AN ABSTRACT OF THE THESIS OF

Shinta W. Kristanto for the degree of Master of Science in Fisheries Science

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Oil and Partially Combusted Crude Oil to Inland Silverside, *Menidia beryllina*.

Abstract approved: *Redacted for Privacy*

Lawrence R. Curtis

As a result of burning oil-wells during 1991 Gulf War in Kuwait, a

substantial amount of crude oil (CO) and partially combusted crude oil (PCO)

were emitted into the environment. Toxicity of the water soluble fraction (WSF)

of CO and PCO were compared based on survival and growth of inland silverside.

Biological effects were assessed as a function of total petroleum hydrocarbon

(TPH) concentration in water. Fish were exposed to 18, 36, 52, and 100% of a

saturated solutions of the WSF of either PCO or CO in 96-hr acute tests. The

concentrations of TPH detected in CO-WSF (10.8-114.8 mg/L) were up to 74 fold

higher than those found in PCO-WSF (0.31-1.55 mg/L). Less than 50% mortality

occurred in PCO exposures whereas CO exposures produced mortality ranging

from 10 to 100%. Growth inhibition was examined in fish exposed in 16-day flow-

through tests. Specific growth rate (SGR) was used as an index of sublethal

effects. SGR reductions were observed in fish exposed to PCO and CO-WSF, but

at TPH concentrations 10-fold higher in CO exposure (67-145 μg/L) than in PCO

exposure (4-12 μg/L). Gas chromatography analysis indicated PAHs contributed

more to the TPH content of the WSF of PCO than CO. Naphthalene and benzo(a)pyrene were the major components in CO and PCO-WSF, respectively. It was concluded, based on TPH content, that PCO-WSF was more toxic than CO-WSF. Possibly, lower molecular weight PAHs in CO-WSF and higher molecular weight PAHs in PCO-WSF were responsible for the toxicity observed.

Toxicity of the Water-Soluble Fraction of Crude Oil and Partially Combusted
Crude Oil to Inland Silverside, *Menidia beryllina*

by

Shinta W. Kristanto

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Dean of Graduate School

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

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Shinta W. Kristanto, Author

وَجَعَلْنَا مِنَ الْمَاءِ كُلَّ شَيْءٍ حَيٍّ

الآيـة ٢٠ مـــورة ٢١ الأنبـيـاء

"... and from water came all life."
THE HOLY QUR'AN: Ayat 30, Sura xxi Anbiya

"This thesis is dedicated to my father and to the loving memory of
my late mother, Kustiah Kristanto"

ACKNOWLEDGEMENT

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Toxicity of the Water-Soluble Fraction of Crude Oil and Partially Combusted Crude Oil to Inland Silverside, *Menidia beryllina*

INTRODUCTION

Petroleum inputs into the marine environment are estimated to be 3 million tonnes per year (NRC, 1985). Intentional or accidental discharges during marine transportation accounts for 49% of this (Couillard & Leighton, 1993). Oil enters the marine environment from other sources such as offshore production, atmospheric deposition and waste water run-off. Crude oil (CO) is of special concern since it is sometimes discharged intentionally during marine transport (Madany et al., 1994).

Inputs of petroleum hydrocarbons into the Arabian Gulf is especially great since over 30% of the world's marine oil transport crosses this small area. Transport of oil across the Gulf contributes to 4.7% of the world's total oil pollution (Madany et al., 1994). Offshore oil exploration, land-base industrial and urban sources, refineries and sewage effluents contribute to the overall pollution in the Gulf's coastal areas. Tanker accidents and burning oil wells due to wars during the 1980's are considered the largest sources of spilled oil in the Arabian Gulf (Madany et al., 1994).

During the 1991 Gulf-War in Kuwait, ignition of land oil wells has added to petroleum sources entering the marine environment. Following the 1991 Gulf-War in Kuwait, nearly 8 million barrels of crude oil were spilled into the marine environment. Release of burning and gushing oil from about 600 damaged wells were estimated at about 600 million barrels (Literathy, 1992; Readman et al., 1992). Large amounts of burning oil were emitted to the atmosphere and associated with

airborne particles. Hahn (1991) estimated that 7.5% of the oil burned was released as smoke and about 70% of the smoke emitted was in the form of elemental carbon (soot). He also concluded that the amount of smoke emitted will have impacts on human health, ecosystems, seawater processing plants, and buildings in the local areas.

Soot is a good adsorber of organic combustion products (Hahn, 1991) and due to frequent dust storms (Foda et al., 1985), petroleum related compounds associated with soot can be resuspended with dust into the atmosphere and deposited in the marine ecosystem. Lack of vegetative cover exacerbates this problem. Literathy (1992) showed that the partially combusted crude oil (PCO) was enriched with the most hazardous, high-boiling point, aromatic compounds, and these compounds were present in the smoke particulates associated with the soot. Photochemical reactions may convert hydrophobic petroleum compounds into hydrophilic structures, having surface-active properties, which will determine the pathways of airborne pollutants after deposition onto the seawater surface. Photochemical degradation products may have higher toxicity than their parent compounds (Payne and Phillips, 1985).

Crude and refined petroleums contain complex mixtures of hydrocarbons including polycyclic aromatic hydrocarbons (PAHs) which are highly toxic (Neff et al., 1976; Payne et al., 1988). Therefore, in most cases, PAH components are measured in assessing the toxicity of petroleum compounds. PAHs are also formed during the high-temperature of incomplete combustion of fossil fuel (Neff, 1985; Oost, 1994). Due to their low water solubility and persistence, marine organisms

may transfer PAHs from low to higher trophic levels (Clements et al., 1994). Lower molecular weight PAHs are generally considered as the most acutely toxic components of crude oil, whereas some of the higher molecular weight components and their metabolites are carcinogenic (Neff,1979).

Many studies have shown that ingested CO causes a variety of toxic effects in seabirds and mammals, including hormonal disturbance, growth retardation, impaired reproduction, and various stress-related changes (Couillard et al., 1991, 1993; Leighton, 1990; Peakall et al., 1982). Peakall et al. (1982) showed that the high-molecular-weight aromatic compounds from Prudhoe Bay CO were responsible for retardation of growth and increase in adrenal and nasal gland weight in nesting gulls. Aquatic organisms have also demonstrated adverse responses as a result of CO exposure. The soluble fractions of petroleum are probably the most acutely harmful to organisms (Dauble et al., 1982; Donahue et al., 1977; NAS, 1975; NRC, 1985). Less-soluble fractions may deposit in the sediment or associate with other particulate matter and eventually be ingested with food particles. Accumulation of persistent PAHs may exert long term biochemical changes in marine and benthic organisms (James and Bend, 1980; Varanasi and Gmur, 1981).

Estuaries are vulnerable to the adverse impacts of chronic oil pollution because they combine high biological productivity with the most severe exposures to wastes (NAS, 1975). Atherenid fishes, native species of estuaries, are commonly used for acute toxicity tests because they are widely distributed indigenous species of all coastal regions of the continental United States (Hemmer et al., 1992). The inland

silverside (*Menidia beryllina*), an atherenid fish, has become a popular test species because their embryonic and larval forms exhibit teratogenic/toxicity responses when exposed to some chemicals (Middaugh et al., 1988, 1993; Genthner and Middaugh, 1992). Histological alterations, such as epithelial hyperplasia and fusion of gill lamellae, and atrophy and necrosis of intrahepatic exocrine pancreatic nodules, occur in adult *Menidia beryllina* exposed to water soluble fraction (WSF) and whole CO (Solangi et al., 1982). Naphthalene, a major constituent of WSF of CO, produced teratogenic effects in *Menidia beryllina* embryos, ranging from slight defect in heart structure to the formation of tube hearts without chambers or valves resulting in greatly reduced circulation (Middaugh et al., 1988). Anderson et al. (1974) tested the toxicity of WSF from two sources of CO (Kuwait and South Louisiana) on six estuarine fish species and crustacea. They found that *Mysidopsis almyra* and *Menidia beryllina* were more sensitive than *Cyprinidon variegatus*, *Fundulus similus*, *Penaeus aztecus* (post larvae), and *Palaemonetes pugio*.

The objective of this study was to compare the toxicity of CO and PCO to inland silversides. Survival and specific growth rate (SGR) were used as an index of toxicity.

MATERIALS AND METHODS

Test animals

Juvenile (0.030-0.45 g) inland silversides, *Menidia beryllina*, were purchased from a commercial source (Aquatic Indicator, St. Augustine, Florida). Prior to experimentation, fish were acclimated for five days to flowing water (50 ml/min) in aquaria (20 L total volume; 22-25°C; salinity 17-19°/oo). During the acclimation period, fish were fed *ad libitum* with freshly hatched brine shrimp (*Artemia nauplii*). Food was withheld for 48 hr prior to 16-day subacute tests. Fish were then weighed and selected for uniform size and placed randomly into aquaria (15 fish per aquaria). During subacute tests fish were fed freshly hatched brine shrimp *ad libitum*. After the 16-day exposures, fish were killed using tricaine methanesulfonate, MS 222, and their final body weight measured.

Test materials

Kuwaiti CO and a composite sample of soil contaminated with PCO were used to make up exposure water for toxicity tests. Burgan CO was provided by Petroleum Division of the Kuwait Institute of Scientific Research. The composite sample of PCO was made from soil samples collected from the northern oil fields of Sabriya and Al-Rawdhatain, and the central oil fields Al-Magwa and Burgan (Figure 1). The zones in oil fields selected for sampling included: 1) oil lake areas

where sand was saturated with oil, 2) tar mat areas within 100-400 m of a well head where the sand was covered by a continuous layer of partially combusted oil, and 3) margin areas of impact zones which received oil ash, but did not display a well developed tar mat. Test materials were stored in a cool, dark room to minimize chemical and photodegradation.

Filtered seawater (36°/oo) used for toxicity tests and for acclimation of tests organisms was obtained from Hatfield Marine Sciences Center (Newport, OR). Seawater was delivered by truck (3600 L) and equally divided into two 3600 L fiberglass storage tanks (1800 L per tank). Seawater in each tank was diluted with well water that was previously processed by reverse osmosis (total dissolved solids reduced by = 95%; hardness = 8-12 mg/L as $CaCO_3$) resulting in a dilution water with salinity of 18°/oo. The diluted seawater (DS) in each storage tank lasted approximately 4 days; storage tanks were refilled twice during 16-day subacute tests. The seawater and DS salinity was confirmed using a temperature compensated salinity refractometer (Aquatic Eco-System Inc.). Storage tanks were equipped with a circulation pump and an airstone to ensure proper mixing of DS during experiments.

96-hr acute tests

Static renewal toxicity tests (96 hr) were conducted to estimate median lethal concentrations (LC50s) and to determine the range of concentrations to be tested in 16-day subacute tests. Five inland silver sides were placed in each 500-
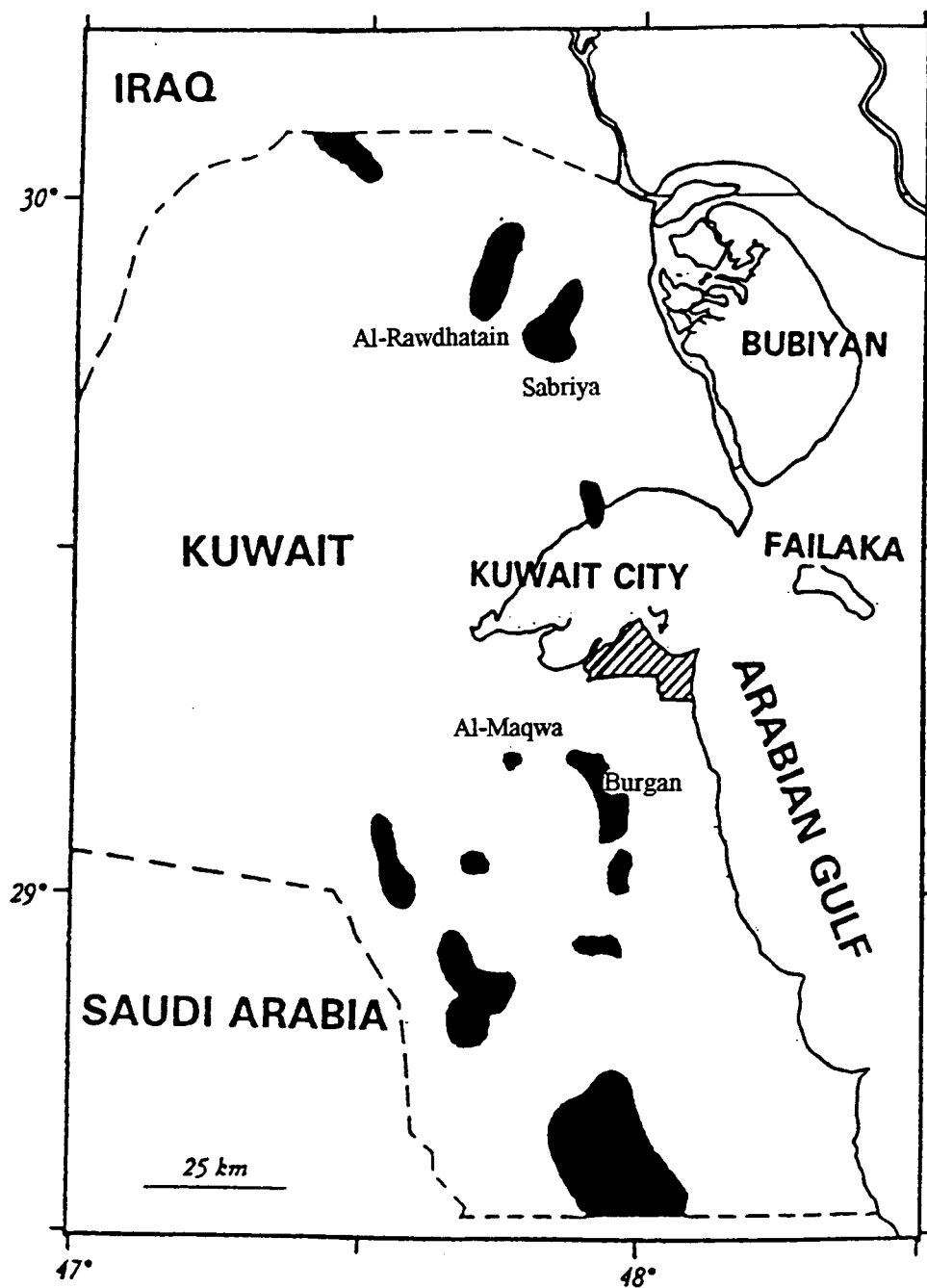
Figure 1. Map of the Kuwait oil fields where the PCO-contaminated soil were collected. (Taken from Al-Yakoob et al., 1993)

ml jar containing 300 ml stock solution of either CO or PCO. Food was withheld during the exposure. Stock solutions of CO were made by mixing 700 ml of DS with 100 ml of CO for 6 min in 1 L separatory funnels. The solution was allowed to settle for 15 min after which the water was removed and stored in a 4 L flask. Stock solutions of PCO were prepared by stirring 600 g of PCO-contaminated soil in a bucket containing 4 L DS, for 24 hr. Fish were exposed to four duplicated 4 concentrations (0, 18, 36, 52, 100 percent of toxicant saturated seawater). Test solutions were changed every 24 hr.

16-day subacute tests

A continuous flow-through device was built as described by Chadwick et al. (1972). A schematic of the apparatus is illustrated in Figure 2. The continuous flow-through diluter system supplied contaminated DS water and DS to aquaria (20 L total volume) at five duplicate concentrations plus control. The 3600 L storage tanks containing DS were equipped with delivery lines in which water was pumped into two head boxes, where temperature was controlled with heating coils. DS from one storage tank was pumped to one of the head boxes equipped with a saturator, an inverted 2 L flask which contained either 600 g of a composite sample of PCO-contaminated soil or 400 ml of crude oil. Oil saturated water (WSF) from the saturator recirculated to the head box from which it flowed to the diluter. DS water was also pumped to the other head box (see Fig.2) and delivered to the diluter. The diluter delivered both stock solution and diluted

seawater to the exposure aquaria. The range of exposure concentrations (0,20,40,60,80 and 100%) of the CO and PCO saturated solution (determined from preliminary static renewal tests) were achieved by varying the ratio of toxicant saturated water to DS. Flow rates of 50 ml/min into 20 L total water volume (volume replacement time = 6.6 h) for each aquaria was sufficient to maintain suitable water quality. Flow rates were monitored daily to maintain the desired toxicant concentrations. To insure uniform toxicant concentration throughout the exposure period, toxicants in the saturator flask were replaced every 48 h during the 16-day period.

Aquaria were housed in a constant temperature room (25°C) with 12 hr light:12 hr dark photoperiod. Water quality was measured every 48 hr (Table 1).

Table 1. Water quality measurement

	PCO Test	CO Test
pH	7.88 ± 0.01[a,b]	7.81 ± 0.04
Dissolved oxygen (DO) (mg/L)	8.63 ± 0.03	8.79 ± 0.02
Temperature (°C)	25.60 ± 0.14	23.32 ± 0.26
Salinity (ppt)	17.75 ± 0.25	18.00 ± 0.45

[a]Mean±SE
[b]Average from four measurements

3600 L Storage Tanks

Head Box

Saturator

DS Water Supply

Reverse Osmosis Unit

Fish Exposure Chambers
(75 Liter Aquaria)

Sea Water

To Head Box ←

PCO Contaminated Soil

Glass Beads

A. PCO Test

Sea Water
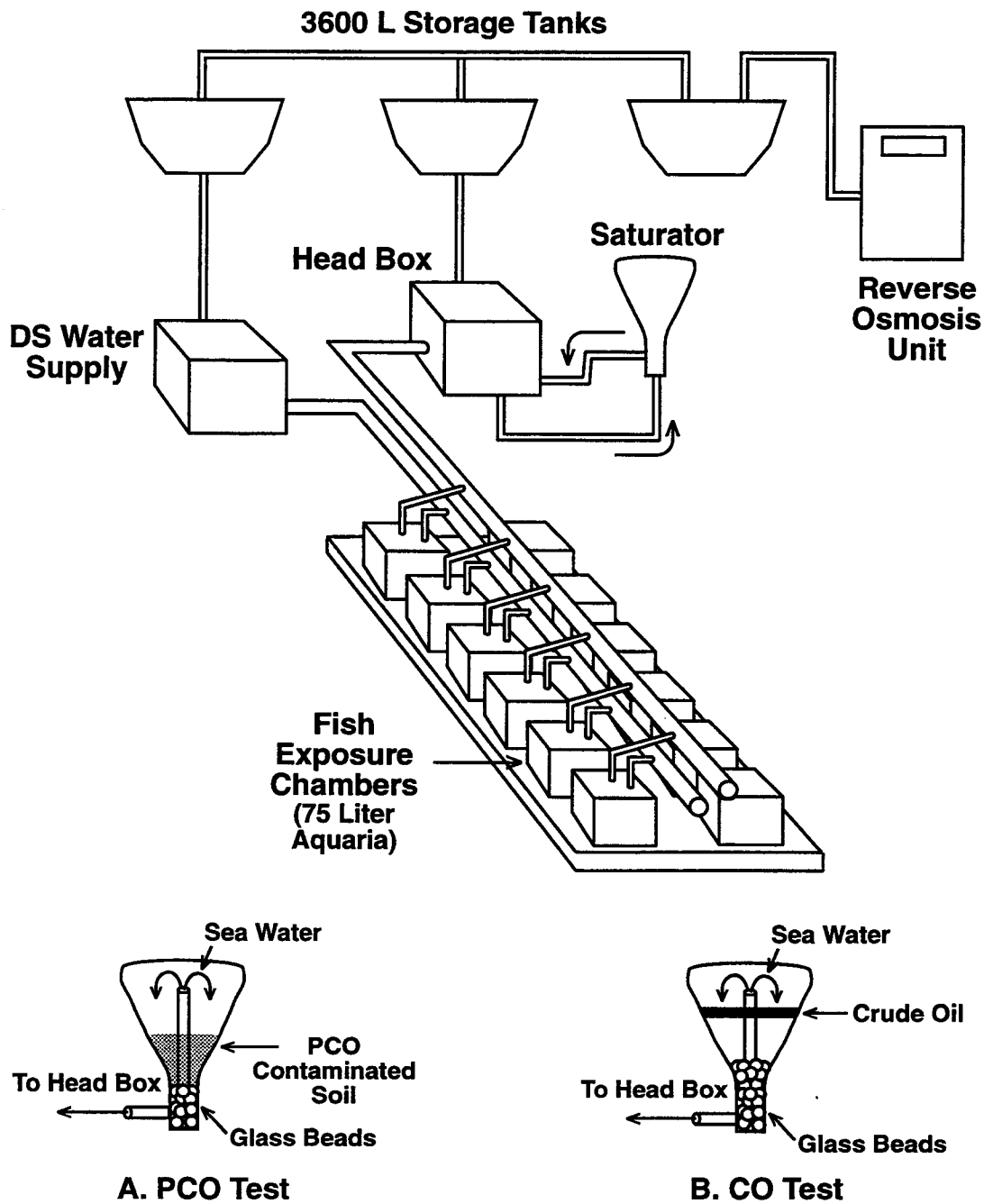
← Crude Oil

To Head Box ←

Glass Beads

B. CO Test

Figure 2. Schematic of 16-day flow-through bioassay apparatus. Saturator represented by either (A) PCO mixture or (B) CO mixture.

Analytical chemistry

Total petroleum hydrocarbons (TPH) and PAH concentrations of exposure solutions were analyzed on day 1, 8, and 16 of experiments. Immediately after sample collection, 500 ml of water containing CO or PCO was extracted twice with spectral grade hexane (20 and then 10 ml) in a 1 L separatory funnel for 2 minutes. Concentrations of TPH were determined spectrophotometrically as described by Picer and Picer (1993) using a Shimadzu RF-551 fluorescense detector, at 290 nm excitation and 390 nm emission. Concentrations of TPH's were determined from a calibration curve obtained using standard solutions of Kuwaiti CO dissolved in hexane.

For PAH analysis, 200 ml of water was sampled from the middle of aquaria and transferred to a 1 L separatory funnel for extraction. Water samples were extracted twice first with 25 and then with 15 ml methylene chloride for 2 min. Extracts were collected and evaporated on a rotary evaporator to approximately 3 ml. The qualitative analysis was carried out using a Hewlett Packard model S 710 gas chromatography equipped with a flame ionization detector (FID) and fitted with PTE-5 QTM capillary column (15 m x 0.53 mm ID with 0.5 um film thickness). Helium was used as a carrier gas (flow rate= 5 cc/min). The oven temperature was programmed as follows : 100°C for 4 min followed by linear increase of 31°C per min for 8 min. Injector and detector temperature were 350°C. PAHs were identified by reference to peak retention time and areas obtained with standards for each compound. PAH standards

containing 15 compounds were purchased from Supelco (4-8905M mixture).

Growth studies

Specific growth rate (SGR) was determined as the end point of toxicity in this study. Growth is a response of the integrated activities of the whole organism, therefore it is often a sensitive parameter of the suitability of the environment (Warren, 1971). Instantaneous or SGR was measured based on the equation presented in Warren (1971) :

$$SGR\ (\%)\ =\ \frac{\ln W_2 - \ln W_1}{days\ of\ exposure}\ x\ 100$$

where W_1 is the initial mean wet weight of all fish in each aquaria at the beginning of the study (t_1) and W_2 is the final wet weight at the conclusion of the growth study (t_2).

Statistical analysis

Median lethal concentration (LC50) in static renewal-acute toxicity tests were estimated using Trimmed Spearman-Karber Method (Hamilton et al., 1978). Simple Linear Regression was used to estimate LC50 for data not meeting requirements of the Spearman-Karber Method. Dunnett's Procedure was used to assess differences between control and treatment means in acute tests (Weber et

al., 1988). Variations among TPH concentrations and growth of inland silversides in different tanks (16-day subacute tests), were analyzed by one-way ANOVA (multiple range tests). Simple linear regression was used to assess the significance of biological responses (SGR) to CO and PCO exposures. Hypothesis testing for different slopes between CO and PCO was performed using multiple regression (Ramsey et al., 1995). A significance level of $p < 0.05$ was used for all statistical comparisons. All statistical analyses were conducted with Statgraphics (version 5.0) statistical software (Statgraphics, 1986).

RESULTS

96-hr acute tests

Cumulative and total mortality of inland silversides exposed to the WSF of PCO and CO for 96-hr is presented in Table 2. Mortality was variable in the 96-hr PCO and CO exposures. There was 100% mortality in the highest treatment concentration of CO in both replicates within 24 hr. The 96-hr mortality in PCO exposures was only 40%. Since mortality in PCO exposures was less than 50%, the confidence limit for that LC50 was not calculated. The 96-hr LC50 for fish exposed to CO was 28.69 with a 95% confidence interval of 17.56-46.85 mg/L. LC50 for fish exposed to PCO-WSF was above the highest TPH concentration tested, 2.85 mg/L. Since mortality was not reliably dose-dependent in either test, these results should be interpreted with appropriate caution. Nonetheless, it is important to note that the range of TPH concentrations resulting in significant toxicity for PCO was much lower than that for CO (Table 3). The lowest exposure concentration of CO-associated TPH (10.8 mg/L) is about 7 times greater than the highest PCO-associated TPH concentration.

TPH concentrations in the WSF of both PCO and CO increased with increasing percent saturation of toxicants to seawater. Figure 3 illustrates the linear trend between percent saturation of exposure solution and TPH concentration. As previously mentioned, the TPH concentration detected in PCO exposure was much lower than those detected in CO-tests. Dunnett's procedure

identified one treatment group in PCO and three groups in CO exposures which were statistically different from controls (Table 3). This test was based on the survival of fish during the 96-hr period. Estimates of the no-effect concentration (NOEC) and LOEC (Lowest Observable Effect Concentration) for CO were 10.8 and 21.00 mg/L. For inland silversides exposed to PCO, the NOEC and LOEC were 0.798 and 1.55 mg/L.

Table 2. Cumulative and total mortality from replicated exposures of *Menidia beryllina* exposed to PCO and CO in 96-hr acute tests.

Test Material	Exposure Concentrations (% saturation)	TPH (mg/L)	Exposure Time (hr)				Total Mortality (%)
			24	48	72	96	
PCO	Control	<0.05	0	0	0	0	0
	Control		0	0	0	0	
	18	0.310	1	1	1	1	30
	18		1	2	2	2	
	36	0.755	0	0	0	0	10
	36		0	0	0	1	
	52	0.798	0	1	2	2	30
	52		0	0	0	1	
	100	1.550[a]	0	0	1	2	40
	100		0	1	1	2	
CO	Control	<0.05	0	0	0	0	0
	Control		0	0	0	0	
	18	10.8	0	0	0	0	10
	18		0	0	0	1	
	36	21.0[a]	0	1	2	4	70
	36		0	0	0	3	
	52	69.6[a]	1	1	1	3	50
	52		0	0	0	2	
	100	114.8[a]	5	5	5	5	100
	100		5	5	5	5	

[a]Survival means are significantly less than control means ($p \leq .05$) by Dunnett's test.
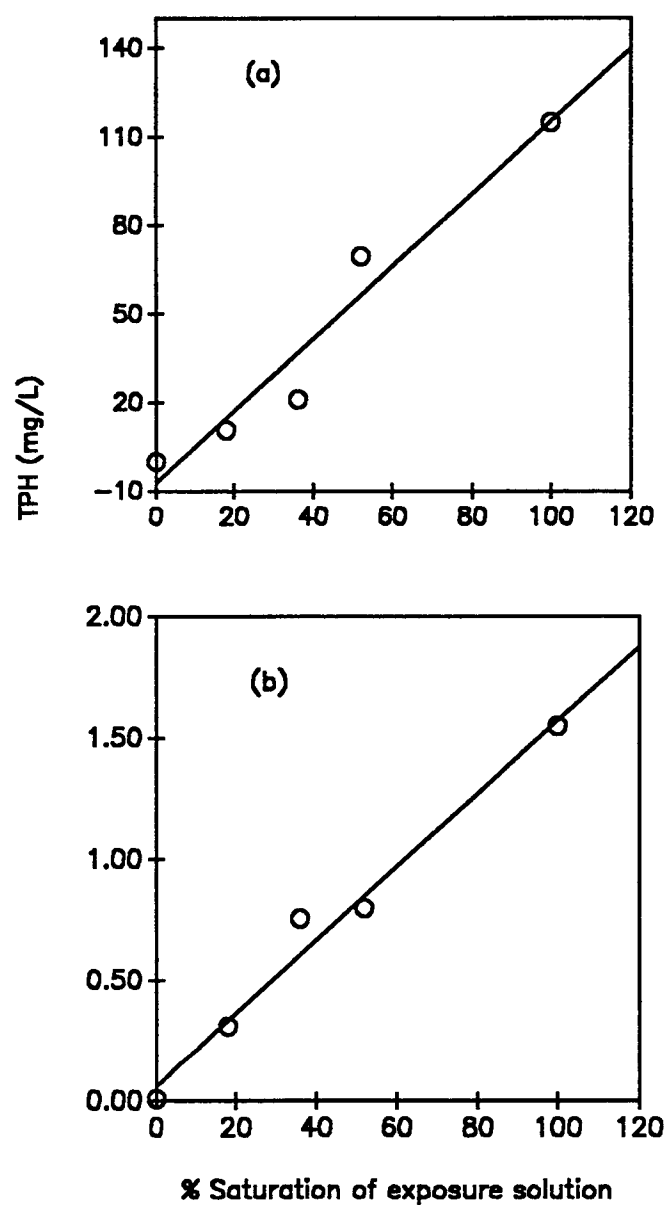
Figure 3. Relationship between % saturation of exposure solution and TPH during 96-hr acute tests in (a) CO test ($R^2=0.95$) and (b) PCO test ($R^2=0.98$).

16-day subacute tests

Homogeneity of average initial fish wet weights for treatment groups were tested using one-way ANOVA and no values were statistically different from each other for either PCO (p=0.91) and CO exposures (p=0.32). One-way ANOVA on final wet weight indicated that mean treatment effect in the PCO test was different than the control mean. Similarly, all mean SGR associated with PCO treatments were different from the control mean (p=0.005). Differences between treatment groups (final wet weight) were inconclusive (p≤0.08) for CO exposures. However, one-way ANOVA on SGR in CO tests suggested that all the treatment levels except the lowest treatment (67.10 μg/L) were different from the control mean (p=0.017). Similar to 96-hr acute tests, TPH concentration in both tests linearly increased with increasing proportion of the toxicant stock solution and the concentration detected in PCO-WSF was much higher than those in CO-WSF (Figure 4). ANOVA indicated that all TPH concentrations between treatments in PCO exposure were different from each other (p≤0.0001), but not all TPH concentrations in CO treatments were different. In both PCO and CO tests, there was a concentration-dependent reduction in SGR (Fig. 5). Final wet weight was significantly reduced in the lowest PCO exposure (3.78 μg/L). Only the highest TPH concentration (144.55 μg/L) for CO exposure reduced mean final wet weight compared to control means. Multiple linear regression comparison of the slopes indicated that the rate of reduction in SGR was significantly different in both tests (2-sided p-value=0.0002 for interaction term).

Gas chromatographic analysis of PAHs in the WSF of PCO and CO showed different proportions of lower and higher molecular weight PAHs. The WSF of CO contained lower molecular weight PAHs, such as naphthalene. In PCO-WSF, higher molecular weight PAH, benzo(a)pyrene, was dominant. Chrysene was found in both WSFs.

Table 3. Average initial and final fish wet weights for each treatment group (30), TPH concentrations (6 measurements), and SGR for tests with PCO and CO during 16-day subacute tests. Results reported as the mean ± SE between replicated exposures.

Test Material	Tank	Initial Wet Weight (g)	Final Wet Weight (g)	SGR (%)	TPH (μg/L)
PCO	Control	0.045 ± 0.002	0.102 ± 0.004	5.13 ± 0.21	<0.05
	1	0.042 ± 0.001	0.081 ± 0.002[a]	4.06 ± 0.40[a]	3.78 ± 0.60
	2	0.044 ± 0.002	0.084 ± 0.003[a]	4.01 ± 0.09[a]	5.74 ± 0.34
	3	0.044 ± 0.002	0.083 ± 0.004[a]	3.92 ± 0.12[a]	7.85 ± 0.25
	4	0.044 ± 0.002	0.074 ± 0.004[a]	3.46 ± 0.26[a]	9.63 ± 0.42
	5	0.044 ± 0.002	0.070 ± 0.003[a]	2.84 ± 0.01[a]	12.24 ± 0.46
CO	Control	0.032 ± 0.001	0.071 ± 0.003	5.08 ± 0.06	<0.05
	1	0.033 ± 0.001	0.070 ± 0.003	4.49 ± 0.11	67.10 ± 11.21
	2	0.034 ± 0.001	0.070 ± 0.003	4.09 ± 0.20[a]	81.45 ± 9.24
	3	0.032 ± 0.001	0.062 ± 0.002	4.23 ± 0.37[a]	101.03 ± 12.26
	4	0.033 ± 0.001	0.064 ± 0.002	4.06 ± 0.16[a]	126.45 ± 20.06
	5	0.034 ± 0.001	0.060 ± 0.002[a]	3.44 ± 0.19[a]	144.55 ± 19.47

[a]Mean significantly less than control mean (ANOVA, p ≤ 0.05)
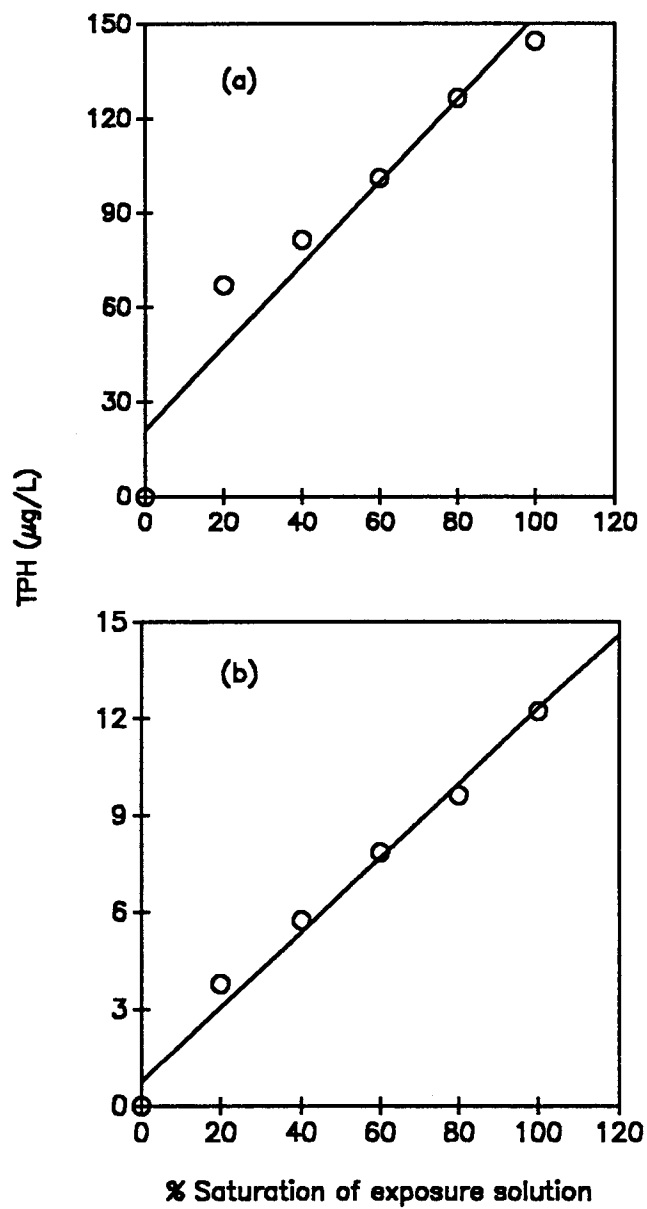
Figure 4. Relationship between % saturation of exposure solution and TPH in 16-day subacute tests in (a) CO test ($R^2 = 0.93$) and (b) PCO test ($R^2 = 0.98$).
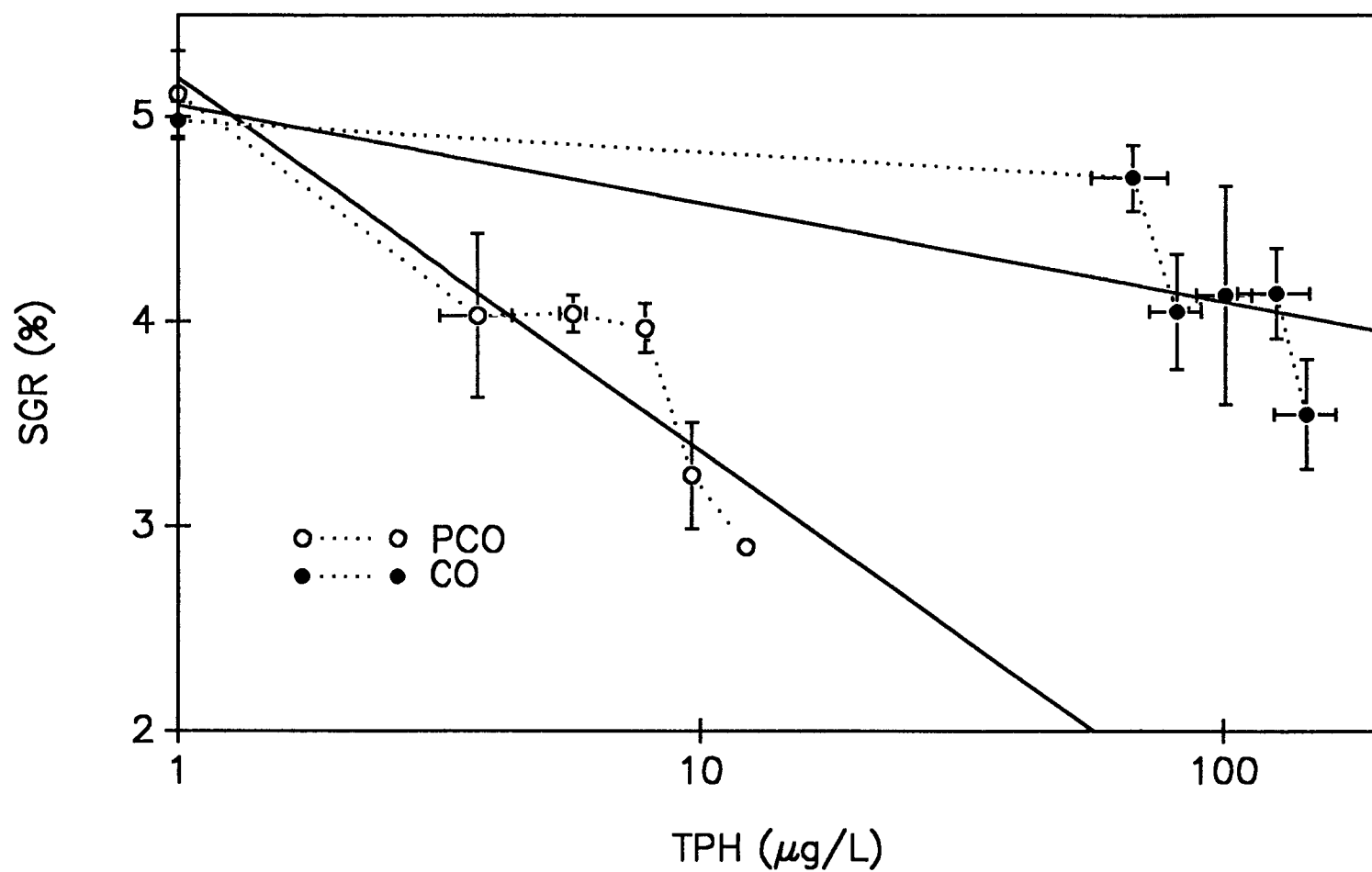
Figure 5. PCO ($R^2 = 0.87$) and CO ($R^2 = 0.73$) reduction in SGR of *Menidia beryllina* in 16-d subacute tests.
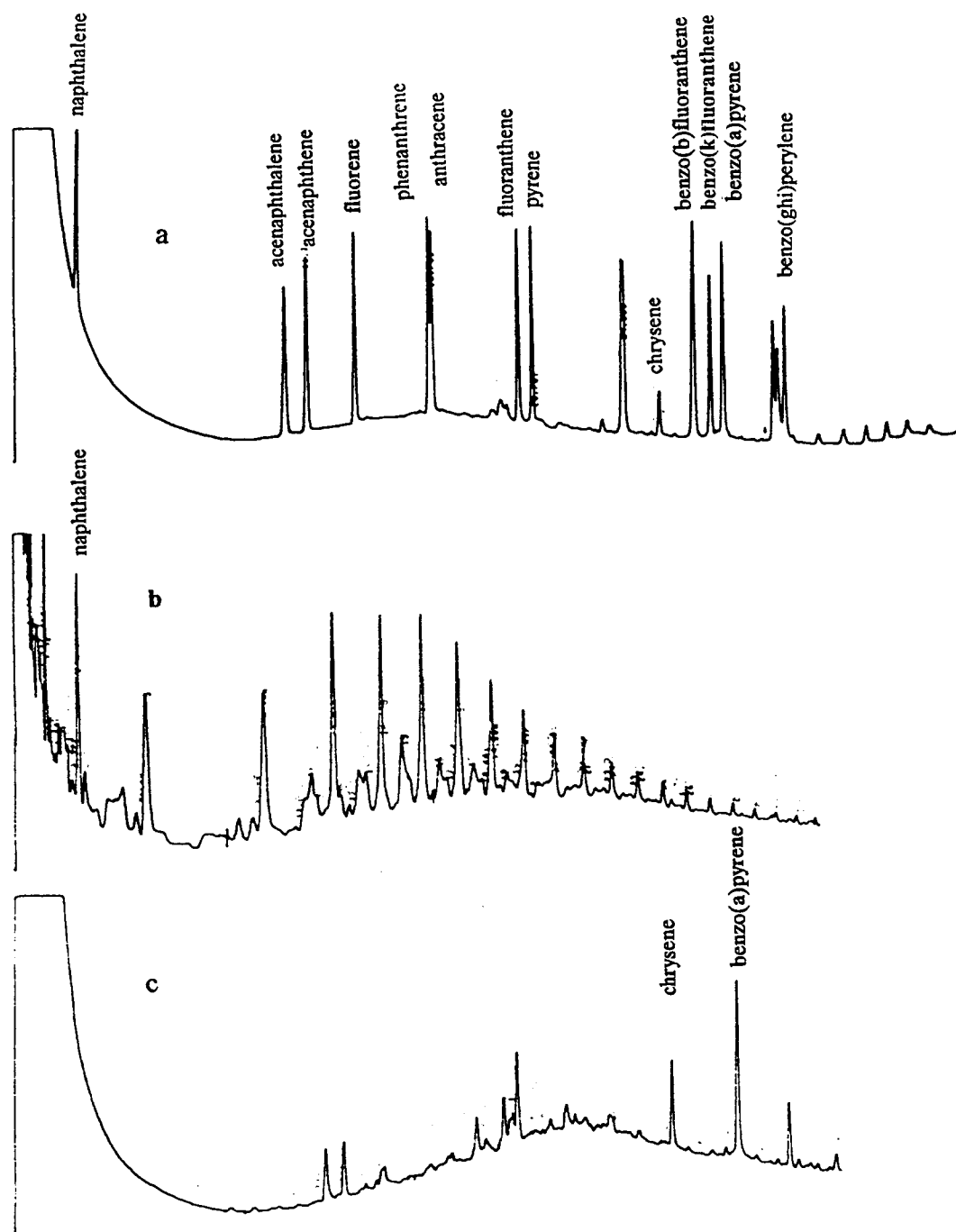
Figure 6. GC profiles for selected PAHs in (a) standard, (b) CO stock and (c) PCO stock solution

DISCUSSION

This study indicated that the WSF of PCO was more toxic than CO-WSF. Although the WSF of CO caused higher acute mortality than that of PCO, it is important to note that TPH concentration in CO exposures was higher than TPH concentration in PCO exposures (Table 2). Additionally, the estimated LC50 of PCO is about 10 times less than for CO (2.85 and 28.69 ppm TPH, respectively). Also, Dunnet's procedure estimation of NOEC and LOEC for survival were much lower for PCO than for CO. The results of acute tests were consistent with results in subacute tests. In our study, both in CO and PCO-tests, SGR of *Menidia beryllina* was reduced with increasing treatment level, particularly in the PCO tests (Table 3). Decreased SGR corresponded to the increasing TPH concentration. A test for different slopes indicated that SGR in PCO and CO tests decreased at different rates, with PCO exposure having higher impact. This indicated that based on TPH concentrations of both toxicants, the WSF of PCO is more toxic than that of CO.

Comparison of our study with others was difficult since different methods for preparation of WSF solutions results in different amounts of TPH. Chemical data obtained in our study was different from others, in which stock solutions (12.5% crude oil in water solution) of crude oil contained 114.8 ppm TPH. Another study found 10% (one part CO and 9 parts seawater) of South Louisiana and Kuwaiti CO mixed 20 hr in water solution contained 19.8 ppm, and 10.4 ppm

of TPH respectively (Anderson et al., 1974). In another study with the same mixing procedure but different analytical methods, 10% WSF of South Louisiana CO contained 6.8 ppm TPH (Solangi et al., 1982). Many factors contribute to differences in the amount of TPH dissolved in water, including duration and mixing procedure and differences in analytical methods (Rice et al., 1977; Lee et al., 1978a). Wide ranges of TPH concentrations in acute (ppm range) and subacute (ppb range) tests in this study indicated PCO and CO stock solutions (acute tests) were supersaturated with TPH. It might be different mixing conditions in acute tests produced oil droplets which may increase the TPH at concentrations exceeding their expected water solubility (Anderson et al., 1974). The actual solubility of total petroleum hydrocarbon in seawater was unclear. A study of hydrocarbons in seawater extracts of crude oil was done by Boylan and Tripp (1971). They mixed 25 ml Kuwaiti crude oil with 1.5 L seawater for 12 hr and found relative concentrations of polar aromatic and total amount of oil in water were 796 and 1453 ppb, respectively.

In our study, lethal effects (10% mortality) of CO to *Menidia beryllina* occurred at the lowest percent saturation of exposure solution (18% = 10.8 ppm TPH; Table 2). This TPH concentration was similar to what Solangi et al. (1982) found in 100 mg whole crude oil/L seawater (10.2 ppm of TPH) that caused about 10% mortality in *Menidia beryllina* in less than 5 days. In their study, all other exposures contained TPH concentrations less than 5 ppm and death did not occur until day 5. No fish survived 96-hr exposures to maximum WSF

concentrations of CO while less than 50% fish died in PCO exposures. This indicated that CO-WSF was acutely more toxic because it contained over 10 fold higher TPH concentrations than PCO-WSF. Sublethal effects of low-level of CO exposure were on growth, feeding, and liver function (Kiceniuk and Khan, 1983), which may affect long-term survival of an organism. Comparisons of our growth study to others on fish was difficult because no clear information of the TPH concentration that affected growth was reported. However, work on *Mytilus edulis* by Widdow et al. (1982) found that scope for growth of the animal decreased as a result of exposures to 7.7 and 68 ppb of TPH for 14 days. These values were similar to what we found in subacute tests, ranging from 3.7 to 144.5 ppb TPH in both PCO and CO-exposures.

CO is a complex mixture of hydrocarbons of varying molecular weight and structure ranging from a light gas (methane) to heavy solids. CO also contains other organic compounds including nitrogen-sulfur-oxygen containing compounds, porphyrins, asphaltenes, and trace metals, the composition of which differs from one CO to another (NRC, 1985). Evaluating the potential adverse effects of such complex mixtures of toxic compounds is a difficult task. When oil enters the marine environment, it is subjected to 'weathering processes', a combined effect of evaporation, oxidation and biodegradation which may enhance or reduce oil toxicity. In addition, the degree to which oil is dissolved into the aqueous phase is strongly dependent on solubility, mixing energy, mixing duration, and oil viscosity (Rice et al., 1977). Based on these physical and chemical characteristics , CO will

either dissolve in the water or form droplets. It is generally agreed that CO toxicity is due more to its WSF than to dispersed droplets (Anderson et al., 1974). Of all petroleum hydrocarbons classes, the aromatic groups are the most toxic found in CO because they are more water soluble than other petroleum components (Neff et al., 1976; Lee et al., 1978b; Solangi et al., 1982). Once dissolved in water, their volatility is decreased, while the dispersed fraction will leave the water more rapidly than those compounds which are present in true solution. Even if the dispersed fraction is ingested by aquatic organisms, metabolism is more rapid. Neff et al., (1976) observed that n-alkanes, which are dominant hydrocarbons found in dispersed oil, are metabolized more rapidly than the aromatics by molluscs because they closely resemble biogenic hydrocarbons.

PAHs are of special concern because their known toxicity to aquatic organisms. Black et al., (1983) studied the toxicity of benzene, naphthalene and phenanthrene to rainbow trout and largemouth bass and found that PAH toxicity was a function of benzene ring number. Kuwaiti CO contains relatively high concentrations of PAHs, sulfur-containing compounds (eg., thiopenes) and heavy metals (Litherathy, 1992; Krahn et al., 1993). Although, CO generally contains relatively low concentrations of PAHs (0.2-7%), high temperature (700°C) pyrolysis of organic materials can form PAHs (Neff, 1985). During the Gulf war in Kuwait, about 600 burning oil wells generated PAHs and enriched them in PCO (Literathy, 1992). This material was deposited on soil with some atmospheric transportation to the marine waters. PAHs formed from CO as a

result of high-temperatures are generally larger molecular weight (≥ 4 aromatic rings) parental (non-alkylated) compounds (Readman et al., 1992). Dibenzoanthracene and benzo(a)pyrene are natural products, resulting from incomplete combustion processes of carbonaceous material (Williams and Weisburger, 1991).

GC analysis of stock solutions indicated predominance of the lower molecular weight PAHs in the WSF of CO and higher molecular weight PAHs contributed more to PCO-WSF. It is generally believed that acute toxicity of the WSF of crude oil is caused by the lower boiling aromatics PAH, such as naphthalene (Lee et al., 1978b). In our study GC analysis on the WSF of CO revealed that naphthalene was present (Figure 5). Lee et al.(1978b) studied the toxicity of 4 aromatic compounds components of petroleum oils (representing volatile compounds, naphthalene and 1,2,4-trimethylbenzene; non-volatile compounds, o-cresol and o-toluidine) to marine amphipods and found that naphthalene was the most toxic substance among the four aromatics tested. The findings were similar to those of Neff et al. (1976), who studied the accumulation and depuration of petroleum-derived aromatic hydrocarbons to marine animals. In most cases, the naphthalenes appeared to be accumulated to the greatest extent and retained for the longest period of time by marine animals. Neff et al. (1976) suggested further that naphthalene was most bioavailable due to its higher water solubility than biphenyls, fluorenes and phenanthrenes. The lower boiling aromatics have higher volatility, thus following an oil spill this group will rapidly

evaporate from solution, with negligible amounts remaining after 24 hr (Lee et al., 1978b). Solangi et al. (1982) observed pathological damage in adult *Menidia beryllina* as a result of exposure to South Louisiana CO. The observed damage included severe gill epithelial hyperplasia, separation of respiratory epithelium from underlying supportive tissues, necrosis of olfactory mucosa and pancreas, and heavy mucus production. Solangi et al. (1982) hypothesized that mortality was the result of gill damage. Prasad (1987) studied the metabolism of freshwater minor carp (*Puntius sophore*) and found that exposure to 200 ppm WSF of Barauni CO decreased respiration rate. It was concluded that decreased oxygen uptake and increased breathing rate exposed gills to more toxicant resulting in the fusion of secondary lamellae, lesions in the respiratory epithelium and enlargement of water-blood diffusion barrier. Transfer of petroleum fractions into fish tissues via the gills may be related to the sensitivity of gills to CO toxicity (Lee et al., 1972). Exposure to naphthalene was also known to cause gill hyperplasia and gill filament hemorrhage (Neff, 1985). There was evidence that the toxicity of naphthalene in mummichogs (*Fundulus heteroclitus*) was on blood components in lethal acute situations (Dimichele and Taylor, 1978). Besides histopathological and morphological damage, exposure to petroleum hydrocarbon (8 days) rapidly produced biochemical changes and altered membrane structure in marine fish (Sabo and Stegemen, 1977).

Based on TPH, the WSF of PCO was more toxic than CO-WSF. GC analysis of PCO stock solution (Figure 6) indicated a higher molecular weight

PAH, such as benzo(a)pyrene (BaP), was likely a major constituent in the WSF

that contributed to the adverse effects observed in the fish. However, since there

is no information on other PCO-related compounds, it was not clear whether TPH

is the major contaminant found in PCO-contaminated soil. It is possible that

mortality and reduced growth observed in PCO tests involved other components

of the soil contaminated-PCO since Kuwaiti crude oil also contains other

compounds such as benzothiophenes and heavy metals. Little is known

concerning the contribution of heavy metals to crude oil toxicity to fish. The

concentration of trace metals in Prudhoe Bay CO, which is of comparable to that

in Kuwaiti CO, did not correlate with the toxicity in seabirds (Miller et al., 1982).

Although, there is no evidence that BaP is an acutely toxic PAH, perhaps there is

high enough concentration of this compound to cause mortality. Goddard et al.

(1987) reported that waterborne BaP at a dose of 3.75 ppm caused 66% mortality

in topminnows (*Poeciliopsis*) within 29-hr exposure. Since BaP has a low water

solubility, it may not be immediately available for bioaccumulation, thus it may

cause delayed adversed effects. Study on the uptake and release of PAHs by

marine fish (mudsucker or sand goby, sculpin, and sand dab) showed rapid

accumulation of both naphthalene and BaP, but naphthalene was taken up 15

times faster than BaP. Also, marine fish excreted naphthalene and its metabolites

at a greater rate than BaP and its metabolites (Lee et al., 1972). BaP is relatively

non-polar and has high partition coefficient (Kow), thus it is slowly removed from

a lipid matrix (Thomas and Rice, 1982).

Sabo and Stegeman (1977) suggested that contamination of marine fish (*Fundulus heteroclitus*) with petroleum hydrocarbons altered membrane structure either intracellularly or at the cell surface. These changes could perturb some membrane functions, via altering membrane fluidity or permeability either physically changing the conformation of the lipid matrix or through oxidative cell injury caused by reactive oxygen species (Varanasi, 1989). This is generally true with lower molecular weight PAHs, particularly naphthalene. A number of studies support the conclusion that a major mode of action of this PAH group is through interference with cellular membrane function (Dimichelle and Taylor, 1978; Levitan and Taylor, 1979; Kiceniuk et al., 1982). Perhaps this would provide an explanation for the decline in SGR of fish exposed to CO-WSF and to the greater extent in PCO-exposure. Leadon et al., (1988) studied the metabolic activation of BaP in human mammary epithelial cell culture and observed metabolic activation of BaP generated reactive oxygen species (i.e. hydrogen peroxide and superoxide anion). The possible mechanism of toxicity of reactive oxygen species may be mitochondrial membrane damage by reacting with double bonds of membrane fatty acid phospholipids. This might further disrupt the potential gradient across the mitochondrial membrane and lower ATP production. Further, energy may also be required to repair membrane and tissue damage. Coupled with these effects, energy required to metabolize PAHs may also decrease efficient energy utilization for growth and fat deposition (Korn et al., 1976). As for lower molecular weight PAHs (as might be expected in CO tests),

the reduction in growth may be due to the latter effects and to their effects on the gills, which reduced oxygen uptake. There is also a possibility that naphthalene in CO might decrease blood oxygen-carrying capacity through methemoglobin formation. Although there is no information that naphthalene can induce formation of methemoglobin (hemolytic anemia) in fish, there is evidence that ingested CO can cause hemolytic anemia in herring gulls (Leighton et al., 1985). In vitro red blood cells assays in rats demonstrated that naphthalene (37%) and petroleum oil (2.9% to 6.9%) induced formation of methemoglobin (Couillard and Leighton, 1993). It was further explained that methemoglobin was formed only in the presence of liver enzymes. Nitrite exposure of fish can oxidize hemoglobin to methemoglobin, thus reducing the oxygen carrying capacity of blood (Leduc, 1984). All of these effects could potentially reduce growth by decreasing energy available to fish exposed to chronic low-level of petroleum oil. Benzene, one of the major water-soluble components of CO, decreased growth, increased mortality and reduced the ability to withstand environmental stress in juvenile striped bass (*Morone saxatilis*). This may be a result of prolonged periods of competitive effects on energy utilization (Korn et al., 1976).

Preliminary analysis of whole fish (*Menidia beryllina*) homogenates indicated neither PCO nor CO induced tissue cytochrome P4501A1 (Gundersen et al., 1995). We believe that using the whole fish homogenates resulted in concentrations near the detection limit. However, there was a suggestion of induction, especially in the highest concentration of PCO-exposed fish, in which

some antibody cross-reacted with a protein band in whole fish homogenates on western blotting. If induction of hepatic P450 1A1 is assumed in fish exposed to PCO, this finding may correlate with the study done by Varanasi and Gmur (1981) who found that the liver of English sole (*Parophrys vetulus*) metabolized BaP more extensively than naphthalene. They explained further that fish hepatic mixed-function oxidases have greater affinity for BaP than naphthalene. It has been shown that BaP induced enzyme activity in freshwater and marine fish (Payne et al., 1976; Goddard et al., 1987). Induction of cytochrome P4501A1 was also observed in cod larvae and juvenile exposed to low-level (ppb range) of CO-WSF (Goksoyr, 1991). This hypothesis could explain the greater reduction of SGR in PCO-exposed fish than those exposed to CO.

There are several points that can be concluded from this study. A substantially higher TPH concentration detected in WSF of CO in both acute and subacute tests indicated that TPH-related compounds are much more water soluble and thus more available than those found in PCO-WSF. The higher water-solubility compounds are likely responsible for the lethal effect in acute tests and the less soluble fractions may be responsible for the subacute effects. PAH analysis indicated that the WSF in CO and PCO tests contained lower and higher molecular weight PAHs, respectively. Different effects produced in acute and subacute tests of both CO and PCO exposures might be due to differences in PAHs constituents of the WSF, TPH concentration, and duration of exposure. Acute toxicity may not adequately reflect the potential of longterm-exposure of

low-level petroleum hydrocarbons. Although accidental spillage provides the largest input of oil into the marine environment, continuous discharges of low-level petroleum hydrocarbons due to oil activities (Madany et al., 1994) may contribute to long-term detrimental effects on aquatic organisms. A continuous flow through bioassay appeared to best simulate chronic oil pollution yet also give stable concentrations of WSF (Brennan et al., 1976; Vanderhorst et al., 1977; Widdows et al., 1982). The TPH data presented in this study (subacute tests) were similar to values found in the natural marine environment. Madany et al.(1994) conducted a comprehensive field study of the levels of aromatic petroleum hydrocarbons in water throughout the coastal areas of Bahrain in the Arabian Gulf. The results of the survey demonstrated that levels of petroleum hydrocarbon (1993) were generally high (16.1-88.5 ppb), indicating a chronic oil pollution problem. They also cited the concentration in Kuwaiti waters following the Gulf war (1991) was 27.04 to 89.80 ppb. Therefore, there is a potential for adverse impacts on marine animals by low-level petroleum hydrocarbon exposure, particularly in confined areas with low energy and shallow water such as Kuwait marine environment. However, the possibility that other constituents of the WSF cause adverse effects was also significant. Therefore, additional study on other WSF-related compounds is important in order to better understand the actual compounds responsible for toxicity. This is may be true for the WSF of PCO since, according to Litherathy (1992), the burning oil may poses different environmental problems. He explained that depending on the completeness of

burning, production of completely oxidized gaseous end products; ashes containing heavy metals; soot; high-boiling, unburnt components; conversion products (e.g., pyrolitic, break-down components); and unchanged oil droplets are possible. There is also evidence of the presence of metals being deposited as a result of oil burning in Kuwait (Sadiq et al., 1992).

BIBLIOGRAPHY

Al-Yakoob, S.N., M.M. Al-Sudairawi, H.A. Nasrallah and N. Al-Majed. 1993. Adsorption of polycyclic aromatic hydrocarbons onto inhalable particulate matter during the Kuwait oil fires. J. Environ. Sci. Health A28(8):1781-1793.

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

Black, J.A., W.J. Birge, A.G. Westerman and P.C. Francis. 1983. Comparative aquatic toxicology of aromatic hydrocarbons. Fund. App. Toxicol. 3:353-358.

Boylan, D.B. and B.W. Tripp. 1971. Determination of hydrocarbons in seawater extracts of crude oil and crude oil fractions. Nature 230:44-47.

Brennan, G., R. Hartung and W.J. Weber, Jr. 1976. A continuous flow bioassay method to evaluate the effects of outboard motor exhaust and selected aromatic toxicants on fish. Water Res. 10:165-169.

Chadwick, G.G., J.R. Palenski and D.L. Shumway. 1972. Continuous-flow dilution apparatus for toxicity studies. Proc. of the Pacific N.W. Industrial Manag. Conference. Portland, Oregon. pp. 101-105

Clements, W.H., J.T. Oris and T.E. Wissing. 1994. Accumulation and food chain transfer of fluoranthene and benzo(a)pyrene in Chironomus riparius and Lepomis macrochirus. Arch. Environ. Contam. Toxicol. 26:261-266

Couillard, C.M., and F.A. Leighton. 1991. Bioassay for the toxicity of petroleum oils in chicken embryos. Environ. Toxicol. Chem. 10:533-538

Couillard, C.M., and F.A. Leighton. 1993. Invitro red blood cell assay for oxidant toxicity of petroleum oil. Environ. Toxicol. Chem. 12:839-845

Curtis, L.R., H.M. Carpenter, R.M. Donohoe, D.E. Williams, O.R. Hedstrom, M.L. Deinzer, M.A. Beilstein, E. Foster and R. Gates. 1993. Sensitivity of cytochrome P450-1A1 induction in fish as a biomarker for distribution of TCDD and TCDF in the Willamette River, Oregon. Environ. Sci. Technol. 27:2149-2157.

Dauble, D.D., W.E. Fallon, R.H. Gray and R.M. Bean. 1982. Effects of coal liquid water-soluble fractions on growth and survival of four aquatic organisms. Arch. Environ. Contam. Toxicol. 11:553-560

Dimichele, L. and M.H. Taylor. 1973. Histopathology and physiological response of *Fundulus heteroclitus* to naphthalene exposure. J. Fish. Res. Board Can. 36:615-620.

Donahue, W.H., R.T. Wang, M. Welch and J.A.C. Nicol. 1977. Effects of water-soluble components of petroleum oils and aromatic hydrocarbons on barnacle larvae. Environ. Pollut. 13:186-202

Foda, M.A., F.I. Khalaf and A.S. Al-Kadi. 1985. Estimation of dust fallout rates in the Northern Arabian Gulf. Sedimentology 32:595-603

Genthner, F.J., and D.P. Middaugh. 1992. Effects of *bauveria bassiana* on embryos of the Inland Silverside (*Menidia beryllina*). App. Environ. Microbiol. 58(9):2840-2845

Goddard, K.A., R.J. Schultz and J.J. Stegeman. 1987. Uptake, toxicity, and distribution of benzo(a)pyrene and monooxygenase induction in the topminnow *Poeciliopsis monacha* and *Poeciliopsis lucida*. Drug Met. Disp. 15(4):449-455.

Goksoyr, A., T.S. Solberg and B. Serigstad. 1991. Immunochemical detection of cytochrome P4501A1 induction in cod larvae and juveniles exposed to a water soluble fraction of North Sea crude oil. Mar. Poll. Bul. 22(3):122-127.

Gundersen, D.T., S.W. Kristanto, L.R. Curtis, A. Al-Yakoob and M. Metwally. 1995. Toxicity and accumulation of the water soluble fraction (WSF) of crude oil (CO) and partially combusted crude oil (PCO) from Kuwaiti oil-fires on the marine fish, *Menidia beryllina*. Abstrack. Society of Toxicology 1995 Annual Meeting.

Hahn, J., 1991, Environmental effects of the Kuwaiti oil field fires. Environ. Sci. Technol. 25(9):1531-1532

Hamilton, M.A., R.C. Russo and R.V. Thurston. 1978. Trimmed Spearman-Karber method for estimating median-lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 12:714-719.

Hemmer, M.J., D.P. Middaugh and V. Comparetta. 1992. Comparative Acute Sensitivity of Larval Topsmelt, *Atherinops affinis*, and inland silverside, *Menidia beryllina*, to 11 chemicals. Environ. Toxicol. Chem. 11:410-408

James, M.O., and J.R. Bend. 1980. Polycyclic aromatic hydrocarbon induction of cytochrome P-450 -dependent mixed-function oxidases in marine fishes. Toxicol. App. Pharmacol. 59:117-133

Kiceniuk, J.W., R.A. Khan, M. Dawe and U. Williams. 1982. Examination of trypanosome infection and crude oil exposure on hematology of the Longhorn sculpin (*Myoxocephalus octodecemspinosus*). Bull. Environ. Contam. Toxicol. 28:435-438.

Kiceniuk, J.W., and R.A. Khan. 1983. Toxicology of chronic crude oil exposure: sublethal effects on aquatic organisms. In: Aquatic Toxicology, edited by J.O. Nriagu. John Wiley & Sons, Inc. USA. pp. 425-436.

Korn, S., J.W. Struksaker and P. Benville, Jr. 1976. Effects benzene on growth, fat content, and caloric content of striped bass, *Morone saxatilis*. Fish. Bull. U.S. 74:694-698.

Krahn, M.M., G.M. Ylitalo, J. Buzitis, J.L. Bolton, C.A. Wigren, S-L. Chan and U. Varanasi. 1993. Analyses for petroleum-related contaminants in marine fish and sediments following the Gulf oil spill. Mar. Poll. Bull. 27:285-292.

Leadon, S.A., M.R. Stamper and J. Bartley. 1988. Production of oxidative DNA damage during the metabolic activation of benzo(a)pyrene in human mammary epithelial cells correlates with cell killing. Proc. Natl. Acad. Sci. 85:4365-4368.

Leduc, G. 1984. Cyanides in water: toxicological significance. In: Aquatic Toxicology, vol.2, edited by L.J. Weber. Raven Press, New York. pp. 153-224.

Lee, R.F., R. Sauerheber and G.H. Dobbs. 1972. Uptake, metabolism and discharge of polycylic aromatic hydrocarbons by marine fish. Mar. Biol. 17:210-208.

Lee, W.Y., K. Winters and J.A.C. Nicol. 1978a. The biological effects of the water-soluble fractions of a no. 2 fuel oil on the planktonic shrimp, *Lucifer faxoni*. Environ. Pollut. 15:167-183.

Lee, W.Y., and J.A.C. Nicol. 1978b. Individual and combined toxicity of some petroleum aromatics to the marine amphipod *Elasmopus pectenicrus*. Mar. Biol. 48:215-222.

Leighton, F.A., Y.Z. Lee, A.D. Rahimtula, P.J. O'Brien and D.B. Peakall. 1985. Biochemical and functional disturbances in red blood cells of herring gulls

ingesting Prudhoe Bay crude oil. Toxicol. App. Pharmacol. 81:25-31.

Leighton, F.A., 1990. The Systemic Toxicity of Prudhoe Bay Crude and other Petroleum Oil to CD-1 Mice. Arch. Environ. Contam. Toxicol. 19:257-262.

Levitan, W.M., and M.H. Taylor. 1979. Physiology of salinity-dependent naphthalene toxicity in *Fundulus heteroclitus*. J. Fish. Res. Board Can. 36:615-620.

Literathy, P., 1992. Environmental consequences of the Gulf war in Kuwait: impact on water resources. Wat. Sci. Tech. 26(1-2):21-30

Madany, I.M., A. Al-Haddad, A. Jaffar, E.-S. Al-Shirbini. 1994. Spatial and temporal distributions of aromatic petroleum hydrocarbon in the coastal waters of Bahrain. Arch. Environ. Contam. Toxicol. 26:185-190

Middaugh, D.P, M.J. Hemmer and E.M. Lores. 1988. Teratological effects of 2,4-dinitrophenol, 'produced water', and naphthalene on embryos of the inland silverside *Menidia beryllina*. Dis. Aqua. Orgs. 4:53-65

Middaugh, D.P., S.M. Resmick, S.E. Lantz, C.S. Heard and J.G. Mueller. 1993. Toxicological assessment of biodegraded pentachlorophenol microtox in fish embryos. Arch. Environ. Contam. Toxicol. 24:162-172

Miller, D.S., D.J. Hallet and D.B. Peakall. 1982. Which components of crude oil are toxic to young seabirds. Environ. Toxicol. Chem. 1:39-44.

NAS, 1975. Petroleum in the Marine Ecosystem. National Academy of Sciences, Washington D.C.

Neff, J.M., B.A. Cox, D. Dixit and J.W. Anderson. 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar. Biol. 38:279-289

Neff, J.M., 1985. PAHs. In: Fundamentals of Aquatic Toxicology, edited by G.M. Rand and S.R. Petrocelli. Taylor & Francis, USA. pp. 416-454

Neff, J.M. 1979. Polycyclic aromatic hydrocarbons in the aquatic environment: sources, fates, and biological effects. Applied Science, London.

NRC, 1985. Oil in the Sea: Inputs, Fates and Effects. National Research Council, National Academy Press. Washington, D.C.

Oost, R.V-D., F.J. Schooten, F. Ariesa, H. Heida, K. Satumalay and N.P.E. Vermeulen. 1994. Bioaccumulation, biotransformation and DNA binding of PAHs in Feral Eel (*Anguilla anguilla*) exposed to polluted sediments: a field study. Environ. Toxicol. Chem. 13(6):859-870

Payne, J.F., 1976. Field evaluation of benzopyrene hydroxylase induction as a monitor for marine petroleum pollution. Science 191:945-946.

Payne, J.F., J. Kiceniuk, L.L. Fancey, V. Williams, G.L. Fletcher, A. Rahimtullah and B. Fowler. 1988. What is a safe level of PAHs for fish: subchronic toxicity study on Winter Flounder (*Pseudopleuronectes americanus*). Can. J. Fish. Aquat. Sci. 45: 1983-1992

Payne, J.R., and C.R. Phillips. 1985. Photochemistry of petroleum in water. Environ. Sci. Technol. 19(7):569-579

Peakall, D.B., D.J. Hallett, J.R. Bend, G.L. Foureman and D.S. Miller. 1982. Toxicity of Prudhoe Bay crude oil and its aromatic fraction to nestling Herring Gulls. Environ. Res. 27:206-215

Picer, M., and N. Picer. 1993. Evaluation of modifications of the simple spectrofluorometry method for estimating petroleum hydrocarbon levels in seawater. Bull. Environ. Contam. Toxicol. 50:802-810.

Prasad, M.S. 1987. Toxicity of crude oil to the metabolism of freshwater minor carp, *Puntius sophore*. Bull. Environ. Contam. Toxicol. 39:188-193.

Ramsey, F., and D. Schafer. 1994. The Statistical Sleuth: a second course in statistic data analysis. Department of Statistics. Oregon State University. Corvallis, Oregon.

Readman, J.W., I.M., S.W. Fowler, J.P. Villeneuve, C. Cattini, B. Oreogioni and L.D. Mee. 1992. Oil and combustion-product contamination of the Gulf marine environment following the war. Nature 358:662-665

Rice, S.D., J.W. Short and J.F. Karinen. 1977. Comparative oil toxicity and comparative animal sensitivity. In: Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms, edited by D.A. Wolfe. Pergamon Press, New York, USA. pp. 78-94.

Sabo, D.J., and J.J. Stegeman. 1977. Some metabolic effects of petroleum hydrocarbons in marine fish. In: Physiological Responses of Marine Biota to Pollutants, edited by F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg. Academic Press, New York. pp. 279-287.

Sadiq, M., K.M. AlThagafi and A.A. Mian. 1992. Preliminary evaluation of metal contamination of soils from the Gulf war activities. Bull. Environ. Contam. Toxicol. 49:633-639.

Solangi, M.A., and R.M. Overstreet. 1982. Histopathological changes in two estuarine fish, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch & Schneider), exposed to crude oil and its water-soluble fractions. J. Fish. Dis. 5:13-35

Statgraphics. 1986. Statistical graphic system by Statistical Graphic Corporation. STSC Inc.

Stegeman, J.J., A.V. Klotz, B.R. Woodin and A.M. Pajor. 1981. Induction of hepatic cytochrome P-450 in fish and the indication of environmental induction in scup (*Stenotomus chrysops*). Aqua. Toxicol. 1:197-212.

Thomas, R.E., and S.D. Rice. 1982. Metabolism and clearance of phenolic and mono-, di-, and polynuclear aromatic hydrocarbons by Dolly Varden char. In: Physiological Mechanism of Marine Pollutants Toxicity, edited by W.B. Vernberg, A. Calabrase, F.P. Thurberg and F.J. Vernberg. Academic Press, New York, NY. pp. 161-167

Vanderhost, J.R., C.I. Gibson, L.J. Moore and P. Wilkinson. 1977. Continuous-flow apparatus for use in petroleum bioassay. Bull. Environ. Contam. Toxicol. 17(5):577-584.

Weber, C.I., W.B. Horning, II, D.J., Klemm, T.W. Neiheisel, P.A. Lewis, E.L. Robinson, J. Mekedick and F. Kessler. eds. 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. 45268. EPA-600/4-87.028. 414pp.

Widdows, J., T. Bakke, B.L. Bayne, P. Donkin, D.R. Livingstone, D.M. Lowe, M.N. Moore, S.V. Evans and S.L. Moore. 1982. Responses of *Mytilus edulis* on exposure to the water-accommodated fraction of North Sea Oil. Mar. Biol. 67:15-31.

Williams, G.M., and J.H. Weisburger. 1991. Chemical carcinogenesis. In: Casarett and Doull's Toxicology: the Basic Science of Poisons, edited by M.O. Amdur, J. Doull and C.D. Klaassen. McGraw-Hill, New York, USA. pp. 172-173

Varanasi, U., and D.J. Gmur. 1981. Hydrocarbons and metabolites in English Sole (*Parophrys vetulus*) exposed simultaneously to (^{3}H) benzo(a)pyrene and (^{14}C) napthalene in oil-contaminated sediment. Aquat. Toxicol. 1:49-67

Varanasi, U., 1989. Metabolism of PAHs in the Aquatic Environment. CRC Press. Florida, USA.