in the estuarine food web (Markle and Grant, 1970; Odum and Heald, 1972; Smith *et al.*, 1984). Mysids are major constituents of the diets of many fish including commercially important species such as the striped bass, *Morone saxatalis* (Gentile *et al.*, 1983).

Mysids are useful to evaluate the toxicity of polluted waters since they are highly sensitive and can be cultured in the laboratory (Nimmo and Hamaker,

1982). Survival, sexual maturity and dry weight (a measure of growth) are endpoints that are most often monitored in toxicity tests. Molting is an important process that may be adversely affected by the presence of pollutants (Lee, 1988). There are few published reports on the effect of pollutants on the molting process of this mysid, although numerous studies have demonstrated toxicant effects on molting in other crustaceans (e.g. Karinen and Rice, 1985).

Three important routes of xenobiotic chemical uptake by aquatic organisms are via water, sediment and food. WSFs are suitable systems in which to conduct tests with this species since water is a major route of uptake. WSFs are a useful system in which to conduct toxicity tests, as they are easy to handle and allow an accurate measurement of exposure concentration.

The objectives of this study were to:

1. Compare the chemical compositions of creosote, Elizabeth River sediment and their respective WSFs;

2. Estimate the short-term toxicities of the WSFs, using a standard EPA test organism (the bay mysid), to determine if compositional differences result in differences in toxicity;

3. Examine the effects of the Elizabeth River sediment (ERS) WSF on three sublethal endpoints: molting, dry weight and attainment of sexual maturity.

CHAPTER II

CHEMICAL COMPARISON OF CREOSOTE, ELIZABETH RIVER SEDIMENT AND THEIR WATER SOLUBLE FRACTIONS.

Introduction

Bioavailability and toxicity of chemicals in a mixture are controlled by the partitioning of constituents within an ecosystem. Thus, it is important to obtain reliable information on the distribution of the components of a mixture (Zurcher and Thuer, 1978). Soluble components may have toxic effects even in minute doses (Jacobson and Boylan, 1973), but are typically diluted and dispersed. In contrast, hydrophobic components may sorb to organic matter and many therefore persist for long periods of time (Jaffe, 1991). However, they are less bioavailable in the sorbed state. By comparing the composition of creosote, ERS and their respective WSFs, the partitioning and behavior of creosote ACs can be better understood.

Materials and methods

ACs in samples of various types were ultimately measured by gas chromatography following suitable

sample preparation and purification (Fig 3). The creosote sample analyzed in this study was obtained from Kopper's Chemical Industries (Pittsburgh, PA). Sediment for this study was collected from a site situated close to Atlantic Wood Industries, until recently an active wood processing plant.

The creosote WSF (1% by volume) was prepared by adding 20 ml of creosote to 2000 ml of artificial sea water of 20 g/l salinity (de-ionized water + Hawaiian Marine Mix, Hawaiian Marine imports, Houston, TX) in a glass beaker. The ERS WSF stock was prepared by adding 125 g of sediment to 2 l of artificial sea water in another beaker. The containers were covered with aluminum foil to minimize loss of chemicals prone to volatilization and photodegradation. Each preparation was mixed for 30 min using a magnetic stirrer. The mixture was allowed to settle for 12 h to achieve phase separation. The aqueous fraction was then carefully decanted. The decanted fraction was filtered through a Whatman A/E glass fibre filter (nominal pore size = 0.7 um).

Known amounts of three surrogate standards (d8 naphthalene, d10 acenaphthene and 1,1' binaphthyl) were added to each sample, prior to any preparatory steps, to assess analyte losses during sample preparation. Amounts of surrogate standard added were

based on estimates of AC concentrations in the samples.

Samples were analyzed using a Varian 3300 gas chromatograph (GC) equipped with a flame ionization detector (FID). Prior to GC injection, samples were extracted with or dissolved in an appropriate solvent such as dichloromethane (DCM). Samples such as the Elizabeth River sediment required additional purification steps before GC injection.

The parent creosote was diluted with DCM and injected onto GC-FID. The WSFs (25 ml of each) were extracted with 15 ml of DCM in a separatory funnel. Extracts were subjected to GC-FID. The ERS was freeze dried, homogenized and soxhlet extracted with DCM. This extract required purification steps. The extract was subjected to rotary evaporation and an aliquot of the sample was used for gravimetric analysis. The remainder was subjected to gel permeation chromatography (ABC Instruments, Columbia, MO, Model 1002 B), which separated larger biogenic molecules from smaller xenobiotic chemicals. The cleaned ERS extract of interest, obtained after GPC, was subjected to open column chromatography with silica gel to remove interfering compounds and separate polar and aromatic fractions. Aliphatic compounds were removed by elution with 25ml of hexane (S1 fraction). The aromatic compounds were eluted with 50 ml of a mixture of hexane

and DCM (4:1). Extract volume was reduced to 0.1ml, using a nitrogen bath, in order to concentrate the sample. The purified fractions were then analyzed by GC-FID. Procedures used were similar to those described by Bieri *et al.* (1986).

An internal standard, p-terphenyl was added to all samples immediately before GC injection. A retention standard consisting of ten PAHs, as well as the surrogate and internal standards, was injected each day to assess instrument performance and determine retention indices. A response factor of one was assumed between the internal standard and the analyte. Injections were made in the splitless mode and the split valve was opened one minute after injection. The initial GC column temperature was 75°C. The column was held at that temperature for a minute. It was then increased to 310°C at the rate of 4°C/min and maintained at 310°C for 10 min. The detector and injector were held at 310°C and 260°C, respectively. Recoveries of surrogate standards were calculated by comparing the area counts of the internal and surrogate standards. Concentrations of the various compounds were corrected for recovery using the appropriate surrogate standard (based on elution time).

Initial identifications were accomplished using an aromatic retention index. Identifications of all major

peaks in the sediment, creosote and each WSF were confirmed using GC, in combination with mass spectrometry (MS) in the electron impact mode. A Dupont 21-492 B magnetic sector mass spectrometer was used.

The precision and accuracy of the analytical techniques were assessed by spiking triplicate blank water samples with known amounts of representative compounds.

Results

Certain components of creosote partitioned selectively into the aqueous phase, while others selectively sorbed to sediment (compare Fig 4 and 6). The creosote WSF was enriched in low molecular weight compounds present in the creosote, such as quinolines and naphthalenes. It contained lower concentrations of intermediate and high molecular weight AC, such as fluorene, pyrene, and benzofluorenes. The ERS sediment, in contrast, contains much lower concentrations of naphthalenes than whole creosote and does not contain any detectable concentrations of quinolines, cresols or phenols. Instead, it is rich in intermediate and high molecular weight AC (Fig 6). The WSF generated from this sediment is enriched in low molecular weight compounds and heterocyclic components and is low in high molecular weight components compared to the

sediment (Fig 5). This is also observed during the generation of the creosote WSF (Fig 4), indicating that the contribution of low molecular weight and heterocyclic compounds generally increases during the process of WSF formation.

Although the identifications of almost all compounds were confirmed using mass spectral data, this could not be done for isoquinolines, methyl quinolines and cresol in the whole creosote sample. In whole creosote, these compounds collectively contributed only 1% of total AC, and were not detected on the mass spectrometer, due to the low sensitivity of this instrument to heterocyclic compounds. These peaks were identified solely on the basis of retention time from the FID data (derived from the creosote WSF runs, which had been MS confirmed).

Discussion

Recoveries of high molecular weight compounds from spiked water samples were low compared to intermediate and low molecular weight compounds (Table 1). However, multiple surrogate standards were added in order to account for such losses. High molecular weight compounds also did not contribute much (only 5% or less) to total AC concentration of water samples. Use of recovery data from high molecular weight standards

Table 1. Recoveries of compounds from water spike experiment (N=3).

Compound	Mean Recovery (%)	Coefficient of variation
1. D8-Naphthalene	76	23
2. Naphthalene	83	12
3. 2-Methylnaphthalene	80	13
4. Biphenyl	83	13
5. 2,6-Dimethyl naphthalene	85	19
6. Acenaphthylene	120	31
7. D10-Acenaphthene	74	21
8. Acenaphthene	85	14
9. 1,6,7-Trimethylnaphthalene	85	14
10. Fluorene	88	15
11. D10-Phenanthrene	100	15
12. Phenanthrene	58	10
13. Anthracene	89	16
14. 1-Methyl Phenanthrene	97	18
15. Fluoranthene	97	18
16. Pyrene	96	14
17. Benzanthracene	79	6.0
18. D12-Chrysene	53	6.0
19. Chrysene	62	6.0
20. Benzo (b) fluoranthene	41	13
21. Benzo (k) fluoranthene	36	12
22. Benzo (e) pyrene	36	14
23. Benzo (a) pyrene	30	6.0
24. D12-perylene	35	10
25. Indeno (1,2,3) pyrene	23	9.0
26. Dibenz (a,h) anthracene	23	7.0
27. Benzo (ghi) perylene	25	8.0

Fig 3. Analytical Procedure.

ANALYTICAL PROCEDURE

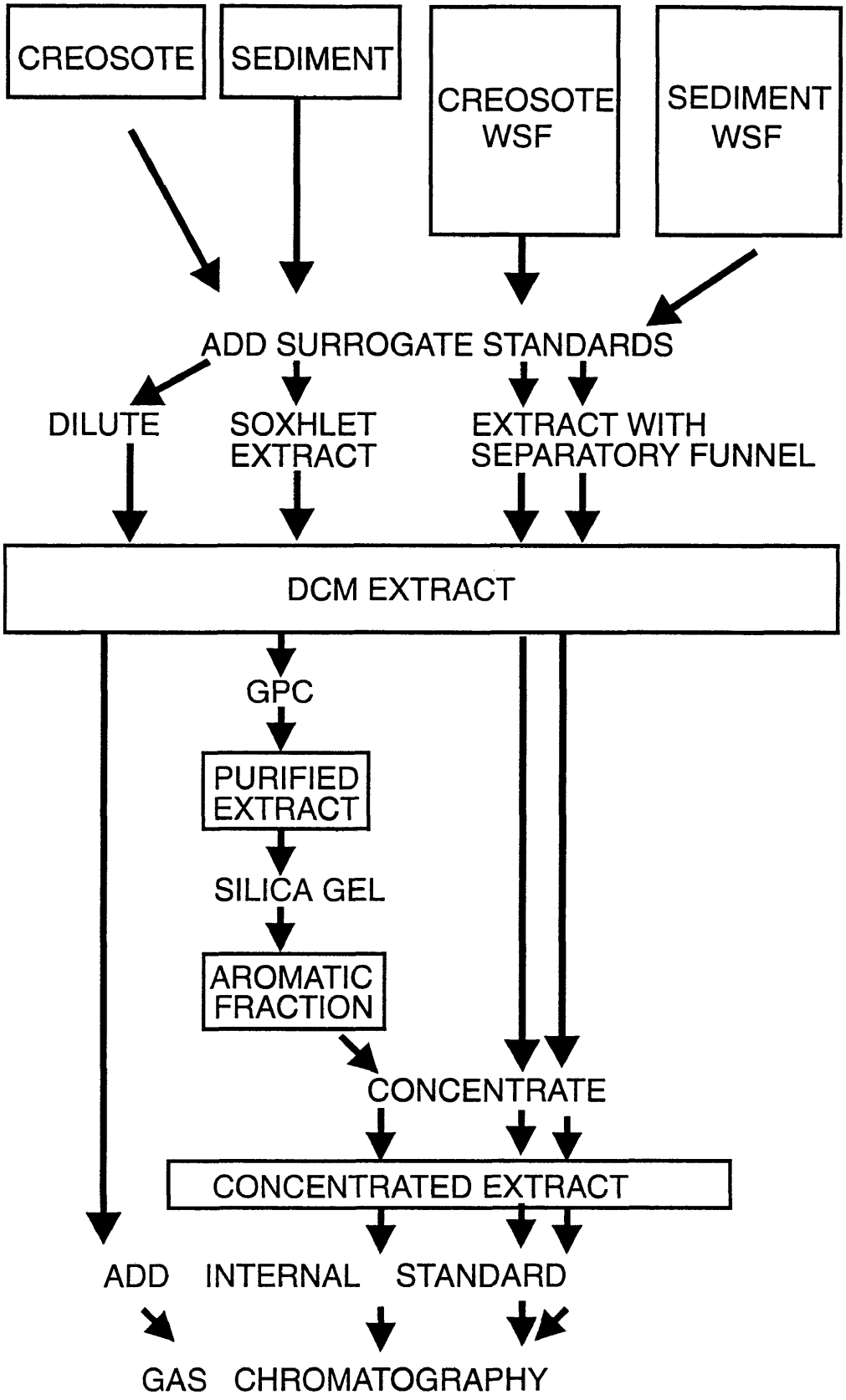


Fig 4. Comparison of dominant ACs determined in creosote and its water soluble fraction (abundances of compounds are expressed as % of total resolved AC).

COMPARISON OF CREOSOTE AND ITS WATER SOLUBLE FRACTION

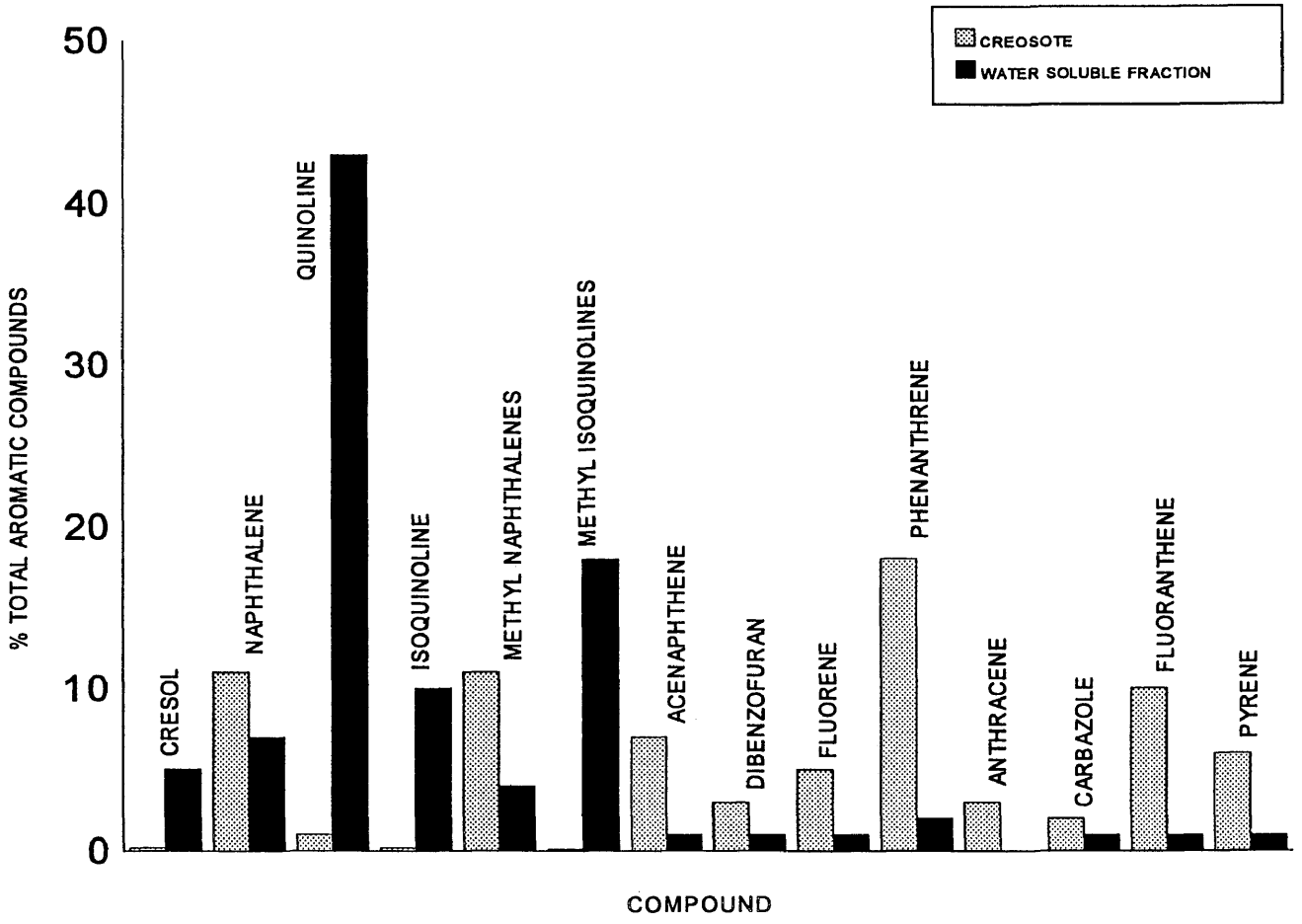


Fig 5. Comparison of dominant ACs determined in Elizabeth River sediment and its WSF (abundances of compounds are expressed in terms of % total resolved AC).

COMPARISON OF SEDIMENT AND ITS WATER SOLUBLE FRACTION

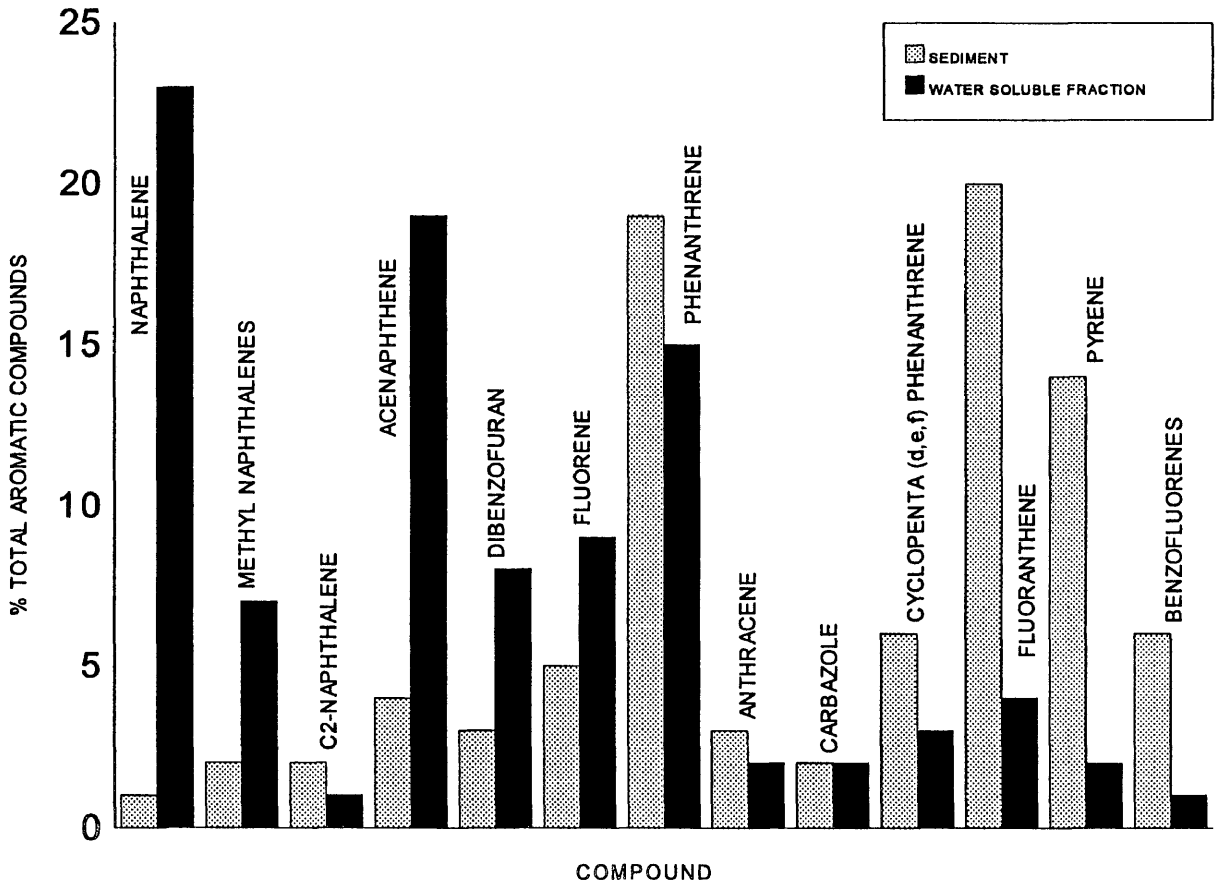
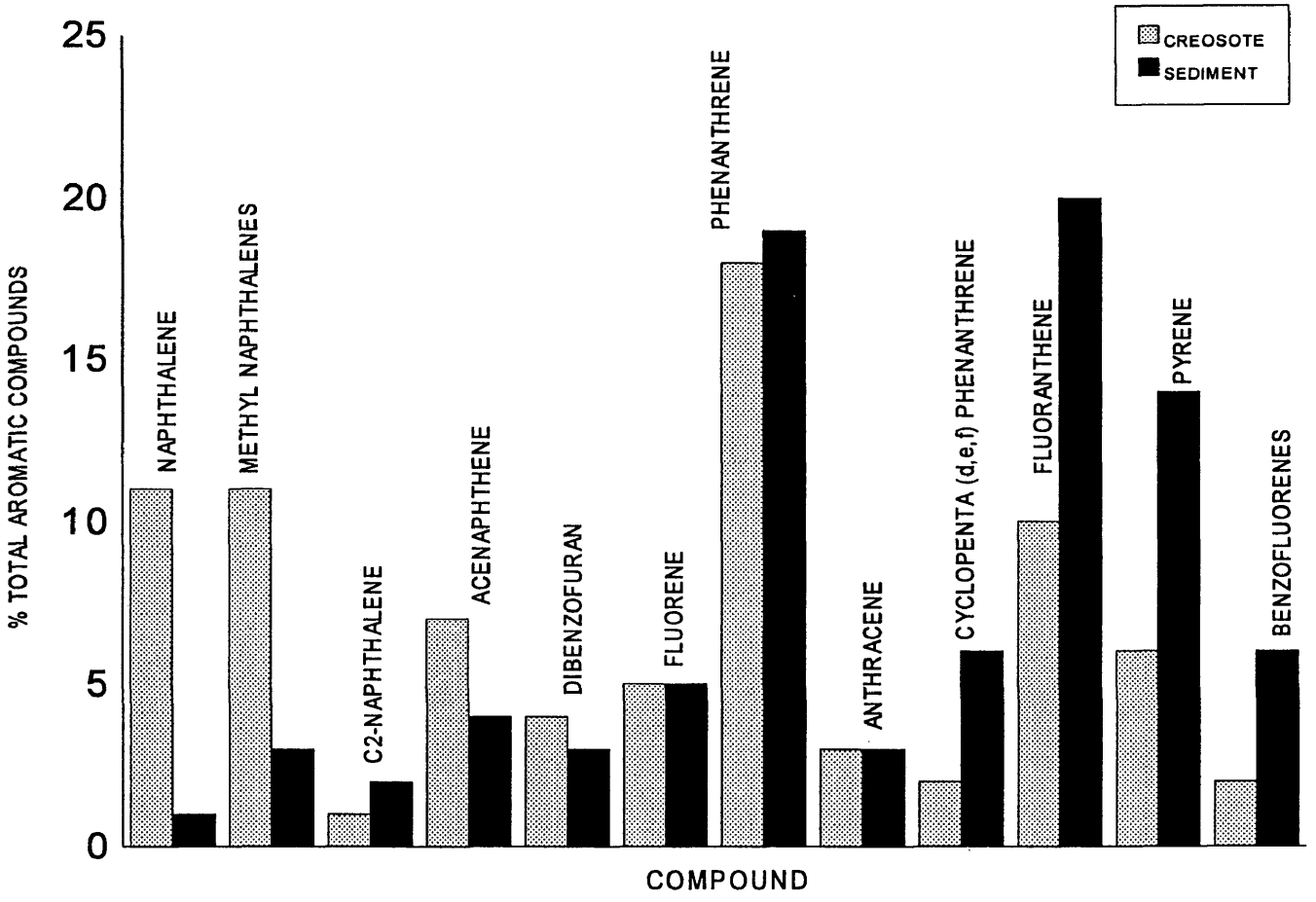


Fig 6. Comparison of dominant ACs determined in creosote with those in creosote-contaminated Elizabeth River sediment (abundances of compounds are expressed as % of total resolved AC).

COMPARISON OF CREOSOTE AND SEDIMENT



should have significantly reduced any bias that may have been introduced by differences in the analytical procedures to which the samples were subjected.

Creosote is defined by the processes by which it is produced, rather than its composition. Thus it may vary slightly from one manufacture to another (Peters and Luthy, 1993). However, the general composition of creosote is known and does not vary greatly. Fewer than 20 of its components individually contribute more than 1% to the total. The composition of the sample used in this study was similar in composition to samples previously described e.g., by Mueller *et al.* (1989).

The composition of the ERS was similar to that of sediments analyzed previously by others (Huggett *et al.*, 1992, Roberts *et al.*, 1989). Phenanthrene, fluoranthene and pyrene were the most abundant of the identified AC. These compounds have previously been identified as the most abundant AC in this sediment (Hargis *et al.*, 1984, Huggett *et al.*, 1992, Roberts *et al.*, 1989). Analysis of the sediment confirms that creosote is the major contaminant. However, contamination from other hydrocarbon pollutants may have further contributed to the levels of high molecular weight ACs present.

Weathering and degradation may have reduced some creosote-derived volatile components of the sediment

used in this study. Naphthalenes were present in the sediment, but were at lower concentrations than in whole creosote. Cresol, a phenolic compound, was identified in the creosote, but not found in the sediment. Phenolic compounds can be degraded both aerobically and anaerobically, and are relatively water soluble. Hence, they are not highly persistent in sediment (Enzminger and Ahlert, 1987). Quinolines were not detected in the sediment. Half lives for these compounds in sediment are also relatively short (Yen *et al* 1991). Higher molecular weight nitrogen heterocyclics, such as carbazole, are more persistent (Mueller *et al.*, 1989) and were present in the sediment.

During WSF generation, low molecular weight and polar compounds selectively enter the aqueous phase (Anderson *et al.*, 1974). AC solubility increases with decreasing size (Neff, 1979). The presence of nitrogen, sulphur or oxygen results in increased solubility of heterocyclics, compared to PAH of similar size and structure. High molecular weight AC have low aqueous solubilities. Mueller *et al.* (1989) reported solubility values as low as 2 ug/l for chrysene, 3 ug/l for benzo(a)pyrene and 7 ug/l for anthracene.

Factors other than solubility may also influence the composition of a WSF. For instance, dissolved

organic matter may increase the apparent solubility of some hydrophobic pollutants by binding to them and thus reduce its bioavailability and toxic effects (Jaffe, 1991).

The data presented here mimic the partitioning of creosote in the environment. Immediately after a creosote spill, water soluble components, such as those seen in the creosote WSF are likely to be present in elevated concentrations. This could lead to mortality in the water column. After low molecular weight components are lost from the system, long-term effects of the mixture may continue to be observed as a result of the more persistent sediment-sorbed pollutants. Storms, anthropogenic activity and bioturbation are processes that can resuspend contaminated sediments, creating environments where WSFs may be formed.

Chemical and physical fractionation of a mixture can result in varying exposures of biota as a function of their ecological niche (Knezovich *et al.*, 1987). Differences in the life history stage of an organism may also result in differential exposure.

CHAPTER III

SHORT-TERM TOXICITY OF WATER SOLUBLE FRACTIONS

Introduction

The first step towards establishing the toxicity of a chemical is the measurement of short-term lethal effects. This is usually expressed in terms of the concentration that causes death in 50% of the population over a specified period of time, such as 48h LC-50. The objective of this part of the study was to compare the short-term lethal effect of WSFs generated from whole creosote and field-collected Elizabeth River sediment to mysids.

Materials and methods

Stock WSFs were prepared as described in Chapter II. The stocks were serially diluted to achieve the desired treatment concentrations. Based on results from range finding tests, four concentrations of creosote WSF (0.03%, 0.009%, 0.0027%, 0.00081%) and four concentrations of ERS WSF (30%, 9.0%, 2.7%, 0.81%) were selected for the study.

A single batch of bay mysid (*Mysidopsis bahia*)

neonates were harvested from the VIMS mysid culture, using the method described by Breteler *et al.*(1982). About 600 adults were placed in harvest baskets and subjected to a sudden temperature increase of 2°C, from 26°C to 28°C. Neonates released from brood pouches of females were collected 24h later. They were grown in artificial sea water (20 g/l) and fed live Artemia (Great Salt Lake strain) until five days of age. They were then moved into the test room and allowed to acclimate to test conditions 24 h before starting the test.

A total of five treatments and one set of controls were used for each WSF. Test organisms were held in 12.9 cm diameter finger bowls. Treatments and replicates were randomly assigned to locations on the test table. There were three replicates per treatment and five mysids per replicate (five mysids per bowl). Mysids were randomly assigned to bowls and bowls were randomly assigned to the treatments. The duration of the experiment was 48h. Solutions were renewed after 24h. Salinity, pH, dissolved oxygen and temperature were measured for one randomly chosen bowl for the lowest, highest and control treatments at the start of the test, at 24h and 48h later. Maximum and minimum temperatures in the test room were recorded. Nitrites were measured in controls to ensure that toxicity due

to nitrite accumulation did not occur and confound results. The photoperiod was controlled (16h daylight and 8h dark). Each mysid was fed ca. 300 live Artemia nauplii per day (ca. 1500 Artemia per bowl per day). Survival was visually monitored at 24h intervals. Samples from the stock, control and highest and lowest concentration treatments were taken each day for chemical analysis. The samples were extracted daily with DCM and analyzed as described in Chapter I.

Data were fitted to probit (Finney, 1971) and simple linear regression models. Residuals were also plotted against the independent variable to determine if the model adequately fit the data. Data was then transformed if required. The pure error lack-of-fit test was conducted to test for randomness in the residuals. Residuals were also checked for normality using normal probability plots.

Results

The 48 h LC-50 for the ERS WSF was 700 ug total resolved AC or 23% WSF (Fig 7). Residuals from the probit analysis of ERS WSF survival data were randomly distributed about the dose, indicating that this model provided a good fit to the data. A simple linear regression also fit these data. The R^2 from the regression was high (76.5%) indicating that a linear

model also fit the data well (Fig 8 a).

The 48 h LC-50 for the creosote WSF was 180 ug total resolved AC or 0.0016% WSF (Fig 7). A plot (not shown) of residuals from this analysis against the independent variable showed that the model fit the data more closely at extreme than at intermediate values. On visual inspection, these data had a steep slope at the lower doses, rising abruptly to an asymptote. Data were coded, log transformed and fitted to a linear regression model. Transformation of data did not result in a significantly improved fit to this model. Data were then split into two parts, and separate linear regressions were performed for the lowest and highest three doses. The use of two separate regression equations for the two halves of data resulted in high R^2 values (87.7% and 66.9%). Analysis of residuals indicated that this provided a good fit of data to the model (Fig 8 b).

Dissolved oxygen was above 60% of saturation in all treatments. Variation in temperature was within 2°C (Appendix 2).

Expected concentrations for total identified AC in the stock, high and low treatments were calculated based on the measured concentration of compounds in the stock (Table 2). The total concentrations for identified ACs at the LC-50 were estimated to be 180

Fig 7. Probit plots of WSF exposures.

PROBIT ANALYSIS ON MORTALITY DATA

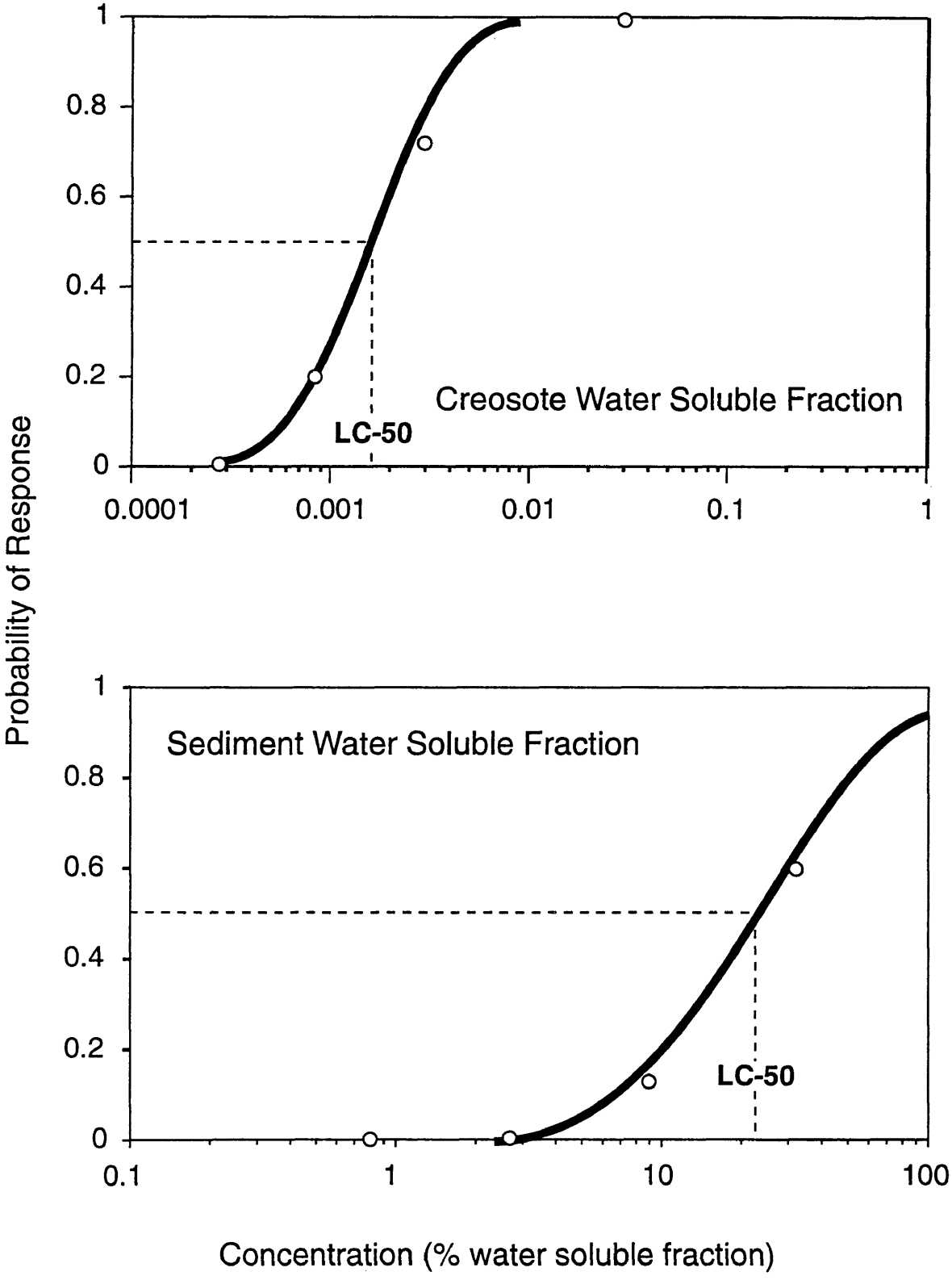


Fig 8 a. Regression plot for ERS
WSF exposure.

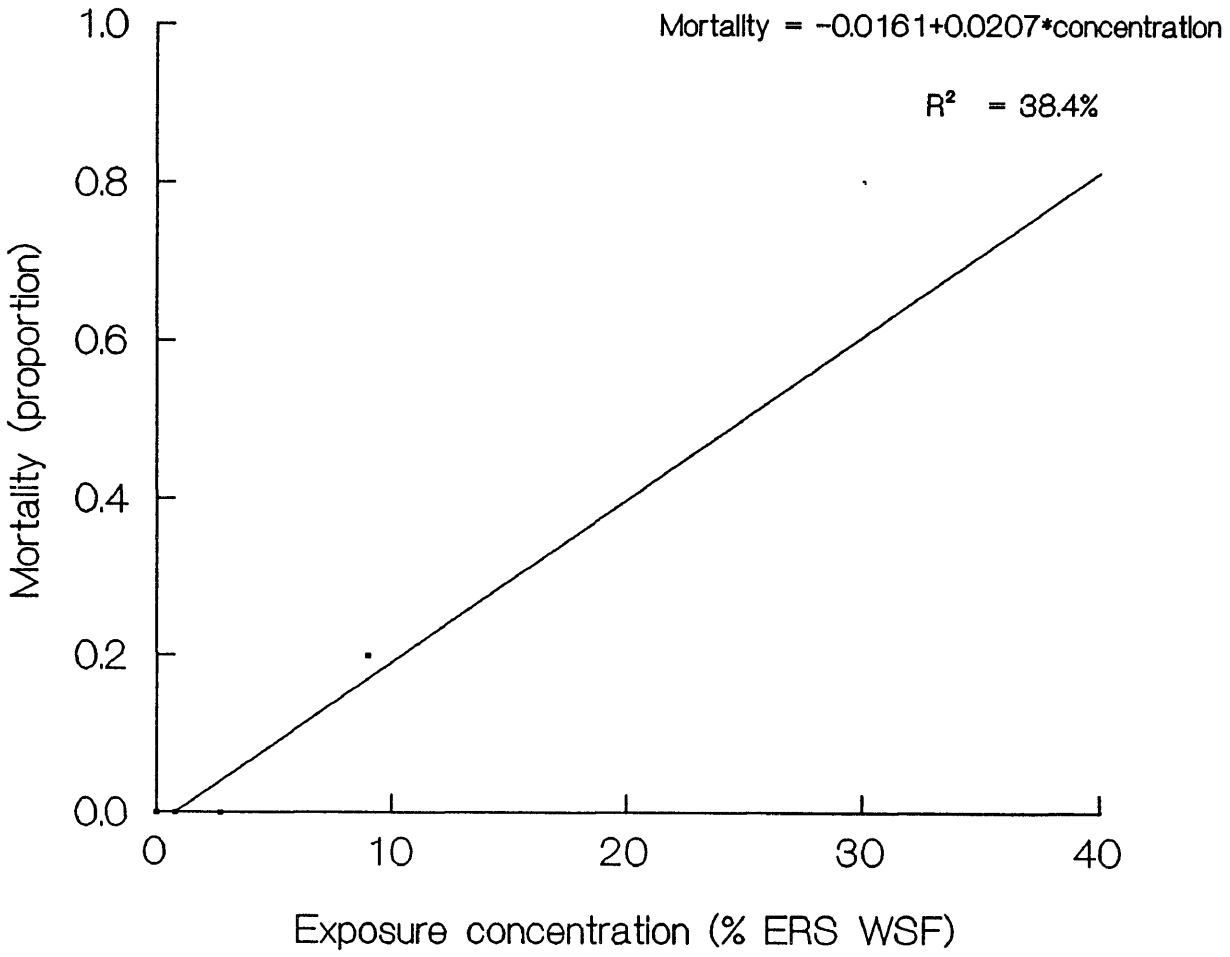
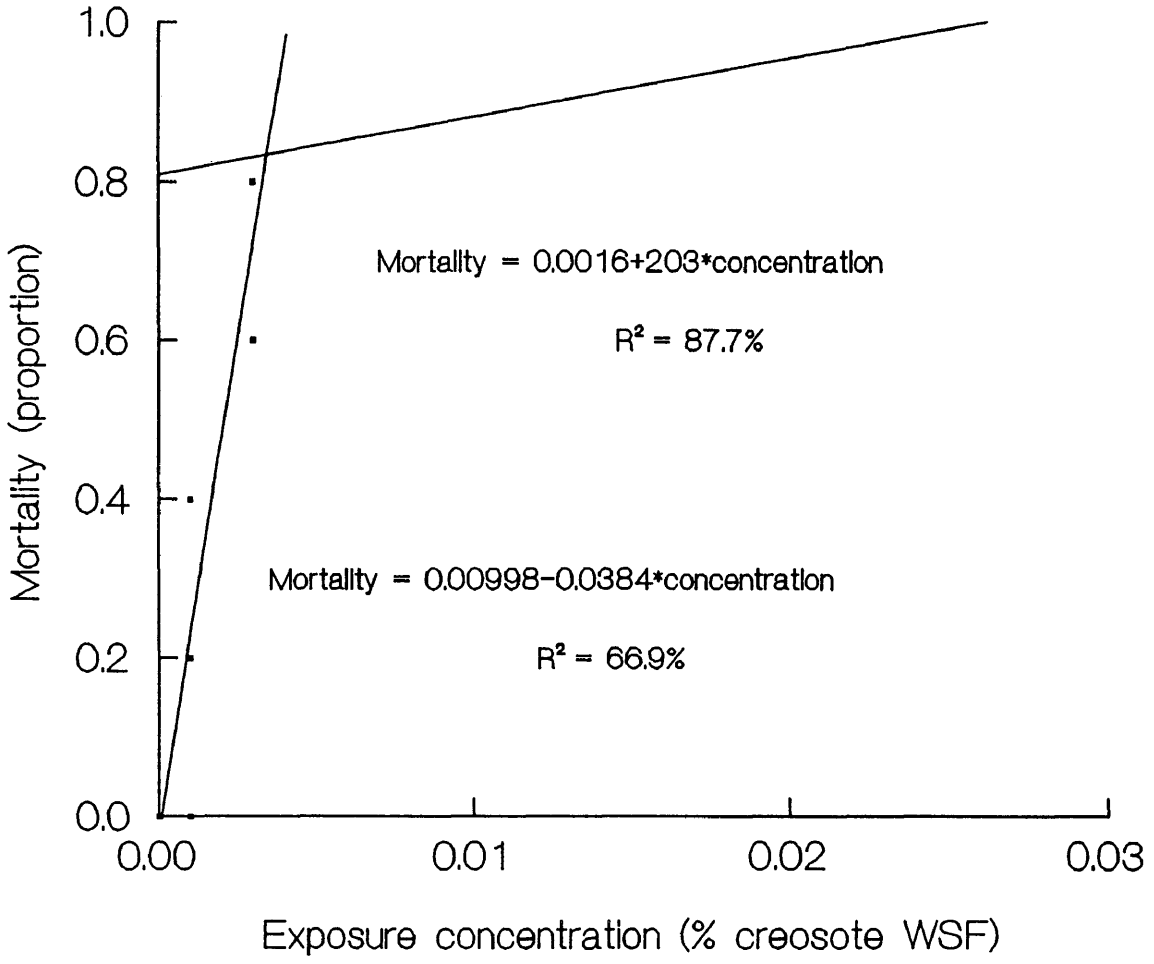

Mortality = -0.0161+0.0207*concentration
R² = 38.4%
1.0
0.8
0.6
0.4
0.2
0.0
Mortality (proportion)
0
10
20
30
40
Exposure concentration (% ERS WSF)

Fig 8 b. Regression plot for creosote WSF exposure.


1.0
0.8
0.6
0.4
0.2
0.0
Mortality (proportion)
Mortality = 0.0016+203*concentration
R² = 87.7%
Mortality = 0.00998-0.0384*concentration
R² = 66.9%
0.00
0.01
0.02
0.03
Exposure concentration (% creosote WSF)

ug/l and 700 ug/l, respectively, for the creosote WSF and ERS WSF. The creosote WSF stock contained a much larger concentration (110,000 ug/l) of total AC than the ERS WSF (3100 ug/l). Thus the WSF percentage for ERS and creosote do not correlate with each other exactly, i.e. a 1% creosote WSF treatment would contain more AC than a 1% ERS treatment. Surrogate standards were recovered in the controls, but no ACs were identified, indicating that the dilution water used was free of contamination.

Important qualitative and quantitative differences were observed based on the GC-FID analysis of the stock WSFs (Appendix 1). The relative concentrations of dominant compounds in each WSF, that accounted for more than 85% of all identified peaks, are compared in Fig 9. Naphthalene, 1-methyl naphthalene, phenanthrene, carbazole, acenaphthene, fluorene, fluoranthene and dibenzofuran were among the top ten for both WSFs. The creosote WSF also contained quinoline, isoquinoline, 2-methyl isoquinoline and cresol. These were absent from the ERS WSF.

The relative concentrations (expressed as percentage of total identified AC) for the various chemical classes of compounds are presented in Figure 10. Compounds were divided into four categories: low molecular weight (one or two aromatic rings),

intermediate molecular weight (three aromatic rings), high molecular weight (four or more aromatic rings), and heterocyclics (sulphur, oxygen or nitrogen containing compounds). The ERS WSF contained a greater proportion of aromatic hydrocarbons, including benzopyrenes and benzofluorenes, that were not identified in the creosote WSF. In contrast, the creosote WSF contained much higher proportions of heterocyclic compounds. Quinolines contributed significantly to the heterocyclics in whole creosote. Benzothiophenes, benzofuran and carbazole were the major heterocyclic compounds identified in the ERS WSF.

In each WSF, fewer than 12 ACs collectively accounted for 85% or more of the total resolved AC. Most compounds were present at concentrations close to or above their nominal concentrations (Table 3 a, b). However, in the ERS WSF, naphthalene was greatly reduced in the first dilution. This loss was not observed in the creosote WSF. Some of the quinolines, which are relatively volatile compounds, appear to have been lost during progressive dilutions of the creosote WSF and were absent from the lowest treatment.

Discussion

The nominal AC concentration in treatments was approximately equal to measured total AC concentrations

Table 2. Total AC concentrations of WSFs (ug/l) for the 48h test. Expected concentrations were calculated based on measured concentrations in the stock. (NA = not applicable).

TREATMENT	MEASURED	EXPECTED
ERS STOCK (100%)	3100	NA
ERS HIGH (30%)	1400	930
ERS LOW (0.81%)	26	25
CRE STOCK (1%)	110,000	NA
CRE HIGH (.03%)	5200	3400
CRE LOW (.00081%)	150	92

Table 3 a. Expected and measured concentrations for dominant compounds detected in the ERS WSF treatments (ug/l). Expected concentrations were calculated based on measured concentrations in the stock.

	HIGHEST TREATMENT (30% WSF)		LOWEST TREATMENT (0.81% WSF)	
COMPOUND	EXPECTED	MEASURED	EXPECTED	MEASURED
1. Naphthalene	210	14	5.7	3.5
2. Methyl naphthalenes	65	63	1.7	4.6
3. Acenaphthene	180	360	4.8	3.5
4. Dibenzofuran	78	150	2.1	0.51
5. Fluorene	82	200	2.2	5.8
6. Phenanthrene	140	290	3.8	2.3
7. Carbazole	22	12	0.58	0.52
8. Cyclopenta (d,e,f) phenanthrene	27	45	0.72	0.72
9. Fluoranthene	38	76	1.0	0.98
10. Pyrene	21	42	0.56	0.52

Table 3 b. Expected and measured concentrations (ug/l) for dominant peaks in the creosote WSF treatments. Expected concentrations were calculated based on measured concentrations in the stock.

	HIGHEST TREATMENT (0.03% WSF)		LOWEST TREATMENT (0.00081% WSF)	
COMPOUND	EXPECTED	MEASURED	EXPECTED	MEASURED
1. Cresol	180	520	4.8	<0.03
2. Naphthalene	220	1400	6.0	22
3. Quinoline	1500	1600	40	5.0
4. Isoquinoline	320	340	8.7	<0.03
5. Methyl isoquinolines	640	740	17	10.2
6. Methyl naphthalenes	140	150	3.7	4.2
7. Acenaphthene	39	49	1.0	14
8. Dibenzofuran	29	22	0.78	3.6
9. Fluorene	30	30	0.80	8.7
10. Phenanthrene	60	100	1.6	35
11. Carbazole	45	49	1.2	1.3
12. Fluoranthene	28	40	1	16.6

Fig 9. A comparison of the dominant ACs determined in the WSFs (expressed as % of total resolved AC).

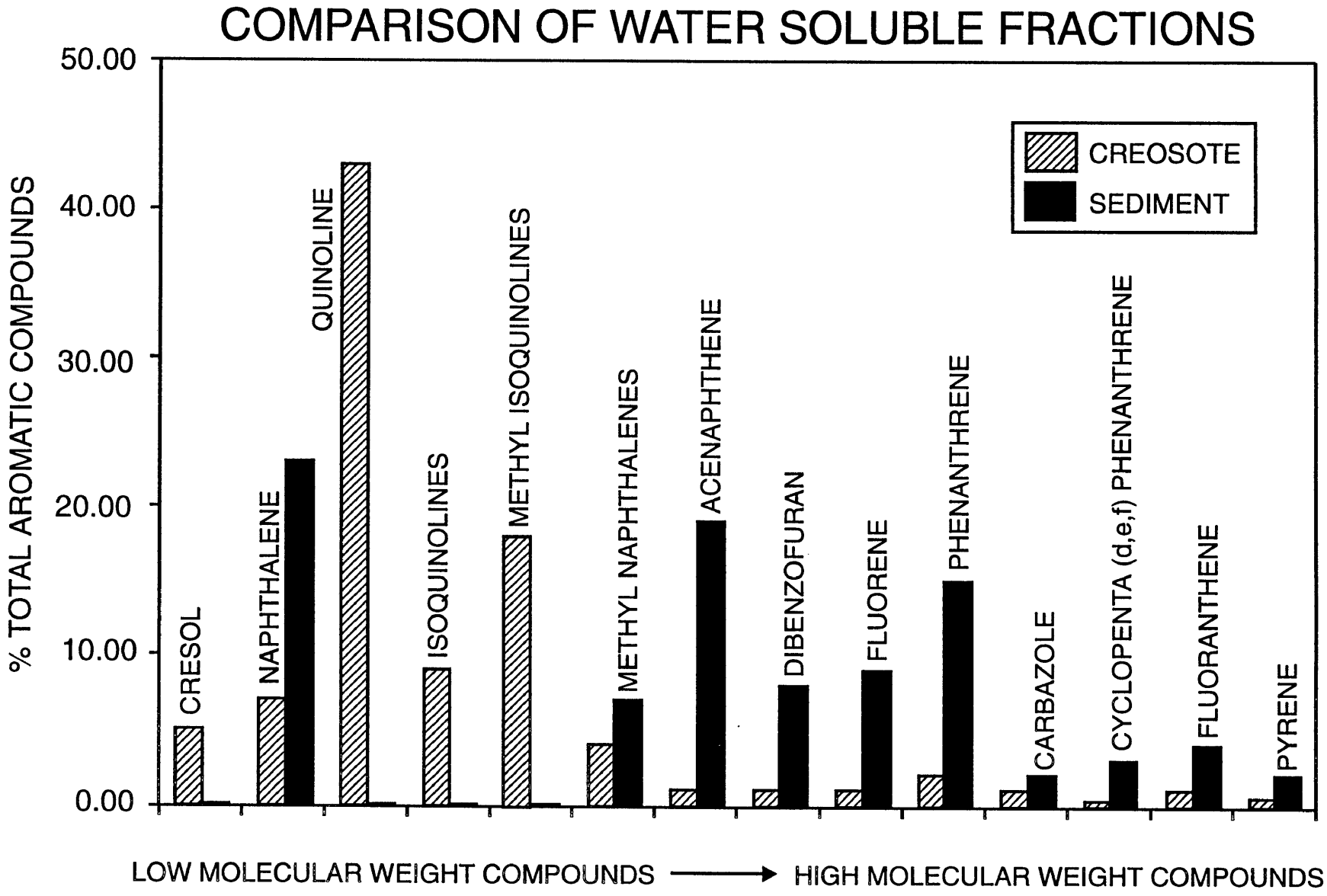
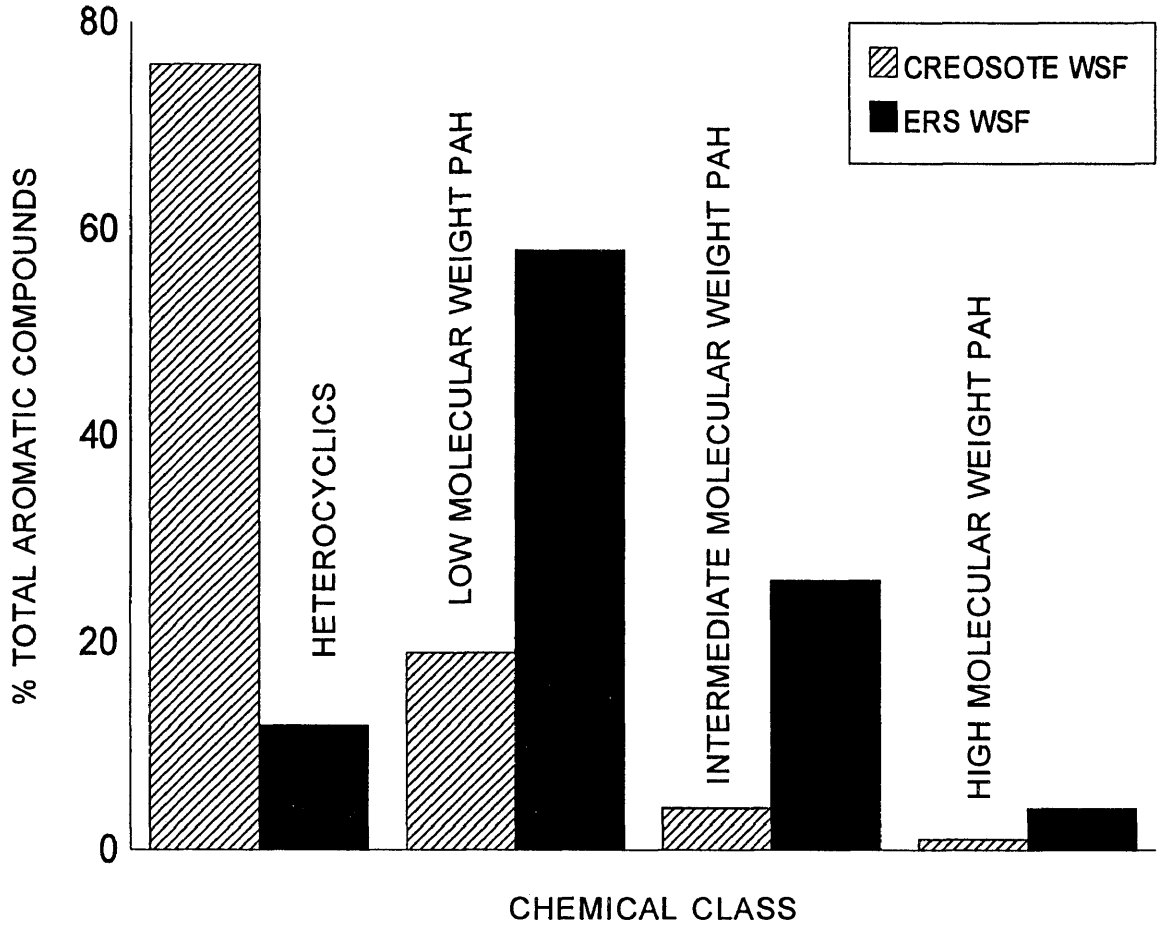

COMPARISON OF WATER SOLUBLE FRACTIONS
% TOTAL AROMATIC COMPOUNDS
50.00
40.00
30.00
20.00
10.00
0.00
CREOSOTE
SEDIMENT
CRESOL
NAPHTHALENE
QUINOLINE
ISOQUINOLINES
METHYL ISOQUINOLINES
METHYL NAPHTHALENES
ACENAPHTHENE
DIBENZOFURAN
FLUORENE
PHENANTHRENE
CARBAZOLE
CYCLOPENTA (d,e,f) PHENANTHRENE
FLUORANTHENE
PYRENE
LOW MOLECULAR WEIGHT COMPOUNDS
HIGH MOLECULAR WEIGHT COMPOUNDS

Figure 10. Relative abundances of chemical classes in the WSFs (expressed as percentage of total resolved AC).

COMPARISON OF CHEMICAL CLASSES



in low and high treatments for both WSFs, indicating that dilutions achieved a systematic reduction in total AC levels. The total AC concentrations of WSFs used in this study (14 ug/l to 5200 ug/l) broadly bracket hydrocarbon concentrations previously reported in polluted areas. Hydrocarbon levels of 200 to 400 ug/l have been reported in the vicinity of oil spills by McAuliffe *et al.* (1975) and Grahl-Nielsen (1978), cited in Pearson *et al.* (1981).

One factor affecting the toxicity of a mixture is the total abundance of ACs. Munoz and Tarazona (1993) reported that ACs at concentrations of 300 ug/l caused mortality in marine species. The LC50 values for creosote and ERS WSF based on total AC concentrations were 180 and 700 ug/l. These values are comparable to those previously reported for *Mysidopsis* species. exposed to AC mixtures. Borthwick and Patrick (1982) reported a 96 h LC50 of 810 ug/l for *M. bahia,* exposed to marine grade creosote dissolved in a tri-ethylene glycol/water mixture. Anderson *et al.* (1974) exposed *Mysidopsis almyra* to various oils and determined that the 48 h LC50 for Bunker C Residual and No.2 Fuel Oil was 900 ug/l.

Total AC concentration is not the only factor affecting mortality. The AC concentration of the ERS WSF at its LC50 is higher than that of the creosote

WSF. This implies that, at a given concentration, the creosote WSF is more toxic than the ERS WSF. This is probably due to qualitative differences between them.

PAH with three or less aromatic rings have a greater short-term effect on aquatic biota than PAH with four or more aromatic rings (Neff, 1979). The ERS WSF had higher proportions of PAH with four or more aromatic rings (4% of total) than the creosote WSF (1% of total). Since the ERS WSF was less toxic, this supports the view that high molecular weight PAH have less influence on short-term lethality.

Amongst the low molecular weight PAH, however, toxicity may increase with increasing size. Naphthalene and acenaphthene have been reported to possess short-term toxicities lower than phenanthrene and anthracene (Esdall 1991; Smith *et al.*, 1986; Munoz and Tarazona, 1993).

A major difference between the WSFs was their proportion of heterocyclic compounds. These constituted the most abundant class of compounds determined in the creosote WSF, but were the second lowest in abundance in the ERS WSF. When heterocyclics are subdivided into high molecular weight (carbazole, dibenzothiophene, dibenzofuran) and low molecular weight (quinolines, isoquinolines, methyl quinolines and dimethyl quinolines), there is a striking difference between the

WSFs (Fig 9). Quinolines make a substantial contribution to total AC in the creosote WSF (71% quinolines in stock, 50% quinolines in high and 10% in low treatments), but are undetectable in the ERS WSF. It is possible that these low molecular weight nitrogen heterocyclics contributed to the greater toxicity of the creosote WSF.

Nitrogen-containing heterocyclics are known to be highly toxic and some are mutagenic (Enzminger and Ahlert, 1987). Birkholz *et al.* (1990) tested the aquatic toxicity of alkyl quinolines using the microtox assay and a static 48 h exposure to fish (*Salmo gairdneri*). This study demonstrated that toxicities varied up to two orders of magnitude and were influenced by the molecular structure and degree of substitution of the compound. Dimethyl quinolines, without a 2-substitution, were found to be particularly toxic.

Compounds that could not be identified by the analytical techniques used may also have contributed to the difference in lethal effects between the WSFs. Included in this category are relatively volatile compounds such as benzene, phenols, toluenes and xylenes. These chemicals have been previously identified in creosote (Peters and Luthy, 1993).

There are two probable reasons for this altered

toxicity. It is possible that some of the toxicants, particularly those that are lipophilic, may be bound to organic matter, altering bioavailability (Knezovich *et al.*, 1987). Altered toxicity may also result from antagonistic or synergistic interactions among compounds in the WSF. Munoz and Tarazona, (1993) reported antagonistic interactions in two-component mixtures of phenanthrene with naphthalene and phenanthrene with anthracene; and synergistic effects in four-component mixtures containing these and acenaphthene. It is presently not possible to accurately predict the outcome of processes that can occur in a mixture such as creosote, which contains more than a 100 individual chemicals.

CHAPTER IV

SUBLETHAL EFFECTS OF ELIZABETH RIVER SEDIMENT WATER SOLUBLE FRACTION

Introduction

A toxicant can exert a wide range of biological effects, ranging from cellular to whole organism to population to system level responses. The type of effect depends on exposure duration and concentration of the toxicant, as well as other factors such as the organism's susceptibility. A toxicant that does not exert a lethal effect may still adversely affect organisms by interfering with major life processes such as reproduction, growth and behavior.

The objective of this part of the study was to examine the long-term sublethal effects of the ERS WSF on growth and reproduction of *Mysidopsis bahia*. Reduced growth or reproduction can have important ecological consequences (Nimmo and Hamaker, 1982). Dry weight gain (a measure of growth), proportion of egg bearing females (an indicator of reproductive health) and molt frequency of the test animals were the endpoints examined in this study.

Materials and Methods

Preparation of the ERS WSF and chemical analysis were conducted as outlined previously. The test was similar to that described in Chapter III. Five ERS WSF treatments were used (10%, 3%, 0.9%, 0.27% and 0.08%). Seven day-old juveniles were exposed to toxicants in finger bowls (12.9 cm diameter). There were eight replicates in each treatment combination, with five mysids per replicate. Treatments and animals were randomly assigned to bowls. The exposures were carried out for seven days. Solutions were renewed daily. Dissolved oxygen, temperature, pH and salinity were measured daily in one randomly chosen replicate at the highest, lowest and control treatments prior to solution renewal. Maximum and minimum temperatures in the test room were recorded. The experiment was terminated after seven days. Mysids from each replicate were examined under the microscope and classified as males, egg-bearing females, egg-less females or juveniles. The mysids were than placed in previously weighed aluminum boats (one boat per replicate), baked at ca. 70°C for 24h and cooled in a desiccator. Dry weights of the mysids were determined to the nearest microgram using a Kahn electromagnetic balance.

Biological data from each end point measured were first visually examined by plotting against dose.

Normality and homogeneity of variance were formally tested using the Shapiro-Wilkes test for normality and both Bartlett's and Cochran's tests for homogeneity of variance at the P=0.01 level. Sexual maturity data (proportion of egg-bearing females) were arc-sin square root transformed to meet the normality assumption. One-Way-ANOVAs were conducted on the data, and further analyses were performed on data which showed significance. One tailed tests (Dunnett's test or the Bonferroni t-test) were performed to determine if treatments differed significantly from controls. Regression analyses were also performed on the data (Fig 11 a, b). Residuals were examined to ensure randomness and normality, as described in the previous chapter. Probit analysis was performed on the data to estimate EC-50 (Fig 12).

Results

The relative abundances of the various chemical classes in the WSF used for the long-term exposure were similar to the ERS WSF used in the 48 h test (Table 4). The WSFs were also comparable in terms of total AC concentrations.

The total concentration of ACs in the highest treatment (10% WSF) was 200 ug/l (Table 5). The total concentration in the lowest treatment was 8.9 ug/l

Table 4. Abundance of major categories of chemicals found in the ERS WSFs used in 7-day exposure (expressed as % total resolved AC). (MW = molecular weight, Low MW = < 3 aromatic ring PAH, intermediate MW = 3 aromatic ring PAH, high MW = >3 aromatic ring PAH).

TREATMENT	LOW MW	INTERMEDIATE MW	HIGH MW	HETEROCYCLICS
ERS STOCK (7-day)	55	25	5	15
ERS STOCK (48 h)	58	26	4	12

Table 5. Total resolved AC concentrations in ug/l for Elizabeth River WSFs used in the 7-day exposure. Expected concentrations were calculated based on measured concentrations in the stock. (NA = not applicable).

TREATMENT	MEASURED	EXPECTED
ERS STOCK (100%)	1400	NA
ERS HIGH (10%)	200	140
ERS LOW (0.081%)	8.9	1.1

Table 6. Expected and measured concentrations for dominant compounds detected in the ERS WSF treatments (ug/l). Expected concentrations were calculated based on measured concentrations in the stock.

	HIGHEST TREATMENT (3% WSF)		LOWEST TREATMENT (0.81% WSF)	
COMPOUND	EXPECTED	MEASURED	EXPECTED	MEASURED
1. Naphthalene	7.1	12	0.06	0.46
2. Methyl naphthalenes	12	13	0.09	5.2
3. Acenaphthene	37	56	0.30	0.21
4. Dibenzofuran	14	14	0.12	0.38
5. Fluorene	17	19	0.14	0.51
6. Phenanthrene	28	34	0.23	0.52
7. Anthracene	3.0	4.4	0.02	< 0.03
8. Cyclopenta (d,e,f) phenanthrene	3.4	4.1	0.03	< 0.03
9. Fluoranthene	4.5	9.3	0.04	0.29
10. Pyrene	2.3	9.3	0.02	0.15

total AC. The quantitation limit achieved was 0.03 ug/l. Individual ACs at or above this amount were identified. No ACs were detected in controls. Table 6 gives the nominal and actual values for dominant AC in the stock, high and low concentration treatments. These compounds accounted for more than 85% of total ACs. A systematic reduction in levels was achieved with close agreement between expected and observed values for most compounds. Some compounds were higher than expected.

A One-Way-ANOVA was conducted on the weight data to test the hypothesis that mean mysid body weights in all treatments was equal. This resulted in rejection of the null hypothesis at the 0.05 level (Table 7 a). Dunnett's procedure was performed to test the hypothesis that mean weight in controls significantly differed from treatments. The mean weight in all toxicant concentrations differed significantly from the mean weight of the control (Table 7 b). The linear regression performed on the weight data was significant, with an R^2 value of 38.4 % (Fig 11 a). Residuals were normally distributed and randomly scattered about the independent variable. The pure error lack of fit test was not significant. This indicates that a linear dose-response relationship could be fitted for the doses examined.

Sexual maturity data were arc-sine square root

transformed to achieve normality. Since the number of females in each replicate was not the same, the proportion of egg-bearing females out of the total females within each replicate was analyzed (rather than the number of mature females). An ANOVA was conducted to test the hypothesis that the proportion of egg-bearing females was equal in all treatments. The data were significantly different (Table 8 a), leading to rejection of the null hypothesis. Bonferroni t-test indicated that the three highest treatments differed significantly from the control (Table 8 b). A probit model fit the data. The EC-50 for the ERS WSF, estimated from the probit analysis, was 1.1% WSF (Fig 12). A linear regression model also provided a significant fit to the data (Fig 11 b), but the R^2 for the regression was low.

Molt data were analyzed as a frequency of molt/animal/day. A One-Way-ANOVA confirmed the null hypothesis of no difference between treatments. The data were not significantly different (Table 9). A regression, which is a more powerful test, was also performed but was not significant.

Variations in salinity and pH were negligible between treatments (Appendix 2). Water temperature varied less than 2°C. Oxygen concentrations in all treatments were greater than 60% of saturation at the

test temperature.

Discussion

Chemical analysis indicates that the WSFs generated for the 48h and 7-day tests were comparable (Table 4). The mean total AC concentration at the highest treatment was 200 ug/l (Table 5). At the lowest concentration, surrogate standards were recovered but some AC, such as naphthalene and dibenzofuran, were not detected in all samples.

The results of the 7-day test indicate that the ERS WSF had sublethal effects on weight and reproduction. (The implicit assumption is made that the presence of more than one mysid within each replicate does not significantly affect the responses of the other individuals within the replicate). Sublethal effects that reduce reproductive success are particularly important, since even small reductions in this parameter may lead to large reductions in populations (Verberg *et al.*, 1978). A delay in the attainment of sexual maturity may directly impact a population by reducing its reproduction. Reduced growth can also impact a population's reproductive capacity. The number of eggs produced in five mysid species was related to the size of females (Jensen, 1958).

Somatic growth (measured as dry weight) was

significantly greater in the controls than in toxicant treatments (Dunnett's test, P=0.05). Mysids are probably the most sensitive estuarine species routinely used in toxicity testing (Lussier *et al.*, 1984, Borthwick and Patrick, 1982). In this study, the total AC concentration at the lowest exposure was extremely low (8.9 ug/l). Nonetheless, mysids were sensitive to toxicant even at this low exposure. The proportional reduction in weight compared to the control at the two lowest exposures was 10% (Fig 13).

The hypothesis that the proportion of egg-bearing females in controls was significantly different from treatments was confirmed using the Bonferroni-t-test (Table 8 b). The EC-50 for the sexual maturity data was estimated at 1.1% WSF using probit analysis. The estimated total AC level at this concentration was 15 ug/l.

The normal time for attainment of sexual maturity for *M. bahia* is 12-14 days (McKenney 1987, Lussier *et al.*, 1988). The data indicate that AC at low ug/l levels cause a definite delay in the attainment of sexual maturity, since the mysids were 14 days old at the termination of the test. It is possible that attainment of sexual maturity may actually have been inhibited, but mysids would have to be observed for a longer period of time to determine whether or not this

was the case.

Previous studies have demonstrated sublethal effects in the low ug/l range for PAH. Swartz *et al.* (1990) report that sublethal effects of fluoranthene can occur at concentrations as low as 8 ug/l. The mean concentrations of fluoranthene in the ERS high treatment was 9.3 ug/l. Proportional reduction in maturity at this treatment was 80% and weight was 40% lower than for control mysids (Fig 13). Although data from pure compound toxicity tests are not directly comparable to those attained with mixtures, they serve as an interesting frame of reference.

Measurement of growth and reproduction in previous tests have indicated close agreement between these two endpoints (Breteler *et al.*, 1982, Nimmo and Hamaker, 1982). The data from this study indicate that proportional reduction in sexual maturity at any treatment was greater than proportional reduction in weight (Fig 13), except at the lowest concentration where both end points were reduced equally (by 10%). This suggests that sexual maturity was more sensitive as an indicator than weight.

Bonferroni's test detected significant differences in maturity from controls only in the three highest treatments (Table 8 b). However, Dunnett's test detected a deviation in weight from controls, even at

the lowest toxicant exposure (Table 7 b). This may be an artifact of experimental design, since the more powerful Dunnett's test could not be used to analyze the sexual maturity data due to unequal sample size. An organism is faced with two options in terms of energy expenditure. It can invest in somatic growth or devote energy to reproduction. It is possible that at the lowest treatment, there was a greater investment in reproduction at the expense of vegetative growth, resulting in a non-significant departure from controls in maturity, but not in weight. At higher treatment concentrations the organisms were probably stressed to the extent that maturity could not be attained, resulting in greater proportional reduction in reproduction than in somatic growth.

Molting in certain crustaceans has been shown to be affected by the presence of hydrocarbons (e.g., Bookhout *et al.*, 1984). However, Percy *et al.* (1978) showed that exposure of isopods to crude oil WSFs at concentrations of 900 ug/l or higher, either had no effect, or failed to show a consistent effect on intermolt period. In this study, molting was not found to be significantly different among treatments (Table 9). The data also failed to fit a linear regression model. Plotting the data did not indicate any recognizable trend. Molting was a highly insensitive

Fig 11 a. Regression plot for weight data.

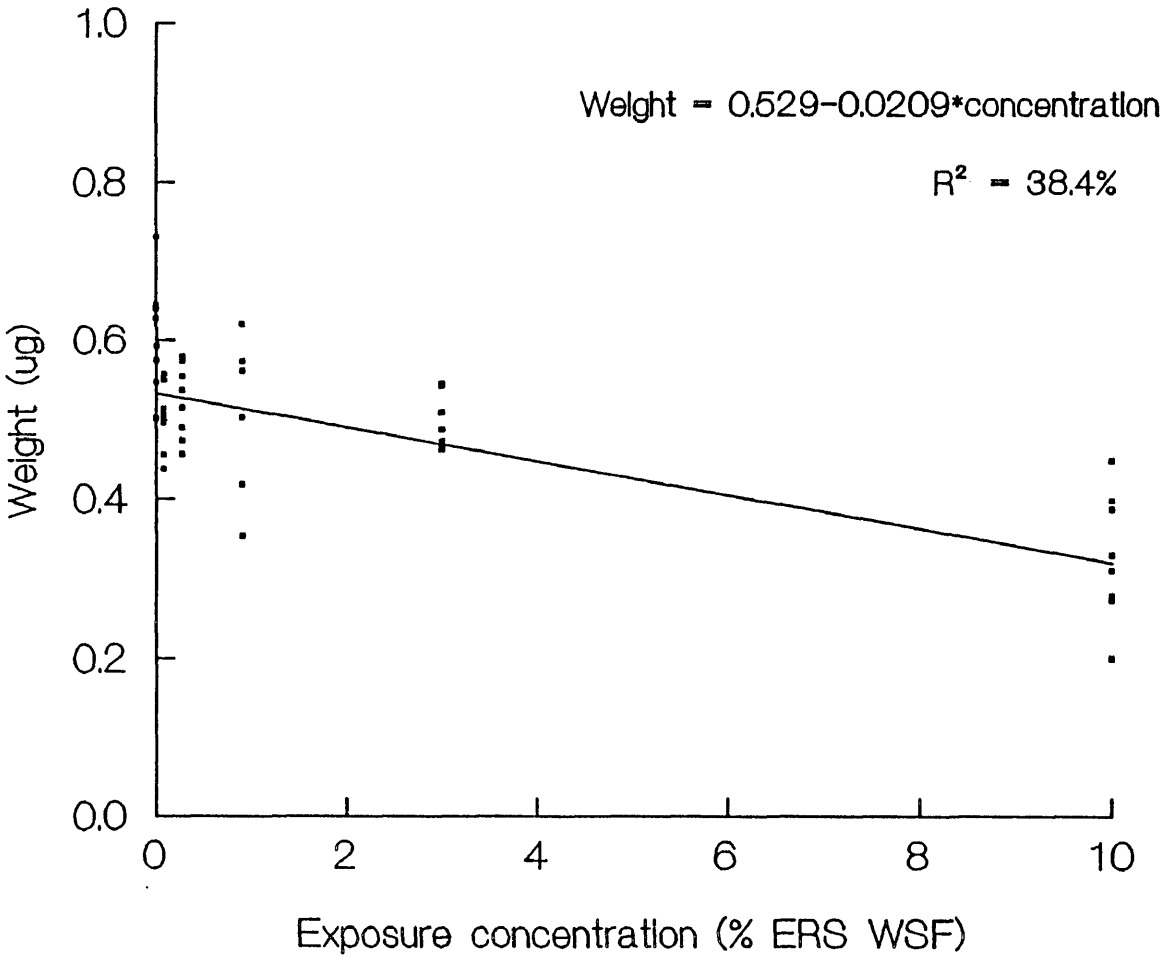

Weight = 0.529-0.0209*concentration
R^2 = 38.4%
Weight (ug)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
Exposure concentration (% ERS WSF)

Fig 11 b. Regression plot for maturity data.

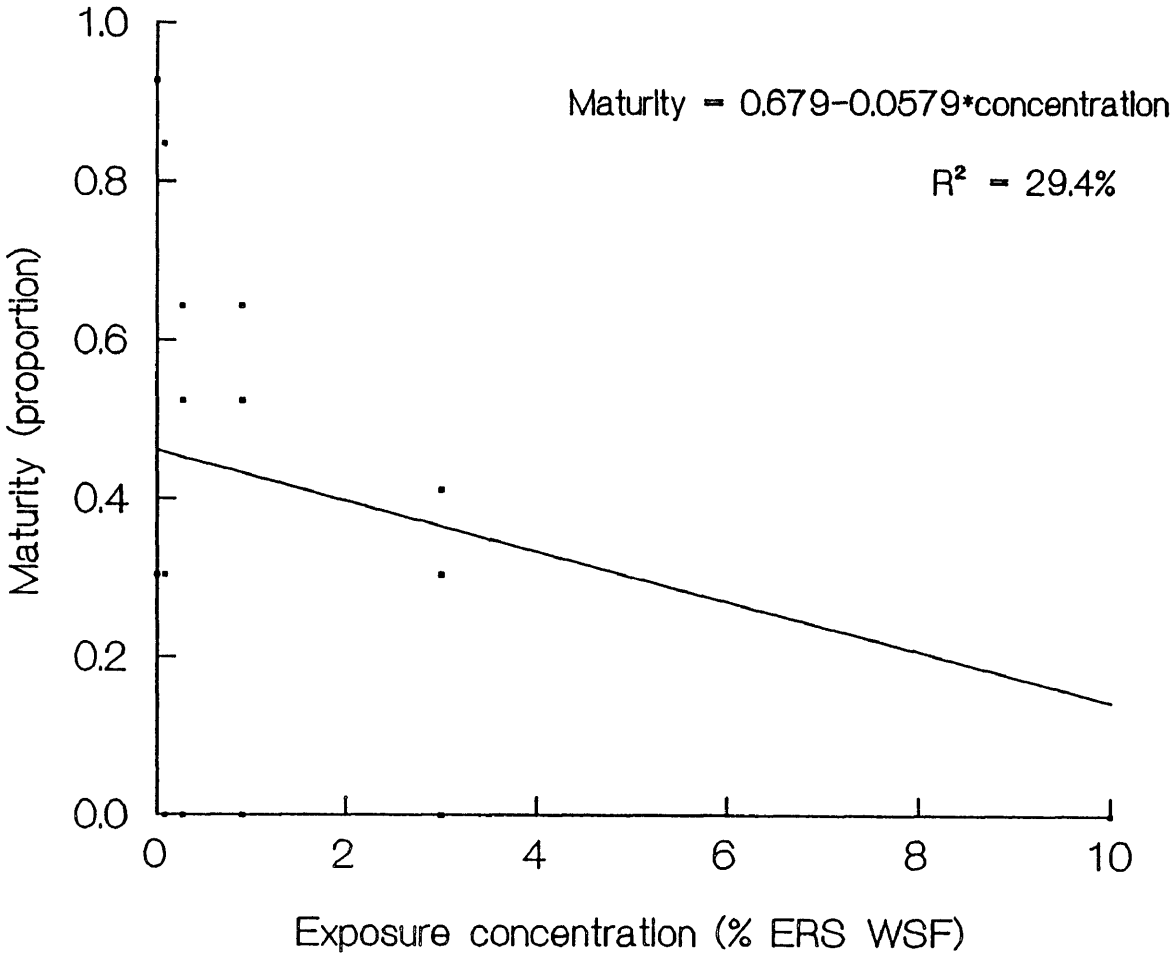

Maturity = 0.679-0.0579*concentration
R² = 29.4%
Maturity (proportion)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
Exposure concentration (% ERS WSF)

Fig 12. Probit analysis on maturity data.

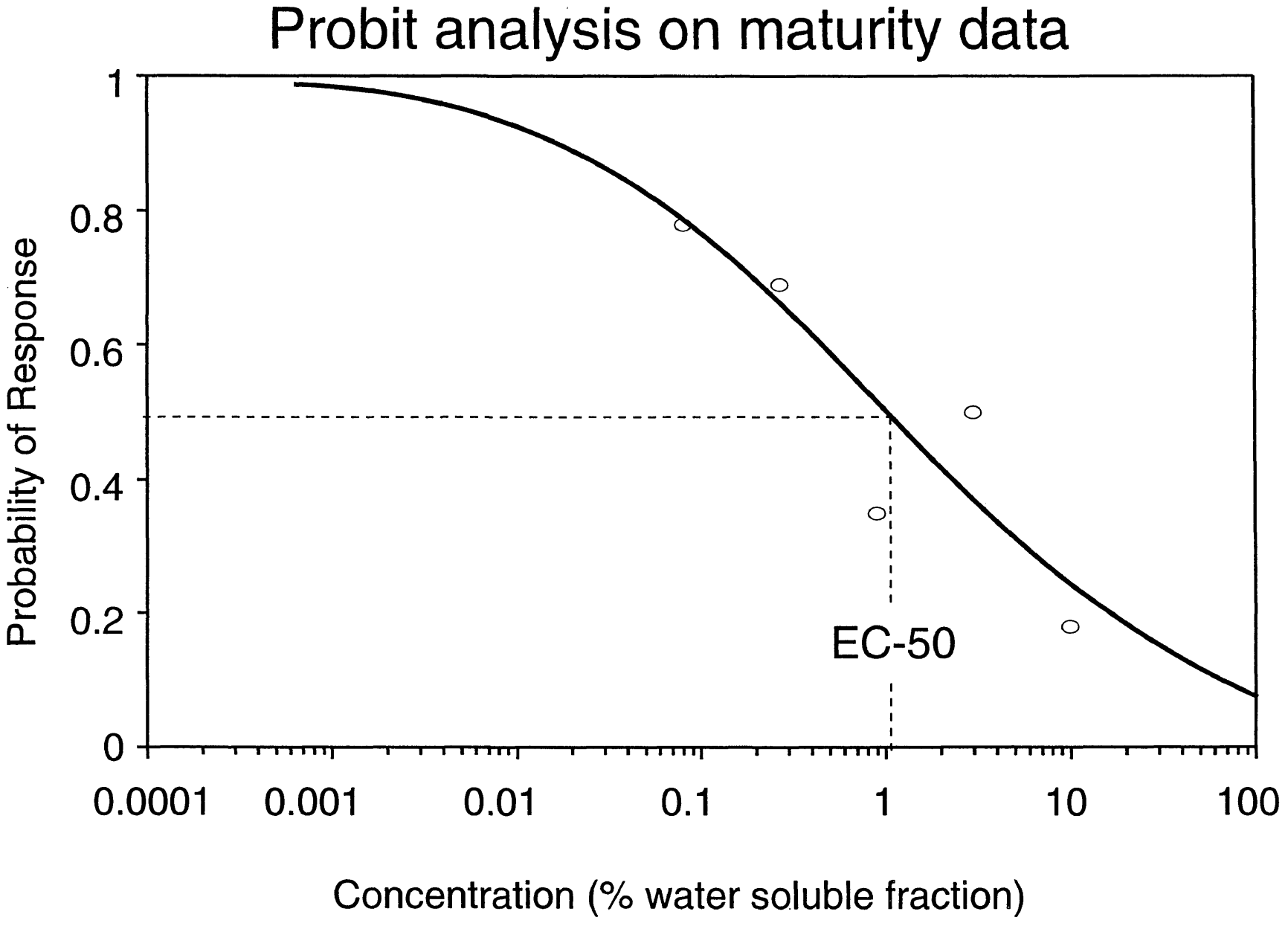

Probit analysis on maturity data
Probability of Response
1
0.8
0.6
0.4
0.2
0
EC-50
0.0001
0.001
0.01
0.1
1
10
100
Concentration (% water soluble fraction)

Fig 13. Proportional reduction in response compared to controls.

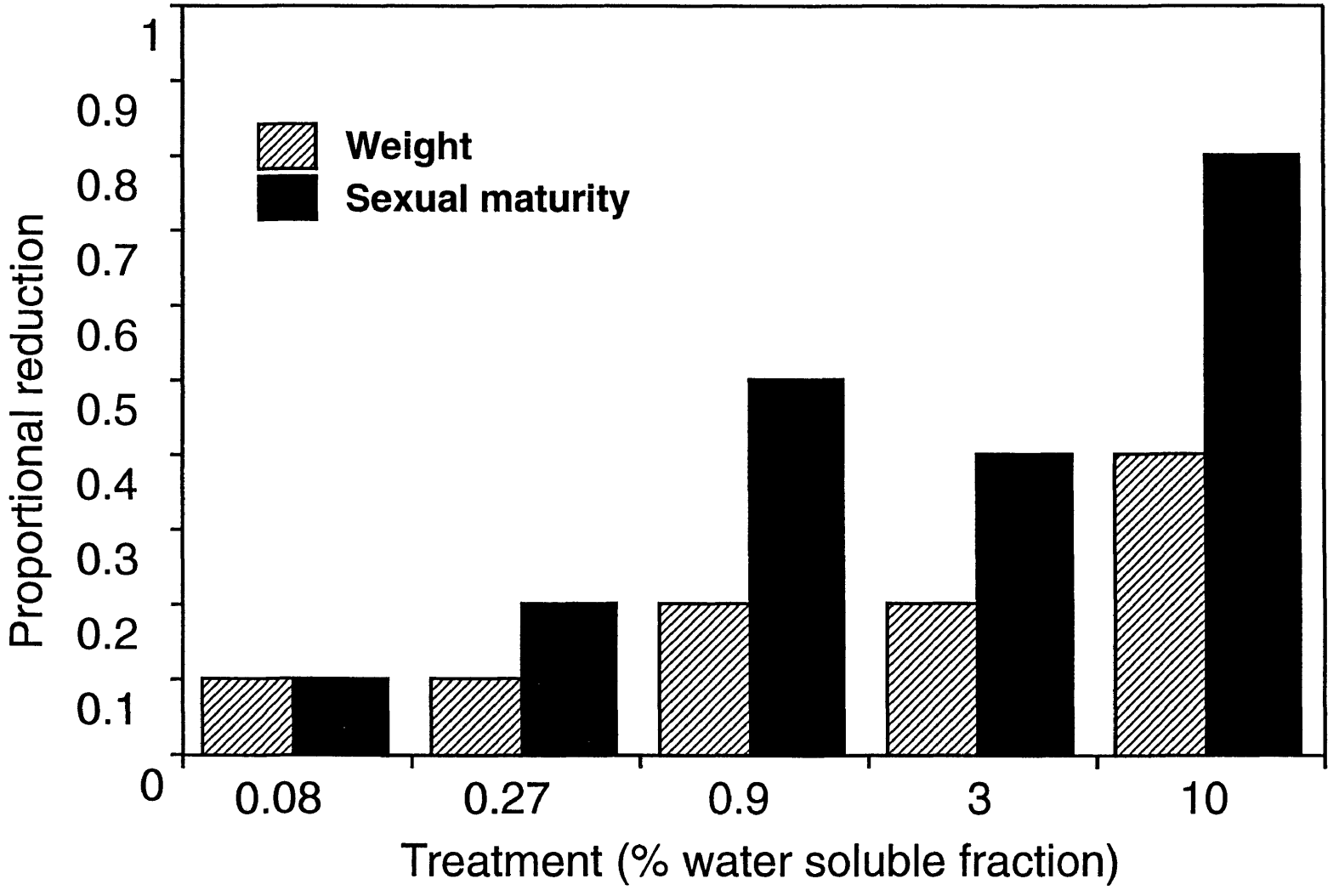

Proportional Reduction in Response
Compared to Controls
Weight
Sexual maturity
Proportional reduction
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.08
0.27
0.9
3
10
Treatment (% water soluble fraction)

Table 7 a. ANOVA table for mean mysid weight (P=0.05, df=5,40).

SOURCE	DF	SS	MS	F
FACTOR	5	0.347	0.069	13.8
ERROR	42	0.207	0.005	
TOTAL	47	0.554		

Table 7 b. Results of Dunnett's test mysid weight data. Dunnett table value = 2.31 (one-tailed value, P=0.05, df=40,5; * indicates significance).

TREATMENT	TEST STATISTIC	MEAN
CONTROL		0.608
0.0081%	* 3.030	0.501
0.27%	* 2.443	0.522
0.9%	* 4.738	0.441
3%	* 2.969	0.503
10%	* 7.897	0.329

Table 8 a. ANOVA for arc-sin square root transformed sexual maturity data (P=0.05, df=5,40).

SOURCE	DF	SS	MS	F
FACTOR	5	3.3438	0.688	6.370
ERROR	40	4.332	0.108	
TOTAL	45	7.769		

Table 8 b. Bonferroni t-test on sexual maturity data. Bonferroni t table value = 2.42. (one-tailed value, P=0.05, df=40,5; * = significant, ~ = not significant).

TREATMENT	TEST STATISTIC	MEAN TRANSFORMED	MEAN ORIGINAL
CONTROL		1.224	0.891
0.0081%	~ 1.124	1.039	0.729
0.27%	~ 1.021	1.050	0.739
0.9%	* 3.358	0.672	0.395
3%	* 2.481	0.816	0.530
10%	* 4.929	0.385	0.143

Table 9. Analysis of variance for molt data (P=0.4, df=5,42).

SOURCE	DF	SS	MS	F
FACTOR	5	0.0492	0.0098	0.97
ERROR	42	0.4275	0.0102	
TOTAL	47	0.4767		

indicator of stress in this experiment. The data suggest that AC at low ug/l concentrations do not have a clear effect on the molt process.

Although these data did not demonstrate an effect on molting, it is possible that creosote contaminated sediment may adversely affect this process. Many xenobiotic pollutants interfere with the mixed function oxygenase (MFO) systems in crustaceans, which play an important role in the molting process (Lee, 1988).

Since ACs were the major pollutant in the sediment, these effects are thought to be primarily due to these compounds. Heavy metal concentrations have been previously measured in the sediment and were found to be low (Chu and Hale, personal communication).

As expected, the sublethal effects were observed at lower total AC levels (e.g. EC-50 for maturity was 15ug/l) of ERS WSF, than the short-term LC-50 (700 ug/l). The two values are not directly comparable due to differences in life stage of mysids used and physical conditions under which the tests were run. However, the order of magnitude difference between EC and LC end points reiterates the importance of measuring effects more sensitive than short term lethality.

CHAPTER V

CONCLUSIONS

WSFs generated from creosote and creosote contaminated sediment were found to be lethal to the bay mysid. The creosote WSF had a 48 h LC-50 (180 ug/l) that was about a quarter of that of the ERS WSF (700 ug/l). The creosote WSF contained a higher percentage of heterocyclic aromatic compounds than the ERS WSF, particularly quinolines (low molecular weight nitrogen heterocyclics). In contrast, the ERS WSF contained greater proportions of high molecular weight compounds, such as pyrene and benzofluorenes. The difference in toxicity may be attributable to this qualitative difference in chemical composition.

The ERS WSF had sublethal effects on the mysid. A seven day exposure to sub-lethal concentrations delayed the attainment of sexual maturity and reduced growth (dry weight), at total AC concentrations as low as 8.9 ug/l. Weights for WSF-exposed mysids were lower than for control mysids at all toxicant concentrations used. Sexual maturity was significantly affected at high concentrations and the EC-50, calculated by probit analysis for this endpoint, was 15 ug/l total

identified AC. Frequency of molting was unaffected at the concentrations used, although toxicant exposure has been shown to affect molting in other crustaceans. The data indicate that at the same level of exposure, different sublethal responses in the same organism can vary in sensitivity.

The composition of the WSFs differed from the materials from which they were generated. WSFs were enriched in low molecular weight and more polar heterocyclic compounds, and had lower concentrations of high molecular weight components than the source materials. High molecular weight compounds from creosote contamination were present in the sediments, while more volatile compounds were largely absent. These qualitative differences result from fractionation and degradation of creosote and are relevant to the fate and biological effects of creosote in the environment.

The data also suggest that the toxicity of a mixture in the aquatic environment cannot be assessed merely by analyzing the constituent chemicals. Interactions within a mixture, and between a mixture and the medium in which it is present, can have important implications on toxicity.

REFERENCES:

Anderson, J. W., J.M. Neff, B.A. Cox, H.E. Tatem and G.M. Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27: 75-88.

Baileys, R. T. and D. A. Webb. 1987. 1958 cooperative creosote project XII: An analysis of creosotes from posts after 26 years in test. American Wood Preserver's Association Proceedings 83: 1-9.

Bennett, D. 1990. Evaluation of the fate of pesticides in water and sediment. in Environmental fate of pesticides. D. H. Hutson and T. R. Roberts, eds., John Wiley and Sons Ltd. pp 150-173.

Bieri, R. H., C. Hein, R. J. Huggett, P. Shou, H. Slone, C. Smith, and C. Su. 1986. Polycyclic aromatic hydrocarbons in surface sediments from the Elizabeth River subestuary. Int. J. Environ. Anal. Chem. 26: 97-113.

Birkholz, D. A., R. T. Coutts, S. E. Hrudley, R. W.

Danell and W. L. Lockhart. 1990. Aquatic toxicity of alkyl quinolines. Water Res. 24: 67-63.

Black, J. J. 1982. Movement and identification of a creosote-derived PAH complex below a river pollution point source. Arch. Environ. Contam. Toxicol. 11: 161-166.

Bookhout, C. G., R. J. Monroe, R. B. Forward, Jr. and J. D. Costlow, Jr. 1984. Effects of soluble fractions of drilling fluids on development of crabs, *Rhithropanopeus harisii* and *Callinectes sapidus*. Water Air Soil Pollut. 21: 183-197.

Borthwick P. W. and J. M. Patrick, Jr. 1982. Use of aquatic toxicology to estimate environmental deactivation of marine-grade creosote in seawater. Environ. Toxicol. Chem. 1: 281-288.

Breteler, R. J., J. W. Williams and R. L. Buhl. 1982. Measurement of chronic toxicity using the oppossum shrimp *Mysidopsis bahia*. Hydrobiologia 93: 189-194.

Enzminger J. D. and R. C. Ahlert. 1987. Environmental fate of polynuclear aromatic hydrocarbons in coal

tar. Environ. Technol. Lett. 8: 269-278.

Esdall, C. C., 1991. Acute toxicities to larval rainbow trout of representative compounds detected in Great Lakes Fish. Bull. Environ. Contam. Toxicol. 46: 173-178.

Finney, D. J., 1971. Probit Analysis. Cambridge University Press, The United Kingdom. pp 333.

Geiger J. G. and A. L. Buikema, Jr. 1982. Hydrocarbons depress growth and reproduction of *Daphnia pulex* (Cladocera). Can. J. Fish. Aquat. Sci. 39: 830-836.

Gentile J. H., S. M. Gentile, G. Hoffman. 1983. The effects of a chronic mercury exposure on survival, reproduction and population dynamics of *Mysidopsis bahia*. Environ. Toxicol. Chem. 2: 61-68.

Hale, R. C. 1988. Disposition of polycyclic aromatic hydrocarbons in blue crabs, *Callinectes sapidus*, from the southern Chesapeake Bay. Estuaries 11: 255-263.

Hargis, W. J., M. H. Roberts Jr. and D. E. Zwerner.

1984. Effects of contaminated sediments and sediment-exposed effluent water on an estuarine fish: acute toxicity. Mar. Environ. Res. 14: 337-354.

Helz, G. R., and R. J. Huggett. 1987. Contaminants in the Chesapeake Bay: a regional perspective. In: Contaminant problems and management of living Chesapeake Bay resources. S. K. Majumdar, L. W. Hall Jr., and H. M. Austin., (eds.), The Pennsylvania Academy of Science. pp 271-297.

Huggett, R. J., M. E. Bender and M. A. Unger. 1984. Polynuclear aromatic hydrocarbons in the Elizabeth River, Virginia. In: Fate and effects of sediment bound chemicals in aquatic systems. K. L. Dickson, A.W. Maki and W. A. Brungs., (eds.), Pergammon Press, New York. pp 328-341.

Huggett, R. J., P. A. Van Veld, C. L. Smith, W. J. Hargis, Jr., W. K. Vogelbein and B. A. Weeks. 1992. The effects of contaminated sediments in the Elizabeth River. In: Contaminated Sediment Toxicity Assessment, G. A. Burton (ed.), Lewis Publishers, New York. pp 403-430.

Jacobson, St. M. and D. B. Boylan. 1973. Effect of seawater soluble fraction of kerosene on chemotaxis in a marine snail, *Nassarius obsoletus*. Nature. 241: 213-215.

Jaffe, R. 1991. Fate of hydrophobic organic pollutants in the aquatic environment: A review. Environ. Pollut. 69: 237-257.

Jensen, J. P. 1958. The relationship between body size and the number of eggs in marine malacostrakes. Meddr. Danm. Fisk-og. Hauvenders 2: 1-25.

Karinen, J. F., and S. D. Rice. 1974. Effects of Prudhoe Bay crude oil on molting tanner crabs, *Chionectes bairdi*. Mar. Fish. Rev. 36: 31-37.

Knezovich, J. P., F. L. Harrison and R. G. Wilhelm. The bioavailibility of sediment-sorbed organic chemicals: a review. 1987. Water Air Soil Pollut. 32: 233-245.

Lee, R. F. 1988. Possible linkages between mixed function oxygenase systems, steroid metabolism, reproduction and pollution in aquatic animals.In: Toxic Contaminants and Ecosystem Health; a Great

Lakes Focus, M. S. Evans (ed.), John Wiley and Sons, Inc., New York. pp 201-213.

Lussier, S. M., A. Kuhn, M. J. Chammas and J. Sewall. 1988. Techniques for the laboratory culture of *Mysidopsis* species (Crustacea: mysidacea). Environ. Toxicol. Chem. 7: 969-977.

Mahoney, M. S. and G. S. Noyes. 1982. Effects of petroleum on feeding and mortality of the American Oyster. Archiv. Environ. Contam. Toxicol. 11: 527-531.

Malins, D. C. and W. T. Roubal. 1985. Free radicals derived from nitrogen containing xenobiotics in the sediments and liver and bile of English sole from Puget Sound, Washington. Mar. Environ. Res. 17: 205-210.

Malins, D. C., M. M. Krahn, M. S. Myers, L. D. Rhodes, D. W. Brown, C. A. Krone, B. B. McCain and S. Chan. 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole *(Parophyrus vetulus)*. Carcinogenesis 6: 1463-1469.

Markle, D. F. and G. C. Grant. 1970. The summer food habits of young-of-the-year striped bass in three Virginia rivers. Chesapeake Sci. 11: 50-54.

McKenney, C. L., Jr. 1987. Optimization of environmental factors during the life cycle of *Mysidopsis bahia*. EPA Environmental Research Brief. USEPA, Gulf Breeze, FL 32561.

Merril, E. G. and T. L. Wade. 1985. Carbonized coal products as a source of aromatic hydrocarbons to sediments from a highly industrialized estuary. Environ. Sci. Technol. 19: 597-603.

Mueller, J. G., P. J. Chapman and P. H. Pritchard. 1989 a. Creosote contaminated sites. Environ. Sci. Technol. 23: 1197-1201.

Mueller, J. G., P. J. Chapman and P. H. Pritchard. 1989 b. Action of a fluoranthene utilizing bacterial community on PAH components of creosote. Appl. Environ. Microbiol. 55: 3085-3090.

Munoz, M.J. and J.V. Tarazona. 1993. Synergistic effect of two and four- component combinations of PAH: phenanthrene, anthracene, naphthalene and

acenaphthene on *Daphnia magna*. Bull. Environ. Contam. Toxicol. 50: 363-368.

Neff, J. M. 1979. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. Applied Science Publishers, London. pp 262.

Neff, J. M. and J. W. Anderson. 1981. Responses of Marine Animals to Petroleum and Specific Petroleum Hydrocarbons. Applied Science Publishers, London. pp 177.

Nimmo, D. R. and T. L. Hamaker. 1982. Mysids in toxicity testing - a review. Hydrobiologia 93: 171-178.

Nylund, L., P. Heikkila, M. Hameila, L. Pyy, K. Linnaimaa and M. Sorsa. 1992. Genotoxic effects and chemical compositions of four creosotes. Mutat. Res. 265: 223-236.

O'Connor, J. M. and R. J. Huggett. 1988. Aquatic pollution problems, North Atlantic Coast, including Chesapeake Bay. Aquat. Toxicol. 11: 163-190.

Odum, W. E. and E. J. Heald. 1972. Trophic analysis of an estuarine mangrove community. Bull. Mar. Sci. 22: 671-738.

Pearson, W. H., S. E. Miller, J. W. Blaylock and B. L. Olla. 1981. Detection of the WSF of crude oil by the blue crab, *Callinectes sapidus*. Mar. Environ. Res. 5: 3-11.

Percy, J. A. 1978. Effects of chronic exposure to petroleum upon the growth and molting of juveniles of the arctic marine isopod crustacean *Mesidotea entomon*. J. Fish. Res. Board Can. 35: 650-656.

Peters, C. A. and R. G. Luthy. 1993. Coal tar dissolution in water miscible solvents: experimental evaluation. Environ. Sci. Technol. 27: 2831-2843.

Roberts, M. H., Jr., W. J. Hargis, Jr., C. J. Strobel and P. F. DeLisle. 1989. Acute toxicity of PAH contaminated sediments to the estuarine fish, *Leiostomus xanthurus*. Bull. Environ. Contam. Toxicol. 42: 142-149.

Smith, S. M., J. G. Hoff, S. P. O'Neil and M. P.

Weinstein. 1984. Community and trophic organization of nekton utilizing shallow marsh habitats, York River, Virginia. Fish. Bull. 82: 455-467.

Smith, B. S., J. F. Savino and M. A. Blouin. 1986. Acute toxicity to Daphnia pulex of six classes of chemical compounds potentially hazardous to Great Lakes aquatic biota. J. Great Lakes Res 14: 394-404.

Sved, D. W. 1991. Mono-oxygenase induction and lethality as endpoints in aquatic toxicology. PhD Dissertation, College of William and Mary, Williamsburg, Virginia.

Swartz, R. C., D. W. Schults, T. H. Dewitt, G. R. Ditsworth and J. O. Lamberson. 1990. Toxicity of fluoranthene in sediment to marine amphipods: A test of the equilibrium partitioning approach to sediment quality criteria. Environ. Toxicol. Chem. 9: 1071-1080.

Tagatz, M. E., Plaia G. R., Christine D. H. and Lores E. M. 1983. Toxicity of creosote-contaminated sediment to field and laboratory-colonized

estuarine benthic communities. Environ. Toxicol. Chem. 2: 441-450.

Verberg, W.B., H. McKellar, Jr., and J.F. Verberg, 1978. Toxicity studies environmental impact assessment. Environ. Manag. 1: 239-243.

Volgelbein, W. K., J. W. Fournie, P. A. Van Veld and R. J. Huggett. 1990. Hepatic neoplasms in the mummichog, *Fundulus heteroclitus* from a creosote-contaminated site. Cancer Res. 50: 5978-5896.

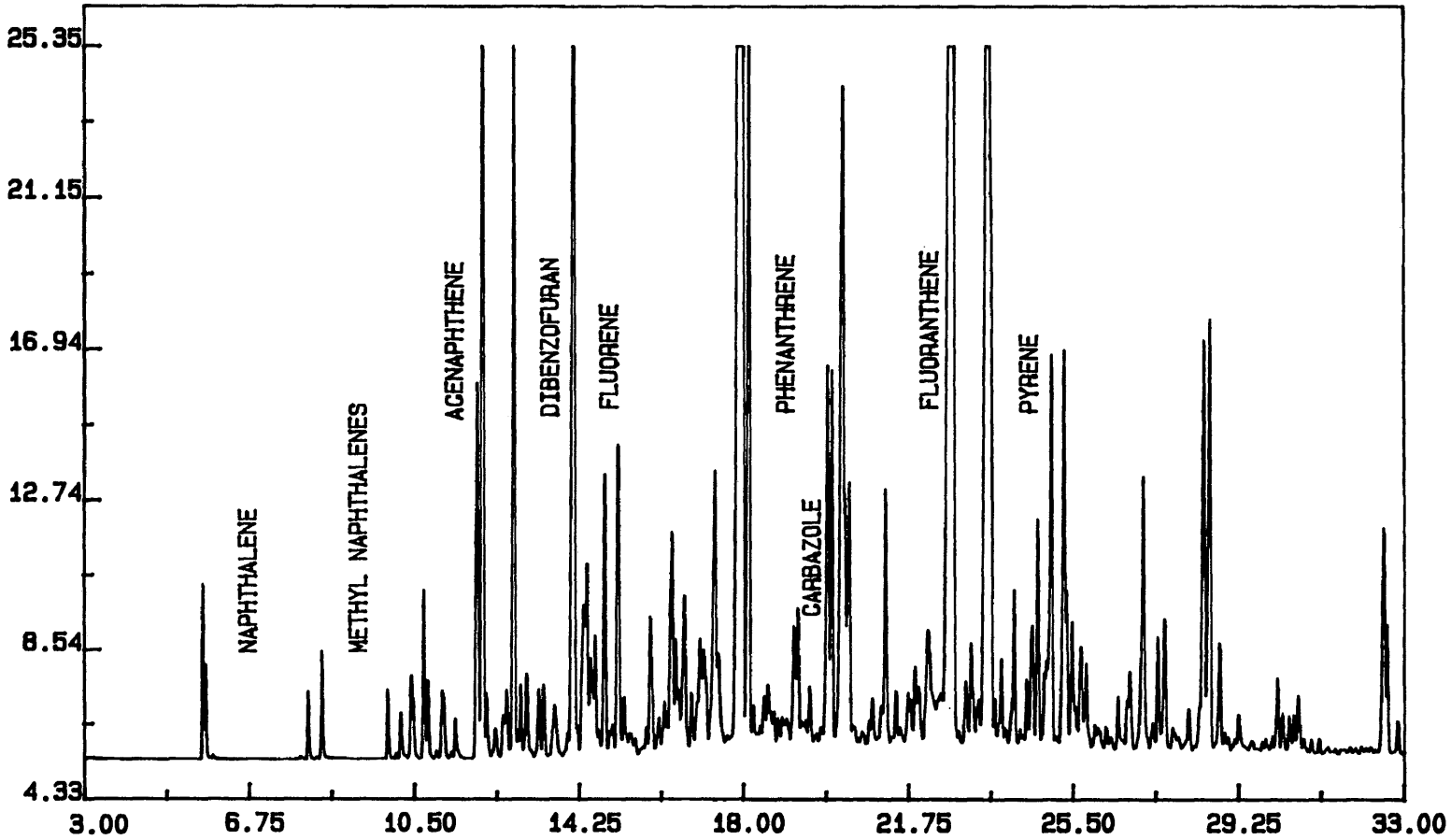
Weeks, B. A. and J. E. Wariner. 1984. Effects of toxic chemicals on macrophage phagocytosis in two estuarine fishes. Mar. Environ. Res. 14: 327-335.

Yen, C. P. C., Perenich, T. A. and G. L. Baughman. 1991. Fate of commercial and disperse dyes in sediments. Environ. Toxicol. Chem. 10: 1009-1017.

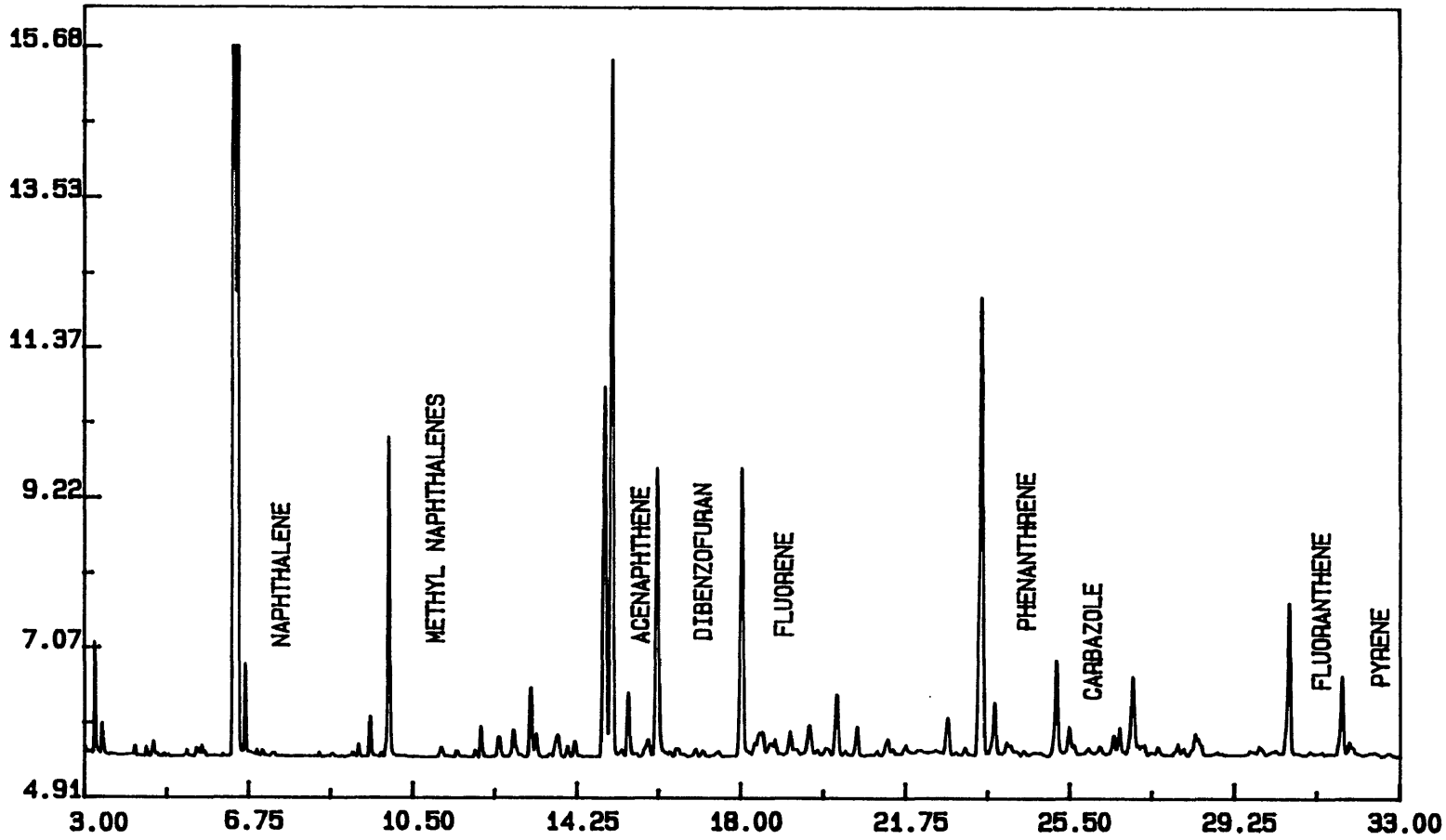
Zurcher, F. and M. Thuer. 1978. Rapid weathering processes of fuel oil in natural waters: analyses and interpretations. Environ. Sci. Technol. 12: 838-843.

APPENDIX 1: CHROMATOGRAMS

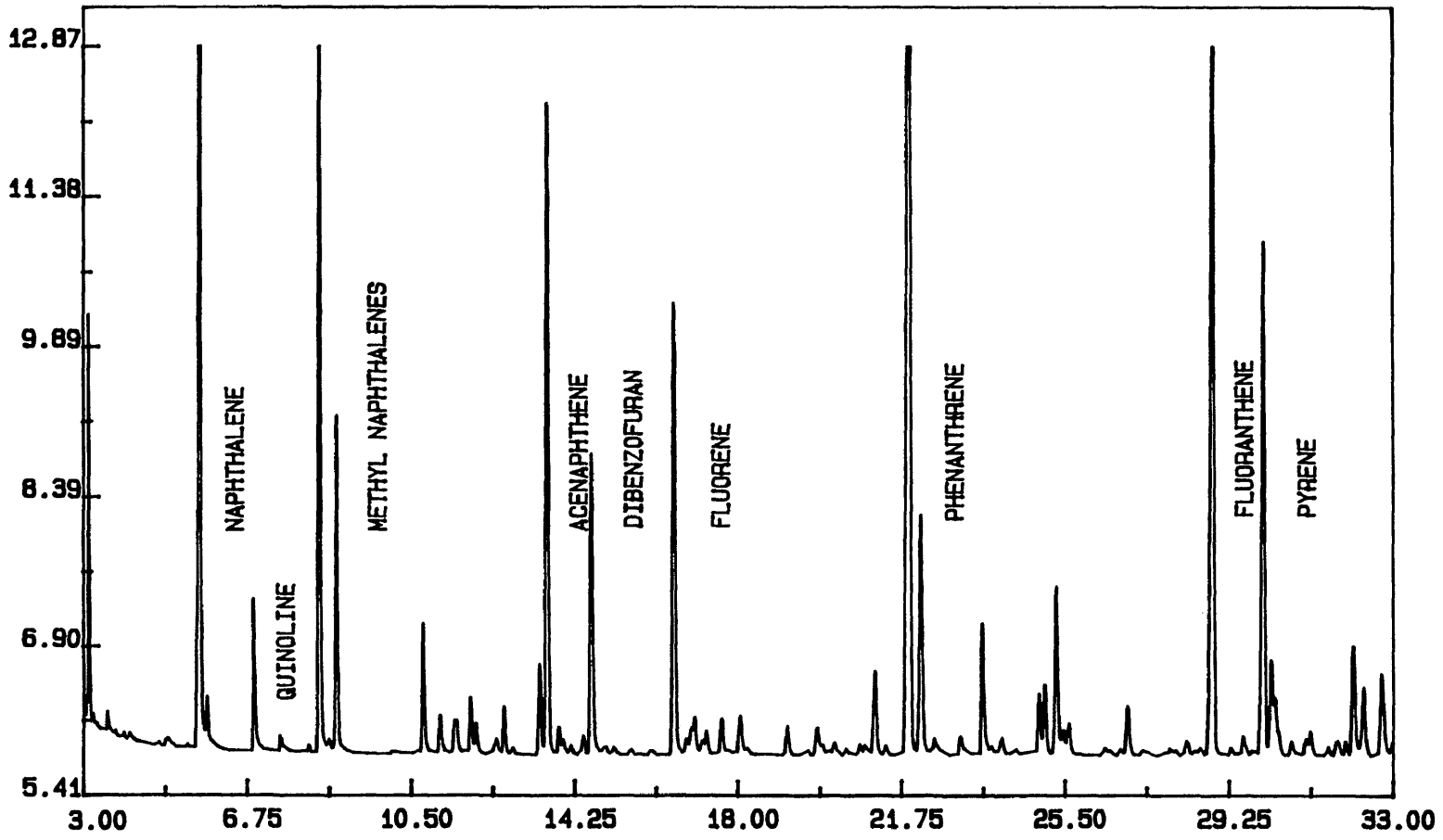
Chromatogram of Elizabeth River sediment. Intensity of electrical response is depicted on the Y-axis and time in minutes is shown on the X-axis.


25.35
21.15
16.94
12.74
8.54
4.33
3.00
6.75
10.50
14.25
18.00
21.75
25.50
29.25
33.00
NAPHTHALENE
METHYL NAPHTHALENES
ACENAPHTHENE
DIBENZOFURAN
FLUORENE
PHENANTHRENE
CARBAZOLE
FLUORANTHENE
PYRENE

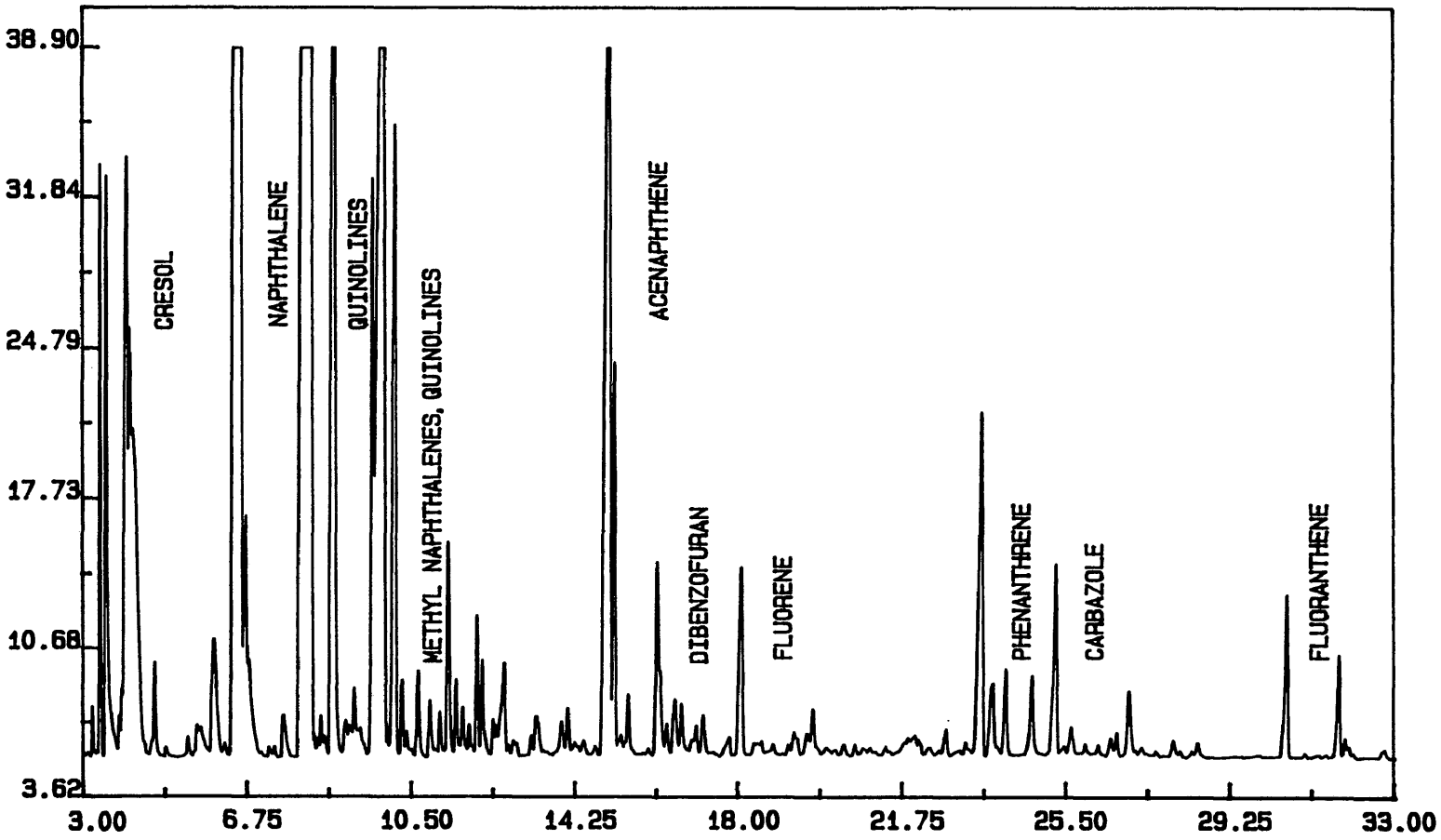
Chromatogram of Elizabeth River sediment water soluble fraction. Intensity of electrical response is depicted on the Y-axis and time in minutes is shown on the X-axis.


15.68
13.53
11.37
9.22
7.07
4.91
3.00
6.75
10.50
14.25
18.00
21.75
25.50
29.25
33.00
NAPHTHALENE
METHYL NAPHTHALENES
ACENAPHTHENE
DIBENZOFURAN
FLUORENE
PHENANTHRENE
CARBAZOLE
FLUORANTHENE
PYRENE

Chromatogram of creosote.
Intensity of electrical response is depicted on the Y-axis and time in minutes is shown on the X-axis.


12.87
11.38
9.89
8.39
6.90
5.41
NAPHTHALENE
QUINOLINE
METHYL NAPHTHALENES
ACENAPHTHENE
DIBENZOFURAN
FLUORENE
PHENANTHRENE
FLUORANTHENE
PYRENE
3.00
6.75
10.50
14.25
18.00
21.75
25.50
29.25
33.00

Chromatogram of creosote water soluble fraction. Intensity of electrical response is depicted on the Y-axis and time in minutes is shown on the X-axis.


38.90
31.84
24.79
17.73
10.68
3.62
3.00
6.75
10.50
14.25
18.00
21.75
25.50
29.25
33.00
CRESOL
NAPHTHALENE
QUINOLINES
METHYL NAPHTHALENES, QUINOLINES
ACENAPHTHENE
DIBENZOFURAN
FLUORENE
PHENANTHRENE
CARBAZOLE
FLUORANTHENE

APPENDIX 2: WATER QUALITY DATA

Water Quality Observations. Mean is reported, with range in parentheses.

TREATMENT	WATER TEMP (°C)	DISSOLVED O_2 (mg/l)	pH	MAX / MIN TEMP
ACUTE TEST:				
CONTROL	21 (21-21)	6.6 (6.5-6.9)	7.62 (7.43-7.81)	
CREOSOTE LOW	21 (20-21)	6.3 (6.0-6.6)	7.86 (7.77-7.93)	
CREOSOTE HIGH	21 (20-21)	6.3 (5.9-6.5)	7.86 (7.77-7.95)	
ERS LOW	21 (20-21)	6.4 (6.3-6.9)	7.84 (7.82-7.86)	
ERS HIGH	21 (20-21)	6.4 (6.3-6.7)	7.85 (7.81-7.90)	
CHRONIC TEST:				
CONTROL	24 (23-25)	5.9 (5.5-6.9)	7.83 (7.70-7.98)	23.0/ 26.5
ERS LOW	24 (23-25)	5.8 (5.5-6.4)	7.83 (7.71-8.04)	
ERS HIGH	24 (23-24)	5.7 (5.4-6.2)	7.86 (7.71-8.06)	

VITA

Padma T. Venkatraman

Born in Madras, India, 13 November 1969. Graduated from St. Michael's Academy, Madras, India, in 1986. Received BSc. in Environmental Science, Chemistry and Botany from St. Joseph's College, Bangalore, India, in 1989. Entered the Master's program at the College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, Virginia in 1990.