

THE NATURE AND BIOLOGICAL EFFECTS  
OF WEATHERED PETROLEUM

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## 1. SUMMARY OF OBJECTIVES, CONCLUSIONS, AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

### 1.1 Summary of Objectives

Determine under laboratory conditions the toxicities of: a) Alaskan crude oil "weathered" by exposure to ultraviolet (UV) light compared to non-UV-exposed Alaskan crude oil, b) Alaskan crude oil weathered under simulated natural conditions compared to nonweathered Alaskan crude oil, and c) No. 2 fuel oil refined from an Alaskan crude oil exposed to UV light, in order to compare crude oil with a material (i.e., fuel oil) known to be capable of extensive photooxidation.

Determine: a) changes in concentrations of total hydrocarbons as a result of exposure to UV light, and b) the nature of oxidized compounds formed from Alaskan crude oils and No. 2 fuel oil and found in the underlying seawater.

Determine the relative uptake by selected marine organisms of hydrocarbons and oxidized components from weathered and nonweathered petroleum materials. Determine the nature of oxidized components in the organisms used for toxicity assays.

### 1.2 Summary of Conclusions

The concentrations of oxidized components released to seawater beneath Cook Inlet crude oil (CICO) and Prudhoe Bay crude oil (PBCO) as the result of UV irradiation were relatively low. UV irradiation of No. 2 fuel oil, in contrast, resulted in the formation and release to underlying seawater of substantially greater concentrations of oxidized products than were formed and released from UV-irradiated crude oils. A study was conducted to determine why a fraction of crude oil (i.e., fuel oil) produced higher concentrations of oxidized compounds after UV irradiation than did the parent crude oil. When polar compounds were removed from Alaskan crude oil and the resulting aromatic/paraffinic (A/P) fraction was subjected to UV irradiation, the concentration of oxidized components increased to levels intermediate between the crude oils and the refined product. These findings suggested that naturally occurring polar compounds in the crude oils inhibit photooxidation.

Experiments with radiotracers showed that petroleum components and oxidized products were substantially bioaccumulated by fish larvae. However, little or no mortality of English sole (Parophrys vetulus) embryos and larvae, or of surf smelt (Hypomesus pretiosus) larvae, resulted from short-term laboratory exposures to environmentally realistic levels of components from either UV-irradiated or nonirradiated crude or fuel oils. Changes in swimming behavior of surf smelt larvae were seen, but only after exposures to unweathered crude oil or fuel oil. Overall, there is no evidence from these studies that photooxidation would significantly enhance the toxicity of petroleum in the marine environment.

### 1.3 Implications with Respect to OCS Oil and Gas Development

The results indicate that, while UV irradiation (sunlight) can produce photooxidized products from CICO and PBCO in seawater, the amounts produced do not appear to be sufficiently large to cause -- at least in the short-term -- marked environmental damage. Environmentally realistic concentrations of crude oil and oxidation products obtained in the seawater-accommodated fractions (SWAF's) did not cause extensive short-term mortality of English sole embryos and larvae, or of surf smelt larvae, under the laboratory conditions used. Swimming behavior of surf smelt larvae was affected only by SWAF's from freshly prepared reference oils.

The implication is, therefore, that it appears unlikely that under most conditions that photooxidized products formed from Alaskan crude oils spilled into the Northeast Pacific Ocean would constitute a serious threat to fish. Environmental irradiation of a refined product, such as No. 2 fuel oil, however, could induce the release of much higher concentrations of soluble components into the water column than would be released from crude oils. These high concentrations could under worst-case conditions cause both mortality and behavioral modifications in fish, especially in early life stages in low energy environments (i.e., sheltered bays and estuaries).

## 2. INTRODUCTION

This two-year study was initiated in FY 82 to investigate: (1) changes in the chemical structures and concentrations of petroleum-derived compounds in the SWAF of petroleum during weathering and (2) the effects of these chemical changes on toxicity to marine species.

During the first year, two types of weathering regimes were employed: (1) CICO was exposed to ambient environmental conditions and (2) CICO and No. 2 fuel oil were exposed to UV light under controlled laboratory conditions in both static and flow-through modes. The SWAF's were analyzed for petroleum components using microgravimetric (MGA) and gas chromatographic (GC) techniques. Radiolabeled compounds (phenanthrene [PHN] and *p*-cresol) were used as markers in weathering, experiments to provide information on oxidative changes. The SWAF's were assayed for acute toxicity and sub-lethal effects on embryos and newly hatched larvae of fish.

It was demonstrated that CICO was only slightly photooxidized under controlled laboratory weathering conditions while No. 2 fuel oil was extensively photooxidized. Technical problems with the ambient environmental weathering system required modification of the protocol to one of laboratory exposures where light intensity, day length, air circulation and temperature could be closely controlled. Thus, during the second year more detailed, controlled studies were conducted and a second Alaska crude oil, Prudhoe Bay crude oil (PBCO), was also examined.

### 3. BACKGROUND

Petroleum entering the oceans undergoes a series of changes (i.e., evaporation, dissolution, photooxidation, microbial degradation) termed "weathering". These processes are believed to alter its toxicity. The oxidized products of petroleum hydrocarbons that are formed during weathering may be more water soluble than the parent hydrocarbons and may reach high concentrations in seawater. However, little is known about the chemical structure of many of these oxidized petroleum hydrocarbons or their concentrations in seawater because they are generally not detected in routine chemical analyses of petroleum-derived compounds.

Numerous oxidized products have been identified from petroleum which has been subjected to UV radiation under both natural (sunlight) conditions and simulated environmental conditions in the laboratory (Hansen 1975, Hendry et al. 1976, Larsen et al. 1977, Patel et al. 1978,). Ahmadjian et al. (1976) demonstrated that petroleum weathered under simulated natural conditions was similar to that weathered in the environment. While the oxidized products of petroleum hydrocarbons are frequently more water soluble than the parent hydrocarbons and oxidation will thus tend to help disperse the oil, it has been reported that some of the oxidized products are more toxic than the parent compounds (Lacaze and Villedon de Naide 1976). There are also reports (Larsen et al. 1977, Patel et al. 1978) that the acute toxicity of underlying water increases with the weathering of crude oils and of hydrocarbon fractions refined from crude oils (e.g., No. 2 fuel oil).

Crude petroleum contains naturally occurring nonhydrocarbons; a few crude oils may contain as much as 50% nonhydrocarbon components. Certain metal lo-organic compounds promote oxidation while others, such as sulfur-containing organic compounds, tend to inhibit oxidation (Clark and MacLeod 1977). Oxygen-containing compounds found in petroleum include acids, phenols, ketones, esters, lactones, ethers, and anhydrides (Clark and Brown 1977).

The oxygen content of the fractions of crude petroleum increases with boiling point, and the greater part of petroleum oxygen is found in high molecular weight distillation fractions boiling above 400°C. Hence, No. 2 fuel oils (boiling range, 185-345°C) usually have negligible amounts of oxygen-containing organic compounds. Oxygen-containing structures may have high molecular weights and long saturated hydrocarbon-type segments which cause their solubilities to be greater in the oil slick than in underlying seawater. This higher volatility in oil than in seawater is not true for many of the lower molecular weight photooxidized products. Hence, high molecular weight polar compounds in crude petroleum tend to be retained in an oil slick whereas photooxidized lower molecular weight polar byproducts of the slick tend to be dissolved into the underlying seawater as soon as they are formed.

#### 4. STUDY AREA

Experiments were performed at either the Northwest and Alaska Fisheries Center (NWAFC) in Seattle, or at the NWAFC's saltwater field station at Mukilteo, WA.

Organisms and seawater were obtained from Puget Sound.

#### 5. METHODS

##### 5.1 Reference Mixtures of Seawater and Oils

The reference mixtures of seawater and CICO, PBCO, and No. 2 fuel oil were prepared under conditions which minimized the oxidation of petroleum hydrocarbons. The seawater was filtered through a 0.45  $\mu\text{m}$  membrane filter and some batches were autoclave. Ten grams of oil were added to 2 L of either nonsterilized or sterilized seawater in 3.8 L clean brown glass bottles. After the bottles were shaken on a mechanical shaker for 2 hr at 4°C, the contents of a bottle were transferred to a 2 L separator funnel and the two phases were allowed to separate for 2 hr at 4°C. The underlying SWAF was then separated from the remaining surface layer of oil and stored in the dark at 4°C.

Radiotracers ( $[^{14}\text{C}]\text{-p-cresol}$ ,  $[^3\text{H}]\text{-phenanthrene}$ , and  $^{14}\text{C}\text{-hexadecane}$ ) were added to the initial preparations so that the amount of time needed for the small oil droplets (unaccommodated oil) suspended throughout the seawater to float to the surface could be readily determined by liquid scintillation counting (LSC). In addition, the radiotracers provided another means for determining the reproducibility of the preparation method. During the preparation, the oil/water mixtures were protected from UV light. The preparations of the SWAF's (Table 1) were then removed and divided into individual aliquots for replicate analyses to determine the precision of the procedures.

All solvents used were "distilled in glass" grade from Burdick and Jackson (Muskegon, Michigan). The  $[9\text{-}^{14}\text{C}]\text{-phenanthrene}$  (specific activity, 9.4 mCi/mmol) and  $[\text{ring-UL-}^{14}\text{C}]\text{-p-cresol}$  (Wizard Laboratories, Davis, CA), as well as  $[\text{UL-}^3\text{H}]\text{-phenanthrene}$  (Moravek, Brea, CA) were purified, immediately prior to use, by silica gel column chromatography (Varanasi and Gmur 1980). The  $n\text{-}[1\text{-}^{14}\text{C}]\text{-hexadecane}$  (specific activity, 53.6 mCi/mmol; Amer-sham, Arlington Heights, IL) was used without further purification.

The total amount of oil incorporated and total extractable organic material was determined by both MGA (Cahn Electrobalance, Model 4700) and GC analyses. Concentrations of selected individual compounds were determined by GC (Malins et al. 1980).

##### 5.1.1 Effects of Separation Time and Filtration on Composition of Reference Mixtures

Two hundred  $\mu\text{L}$  of a standard solution of  $[^{14}\text{C}]\text{-hexadecane}$  were added to each of three preweighed Erlenmeyer flasks containing (1) 10.6 g of CICO, (2) 10.7 g of No. 2 fuel oil, or (3) 10.6 g of PBCO. The hexadecane was

TABLE 1. Codes for the seawater-accommodated fractions (SWAF's) sampled from beneath oils and phenanthrene in static, agitated, and shaken reference oil/seawater experiments.

Code	Description
[ICICO <sub>S120</sub> ]	Irradiated, Cook Inlet crude oil, static, 120-hr exposure.
[ICICO A/P <sub>S120</sub> ]	Irradiated, Cook Inlet crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[I PBCO <sub>A120</sub> ]	Irradiated, Prudhoe Bay crude oil, agitated, 120-hr exposure.
[I PBCO <sub>S120</sub> ]	Irradiated, Prudhoe Bay crude oil, static, 120-hr exposure.
[I PBCO A/P <sub>S120</sub> ]	Irradiated, Prudhoe Bay crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[IFO <sub>A120</sub> ]	Irradiated, No. 2 fuel oil, agitated, 120-hr exposure.
[IFO <sub>S120</sub> ]	Irradiated, No. 2 fuel oil, static, 120-hr exposure.
[IFO <sub>S475</sub> ]	Irradiated, No. 2 fuel oil, static, 475-hr exposure.
[I PH <sub>S120</sub> ]	Irradiated, Phenanthrene, static, 120-hr exposure.
[NC ICO <sub>S120</sub> ]	Nonirradiated, Cook Inlet crude oil, static, 120-hr exposure.
[NCICO A/P <sub>S120</sub> ]	Nonirradiated, Cook Inlet crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[NPBCO <sub>A120</sub> ]	Nonirradiated, Prudhoe Bay crude oil, agitated, 120-hr exposure.
[NPBCO <sub>S120</sub> ]	Nonirradiated, Prudhoe Bay crude oil, static, 120-hr exposure.
[NPBCO A/P <sub>S120</sub> ]	Nonirradiated, Prudhoe Bay crude oil, aromatic/paraffinic fraction, static, 120-hr exposure.
[NIFO <sub>A120</sub> ]	Nonirradiated, No. 2 fuel oil, agitated, 120-hr exposure.
[NIFO <sub>S120</sub> ]	Nonirradiated, No. 2 fuel oil, static, 120-hr exposure.
[NIFO <sub>S475</sub> ]	Nonirradiated, No. 2 fuel oil, static, 475-hr exposure.
[NIPH <sub>S120</sub> ]	Nonirradiated, Phenanthrene static, 120-hr exposure.
[RCICO]	Reference seawater solution Cook Inlet crude oil, (14 C-hexadecane).
[RPBCO]	Reference seawater solution, Prudhoe Bay crude oil, (14 C-hexadecane).
[RFO]	Reference seawater solution, No. 2 fuel oil, (14 C-hexadecane).

thoroughly mixed into the oil by swirling. Each flask was poured into its designated 3.8 L bottle containing filtered (0.45  $\mu\text{m}$ ) and autoclave (sterilized) seawater; the air above the water was then displaced with dry  $\text{N}_2$ .

The bottles were shaken for 2 hr in an Eberbach shaker which was placed in a dark, 4°C walk-in refrigerator. The contents of each bottle were then poured into a 2 L separator funnel. Zero time samples of 1-2 mL were immediately withdrawn from the bottom of the separator funnel into 20 mL scintillation vials; additional samples were taken at 15, 30, 45, 60, 120, 180, and 240 min.

After 240 minutes, the seawater was filtered to determine if the residual radioactivity was removable as entrained oil. The water was slowly drained from the separator funnel through a 2000 mL Buchner funnel containing a coarse (40-60  $\mu\text{m}$ ) frit into a clean,  $\text{N}_2$ -filled 3.8 L bottle.

### 5.1.2 Effects of Storage on Composition of [RCIC0]

A preparation of [RCIC0] [Table 1] was divided into three 500 mL aliquots and stored at 4°C for up to 65 days under four separate conditions. These conditions were: (1) [RCIC0] made with sterilized seawater, (2) [RCIC0] made from sterilized seawater to which 20 mL of methylene chloride ( $\text{CH}_2\text{Cl}_2$ ) and 1 mL of 6N hydrochloric acid (HCl) had been added, (3) [RCIC0] made with nonsterilized seawater, and (4) [RCIC0] made with nonsterilized seawater to which 20 mL of  $\text{CH}_2\text{Cl}_2$  and 1 mL of 6N HCl had been added. All bottles were rinsed with 1N HCl, methanol ( $\text{CH}_3\text{OH}$ ), and  $\text{CH}_2\text{Cl}_2$  consecutively, before use.

After storage for up to 65 days the [RCIC0] was extracted and analyzed as described in Section 5.2.4.

## 5.2 Weathering of Oils and Hydrocarbon Fractions of Oils

Several different modes of weathering were used to expose C10, PBC0, No. 2 fuel oil, and the A/P (aromatic/paraffinic; see Section 5.2.3) fractions of crude oil. In some experiments,  $^{14}\text{C}$ -phenanthrene,  $^3\text{H}$ -phenanthrene, or  $^{14}\text{C}$ -*p*-cresol were added to the test oil prior to the weathering to facilitate characterization of oxidation products formed. The radiotracers were added on the assumption that they would be affected in a manner representative of compounds of their respective classes (i.e., phenanthrene representative of mid-range aromatic hydrocarbons; *p*-cresol representative of phenols).

### 5.2.1 Flow-through Conditions

A 25 L and a 5 L wave machine were constructed from stainless steel and used to produce waves by the movement of a motor-driven paddle within the containers. These machines were placed outdoors where the oils were weathered under simulated natural environmental conditions in a water bath maintained at ambient Puget Sound water temperature (10-12°C). In addition, a second 25 L container and 5 L container, identical to the above wave machines with the exception of not having a wave-producing paddle,

were placed in a laboratory water bath maintained at ambient Puget Sound water temperature and irradiated with filtered UV light (six, 1.2 m long sunlamps, Sylvania FS 40 placed 25 cm above the oil-water surface). To simulate natural sunlight, cellulose triacetate film was used to filter out wavelengths below 270 nm (Hansen 1975). The filter was changed after each experimental run.

In addition, a third 25 L container and 5 L container identical to the second pair of containers were kept in the dark. All containers were provided with a constant flow of unfiltered, unsterilized Puget Sound seawater (salinity, 28-29 ‰; temperature, 10-12°C). In each system, the 25 L container was used for weathering oil without added radiotracers, while the 5 L container was used for weathering oil which contained radiotracers.

The weathering experiments during the first year were initiated by layering C10 (5 g C10 in pentane/L seawater) over the unfiltered, non-sterilized seawater in each container. The flow of seawater was started after 10 min by which time most of the pentane had evaporated. The oil in the 5 L containers contained 3H-phenanthrene and <sup>14</sup>C-p-cresol. Water samples from the outlet pipe, which drew water from the bottom of the water column of the 5 L tanks, were collected at various intervals during the weathering processes to assess incorporation of radioactivity from oil into the underlying seawater. The radioactivity was determined by LSC.

### 5.2.2 No-flow Conditions

5.2.2.1 Static: The weathering of oils under laboratory conditions was carried out so that the primary weathering factor was photo-oxidation by UV light. Two Pyrex glass trays (19 X 30 cm), each containing 1 L of filtered, sterilized seawater, were placed in a water bath maintained at 12°C. The water bath was enclosed in a wooden box containing six 0.6 m long sunlamps (Sylvania FS 20 placed 25 cm above the oil-water surface), filtered as previously mentioned in 5.2.1.

During the first year, a solution consisting of 5 g of oil (C10 or No. 2 fuel oil, in pentane or hexane) was poured on the surface of the filtered, sterilized seawater in each tray. After 10 min, by which time most of the added solvent had evaporated, one tray was covered with aluminum foil, the other tray was left uncovered and the sunlamps were then energized for the duration of the experiment.

During subsequent studies in the second year, the oil was poured directly onto the seawater surface and a hexane rinse of the oil container was added. The wooden box was redesigned so that only one tray was irradiated with the light; the other tray was kept dark without having to be covered with aluminum foil. The light was filtered through cellulose triacetate as described in Section 5.2.1. One experiment was conducted in which only a mixture of radioisotopically labelled and nonlabelled phenanthrene dissolved in pentane was layered onto the seawater. The duration of all exposures was 120 hr.

5.2.2.2 Agitated: During the second year, two 5 L wave machines were used and they were located outdoors in a water bath maintained at ambient Puget Sound water temperature (10-12°C). Both wave machines were covered with a lightproof and waterproof wooden box; one box, however, contained four 0.6 m long Sylvania FS 20 sunlamps located 21 cm above the oil/water surface. The light was filtered as previously described. The same oil/water ratios and times were used as those for the laboratory static exposures (5 g/L and 120 hr). It had been determined during experiments using radiotracers that the time required for settling or separation of the aqueous and oil phases was 2-4 hr, depending on the oil used. At the conclusion of the exposures, therefore, the two phases were allowed to separate for a minimum of 4 hr.

#### 5.2.3 Removal of Polar Compounds from Crude Oils Prior to Irradiation (A/P Fractions)

Twenty grams of PBCO or 20 grams of CICO were mixed with 20 mL of pentane and passed through 500 mL attapulgitic clay (Harrison and Crossfield, Los Angeles, CA) columns to separate the aromatic/paraffinic (A/P) fraction from the asphaltene and naturally occurring crude oil polar compounds which were retained on the column (Pancirov 1974). After removal of the eluting solvent, pentane, 13.7 g of the PBCO and 14.7 g of the CICO remained in the form of a moderately viscous, clear yellow A/P fraction of the original crude oil.

#### 5.2.4 Extraction of Seawater-Accommodated Fractions

Seawater-accommodated fractions (SWAF's) were placed in separator funnels, the pH was adjusted to 2 with 1N HCl and the seawater was extracted 3 times with CH<sub>2</sub>Cl<sub>2</sub> (seawater: CH<sub>2</sub>Cl<sub>2</sub> = 9:1). The extract was concentrated to 15 mL in a water bath at 65°C, then to 1 mL in a concentrator tube with a tube heater (Kontes, Vineland, NJ). A 100 µL portion of the concentrated extract was set aside for chemical analysis. The remaining 900 µL were chromatographed on silica gel (Malins et al. 1980) to yield fractions containing mainly (1) alkanes, (2) aromatic hydrocarbons and olefins, and (3) compounds containing oxygen. Samples were analyzed by GC and MGA.

### 5.3 Characterization of Oxidation Products of Radiotracers

In experiments in which radiotracers were added prior to the weathering, thin layer chromatography (TLC) analyses were conducted on all SWAF extracts and on the remaining oil (surface slicks). All samples were spotted on channelled, 5 x 20 cm silica gel plates (KL6DF, Whatman, Clifton, NJ).

During analysis of the <sup>14</sup>C-phenanthrene and its oxidized products, the TLC plate was first developed in 100% toluene. The plate was removed; the solvent was allowed to evaporate, and a 4 cm band (R<sub>f</sub> 1.00 to 0.75) containing the parent hydrocarbon was scraped from the plate. The plate was redeveloped in toluene:ethyl alcohol (92:8 v/v) which separated the different classes of oxidized products. The remaining silica gel was then scraped off the plate in 2 cm bands. Phenanthrene, 9-fluorenone, phenanthraquinone, and diphenic acid were used as TLC standards.

Infrared analyses of the extracts were conducted using a Perkin-Elmer Infrared Spectrophotometer (Model 681). Microprocessor-controlled ordinate scale expansion was used to detect low levels of oxygenated components.

#### 5.4 Uptake Experiments

The bioaccumulation studies with newly hatched surf smelt larvae were conducted using SWAF's from either fresh CICO ([RCICO]) or weathered CICO ([ICICO<sub>S120</sub>]) PLUS the added radiotracers. Duplicate static exposures were used, each having an initial <sup>3</sup>H-phenanthrene concentration of 140 pg phenanthrene equivalents/g of [RCICO] or [ICICO<sub>S120</sub>]. Samples were taken at various intervals during the uptake experiments to determine the amount of radiolabeled compounds in the SWAF's and in the surf smelt tissue.

The larvae were removed from the SWAF and placed in uncontaminated flowing seawater to flush away radioactivity not directly associated with the larvae. An aliquot of the final rinse water was also collected and analyzed by LSC; any residual radioactivity present in this final rinse water was subtracted from the total radioactivity for the larvae to correct for contaminated water associated with the larvae as a result of sampling. The larvae in 1-2 mL seawater were then placed in a vial containing approximately 200 µL tricaine methanesulfonate and counted. The tissue, after being either homogenized with a Brinkman Polytron Homogenizer or solubilized chemically by Soluene 350, was then analyzed by LSC. At various intervals during the experiments approximately 100 larvae were sampled, washed and frozen for future extraction of tissue and analysis by TLC.

Newly hatched surf smelt were collected on a 64 µm screen. Three groups of ten larvae were removed from the screen and weighed. The mean weight of the larvae was determined to be  $320 \pm 30 \mu\text{g}$  ( $\bar{X} \pm \text{S.D.}$ ).

#### 5.5 Toxicity Assays

##### 5.5.1 Surf Smelt Larvae

Surf smelt eggs were collected from Puget Sound 1-2 days after deposition and 14-20 days prior to each bioassay. Eggs and sand-and-gravel substrate to which the eggs were attached were transferred to the Mukilteo Field Facility for incubation, where holding containers with the eggs were flooded with unfiltered ambient seawater (10-12°C and 28-29°/00) for 3-5 hr per day (Misitano 1977).

Nine hundred mL of undiluted SWAF of each of the three different oils (CICO, PBCO, No. 2 fuel oil) and duplicate dilutions of each SWAF (50, 25, 12, and 6% of original SWAF in filtered [5 µm] seawater) were placed in 1 L glass beakers (11 cm water depth). A known number of newly hatched larvae (100-200) were added to each beaker, and the beakers were placed in a 10°C water bath for the duration of the 48 hr exposure period. The bioassay included 6 control groups (larvae held in uncontaminated filtered [5 µm] seawater) interspersed among 30 oil-exposed groups.

At 2 and 24 hr after the start of exposure, visual estimates were made of the percent larvae swimming in the upper 1-2 cm of the water column. In addition, at the termination of the 48-hr exposure, all larvae were counted and assigned to one of four categories: (1) positive phototaxis with active swimming in the upper 1-2 cm of the water column; (2) active, but uncoordinated swimming on the bottom of the holding container; (3) inactive, with heart beat, and usually partly opaque; (4) dead, i.e., no heart beat. All larvae except those inactive or dead were then transferred to 900 ml of filtered (5  $\mu$ m) uncontaminated seawater and held for an additional 72-96 hr. At termination of the bioassay (120-144 hr after start of the exposure), the larvae were again counted and categorized on the basis of swimming behavior and survival.

### 5.5.2 English Sole Embryos

Mature English sole were caught by trawl in Port Orchard, Puget Sound, Washington. Eggs were immediately stripped from ripe females and mixed with sperm from several males. The fertilized eggs were held at 8-10°C during transportation to the Mukilteo Field Facility where the eggs were incubated in 8°C seawater in 3 L containers, each containing 2,000-4,000 eggs. Approximately 100 three-day old viable embryos were transferred to 200 mL beakers containing 50 mL of filtered (0.45  $\mu$ m) seawater in an 8°C water bath. Crude oil SWAF's or No. 2 fuel oil SWAF's were added so as to achieve a desired dilution in a total of 150 mL of liquid.

All exposures were conducted in triplicate. The embryos in test and control media were incubated at 8°C. After 48 hr, each beaker was examined under a dissecting microscope for (1) dead eggs, larvae, or embryos, (2) moribund individuals, and (3) viable embryos of larvae. Moribund individuals included embryos and larvae having a whitish color, larvae on the bottom of the container, and larvae that were unresponsive to touch.

### 5.5.3 English Sole Larvae

Larvae hatched from five-day old eggs held at 8°C were exposed and evaluated in a fashion similar to that described above (Sec. 5.5.2).

Data were statistically analyzed using either a chi-square analysis or analysis of variance with a Student-Newman-Keuls multiple comparison test.

## 5.6 Cytopathology

Samples of surf smelt larvae for histopathological and ultrastructural analyses were collected after 48 hr of exposure to the CICO SWAF and again at the termination of each bioassay experiment (120 hr). Larvae were fixed in a solution containing 0.75% glutaraldehyde, 3% formalin, 0.5% acrolein and 0.1 M sodium cacodylate buffer (pH 7.4) with 0.02% calcium chloride, and 5.5% sucrose. Larvae were postfixed in 1% osmium tetroxide in buffer, dehydrated with ethanol, embedded in Spurr medium (Spurr 1969) and sectioned with glass or diamond knives. Semi-thin (1  $\mu$ m) sections were stained with Richardson's mixture. Tissues were first examined by light microscopy. Selected larvae were then thin-sectioned (80 nm), triple-stained with lead citrate, uranyl acetate, and again with lead citrate, and examined by transmission electron microscopy (TEM).

## 6. RESULTS

### 6.1 Reference Mixtures of Seawater and Oils

The data from GC and MGA for total organic extractable material and from GC for selected individual compounds indicated that the protocol was suitable for preparing reproducible concentrations of essentially non-weathered and nonoxidized reference mixtures. In subsequent discussions, the use of MGA for the total extractable organic material is assumed unless otherwise stated.

The total hydrocarbon (GC) concentrations in undiluted SWAF's for three reference oils used for the surf smelt larvae assays were: 2.2  $\mu\text{g}/\text{mL}$  for PBCO; 3.4  $\mu\text{g}/\text{mL}$  for CICO; and 3.9  $\mu\text{g}/\text{mL}$  for No. 2 fuel oil (see Fig. 1).

#### 6.1.1 Effects of Separation Time and Filtration on Composition of Reference Mixtures

Only 2-4% of the total  $^{14}\text{C}$ -hexadecane-derived radioactivity added to the nonweathered crude oils was transferred to the aqueous phase SWAF's after 2 hr of mechanical shaking and 2 hr of separation of the two phases (Fig. 2). However, the more soluble No. 2 fuel oil had 13% of the total radioactivity remaining in the seawater under the same conditions. After 4 hr of settling, filtration did not change the amount of residual radioactivity in the SWAF's.

#### 6.1.2 Effects of Storage on Composition of [RCICO]

The data in Table 2 and Appendix Tables 1A and 2A, show that [RCICO] can be stored for up to 15 days at 4°C with little change in the concentration of total extractable organic material as determined by GC and MGA or in the concentration (GC) of selected individual compounds -- except for naphthalene in the nonsterilized, nonpreserved sample. Major differences were not seen in these parameters between preparations made with sterilized and nonsterilized seawater and between preserved and nonpreserved samples. However, there were differences in the absolute values of total extractable organic material determined by GC compared to those determined by MGA. This was not unexpected because MGA is a measure of total extractable organic material extracted from the SWAF's, whereas the GC resolves only those components which can be injected into a gas chromatography and resolved by flame-ionization detection.

After 65 days of storage, there were no changes in nonsterilized, nonpreserved [RCICO] based on the results from MGA; however, there were sharp reductions in the extractable material based on the results from GC and GC for selected individual compounds. Specifically, total aromatic hydrocarbon concentrations were sharply reduced after 65 days of storage, whereas concentrations of selected phenolic compounds did not change, except for *o*-cresol which increased.

TABLE 2. Effects of storage at 4°C on [RCICO] (reference preparation of seawater and CICO),<sup>a</sup>

[RCICO]	Total Extractable (Pre-chromatography)		Total Aliphatic (Compounds)		Total Aromatic (Compounds)		Total Polar (Compounds)	
	GC	MGA	GC	MGA	GC	MGA	GC	MGA
	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$	$\mu\text{g/mL}$
<b>Sterilized seawater</b>								
0 days, preserved	<b>2.5 ± 0.1<sup>b</sup></b>	2.6 ± 0.1	0.14 ± 0.01	0.38 ± 0.01	<b>1.5 ± 0.1</b>	0.46 ± 0.03	0.32 ± 0.03	1.7 ± 0.1
0 days, non-preserved	2.4 ± 0.1	<b>2.7 ± 0.1</b>	0.15 ± 0.01	0.39 ± 0.10	1.4 ± 0.1	0.37 ± 0.17	0.24 ± 0.03	<b>1.5 ± 0.1</b>
Blank	0.07	0.44	0.001	0	0.002	0.01	0.11	0.60
15 days, preserved	<b>2.8 ± 0.1</b>	3.7 ± 0.4	0.10 ± 0.01	0.29 ± 0.08	<b>1.6 ± 0.0</b>	0.41 ± 0.03	0.37 ± 0.04	1.2 ± 0.2
15 days, non-preserved	2.8 ± 0.2	3.7 ± 0.3	<b>0.10 ± 0.02</b>	0.30 ± 0.04	<b>1.4 ± 0.1</b>	0.42 ± 0.03	0.30 ± 0.02	<b>1.1 ± 0.1</b>
Blank	0.06	0.89	0.001	0	0.01	0	0.09	0.24
<b>Non-sterilized seawater</b>								
0 days, preserved	<b>2.1 ± 0.2</b>	1.5 ± 0.0	0.13 ± 0.01	0.40 ± 0.07	<b>1.4 ± 0.1</b>	0.56 ± 0.05	0.30 ± 0.01	0.84 ± 0.11
0 days, non-preserved	2.0 ± 0.1	1.7 ± 0.2	0.14 ± 0.01	<b>0.33 ± 0.06</b>	<b>1.4 ± 0.1</b>	<b>0.39 ± 0.04</b>	<b>0.25 ± 0.01</b>	0.93 ± 0.18
Blank	0.01	0.27	0.001	0	0.004	0	0.02	0
15 days, preserved	3.0 ± 0.4	2.0 ± 0.0	0.09 ± 0.01	0.30 ± 0.05	1.6 ± 0.2	0.33 ± 0.12	0.25 ± 0.01	0.90 ± 0.09
15 days, non-preserved	<b>2.6 ± 0.1</b>	<b>2.0 ± 0.1</b>	0.04 ± 0.01	<b>0.21 ± 0.02</b>	1.5 ± 0.0	0.32 ± 0.07	0.33 ± 0.03	<b>0.94 ± 0.16</b>
Blank	0.07	0.47	0.0003	0.03	<b>0.003</b>	0.01	0.06	0.05
65 days, non-preserved	0.92	2.4	0.03	0.29	0.37	0.36	0.45	1.5

a For concentration data for selected individual compounds, see Appendix, Tables 1A and 2A.

b  $\bar{x} \pm S.D.$ , n = 3.

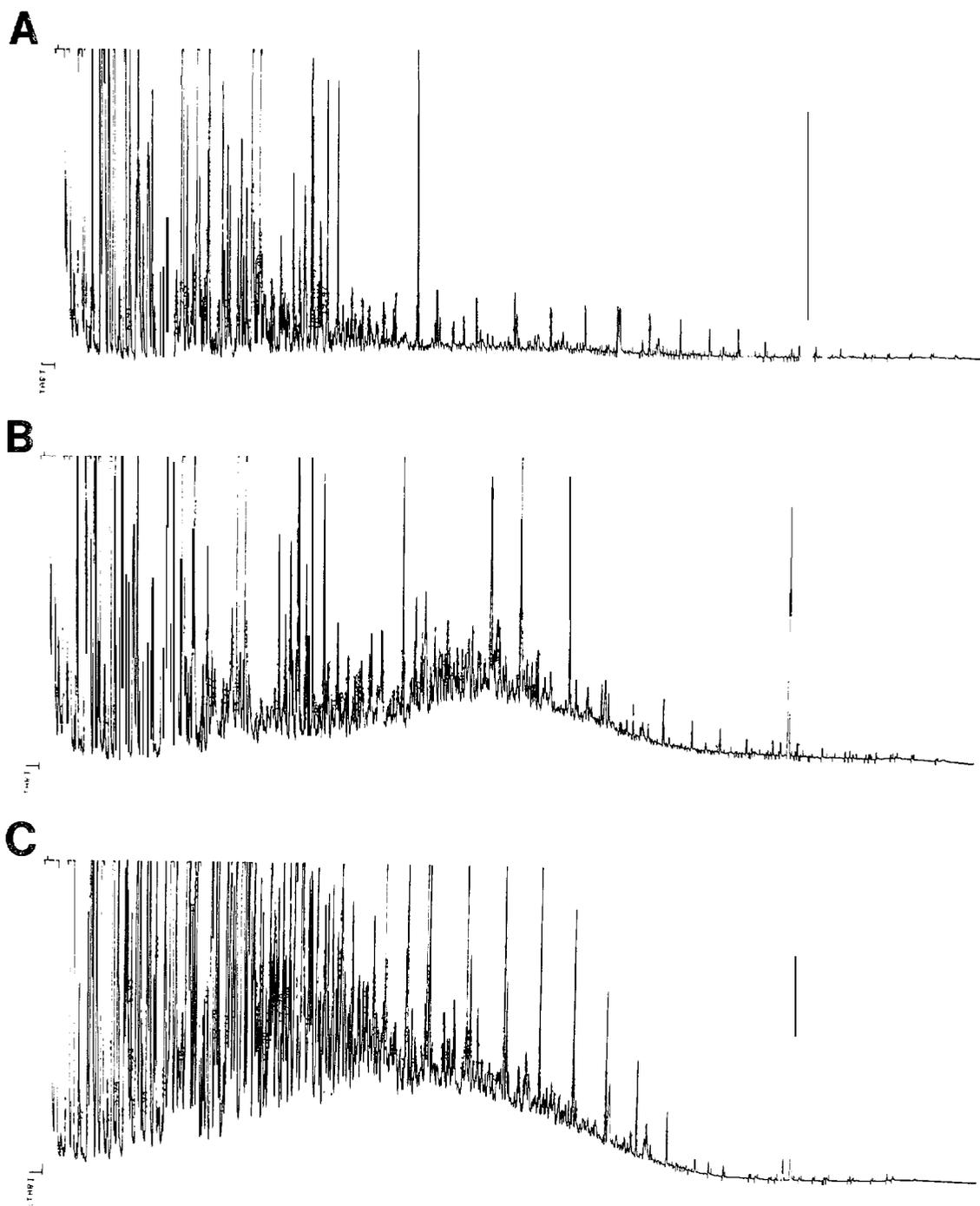


FIGURE 1. Gas chromatograms of the reference SWAF's of Prudhoe Bay crude oil [RPBCO] (A), Cook Inlet crude oil [RCICO] (B), and No. 2 fuel oil [RFOI] (C).

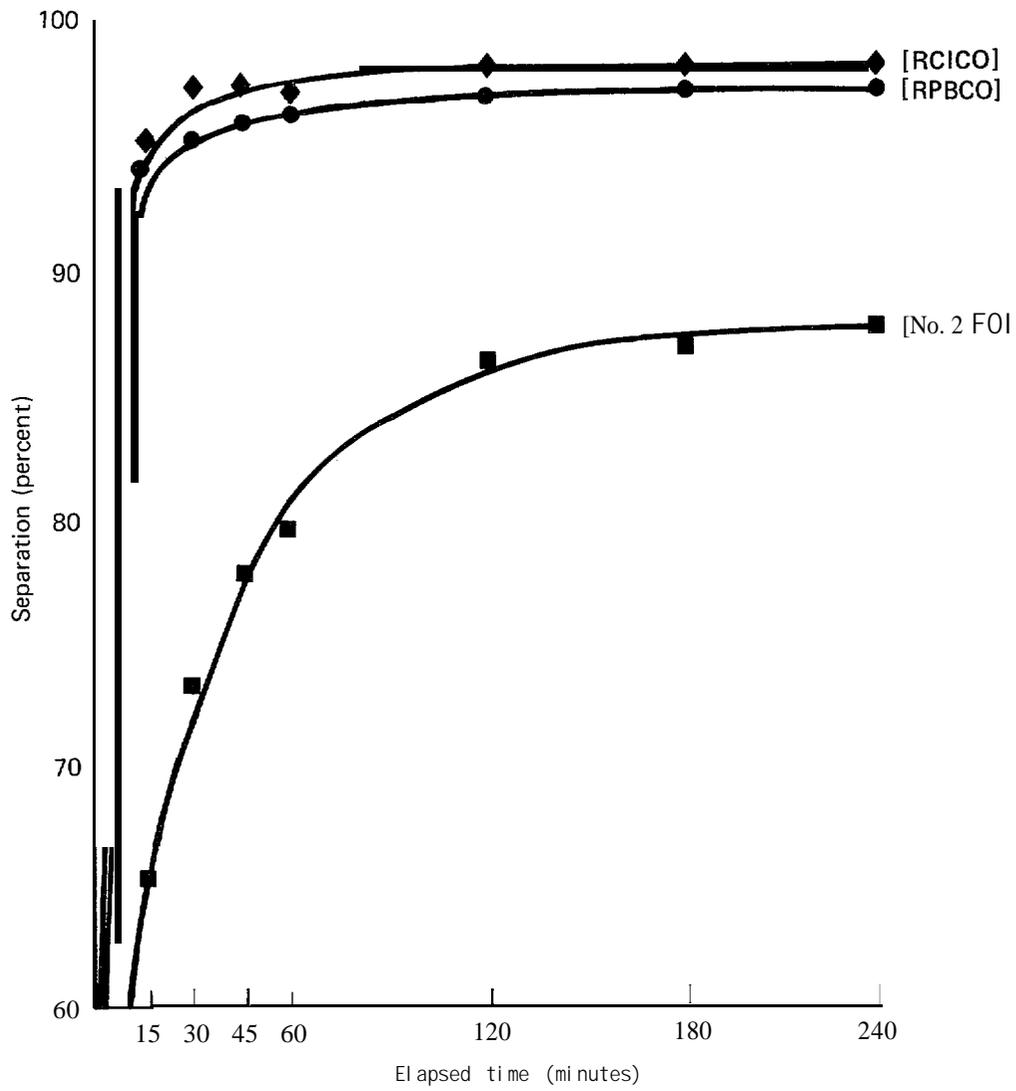


FIGURE 2. Separation times for reference SWAF's of Prudhoe Bay Bay crude oil [RPBCO], Cook Inlet crude oil [RCICO], and No. 2 fuel oil [RFO].

## 6.2 Weathering of Oils and Hydrocarbon Fractions of Oils

### 6.2.1 Flow-through Conditions

The MGA-determined total extractable organic material in the CICO SWAF produced by environmental weathering was markedly more variable than the content of this material present in any other preparation. High levels of total extractable organic material present in the seawater after environmental weathering appear to be due to unaccommodated oil. Chemical analyses revealed high concentrations of total aliphatic compounds in seawater environmental weathering compared to low concentrations in the other preparations.

### 6.2.2 No-flow Conditions

6.2.2.1 Static: The amount of total extractable organic material entering the seawater varied greatly depending on the type of oil layered on the surface and the presence of UV light (Table 3). There were also differences in the physical appearance between irradiated and nonirradiated oil. For instance, nonirradiated CICO spread out evenly over the entire surface of the seawater, whereas the irradiated CICO formed a layer on only a portion of the water's surface. In the case of No. 2 fuel oil, both irradiated and nonirradiated oil spread out as a thin film over the entire surface of the seawater, whereas the nonirradiated fuel oil remained clear while the irradiated fuel oil and A/P fractions of crude oils turned a dark yellowish brown with a concomitant increase in the surface film tensile strength.

After 120 hr of exposure with no irradiation, the amount of total extractable organic material recovered from the seawater underlying the No. 2 fuel oil ([NIFO<sub>S120</sub>]) was only slightly more than that extracted from the CICO SWAF ([NCICO<sub>S120</sub>]) (Table 3); however, after 120 hr of weathering with irradiation of the oils, the amount of total extractable organic material recovered from the No. 2 fuel oil system ([IFO<sub>S120</sub>]) was nearly 14 times more than the amount extracted from the CICO system ([ICICO<sub>S120</sub>]).

After 120 hr of weathering, the seawater beneath irradiated No. 2 fuel oil contained about 24 times as much total extractable organic material as did the seawater beneath the nonirradiated fuel oil. In contrast, the CICO SWAF contained about 3 times more material in the irradiated SWAF and PBCO was similar with 5 times more material in the irradiated SWAF than in non-irradiated SWAF.

When <sup>14</sup>C-phenanthrene was added to the oils, the ratios of radioactivity in irradiated versus nonirradiated SWAF's were similar to ratios of total extractable organic material determined by MGA (Table 3). When <sup>14</sup>C-phenanthrene was added to CICO, 0.4% of the phenanthrene-derived radioactivity was incorporated into [ICICO<sub>S120</sub>], and 0.2% was incorporated into [NCICO<sub>S120</sub>] (Table 3 and Fig. 3). Similarly for PBCO, 0.7% of the radioactivity was incorporated into [IPBCO<sub>S120</sub>] and 0.2% into [NPBCO<sub>S120</sub>]. However, when <sup>14</sup>C-phenanthrene was added to No. 2 fuel oil, 11.6% of the phenanthrene-derived radioactivity was incorporated into [IFO<sub>S120</sub>] compared to 1.2% for [NIFO<sub>S120</sub>] (Table 3 and Fig. 3).

TABLE 3. Concentrations of total extractable organic material and percent radioactivity incorporated in seawater underlying oil. Tests conducted with and without exposure to UV light and under static conditions.

Surface Layer	Exposure Time (hr)	Source of $^{14}\text{C}$	Incorporation of $^{14}\text{C}$ (%)	Total Extractable organic material ( $\mu\text{g/mL}$ )	Radioactivity extraction efficiency (%)
Cook Inlet crude oils	Irradiated [ICICO <sub>S120</sub> ]	Phenanthrene	(-).4	25.7	76.3
	Nonirradiated [NCICO <sub>S120</sub> ]	Phenanthrene	0.2	9.2	98.0
Cook Inlet crude oil	Irradiated [ICICO <sub>S120</sub> ]	<u>p</u> -cresol	53.3	14.9	77.6
	Nonirradiated [NCICO <sub>S120</sub> ]	<u>p</u> -cresol	67.1	4.2	78.0
Cook Inlet A/P fraction	Irradiated [ICICO A/P <sub>S120</sub> ]	Phenanthrene	5.7	147	97.4
	Nonirradiated [NCICO A/P <sub>S120</sub> ]	Phenanthrene	0.9	29.0	100.0
Prudhoe Ray crude oil	Irradiated [IPRCO <sub>S120</sub> ]	Phenanthrene	0.7	12.0	73.2
	Nonirradiated [NPBCO <sub>S120</sub> ]	Phenanthrene	0.2	2.3	99.9
Prudhoe Ray crude oil	Irradiated [IPBCO A/P <sub>S120</sub> ]	Phenanthrene	5.3	161	97.6
	Nonirradiated [NPBCO A/P <sub>S120</sub> ]	Phenanthrene	0.5	6.9	100.0
No. 2 fuel oil (2) <sup>a</sup>	Irradiated [IFCO <sub>S120</sub> ]	Phenanthrene	11.6	356	67.7
	Nonirradiated [NIFCO <sub>S120</sub> ]	Phenanthrene	1.2	12.5	83.9
Phenanthrene without oils	Irradiated [IPH <sub>S120</sub> ]	Phenanthrene	3.9	3.6	68.5
	Nonirradiated [NIPH <sub>S120</sub> ]	Phenanthrene	0.6	1.2	98.7

<sup>a</sup> These data used in Figure 3.

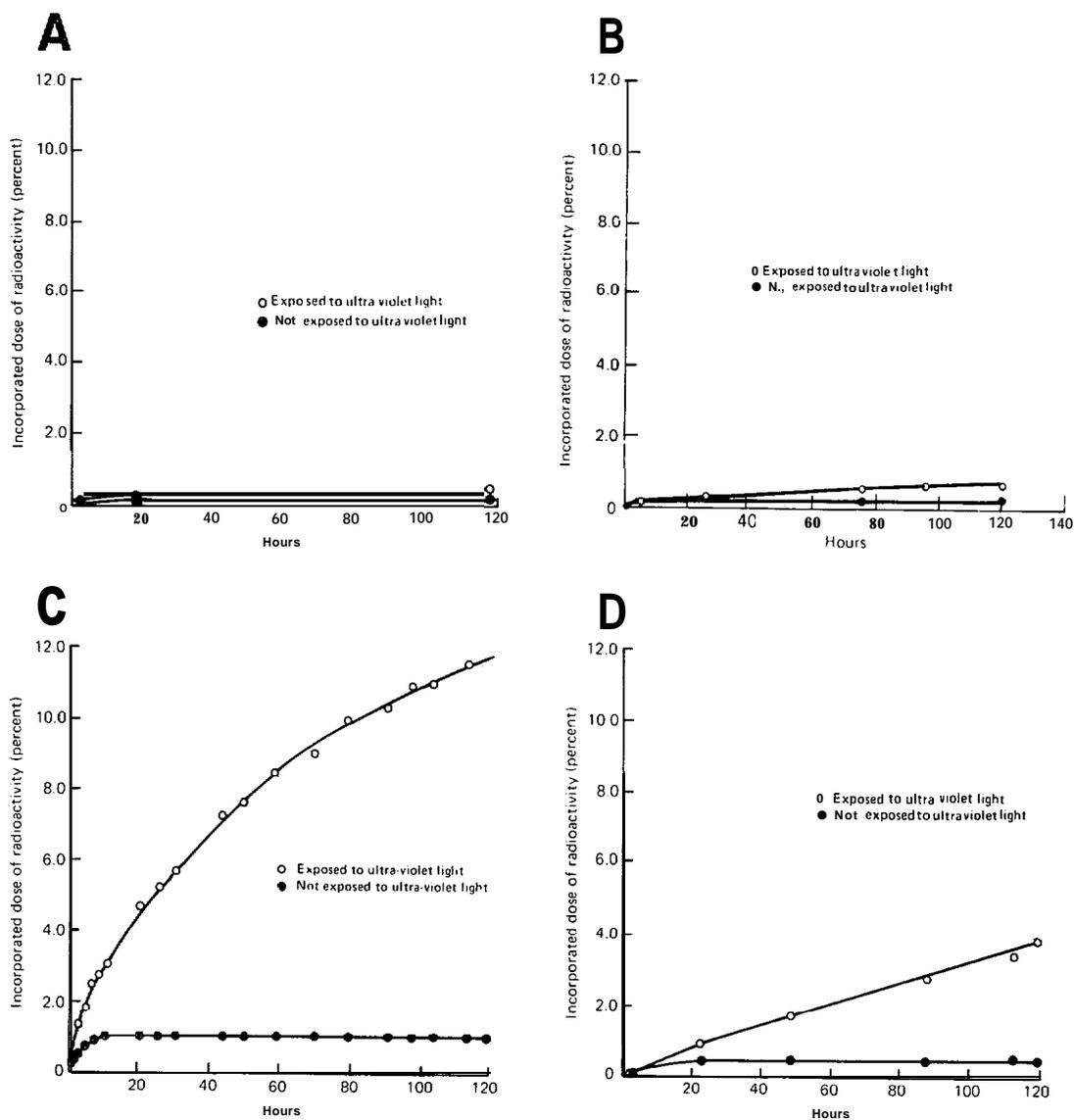


FIGURE 3. Incorporation of  $^{14}\text{C}$ -phenanthrene-derived radioactivity into sterilized seawater with time under static conditions. The phenanthrene was layered on the seawater either mixed with CIC0 (A), PBC0 (B), No. 2 fuel oil (C) or without oil (D) with and without exposure to UV irradiation.

When  $^{14}\text{C}$ -phenanthrene (in pentane) was layered onto seawater in the absence of petroleum, the amount of radioactivity recovered in the total extractable organic material from the seawater following irradiation ([IPH<sub>S120</sub>]) was nearly seven times higher than from the material from the nonirradiated system ([NIPH<sub>S120</sub>]).

When  $^{14}\text{C}$ -p-cresol, a polar component of crude oil, was added to CICO and then layered onto seawater, the incorporation of radioactivity into the underlying water was rapid, being slightly faster and greater in the non-irradiated system (Fig. 4). Within 4-5 hr the underlying seawater of both irradiated and nonirradiated CICO contained over 50% of the added radioactivity and a maximum was attained within 20 hr.

The extraction efficiency was determined by monitoring the amount of radioactivity remaining in the seawater after solvent extraction (Table 3). The extraction efficiency for the irradiated samples was considerably lower than for the samples which were not irradiated. The determination of the chemical composition of the nonextractable (using  $\text{CH}_2\text{Cl}_2$ , at pH = 2) radioactivity was beyond the scope of this study.

6.2.2.2 Agitated: Preliminary experiments to establish suitable periods of mixing suggested that UV-irradiated SWAF contained a relatively constant level of total extractable organic material with only 10 hr of wave machine agitation. However, a 120-hr mixing period and a 4-hr separation time (Fig. 5) for the disappearance of dispersed petroleum droplets from the water column were experimentally determined to be suitable.

In a radiotracer study of No. 2 fuel oil, the nonirradiated SWAF ([NIFO<sub>A120</sub>]) contained more  $^{14}\text{C}$ -phenanthrene-derived radioactivity than did the irradiated SWAF ([IFO<sub>A120</sub>]); however, the total organic extractable material from the irradiated SWAF was twice that from the nonirradiated SWAF. The amounts of total extractable organic material found in the agitated systems were markedly different (21.4  $\mu\text{g}/\text{mL}$  for nonirradiated and 41.5  $\mu\text{g}/\text{mL}$  for irradiated SWAF) from those found in the static system (12.5 and 356  $\mu\text{g}/\text{mL}$ , respectively). When PBCO was weathered for 120 hr in the wave machine, concentrations of total extractable organic material from nonirradiated ([NPBCO<sub>A120</sub>]) and irradiated ([IPBCO<sub>A120</sub>]) SWAF were 3.2 and 11.2  $\mu\text{g}/\text{mL}$ , respectively. For comparison, a reference No. 2 fuel oil SWAF contained 3.9  $\mu\text{g}/\text{mL}$  total extractable organic material.

#### 6.2.3 Removal of Polar Compounds from Crude Oil Prior to Irradiation (A/P Fraction)

The two crude oils were subjected to a further chromatographic separation producing an A/P fraction which was essentially free of naturally occurring polar compounds. This fraction was then investigated in the same way as were the whole oils.

When the A/P fractions of CICO and PBCO were layered onto seawater, the amounts of extractable material entering the underlying seawater in both irradiated and nonirradiated systems were considerably higher than those for the unfractionated oils (Table 3). The total extractable organic material, 147 and 161  $\mu\text{g}/\text{mL}$ , respectively, in both [ICICO A/P<sub>S120</sub>] and [IPRCO A/P<sub>S120</sub>] approached the values obtained with the irradiated No. 2

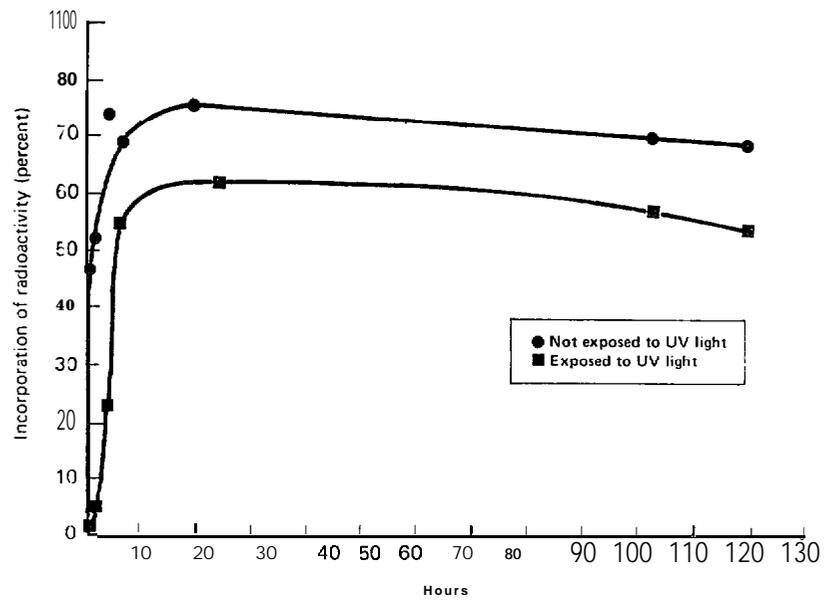


FIGURE 4. Incorporation of  $^{14}\text{C}$ -p-c resol-derived radioactivity under static conditions into sterilized seawater underlying CICO with and without exposure to UV irradiation.

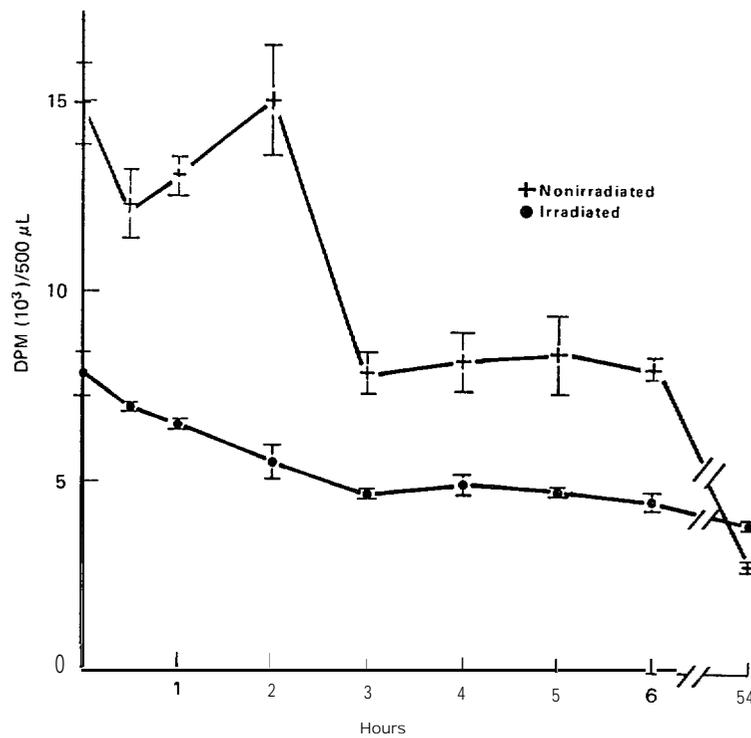


FIGURE 5. Radioactivity ( $^{14}\text{C}$ -phenanthrene-derived) in sterilized seawater after various periods of separation following 23 hr of no-flow agitated mixing. The phenanthrene was initially mixed with No. 2 fuel oil and layered onto the seawater, with and without exposure to UV irradiation ( $\bar{X} \pm \text{S. D.}$ ).

fuel oil [IFO<sub>S120</sub>] (356 µg/mL). The incorporation of <sup>14</sup>C-phenanthrene into the irradiated SWAF's increased to 5.7% for [ICICO A/P<sub>S120</sub>] and 5.3% for [IPBCO A/P<sub>S120</sub>], respectively. This represents an increase of approximately 14 and 8 times over the corresponding values for the respective irradiated whole crude oils. However, the incorporation of <sup>14</sup>C-phenanthrene from A/P fractions into the nonirradiated SWAF's remained under 1%.

The results of the incorporation of <sup>14</sup>C-phenanthrene in the seawater under irradiated and nonirradiated A/P crude oil fractions are given in Table 4. A greater amount (3.4x and 11x) of the radioactivity was incorporated from the A/P fractions into the underlying seawater than from the irradiated whole crude-oils; the nonirradiated enrichments from the A/P SWAF's were less (ea. 2x). In the case of the irradiated A/P fractions, nearly half (45%) of the radioactivity remained in the seawater after the methylene chloride extraction; however, in the nonirradiated SWAF's no radioactivity remained.

The TLC analyses of the extracts of the A/P SWAF's of the two crude oils showed that the bulk (92-97%,  $R_f > 0.62$ ) of the radioactivity in the non-irradiated SWAF's was present as the parent <sup>14</sup>C-phenanthrene (Fig. 6). The disposition of the radioactivity in the irradiated SWAF's of the two crude oil A/P fractions was nearly identical. The broader distributions of radioactivity in the irradiated SWAF's suggested the possible formation of several oxidized products ( $R_f < 0.5$ ) from these fractions, such as diphenic acid and quinone components.

### 6.3 Characterization of Oxidation Products of Radiotracers

Studies were made of the chemical composition of the radioactive compounds in the surface oil slick and in the total extractable organic material from the underlying seawater. The TLC analyses of the surface oil slicks ([NCICO<sub>S120</sub>] and [NIFO<sub>S120</sub>]) and phenanthrene without oil ([NIPH<sub>S120</sub>]) showed that the radioactivity was largely ( $\geq 99\%$ ) associated with the parent compound, phenanthrene, under static, nonirradiated conditions (Table 5). After irradiation with UV light, the CICO ([ICICO<sub>S120</sub>]) and phenanthrene without oil ([IPH<sub>S120</sub>]) underwent a 5- to 9-fold increase in the amount of oxidized materials, although the concentrations were low in the surface slick after 120 hr of exposure. The No. 2 fuel oil ([IFO<sub>S120</sub>]) showed a 15-fold increase in oxidized products in the irradiated surface slick.

The distribution of radioactivity between parent compound and oxidized products extracted from the underlying seawater under static conditions (Table 5) was quite different from the distribution in the surface oils. After irradiation with UV light the proportion of radioactivity due to oxidized products of phenanthrene in [ICICO<sub>S120</sub>], [ICICO A/P<sub>S120</sub>], [IPBCO A/P<sub>S120</sub>], [IPH<sub>S120</sub>], and [IFO<sub>S120</sub>] was noticeably increased.

Infrared analysis confirmed the presence of oxygenated compounds, and gas chromatographic/mass spectrometric (GC/MS) analyses identified several specific phenanthrene-derived oxidized products in [IPH<sub>S120</sub>], such as carbonyl, quinone, and carboxylic acid structures (Fig. 7).

TABLE 4. Incorporation of  $^{14}\text{C}$ -phenanthrene-derived radioactivity in seawater beneath irradiated and nonirradiated slicks of aromatic/paraffinic (A/P) fractions of two crude oils.

	Incorporated into SWAF's			Whole oil (%)	Ratio of SWAF/Whole oil
	Total extractable (using $\text{CH}_2\text{Cl}_2$ ) (%)	Remaining in seawater (%)	Total (%)		
<u>IRRADIATED</u>					
Cook Inlet crude oil [ICICO A/P <sub>S120</sub> ]	3.1	2.6	5.7	1.7	3.4
Prudhoe Bay crude oil [IPBCO A/P <sub>S120</sub> ]	2.9	2.4	5.3	0.5	11
<u>NONIRRADIATED</u>					
Cook Inlet crude oil [NCICO A/P <sub>S120</sub> ]	0.9	0	0.9	0.5	2
Prudhoe Bay crude oil [NPBCO A/P <sub>S120</sub> ]	0.5	0	0.5	0.2	2

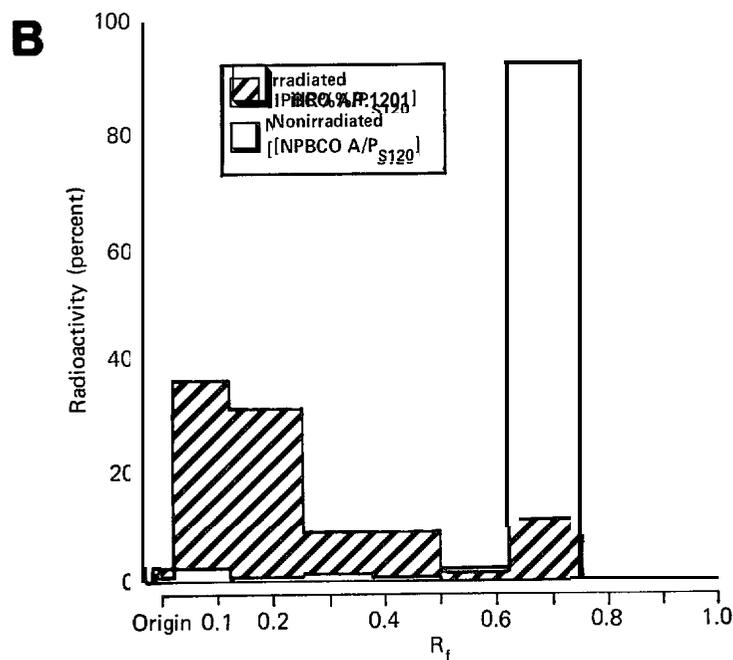
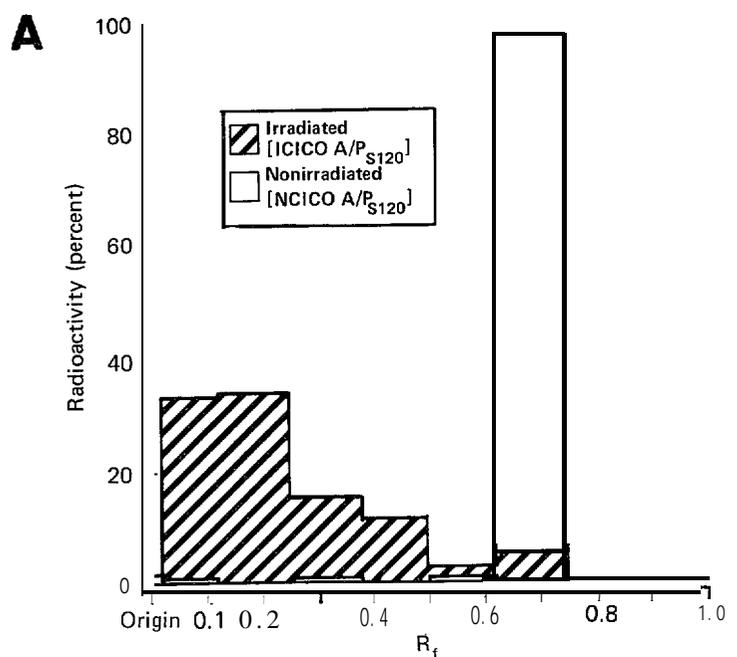


FIGURE 6. Radioactivity in TLC extracts of SWAF's from irradiated and nonirradiated A/P fractions of Cook Inlet crude oil (A) and Prudhoe Bay crude oil (B).

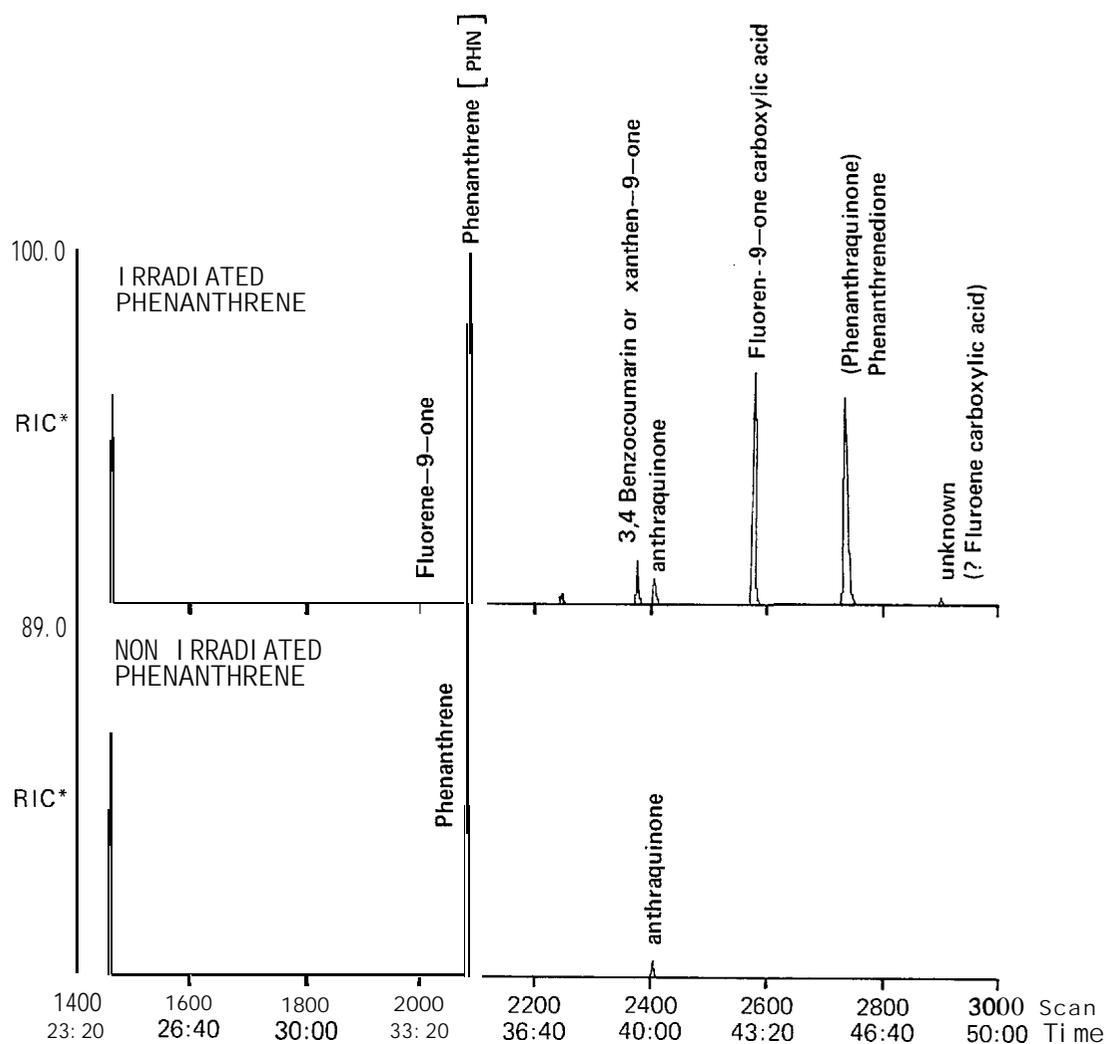


FIGURE 7. Reconstructed ion chromatograms (RIC) of phenanthrene-derived products extracted from seawater underlying phenanthrene with and without exposure to UV irradiation.

In the wave machine-produced SWAF's, the ratios of parent hydrocarbon: oxidized products were slightly different from the static system ratios; however, the overall profiles of the phenanthrene-derived oxidized products were similar. For instance, in the No. 2 fuel oil SWAF, 95% of the non-irradiated  $^{14}\text{C}$ -phenanthrene remained whereas 75% remained unoxidized in the irradiated system.

In summary, the nonirradiated surface oil slicks and SWAF's generally had lower levels of oxidized products than did the irradiated slicks and SWAF's (Table 5). In the case of the higher levels of radioactivity associated with "oxidized" material detected in the nonirradiated No. 2 fuel oil SWAF infrared analyses failed to detect the presence of any oxygenated compounds in the solution ([NIFOS<sub>120</sub>]). Moreover, the GC/MS of [NIPH<sub>5120</sub>] (Fig. 7) showed no oxygenated compounds except for trace amounts of anthraquinone.

#### 6.4 Uptake Experiments

Larval surf smelt were exposed to [RCICO] which contained added  $^3\text{H}$ -phenanthrene (Fig. 8). Duplicate static exposures were used, each having an initial  $^3\text{H}$ -phenanthrene concentration of 140 pg phenanthrene equivalents/g [RCICO]. The concentration of  $^3\text{H}$ -phenanthrene in the SWAF of [RCICO] did not change markedly over the 24 hr exposure period (Fig. 8). The concentration of  $^3\text{H}$ -phenanthrene associated with the surf smelt larvae (pg phenanthrene equivalents/g wet weight larvae) increased rapidly for the first 2 hr, and then remained constant at approximately 3,000 times the concentration of phenanthrene (pg phenanthrene equivalents/g water) in the water for the remainder of the 24-hr exposure.

Larval surf smelt were exposed to SWAF's of CICO from low-flow weathering experiments conducted during the first year. Prior to 24 hr of weathering,  $^3\text{H}$ -phenanthrene and  $^{14}\text{C}$ -*p*-cresol were added to the CICO (see Section 5.2.1). During the 24-hr uptake experiment, concentrations of  $^3\text{H}$ -phenanthrene and  $^{14}\text{C}$ -*p*-cresol-derived material in the irradiated and nonirradiated SWAF's remained relatively constant at 1.5 pg phenanthrene equivalents/g of seawater and 24 ng *p*-cresol equivalents/g of seawater, respectively (Fig. 9). The concentration of  $^3\text{H}$ -phenanthrene-derived material in the fish exposed to the CICO increased to a maximum value of 560 pg phenanthrene equivalents/g wet weight larvae after 6 hr of UV irradiation, compared to a maximum concentration (19 hr) of 240 pg phenanthrene equivalents/g larvae exposed to non-irradiated CICO SWAF. Similarly, the concentration of  $^{14}\text{C}$ -*p*-cresol-derived material reached a maximum value of 400 ng *p*-cresol equivalents/g wet weight of larvae after 19 hr of exposure in the irradiated CICO, compared to the value 150 ng *p*-cresol equivalents/g wet weight larvae in the nonirradiated CICO SWAF.

#### 6.5 Toxicity Assays

##### 6.5.1 Surf Smelt Larvae

6.5.1.1 Mortality: The survival of surf smelt larvae was routinely high until concentrations of total hydrocarbons (GC) exceeded about 1  $\mu\text{g}/\text{mL}$  (Fig. 10). Approximately 80% of the mortality occurred within the

TABLE 5. Characterization of phenanthrene-derived radioactivity in the surface oil and SWAF's with and without exposure to UV light.<sup>a</sup>

Surface Layer	Exposure Time (hr)	Surface oil slick		Underlying seawater (SWAF's)		
		<sup>14</sup> C as phenanthrene (%)	<sup>14</sup> C as oxidized products (%)	<sup>14</sup> C as phenanthrene (%)	<sup>14</sup> C as oxidized products (%)	
Cook Inlet crude oil	Irradiated [ICICO <sub>S120</sub> ]	120	99.1	0.9	57.5	42.5
	Nonirradiated [NCICO <sub>S120</sub> ]	120	99.9	0.1	96.6	3.4
Cook Inlet A/P fraction	Irradiated [ICICO A/PS <sub>120</sub> ]	120			7.3	92.7
	Nonirradiated [NCICO A/PS <sub>120</sub> ]	120			97.7	2.3
Prudhoe Bay A/P fraction	Irradiated [IPBCO A/PS <sub>120</sub> ]	120			12.3	87.7
	Nonirradiated [NPBCO A/PS <sub>120</sub> ]	120			94.4	5.6
No. 2 Fuel Oil	Irradiated [IFO <sub>S120</sub> ]	120	90.9	9.1	8.2	91.8
	Nonirradiated [NIFO <sub>S120</sub> ]	120	99.4	0.6	51.9	48.1
Phenanthrene without oil	Irradiated [IPH <sub>S120</sub> ]	120	99.5	0.5	17.9	82.1
	Nonirradiated [NIPH <sub>S120</sub> ]	120	99.9	0.1	89.8	10.2

<sup>a</sup>Analyses performed by TLC (see Methods).

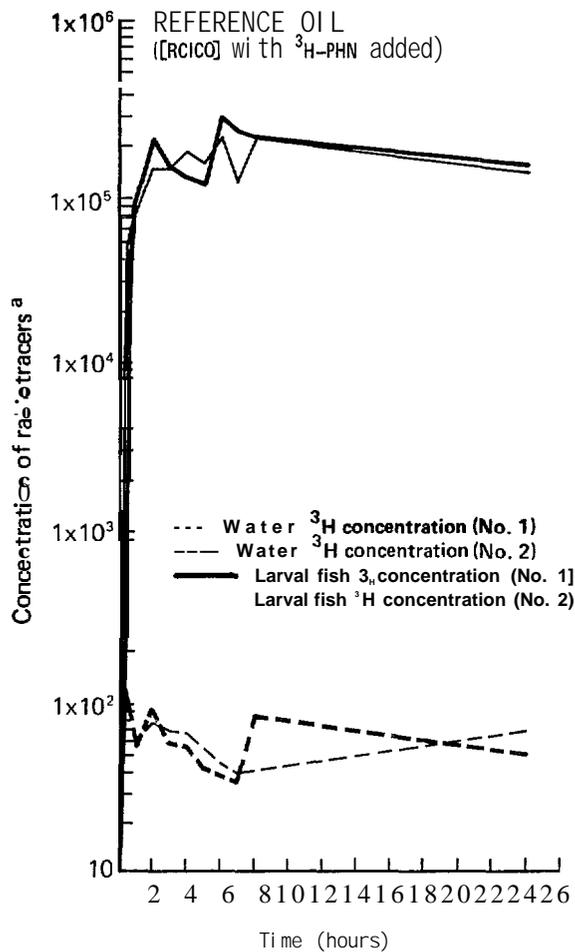


FIGURE 8. Uptake of <sup>3</sup>H-phenanthrene-derived material by surf smelt larvae from [RCICO]. <sup>3</sup>H-phenanthrene was added after [RCICO] was prepared.

<sup>a</sup>The concentrations of phenanthrene-derived radioactivity in seawater are expressed as pg ( $10^{-12}$ g) phenanthrene equivalent/mL of seawater and in larval fish tissues as pg phenanthrene equivalents/g of larvae (wet weight).

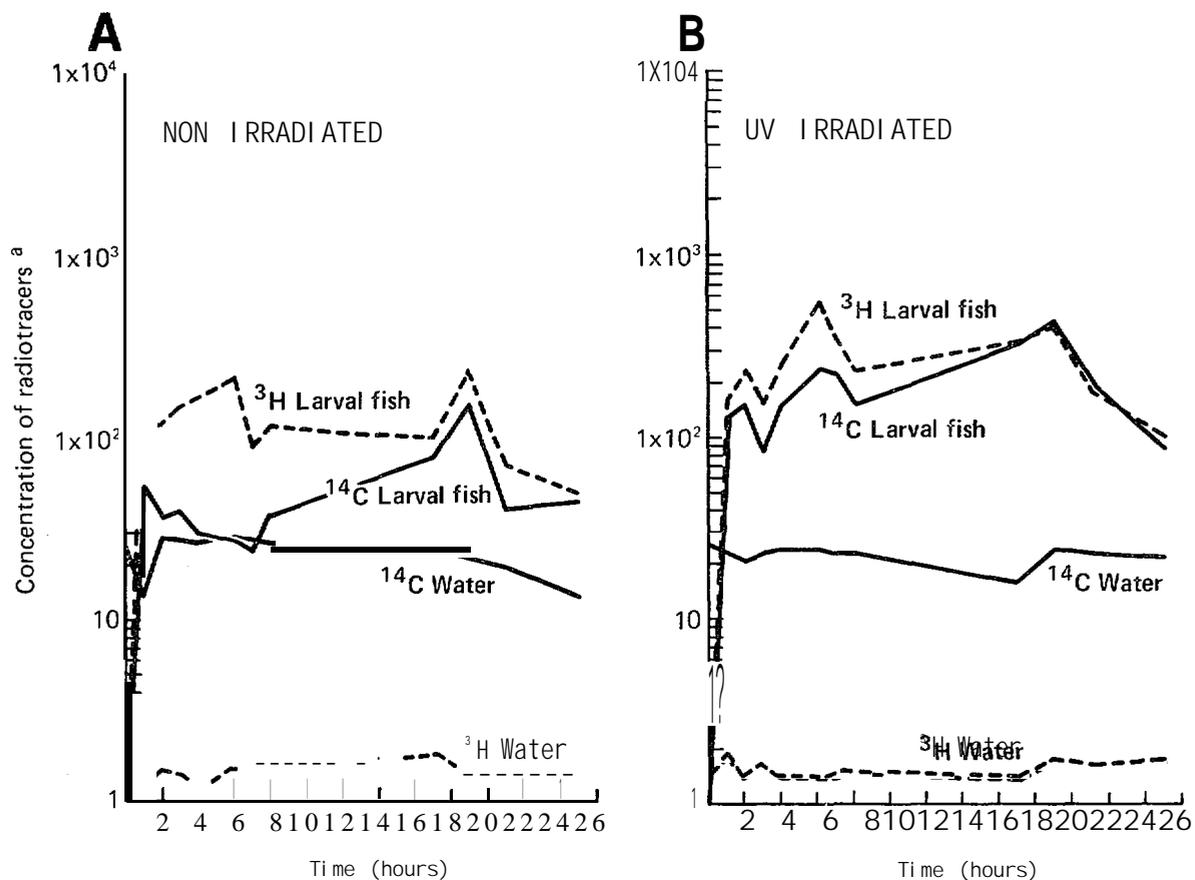


FIGURE 9. Uptake of <sup>3</sup>H-phenanthrene-derived and <sup>14</sup>C-p-cresol-derived radioactivity by surf smelt larvae from low-flow, weathered nonirradiated (A) and irradiated (B) Cook Inlet crude oil.

<sup>a</sup> Concentrations of phenanthrene-derived radioactivity in seawater are expressed as pg (10<sup>-12</sup>g) phenanthrene equivalents/mL of seawater and in larval fish tissues as pg phenanthrene equivalent/g of larvae (wet weight). Concentrations of cresol-derived radioactivity in seawater are expressed as ng (10<sup>-9</sup>g) cresol equivalent/mL of seawater and in larval fish tissues as ng cresol equivalent/g of larvae (wet weight).

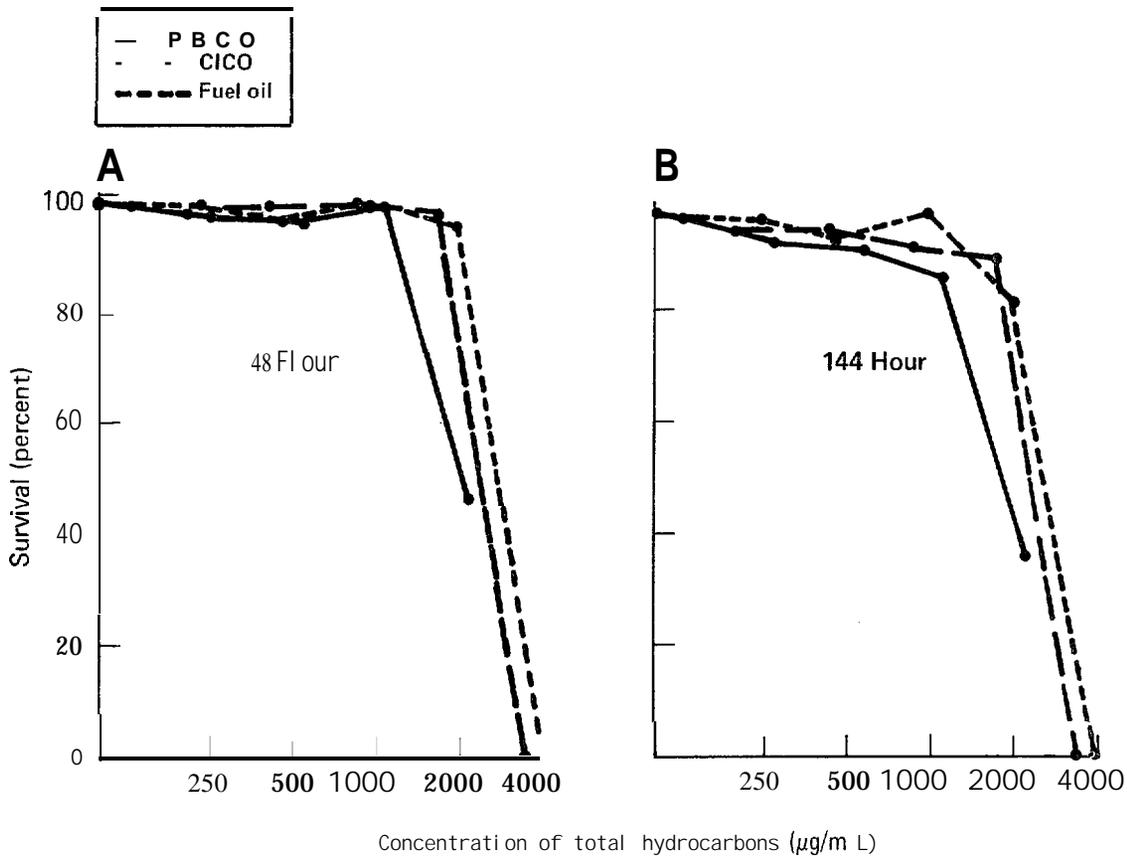


FIGURE 10. Percent survival of surf smelt larvae after (A) 48-hr exposure to the reference SWAF's of PBCO, CICO, and No. 2 fuel oil and (B) 96 hr of deputation following exposure (144 hr total). Concentrations of total hydrocarbons determined by GC analysis. Each data point represents an average of the percent survival in duplicate tests. The average percent differences between duplicate oil-exposed groups was 4.4% ( $\pm$  3.7% S.D.), and 1.1% ( $\pm$  0.7% S.D.) for controls.

initial 48 hr of exposure. The estimated 48-hr  $EC_{50}$  (value where 50% of the larvae died) varied from 2.1  $\mu\text{g}/\text{mL}$  total hydrocarbons for fresh, non-weathered PBCO SWAF ([RPBCO]) to 2.7  $\mu\text{g}/\text{mL}$  for fresh, nonweathered No. 2 fuel oil ([RFO]).

6.5.1.2 Swimming Behavior: The position of surf smelt larvae in the water column was evaluated after 2, 24, and 48 hr of exposure and again after 96 hr of deputation. The results shown in Figure 11A indicated that after 2 hr of exposure to nonweathered and undiluted SWAF's of PBCO, CICO or No. 2 fuel oil, 97-100% of the larvae had developed a pronounced change in swimming behavior, called ataxia, whereby they were unable to maintain their usual position in the water column. The estimated hydrocarbon concentration at which 50% of the larvae exhibited ataxia ( $EC_{50}$ ) ranged from 0.5 to 1.0  $\mu\text{g}/\text{mL}$ . By 24 hr (Fig. 11B), the estimated  $EC_{50}$  had dropped to 0.25 to 0.35  $\mu\text{g}/\text{mL}$  (roughly 250-350 ppb). Thereafter, the  $EC_{50}$ 's increased, possibly as the more volatile and toxic hydrocarbons were lost from the water column by evaporation (Fig. 11C). After 96 hr of deputation (Fig. 11D), over 75% of the larvae previously exhibiting ataxia had regained normal swimming behavior.

### 6.5.2 English Sole Embryos

6.5.2.1 Hatching Success: When fertilized eggs of English sole were exposed to high concentrations of fuel oil SWAF's ([IFO<sub>A120</sub>]) and [NIFO<sub>A120</sub>] for 48 hr, the hatching success was significantly ( $p < 0.05$ ) different from the 98% hatching success in control seawater (Fig. 12). In this experiment, the nonirradiated fuel oil SWAF ([NIFO<sub>A120</sub>]) reduced hatching success more than the irradiated SWAF ([IFO<sub>A120</sub>]). The differences were statistically different over the concentration ranges 1-5, 6-10, 11-15, and  $>15 \mu\text{g}/\text{mL}$ . The hatching success of eggs exposed to the nonirradiated fuel oil was not markedly different from the hatching success of the controls at SWAF concentrations of the total extractable organic material below 4  $\mu\text{g}/\text{mL}$ . Under static conditions with UV-irradiated PBCO, hatching success of English sole eggs was less than 50% in the 1-2  $\mu\text{g}/\text{mL}$  of total extractable organic material from both the irradiated SWAF ([IPBCO<sub>S120</sub>]) and the nonirradiated SWAF ([NPBCO<sub>S120</sub>]).

6.5.2.2 Mortality: The toxicities of SWAF's from irradiated and nonirradiated wave machine-weathered No. 2 fuel oil were not statistically significantly different, based on mortalities from 48-hr exposures of English sole embryos (Table 6). Mortalities were not different from controls (2%) when the embryos were exposed for 48 hr to concentrations of 21  $\mu\text{g}/\text{mL}$  or less of total extractable organic material from the UV-irradiated SWAF ([IFO<sub>S120</sub>]) or nonirradiated SWAF ([NIFO<sub>S120</sub>]). An environmental unrealistic level of 35  $\mu\text{g}/\text{mL}$  from the irradiated SWAF ([IFO<sub>S120</sub>]) caused 26% mortality. This concentration of No. 2 fuel oil SWAF materials probably reflects a hydrocarbon content of 10-15  $\mu\text{g}/\text{mL}$  which is at the high end of laboratory-prepared solutions (Anderson et al. 1974b) and several orders of magnitude higher than levels routinely measured after oil spills (Clark and MacLeod 1977). Until these high levels were reached, English sole embryos were not detectably affected during 48-hr assays.

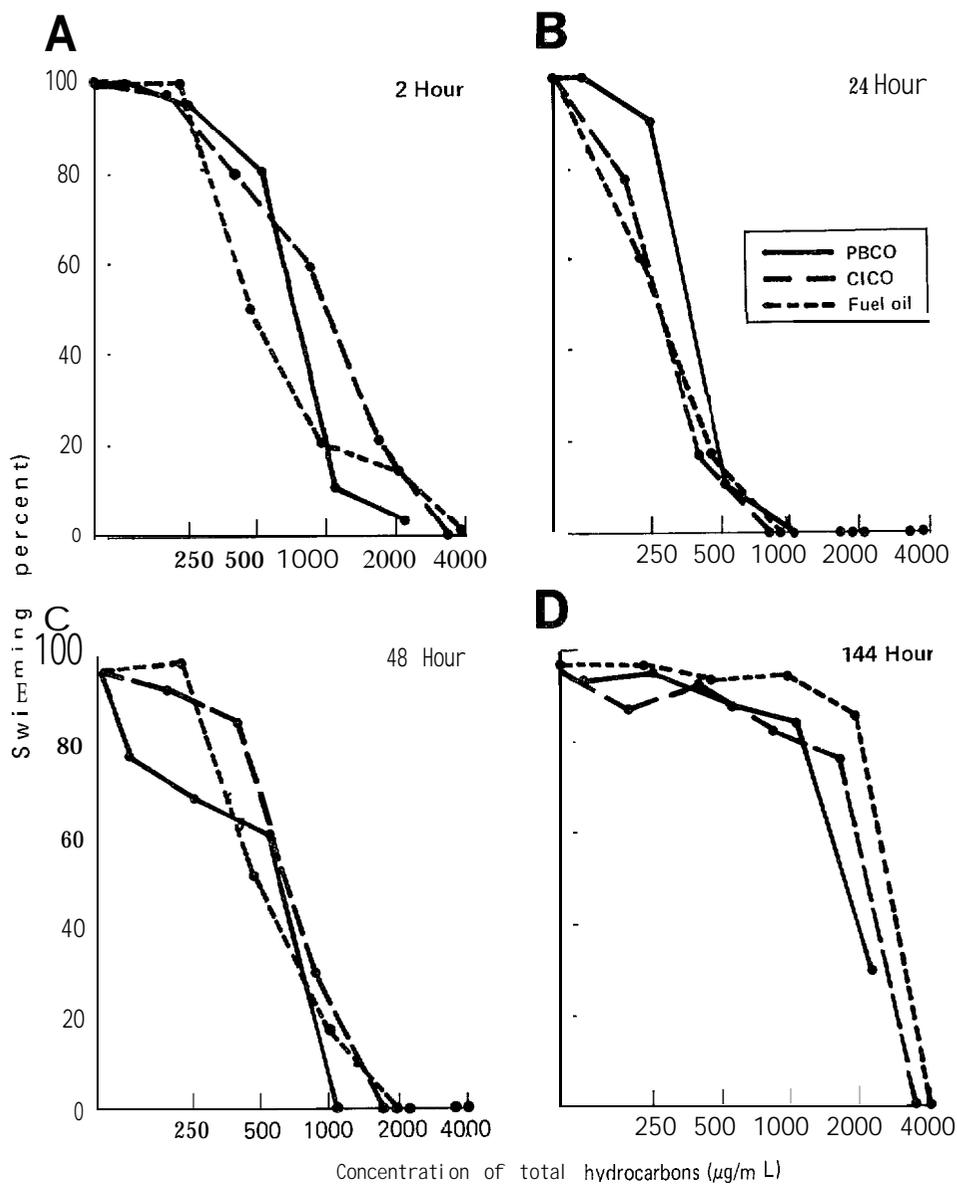


FIGURE 11. Percent of surf smelt larvae swimming in the upper 2-4 cm of the water column and respective total hydrocarbon concentrations in the reference SWAF's of PBCO, CICO, and No. 2 fuel oil: (A) 2-hr exposure, (B) 24-hr exposure, (C) 48-hr exposure, and (D) 96-hr deputation following 28-hr exposure (144 hr total). Concentrations of total hydrocarbons determined by GC analysis. Data points include mortalities, and each point is an average of the percent swimming in duplicate tests. The average percent difference between duplicate oil-exposed groups was 10.5% ( $\pm 14.7\%$  S. D. ), and 1.2% ( $\pm 1.2\%$  S. D. ) for controls.

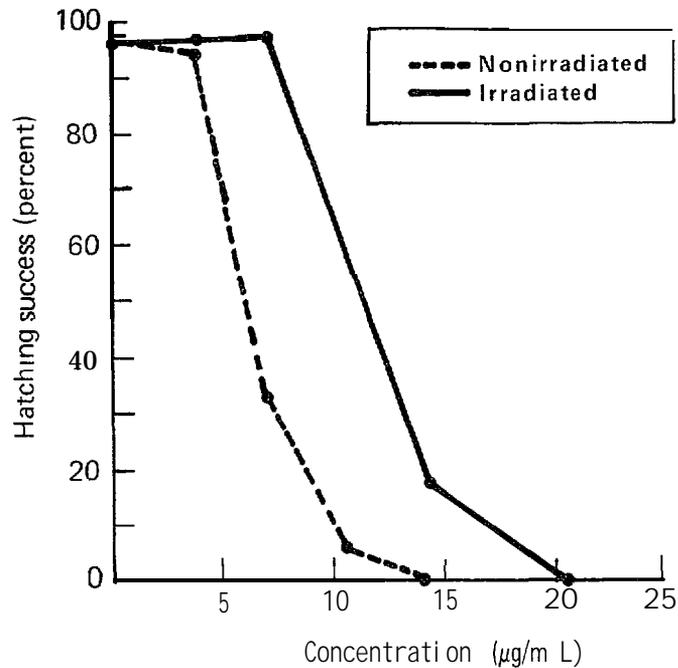


FIGURE 12. Hatching success of English sole eggs after exposure to irradiated [I FO<sub>S120</sub>] and non-irradiated [NIFO<sub>S120</sub>] SWAF's of No. 2 fuel oil under static conditions.

Very high levels of total extractable organic material were obtained from the static UV-irradiated No. 2 fuel oil weathering system (Table 6). In order to accommodate levels as high as 160 µg/mL of SWAF without having dispersed oil droplets, most of the total extractable organic material must have consisted of oxidized products.

English sole embryos exposed for 48 hr to SWAF's from wave machine-agitated UV-irradiated PBCO ([IPBCO<sub>A120</sub>]), which contained considerably lower levels of extractable organic material than fuel oil SWAF's, had a mortality not markedly different from the mortality seen in the controls (Table 7). Similar results were seen for PBCO SWAF obtained under static conditions. The highest levels of total extractable organic material obtained from PBCO SWAF under these two different weathering regimes were similar.

When the nonweathered, nonirradiated reference preparations of No. 2 fuel oil ([RFO]) and PBCO ([RPBCO]) obtained by mechanical shaking (Section 5.1) were used in the English sole embryo assay, the mortalities were not markedly different from those for the controls (Table 8). The highest concentrations of total extractable organic material obtained in the reference preparations were one to two orders of magnitude lower than those obtained either in the static or agitated controlled irradiation systems.

TABLE 6. Mortality of English sole embryos exposed for 48 hr to irradiated or nonirradiated SWAF's of No. 2 fuel oil produced under agitated or static conditions.

	Concentration ( $\mu\text{g/mL}$ )	Animals		Mortality (%)
		Tots	Dead	
<u>AGITATED CONDITIONS</u>				
Irradiated [IFO <sub>A120</sub> ]	3	333	4	1
	7	319	7	2
	14	302	14	5
	21	329	9	3
	28	304	7	2
	35 <sup>a</sup>	312	81	26
Nonirradiated [NIFO <sub>A120</sub> ]	4	286	8	3
	7	349	13	4
	11	292	13	4
	14	298	11	4
	18	341	13	4
	21 <sup>a</sup>	165	5	3
Control	0	680	13	2
<u>STATIC CONDITIONS</u>				
Irradiated [IFO <sub>S120</sub> ]	13	293	52	18
	25	272	147	54
	54	289	218	75
	107	310	310	106
	161 <sup>a</sup>	303	303	10(-1)
Nonirradiated [NIFO <sub>S120</sub> ]	2	181	4	2
	3	204	20	10
	6	312	34	11
	7 <sup>a</sup>	327	67	20
Control	0	507	55	11

<sup>a</sup> Maximum concentration of total extractable organic material removed from the SWAF's.

TABLE 7. Mortality of English sole embryos exposed for 48 hr to irradiated or nonirradiated SWAF's of Prudhoe Bay crude oil produced under agitated or static conditions.

	Concentration ( $\mu\text{g/mL}$ )	Animals		Mortality (%)
		Total	Dead	
<u>AGITATED CONDITIONS</u>				
Irradiated [IPBCO <sub>A120</sub> ]	2	280	14	5
	4	310	24	8
	6	310	14	5
	8 <sup>a</sup>	290	27	9
Nonirradiated [NPBCO <sub>A120</sub> ]	0.5	250	7	3
	1	310	17	5
	2	310	20	6
	2.1 <sup>a</sup>	260	10	4
Control	0	507	55	11
<u>STATIC CONDITIONS</u>				
Irradiated [IPBCO <sub>S120</sub> ]	1	281	13	5
	2	320	8	3
	4	290	17	6
	6	310	12	4
	8 <sup>a</sup>	210	13	6
Nonirradiated [NPBCO <sub>S120</sub> ]	0.2	270	9	3
	0.4	330	12	4
	0.8	320	18	6
	1	310	11	4
	2 <sup>a</sup>	230	14	6
Control	0	680	13	3

<sup>a</sup> Maximum concentration of total extractable organic material removed from the SWAF's.

TABLE 8. Mortality of English sole embryos exposed for 48 hr to reference SWAF's from No. 2 fuel oil or Prudhoe Bay crude oil.

	Concentration ( $\mu\text{g}/\text{mL}$ )	Animals		Mortality (%)
		Total	Dead	
No. 2 fuel oil [RFO]	0.2	297	7	2
	0.4	307	3	1
	0.6	323	6	2
	0.7	291	11	4
	0.9 <sup>a</sup>	267	6	2
Prudhoe Bay crude oil [RPBCO]	0.07	322	7	2
	0.1	327	7	2
	0.2	290	9	3
	0.3	250	6	2
	0.33 <sup>a</sup>	250	9	4

<sup>a</sup> Maximum concentration of total extractable organic material removed from the reference SWAF's.

### 6.5.3 English Sole Larvae

In the experiment where newly hatched English sole larvae were exposed to irradiated ([IFO<sub>A120</sub>]) or nonirradiated ([NIFO<sub>A120</sub>]) fuel oil SWAF's, the mortalities differed significantly (ANOVA, Student-Newman-Keuls  $p \leq 0.05$ ) from those for the seawater control (Table 9). The mortalities of the larvae occurred at higher levels of SWAF than the levels necessary to produce mortality in the apparently more resistant English sole embryos. For instance, larvae exposed for 48 hr to starting total extractable organic material concentrations of 4  $\mu\text{g}/\text{mL}$  and 2  $\mu\text{g}/\text{mL}$  from the irradiated SWAF ([IFO<sub>A120</sub>]) experienced 84% and 34% mortalities respectively, compared to 8% for controls. Exposure of the larvae to the nonirradiated SWAF ([NIFO<sub>A120</sub>]) from No. 2 fuel oil produced 100% mortality at concentrations of 2  $\mu\text{g}/\text{mL}$  and 18% mortality at 0.6  $\mu\text{g}/\text{mL}$  (roughly 600 ppb).

TABLE 9. Mortality of English sole larvae exposed for 48 hr to irradiated or nonirradiated SWAF's of No. 2 fuel oil produced by a wave machine.

	Concentration ( $\mu\text{g}/\text{mL}$ )	Animals		Mortality (%)
		Total	Dead	
Irradiated [I FO <sub>A120</sub> ]	2	210	72	34
	4	120	101	84
	6	227	227	100
	8 <sup>a</sup>	153	153	100
Nonirradiated [NI FO <sub>A120</sub> ]	0.6	209	37	18
	1	187	111	59
	2	216	216	100
	2.3 <sup>a</sup>	198	198	100
Control	0	203	16	8

<sup>a</sup> Maximum concentration of total extractable organic material removed from the SWAF's.

### 6.6 Cytopathology

Eight control surf smelt larvae and 25 larvae exposed to 2.7  $\mu\text{g}/\text{mL}$  of CICO ([RCICO]) were examined by light microscopy. Tissues from larvae which were actively swimming on the bottom or in the water column following a 48-hr exposure to reference oil SWAF ([RCICO]) appeared normal and similar to controls (Fig. 13A & B). Ninety-one percent (10 of 11) of the inactive larvae exhibited necrotic neurons in either the eye, brain, and/or olfactory placode (Fig. 13C). Eighty-two percent (9 of 11) of the inactive larvae also had compressed muscle bundles that were more intensely stained with Richardson's solution (Fig. 13D) than were those of controls. Limited examination with TEM showed that neurons of the eye and brain of inactive larvae exhibited condensed chromatin and numerous lysosomes, which is indicative of necrosis, and that there were severe disruptions in regions of myofibrils and sarcomeres of the muscle.

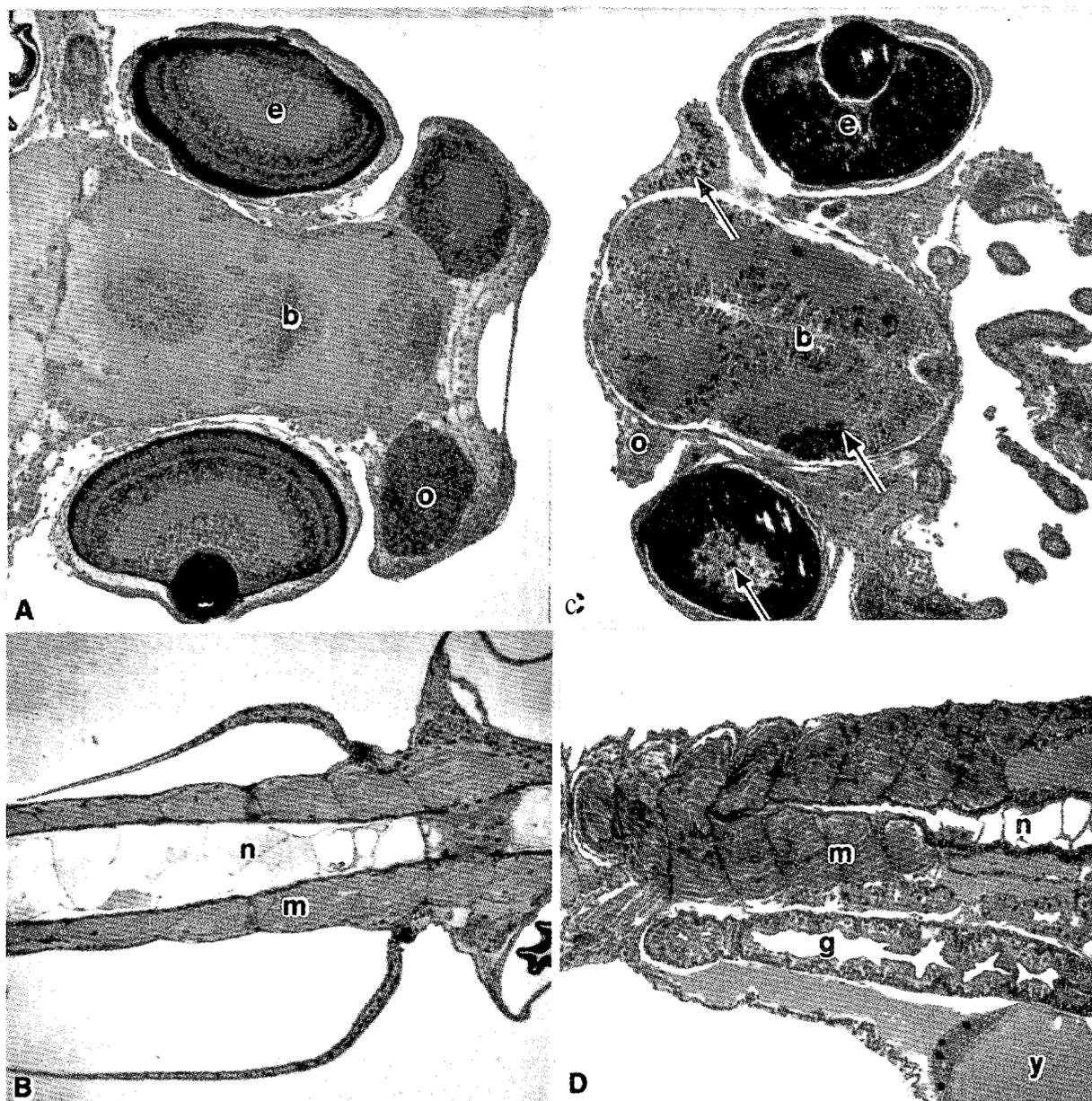


FIGURE 13. Microphotograph of a surf smelt larva. Frontal sections, X160. (A) Head of control larva with normal eye (e), brain (b), and olfactory placode (o). (B) Normal body musculature (m) and notochord (n) of a control larva. (C) Head of inactive surf smelt larva exposed to the reference SWAF of Cook Inlet crude oil [RCICO] for 48 hr showing dark staining necrotic cells (arrows) in the eye (e), brain (h), and olfactory placodes (o). (D) Compressed muscle bundles (m) of an inactive larvae with notochord (n), yolk (y), and gut (g) visible.

## DISCUSSION

### 7.1 Reference Mixtures of Seawater and Oils

Detailed studies with CICO SWAF's ([RCICO]) showed that the [RCICO] was essentially stable when stored at 4°C for up to 15 days, when sterilized or when a preservative was added. This suggested that samples of seawater collected from under an oil spill can be stored at 4°C for up to 15 days before chemical analyses without any marked changes occurring. In the absence of sterilization or a preservative, considerable loss of naphthalene occurred from the SWAF even when samples were stored at 4°C. Microbial degradation of hydrocarbons occurs even at low temperature (Karrick 1977) and, therefore, the addition of preservatives is necessary during storage.

The results from partitioning <sup>14</sup>C-hexadecane into seawater showed that 2 hr of separation was sufficient for the preparation of crude oil SWAF's; however, for No. 2 fuel oil a longer separation time was necessary. It was determined that a 4 hr separation time was suitable for preparing stable SWAF's from all three oils. Moreover, it was noted that filtration of the SWAF's did not alter the amount of <sup>14</sup>C-hexadecane-derived radioactivity in the seawater thereby indicating that SWAF's did not contain appreciable amounts of oil droplets.

### 7.2 Weathering of Oils and Hydrocarbon Fractions of Oils

#### 7.2.1 Flow-through Conditions

Studies conducted with CICO during the first year of this investigation revealed that high concentrations of total extractable organic material were present in the seawater from the agitated, environmentally weathered system. However, the high values may be due to the presence of oil droplets as indicated by high concentrations of aliphatic hydrocarbons in the SWAF. Moreover, the variability of natural exposure conditions (i.e., sunlight, wind, rain, temperature), low production of photooxidized products from CICO, and the rapid flushing out of these oxidized products in the flow-through system prompted us to replace this system during the second year with a no-flow laboratory weathering system.

#### 7.2.2 No-flow Conditions

Two modes of no-flow, UV irradiated exposures were selected: (1) static and (2) wave machine agitated. These modes were designed to simulate in the laboratory two important natural environments: (1) highly sheltered bays and estuaries where wave and wind action is restricted and spilled oil could spread out in a thin film as was simulated in the static system, and (2) open water areas, where the action of wind and waves could break up the oil into droplets which would be suspended in the water column as occurs in the water column of the wave machine.

The radiometric and gravimetric analyses with No. 2 fuel oil suggest that the effects of UV light on oil are markedly dependent on the conditions of exposure. The intensity of UV light in seawater rapidly decreases with depth. For extensive photooxidation of oils to take place, it was important

to have an undisturbed surface oil film. For example, after 120 hr of irradiation under static conditions, the seawater underlying the fuel oil film contained 7 times more total extractable organic material than did seawater beneath wave machine-agitated fuel oil. This was consistent with proposed mechanisms of photooxidation reactions of air/oil and oil/water interfaces (Aksnes and Iversen 1983).

It was shown that compared to either CICO or PBCO, UV irradiation of No. 2 fuel oil produced SWAF's with higher amounts of oxidized products. Because aromatic hydrocarbons are in general more soluble in seawater and are more easily photooxidized than paraffins (Atlas 1981), UV irradiation would be expected to be more pronounced on oils having the highest concentration of aromatic hydrocarbons. The No. 2 fuel oil contained a higher percentage of aromatic hydrocarbons than did CICO and PBCO (Pancirov 1974).

### 7.2.3 Removal of Polar Compounds from Crude Oils Prior to Irradiation (A/P Fraction)

The low concentrations of photooxidized products from irradiated CICO observed during the first year of this investigation suggested that certain physico-chemical characteristics of CICO had an inhibitory effect on the oxidation of crude oil. To test this hypothesis, A/P fractions separated from CICO and PBCO were subjected to UV irradiation; the amounts of total extractable organic material in the underlying seawater was considerably greater than that found in seawater under irradiated whole crude oils (Table 10).

The precise reasons for the increased oxidation of A/P fractions compared with oxidation of the whole crude oils are unknown; however, it appears that some of the naturally occurring polar components in these crude oils inhibit the photooxidation process in some way, such as acting as scavengers for free radicals in UV light-induced oxidations (Parker et al. 1971).

### 7.3 Characterization of Oxidation Products of Radiotracers

The extremely complex chemical nature of crude oils and refined products, such as No. 2 fuel oils (Clark and Brown 1977), makes it essentially impossible to (1) monitor photooxidative changes on an individual compound basis and (2) assess uptake of oxidized products by organisms used in toxicity studies. Therefore, we devised a radiotracer study using  $^{14}\text{C}$ -phenanthrene as a model petroleum compound which was added to the oils being weathered. There is precedence for this approach. In previous studies, single compounds were exposed to UV light in the presence of seawater and oxidative products were determined: Aksnes and Iversen (1983) used diphenylmethane and 1,2,3,4-tetrahydronaphthalene and Patel et al. (1978, 1982) used phenanthrene. Chromatographic and mass spectral analyses revealed the presence of several carboxyl derivatives, quinones, and acids of phenanthrene in seawater underlying irradiated phenanthrene. These results are in agreement with those of Patel et al. (1978, 1982) who identified similar photooxidation products of phenanthrene. The presence of oxidized products of phenanthrene in seawater suggested that oxidized products of other aromatic hydrocarbons may also be present in SWAF's from weathered oils.

TABLE 10. Amounts of total extractable organic material in SWAF's from various oils under different weathering conditions (120 hr, unless noted).

	Total extractable organic material		Ratio:
	Irradiated ( $\mu\text{g/mL}$ )	Nonirradiated ( $\mu\text{g/mL}$ )	$\frac{\text{Irradiated}}{\text{Nonirradiated}}$
<u>STATIC CONDITIONS</u>			
Cook Inlet crude oil - whole	25.7	9.2	2.8
- A/P fraction	147	29.0	5.1
Prudhoe Bay crude oil - whole	12.0 & 8.0	2.3 & 1.9	5.2 & 4.2
- A/P fraction	161	6.9	23
No. 2 fuel oil			
whole	356	12.5	28
whole <sup>a</sup>	474	17.9	26
<u>AGITATED CONDITIONS</u>			
Cook Inlet crude oil - whole	1.3	0.6	2.2
Prudhoe Bay crude oil - whole	7.5	2.1	3.6
No. 2 fuel oil			
whole	35	21	1.7

<sup>a</sup> After 425 hr of weathering.

## 7.4 Uptake Experiments

Uptake of two radiotracers,  $^3\text{H}$ -phenanthrene and  $^{14}\text{C}$ -p-cresol, from SWAF's of weathered CICO was studied during the first year of this investigation. Both radiotracers, when added directly to [RCICO] SWAF or when present as oxidized products in SWAF's of weathered oils, were taken up by surf smelt larvae. However, considerable differences in bioconcentration values were noted. For example, the uptake of  $^3\text{H}$ -phenanthrene from [RCICO] SWAF by surf smelt larvae was dramatically different from the uptake of phenanthrene-derived radioactivity from low-flow environmentally weathered CICO preparations. Larval fish accumulated maximum concentrations of  $^3\text{H}$ -phenanthrene equivalents of approximately 3,000 times the seawater concentrations when exposed to [RCICO] containing  $^3\text{H}$ -phenanthrene. However, in fish exposed to phenanthrene-derived radioactivity in the environmentally weathered preparation, the concentrations reached were never greater than 250 times the water concentration. This difference in uptake by the larval fish could be due to a number of factors; however, differences in lipophilicity between phenanthrene and its weathered products may be of primary importance.

The extent of uptake by larval surf smelt of phenanthrene-derived radioactivity was much greater than the uptake of  $^{14}\text{C}$ -p-cresol-derived radioactivity. The maximum concentration of p-cresol-derived radioactivity in the larval fish was 18 times the concentration of p-cresol-derived radioactivity in seawater. The maximum concentration of  $^3\text{H}$ -phenanthrene-derived radioactivity in the same larvae was 400 times the concentration of phenanthrene-derived radioactivity in seawater. Whether or not p-cresol and its weathered products were taken up, rapidly metabolized and excreted, or were less bioavailable than phenanthrene and its weathered products requires further investigation.

## 7.5 Toxicity Assays

### 7.5.1 Surf Smelt Larvae

Based on the results of radiotracers added to nonweathered, nonirradiated reference oils, it was demonstrated that larval surf smelt could bioconcentrate selected petroleum hydrocarbons during exposure in a static system. At initial concentrations of 2-3  $\mu\text{g}/\text{mL}$  (ea. ppm) of total extractable organic material in the SWAF's, short-term (48 hr) survival of the larvae was high (>95%). However, an order of magnitude lower concentration (0.25-0.35  $\mu\text{g}/\text{mL}$ ) caused a pronounced sublethal effect involving altered swimming (ataxia). These observations point out the need to differentiate between acute toxicity test results and long-term sublethal impacts at environmentally realistic levels. Reduced swimming activity and/or ataxia in marine organisms following exposure to petroleum hydrocarbons is well documented (Percy 1976, Patten 1977). In our experiments, the observed ataxia was reversible; however, if organisms are so affected and sink out of their usual position in the water column, they may lose their accustomed food supply as well as be subject to increased predation (Frank and Leggett 1982). Though the exact mechanism(s) involved in this swimming behavioral change is not clear, it is generally thought to be induced by aromatic compounds (Anderson et al. 1974b, Johnson 1977). The SWAF's of each of the

oils tested contained large quantities of aromatic compounds, and the reference (nonweathered) preparations of all the oils affected the larval swimming behavior to a similar degree.

### 7.5.2 English Sole Embryos

Hatching success of English sole eggs was affected only at levels of total extractable organic material exceeding 1  $\mu\text{g}/\text{mL}$ . Alterations in the hatching parameters can be caused by a number of stresses other than toxicant stresses, including changes in temperature, changes in salinity, and lowered oxygen concentrations (Rosenthal and Alderdice 1976). The protocol used in the assays in the present studies was designed, therefore, to minimize stresses related to temperature, salinity, oxygen and light.

Mortality of English sole embryos from SWAF's of different oils was low at environmentally high levels ( $>20 \mu\text{g}/\text{mL}$ ) of total extractable organic material. Clark and MacLeod (1977) reported ambient levels of petroleum hydrocarbons in the world's oceans to be a few  $\mu\text{g}/\text{L}$  (ea. ppb) but in close proximity to large oil spills, the levels reach several  $\mu\text{g}/\text{mL}$  (ea. ppm). One difficulty with comparing published data to our experimental results is that different investigators have reported their values based on different parameters (e.g., total extractable material using different solvents, total hydrocarbons, saturates, n-alkanes, aromatics, total and unresolved envelope by GC, selected individual compounds). While 5-20  $\mu\text{g}/\text{mL}$  appears to be an upper range for laboratory-produced SWAF's (as petroleum hydrocarbons) depending on the petroleum used, the total extractable organic material content can be several times this value. The exact amount found in a SWAF is a function of the specific petroleum, duration and intensity of photooxidation, type and degree of mixing, and other weathering processes unique to each experiment or spill. Therefore, while the absolute concentrations of total extractable organic material produced in our experiments may appear to be high when compared to petroleum hydrocarbon content, they are undoubtedly attainable in actual oil spill situations, especially in high energy locations in close proximity to a spill site. Under open ocean conditions characteristic of the Northeast Pacific, dispersed petroleum would form only a portion of the total extractable organic material and levels of petroleum would probably only reach ppm levels in close proximity to a major spill.

### 7.5.3 English Sole Larvae

Newly hatched English sole larvae were more sensitive in these assays than the embryos to both irradiated ([IF0<sub>S120</sub>]) and nonirradiated ([NIF0<sub>S120</sub>]) No. 2 fuel oil. This is not surprising because larval organisms are frequently more sensitive to toxicants than are other life stages; however, there is considerable variability in this regard among species (Rosenthal and Alderdice 1976, Rice et al . 1979).

## 8. CONCLUSIONS

The concentrations of oxidized components released to the underlying seawater beneath Cook Inlet crude oil (CICO) and Prudhoe Bay crude oil (PBCO) during UV irradiation were relatively low. In contrast, UV irradiation of No. 2 fuel oil resulted in the formation and release to underlying seawater of substantially greater amounts of oxidized components. An attempt was made, therefore, to determine why a fraction of crude oil (i.e., fuel oil) produced much higher concentrations of oxidized compounds after UV-irradiation than did the parent crude oil. When polar compounds were removed from Alaskan crude oils and the resulting aromatic/paraffinic (A/P) hydrocarbon fractions subjected to UV irradiation, the concentration of oxidized components increased to levels intermediate between the crude oils and the comparable, refined product. These results suggest that naturally occurring polar materials in crude oils inhibit crude oil photooxidation.

The simulated natural weathering protocol tested during the first year of this two-year investigation was modified because of unresolved technical problems which resulted in unacceptable variations. The successful weathering protocol used during the second year involved placing crude oil and No. 2 fuel oil slicks under (1) static or (2) agitated conditions with or without exposure to controlled UV irradiation under no-flow conditions. UV irradiation under these conditions induced measurable increases (2-5x nonirradiated levels) in total extractable organic material in the seawater-accommodated fractions (SWAF's) from CICO or PBCO. However, UV irradiation of No. 2 fuel oil under static, but not under agitated, conditions resulted in much higher concentrations (>100  $\mu\text{g}/\text{mL}$ ) of total extractable organic material in SWAF's compared to the concentrations in the nonirradiated No. 2 fuel oil SWAF's. As indicated above, after removal of polar materials from crude oils, UV-irradiation (static conditions) resulted in a clear increase in total extractable organic material in SWAF's compared to the concentration in the nonirradiated SWAF's.

Newly hatched surf smelt larvae and the embryos and larvae of English sole exposed to concentrations below 1  $\mu\text{g}/\text{mL}$  (ea. 1 ppm) of total extractable organic material in the seawater from beneath an irradiated oil slick did not undergo mortalities statistically different from mortalities of non-oil-exposed controls. However, at sublethal total hydrocarbon levels (0.25-0.35  $\mu\text{g}/\text{mL}$ ), pronounced effects on the swimming behavior of surf smelt larvae were observed during exposure to reference SWAF's (nonirradiated and nonweathered) from both crude oil and No. 2 fuel oil. Cytopathological examination of the affected larvae showed both necrosis of sensory tissues and compressed muscle bundles.

English sole embryos exposed to environmentally high levels (>20  $\mu\text{g}/\text{mL}$  of total extractable organic material) in the SWAF of No. 2 fuel oil still exhibited short-term toxic effects, but only in the static exposures. Newly hatched larvae showed a 50% mortality in the range of 1-3  $\mu\text{g}/\text{mL}$  with no significant difference in mortality of larvae between irradiated or nonirradiated SWAF's. Exposure to No. 2 fuel oil SWAF reduced the hatching success of English sole embryos, but again, only when exposed to high concentrations of oil-derived material.

Radiotracer studies were used to determine the nature of the oxidized products formed as a result of several of the weathering regimes and to measure the relative uptake of hydrocarbons and oxidized components by selected marine organisms. The highest concentration of weathered  $^3\text{H}$ -phenanthrene-derived material bioaccumulated in larval surf smelt was 370 times the concentration in the seawater. The maximum uptake of  $^{14}\text{C}$ -*p*-cresol-derived material in the larval fish was only 18 times that in the seawater.

Finally, although UV irradiation of the two crude oils and the fuel oil clearly induced formation of a variety of oil-derived oxidized products, there is no evidence from the present studies to lead to a conclusion that photooxidation under most natural conditions would significantly enhance the toxicity of petroleum in the marine environment of the Northeast Pacific Ocean.

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APPENDI X

TABLE 1A. Effects of storage at 4°C on the concentration of selected individual compounds in reference SWAF of CICO [RCICO] prepared from sterilized seawater and determined by GC analysis.

Compound	Time of Storage			
	0 Days		15 Days	
	Preserved (ng/mL)	Non- Preserved (ng/mL)	Preserved (ng/mL)	Non- Preserved (ng/mL)
Isopropyl benzene	15 $\bar{+}$ 1 <sup>a</sup>	14 $\bar{+}$ 1	15 $\bar{+}$ 0	14 $\bar{+}$ 1
n-propyl benzene	24 $\bar{+}$ 2	22 $\bar{+}$ 2	24 $\bar{+}$ 1	22 $\bar{+}$ 1
Indan	11 $\bar{+}$ 1	11 $\bar{+}$ 1	11 $\bar{+}$ 1	11 $\bar{+}$ 0
1,2,3,4-tetramethyl benzene	10 $\bar{+}$ 1	9.5 $\bar{+}$ 0.3	9.9 $\bar{+}$ 0.2	9.5 $\bar{+}$ 0.2
Naphthalene	83 $\bar{+}$ 6	81 $\bar{+}$ 8	84 $\bar{+}$ 1	82 $\bar{+}$ 1
Benzothiophene	2.5 $\bar{+}$ 0.2	2.4 $\bar{+}$ 0.2	2.6 $\bar{+}$ 0.1	2.5 $\bar{+}$ 0.1
2-methylnaphthalene	45 $\bar{+}$ 3	46 $\bar{+}$ 1	45 $\bar{+}$ 1	45 $\bar{+}$ 1
1-methylnaphthalene	29 $\bar{+}$ 2	29 $\bar{+}$ 1	30 $\bar{+}$ 1	30 $\bar{+}$ 1
Biphenyl	4.8 $\bar{+}$ 0.4	5.0 $\bar{+}$ 0.3	5.0 $\bar{+}$ 0.1	5.0 $\bar{+}$ 0.2
2,6-dimethylnaphthalene	6.4 $\bar{+}$ 0.5	6.8 $\bar{+}$ (-).4	8.7 $\bar{+}$ 0.2	8.5 $\bar{+}$ 0.2
Acenaphthene	1.77 $\bar{+}$ 0.2	1.8 $\bar{+}$ 0.1	0.15 $\bar{+}$ 0.01	0.15 $\bar{+}$ 0.01
2,3,5-trimethylnaphthalene	1.1 $\bar{+}$ 0.1	1.2 $\bar{+}$ 0.1	1.7 $\bar{+}$ 0.1	1.7 $\bar{+}$ 0.1
Fluorene	1.3 $\bar{+}$ 0.1	1.6 $\bar{+}$ 0.2	1.5 $\bar{+}$ 0.1	1.5 $\bar{+}$ 0.1
Phenanthrene	0.9 $\bar{+}$ 0.1	1.3 $\bar{+}$ 0.3	1.3 $\bar{+}$ 0.1	1.3 $\bar{+}$ 0.1
o-cresol	21 $\bar{+}$ 1	21 $\bar{+}$ 1	23 $\bar{+}$ 0	25 $\bar{+}$ 1
m- and p-cresol	9.77 $\bar{+}$ 0.3	9.7 $\bar{+}$ 0.5	11 $\bar{+}$ 1	11 $\bar{+}$ 1
2-ethylphenol	3.67 $\bar{+}$ 0.2	3.9 $\bar{+}$ 0.1	5.0 $\bar{+}$ 0.3	4.7 $\bar{+}$ 0.2
2,4- and 2,5-dimethylphenols	26 $\bar{+}$ 4	29 $\bar{+}$ 4	39 $\bar{+}$ 1	42 $\bar{+}$ 2
3- and 4-ethylphenols and 3,5-dimethylphenol	7.07 $\bar{+}$ 0.3	7.0 $\bar{+}$ 0.3	5.4 $\bar{+}$ 0.4	6.7 $\bar{+}$ 0.5
2,3-dimethylphenol	4.1 $\bar{+}$ 1.3	3.3 $\bar{+}$ 1.6	4.6 $\bar{+}$ 1.2	4.6 $\bar{+}$ 1.2
3,4-dimethylphenol	2.9 $\bar{+}$ 0.2	3.3 $\bar{+}$ 0.3	3.4 $\bar{+}$ 0.1	3.9 $\bar{+}$ 0.2
2,4,6-trimethylphenol	6.7 $\bar{+}$ 1.9	9*3T $\bar{+}$ 3.5	21 $\bar{+}$ 4	21 $\bar{+}$ 0
2-n-propylphenol	1.6 $\bar{+}$ 0.3	1.8 $\bar{+}$ 0.0	1.8 $\bar{+}$ 0.2	1.9 $\bar{+}$ 0.1
4-n-propylphenol	3.7 $\bar{+}$ 0.3	3.6 $\bar{+}$ 0.2	3.9 $\bar{+}$ 0.1	4.2 $\bar{+}$ 0.2
2,4,5-trimethylphenol	5.5 $\bar{+}$ 1.5	6.7 $\bar{+}$ 1.3	9.7 $\bar{+}$ 0.4	10 $\bar{+}$ 1
2,3,5-trimethylphenol	1.9 $\bar{+}$ 0.6	1.5 $\bar{+}$ 0.6	2.6 $\bar{+}$ 0.2	3.0 $\bar{+}$ 0.4
2,6-dimethylphenol	11 $\bar{+}$ 2	13 $\bar{+}$ 2	19 $\bar{+}$ 1	19 $\bar{+}$ 2
2,3,6-trimethylphenol	2.2 $\bar{+}$ 0.8	2.9 $\bar{+}$ 0.6	13 $\bar{+}$ 1	14 $\bar{+}$ 1

a  $\bar{X} \pm$  S.D., n = 3.

TABLE 2A. Effects of storage at 4°C on the concentration of selected Individual compounds in reference SWAF of CICO [RCICO] prepared from nonsterilized seawater and determined by GC analyses.

Compound	Time of Storage				
	0 Days		15 Days		65 Days
	Preserved (ng/mL)	Non- Preserved (ng/mL)	Preserved (ng/mL)	Non- Preserved (ng/mL)	Non- Preserved (ng/mL)
Isopropylbenzene	13 ± 1 <sup>a</sup>	14 ± 2	16 ± 2	16 ± 0	8.2
n-propyl benzene	22 ± 2	23 ± 2	26 ± 3	25 ± 0	1.4
Indan	11 ± 1	11 ± 1	12 ± 2	12 ± 0	5.8
1, 2, 3, 4-tetramethyl benzene	9.4 ± 0.6	9.6 ± 0.4	10 ± 1	11 ± 0	10
Naphthalene	80 ± 4	82 ± 4	90 ± 14	1.3 ± 0.5	0.43
Benzothiophene	2.4 ± 0.1	2.5 ± 0.2	2.8 ± 0.3	2.6 ± 0.1	3.7
2-methylnaphthalene	45 ± 2	45 ± 2	49 ± 7	45 ± 2	1.6
1-methylnaphthalene	29 ± 1	29 ± 1	32 ± 4	30 ± 1	0.87
Biphenyl	5.1 ± 0.1	4.97 ± 0.2	5.2 ± 0.6	4.8 ± 0.1	<0.07
2,6-dimethylnaphthalene	8.5 ± 0.4	8.3 ± 0.2	8.8 ± 0.9	8.4 ± 0.2	<0.09
Acenaphthene	1.5 ± 0.2	1.67 ± 0.2	0.13 ± 0.01	0.11 ± 0.02	0.06
2,3,5-trimethyl naphthalene	1.1 ± 0.1	1.0 ± 0.0	1.7 ± (-).2	1.7 ± 0.1	0.45
Fluorene	1.4 ± 0.2	1.3 ± 0.0	1.3 ± (-).1	1.3 ± 0.1	<0.07
Phenanthrene	1.3 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	<0.07
o-cresol	19 ± 1	20 ± 2	19 ± 2	26 ± 1	110
m- and p-cresol	6.9 ± 3.4	7.37 ± 3.4	9.4 ± 0.6	10 ± 0	9.9
2-ethylphenol	3.6 ± 0.0	3.9 ± 0.2	4.2 ± 0.3	4.2 ± 0.2	6.6
2,4 and 2,5-dimethyl phenols	39 ± 1	39 ± 2	39 ± 3	24 ± 1	9.1
3- and 4-ethylphenols and 3,5-dimethyl phenol	7.9 ± 0.1	7.8 ± 0.1	6.7 ± 0.4	7.8 ± 0.2	4.1
2,3-dimethyl phenol	4.9 ± 0.2	4.8 ± 0.0	3.8 ± 0.3	3.7 ± 0.2	4.0
3,4-dimethyl phenol	3.4 ± 0.1	3.5 ± 0.1	3.0 ± (1.2)	3.4 ± 0.1	2.3
2,4,6-trimethyl phenol	27 ± 1	25 ± 1	20 ± 2	22 ± 1	19
2-n-propylphenol	1.57 ± 0.0	1.57 ± 0.0	1.9 ± 0.2	2.0 ± 0.1	2.4
4-n-propylphenol	4.47 ± 0.2	4.2 ± 0.1	3.5 ± 0.3	3.8 ± 0.1	10
2,4,5-trimethyl phenol	12 ± 1	11 ± 1	10 ± 0.9	11 ± 0	15
2,3,5-trimethyl phenol	3.9 ± 0.2	3.57 ± 0.7	3.0 ± 0.6	4.4 ± 0.2	4.2
2,6-dimethyl phenol	17 ± 0	17 ± 1	17 ± 1	17 ± 1	11
2,3,6-trimethyl phenol	7.9 ± 0.2	7.8 ± 0.3	NA <sup>b</sup>	NA	5.5

a  $\bar{X} \pm S.D.$ , n = 3.  
b NA = not analyzed