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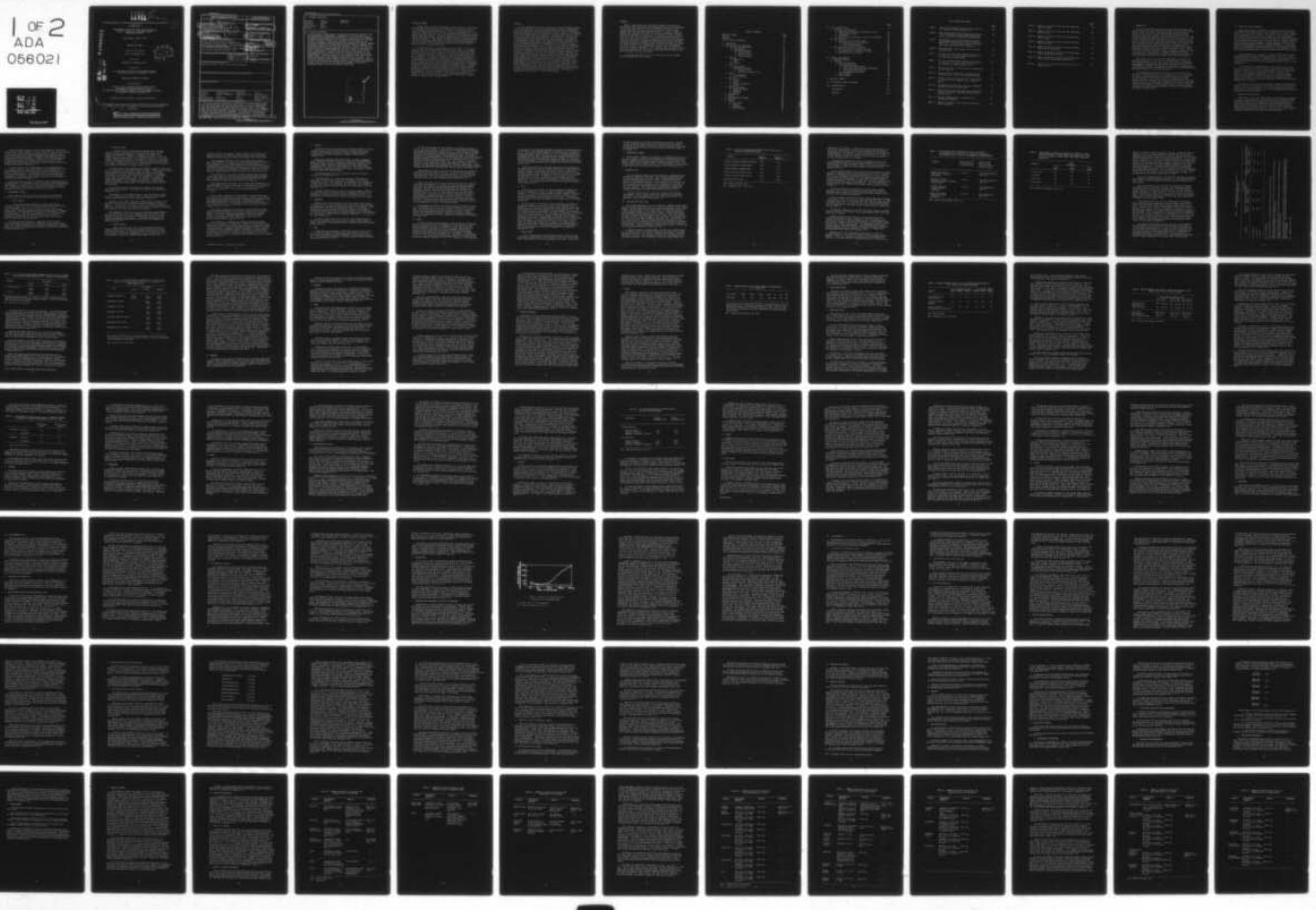

A Literature Review - Problem Definition Studies on Selected Toxic Chemicals

Volume 8 of 8

ENVIRONMENTAL ASPECTS OF DIESEL FUELS AND FOG OILS SGF NO. 1 AND SGF NO. 2 AND SMOKE SCREENS GENERATED FROM THEM

Final Report - April, 1978

by

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In this literature review (117 references) on the environmental aspects of fog oils and diesel fuel and the smoke screens, or fogs, generated from them, the topics which are investigated include the effects of petroleum fuels and lubricants on waterfowl and birds, insects, plants, soil nematodes, fish, marine worms, molluscs, crustaceans, and other marine species, phytoplankton, microorganisms and zooplankton. In addition to acute toxicity of these petroleum oils in most species, adverse effects on reproduction, carcinogenicity, chemically-mediated behavior disruption, and inhibition

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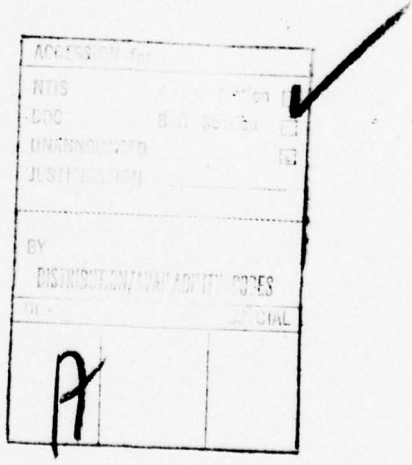
of photosynthesis, among others, are reported for various organisms. Factors influencing the atmospheric dispersion of the oil smokes, and the dispersion and persistence of the oil films on soil, water, and vegetation resulting from the settling of the oil smoke to ground level are reviewed, as well as pathways by which these petroleum oils are chemically and biologically degraded, and their uptake and accumulation in species ranging from algae through fish and shellfish to humans. Current techniques for sampling and analysis of fog oils and diesel fuel in water, soil and biological media are presented. Because the actual levels of atmospheric and ground contamination with diesel fuel and fog oil smoke and its fallout after repeated smoke screening operations have not been measured, it is recommended that this be done in order to accurately assess the environmental impact. A tentative model has been offered in which their environmental contamination levels under set conditions were calculated. The conclusion reached is that it may be possible to expect fog oil to persist in soil, harm vegetation and pollute bodies of water sufficiently enough to introduce the oils into the food chain and possibly to harm aquatic organisms feeding or living in smoke screening areas.



EXECUTIVE SUMMARY

In this report, studies are reviewed on topics related to the environmental damage caused by diesel fuel and fog oils. The oils are used in U.S. Army smoke screening operations. Both of these petroleum oils, in sufficient quantity, are toxic to ducks, freshwater and marine fish, clams, shrimps and other shellfish, algae, soil and water microorganisms, soil and marine worms, and insects. They cause harmful effects in trees and plants, such as yellowing of leaves, dwarfing, and interference with photosynthesis and fruit production. Other effects in animals include contamination of feathers of ducks, egg abnormalities in quail, equilibrium disturbances in fish, cancer in soft shell clams and interference with food finding behavior in snails and oceanic bacteria.

Oil smoke consists of tiny droplets of fog oil, which are carried as far as 10 km by air currents before falling back to earth. As the smoke droplets settle, an oil film forms over soil, bodies of water and plants. Oil seeps into soil, where it remains to be slowly biotransformed by bacteria. Rainfall runoff may carry some oil into drinking water supplies and other waters. Oil forms a film when it falls on water, and some of the oil may dissolve in the water, be photolyzed by sunlight, metabolized by microorganisms in the water and bioconcentrated by organisms living in the water. In this way the oil can bioaccumulate through secondary consumption, reaching humans eating fish taken from the contaminated water. The amounts of fog oil or diesel fuel which contaminate the ground, plants and waters in smoke screening areas need to be measured in order to present a more definitive evaluation of the environmental impact of U.S. Army smoke screening operations. It may be possible to expect fog oil to persist in soil, harm vegetation and pollute bodies of water sufficiently enough to introduce the oils into the food chain and possibly harm aquatic organisms feeding or living in smoke screening areas.

ABSTRACT

In this literature review (117 references) on the environmental aspects of fog oils and diesel fuel and the smoke screens, or fogs, generated from them, the topics which are investigated include the effects of petroleum fuels and lubricants on waterfowl and birds, insects, plants, soil nematodes, fish, marine worms, molluscs, crustaceans, and other marine species, phytoplankton, microorganisms and zooplankton. In addition to acute toxicity of these petroleum oils in most species, adverse effects on reproduction, carcinogenicity, chemically-mediated behavior disruption, and inhibition of photosynthesis, among others, are reported for various organisms. Factors influencing the atmospheric dispersion of the oil smokes, and the dispersion and persistence of the oil films on soil, water, and vegetation resulting from the settling of the oil smoke to ground level are reviewed, as well as pathways by which these petroleum oils are chemically and biologically degraded, and their uptake and accumulation in species ranging from algae through fish and shellfish to humans. Current techniques for sampling and analysis of fog oils and diesel fuel in water, soil and biological media are presented. Because the actual levels of atmospheric and ground contamination with diesel fuel and fog oil smoke and its fallout after repeated smoke screening operations have not been measured, it is recommended that this be done in order to accurately assess the environmental impact. A tentative model has been offered in which their environmental contamination levels under set conditions were calculated. The conclusion reached is that it may be possible to expect fog oil to persist in soil, harm vegetation and pollute bodies of water sufficiently enough to introduce the oils into the food chain and possibly to harm aquatic organisms feeding or living in smoke screening areas.

FOREWORD

The U.S. Army Research and Development Command is assessing the occupational health and safety, and environmental impact of various chemicals to which Army personnel and the environment are exposed. This problem definition study has been prepared as part of this research program under contract number DAMD-17-77C-7020, to provide a review and analysis of literature relating to the environmental effects of diesel fuel and fog oils SGF No. 1 and SGF No. 2 and the white smokes generated from them. The topics included in this problem definition study are: effects on fauna and flora living in aquatic and terrestrial environments; occurrence, persistence, dispersion, biotransformation, bioconcentration and bioaccumulation; environmental impact assessment; methods of sampling and analysis applicable to environmental research; and recommendations for further studies. The appendix lists sources which were examined to locate the relevant information in the literature, and includes the various persons and organizations contacted in obtaining important data on fog oils and diesel fuel, and their smokes.

This problem definition study is the eighth in a series of eight reports prepared under this contract.

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I. INTRODUCTION

This problem definition study reviews the effects of diesel fuel and fog oils on flora and fauna and the aquatic and terrestrial environments in which they reside. The present volume follows two other reports in this series: Volume 1, Occupational Health and Safety Aspects of Diesel Fuel and White Smoke Generated from It (1); and Volume 4, Occupational Health and Safety Aspects of the Fog Oils SGF No. 1 and SGF No. 2 and Smoke Screens Generated from Them (2). These two reports contain in-depth reviews of the physical and chemical properties of Fog Oils and diesel fuel, and their relationship to fuel oils, lubricating oils, kerosene and other petroleum fuels and lubricants which are discussed in the present review. In addition to physical and chemical properties, Volumes 1 and 4 also review the literature on the effects of exposure of humans, laboratory animals and domestic animals to diesel fuel, fog oils and their smokes, as well as mammalian metabolism, carcinogenicity and mutagenicity. Methods of sampling and analysis of oils and smoke in the air and in biologic media are also presented.

This volume does not reiterate the information contained on the above-mentioned topics. It covers the following areas: effects on birds and waterfowl, insects, fish, crustaceans, molluscs, zooplankton, phytoplankton, microorganisms and plants; occurrence, dispersion and transformation in aquatic and terrestrial environments, bioaccumulation and environmental impact; and sampling and analysis of petroleum fuels and lubricants in water, soil and biologic media.

The environmental effects of petroleum fuels and lubricants are assessed by analyzing literature on accidental fuel oil or lubricating oil spills, crop spraying with fuel and lubricating oils, bioassays of petroleum fuels and lubricants in various organisms, and other relevant research. Due to a lack of data on actual amounts of fog oils released into the environment and contaminating the areas in which smoke screening operations are performed, hypothetical fog oil concentrations likely to be encountered in the atmosphere, ground and water in smoke screening areas were estimated in order to construct an environmental and impact assessment model.

II. EFFECTS ON LIVING ORGANISMS

Operations involving the generation of smoke screens from fog oils, and possibly diesel fuel, result in oil contamination to the flora and fauna in the area being screened. This occurs as the oil smoke, consisting of microdroplets of the fog oil, settles in a film on the terrain. No literature is presently available concerning actual measurements of fog oil concentrations in air, on land or in water as a result of settling of oil droplets from smoke screens. However, based on available information on the operation and placement of smoke generators (3) as well as personal communications with smoke screening personnel (4), it was estimated that one M3A3 smoke generator operating for one hour could theoretically create atmospheric smoke concentrations of 13 to 2000 mg/m^3, and fallout concentrations on soil, water or foliage surfaces of 6 to 60 g/m^2.

These estimates fail to account for either meteorological factors (wind speed and direction; temperature gradient) or loss of oil due to evaporation prior to settling. In addition, smoke screening operations may be conducted many times in a single location, magnifying the quantity of diesel fuel or fog oils contaminating the air, water and soil considerably.

The fog oils are petroleum products of two specific types. SGF No. 1 is a distillate of crude oil which is physically and chemically similar to both No. 2 fuel oil (a home heating oil) and to diesel fuel. SGF No. 2 is physically and chemically similar to 10W motor oil, which is a low viscosity lubricating oil. See the chapter, Physical and Chemical Properties in Volume 4, Occupational Health and Safety Aspects of the Fog Oils SGF No. 1 and SGF No. 2 and Smoke Screens Generated from Them, for an in-depth discussion of physical and chemical properties of fog oils (2).

The toxicity of fog oils and diesel fuel in humans, laboratory and domestic animals and livestock has previously been reviewed in Volumes 1 and 4 of this series of reports (1,2). In this chapter, the effects of petroleum fuel and lubricating oils on flora and nonmammalian terrestrial and aquatic fauna are reviewed.

A. WATERFOWL AND OTHER BIRDS

The effects of fuel oils, diesel fuel, and lubricating oils similar to SGF No. 2 fog oil in ducks, geese and other types of birds and fowl have been observed after oil spills and have been investigated in the laboratory. Because of the difficulty of calculating the amount of fog oil smoke which settles onto beaches and areas where waterfowl might reside, the literature which is presented covers a wide range of pollution levels which may be greater or less than the fog oil levels encountered during and after smoke screening operations.

1. FIELD STUDIES

The following literature illustrates the damage caused by oil spills and industrial oil pollution to waterfowl in freshwater and marine environments.

A spill of over 750,000 liters of No. 2 diesel fuel oil into Puget Sound in Washington, in 1971, severely affected the seabirds in the area. In addition to approximately 10,000 casulaties, a State Game representative estimated that 30,000 Black Brent geese (25% of the entire population in the Pacific flyway), as well as ducks, loons, cormorants, grebes and mergansers were in the area at the time of the spill, although the consequences to these species were not reported (5).

The 1976 grounding of a trawler and a coaster on a northeast Scotland beach caused the release of approximated 2900 liters of diesel fuel onto the beach. The tide carried oil into an estuary and to a sea-front area. A "handful" of the 4,000 Eiders which were breeding immediately opposite the wrecks were killed. At least 6 of 70 pairs of nesting Sandwich terns became oiled. Other effects had not yet been assessed. A previous oil spill which contaminated Sandwich terns caused a failure of eggs to hatch. No other information was provided, however (6).

Industrial oil pollution to the Detroit River and part of Lake Erie adjacent to the river's mouth was partly responsible for the death of an estimated 10,000 ducks, including canvasbacks, scaups, redheads, and black ducks in the winter of 1948. Oils and greases tended to accumulate in eddies and backwaters, and were flushed from these areas in extensive flows during periods of unusually heavy surface runoff. Industrial effluent also caused a gradual warming of the river, preventing winter freezing. The unfrozen water was a food source which attracted migrant waterfowl. Actual causes of death were cold weather, starvation and oil contamination (7).

Waterfowl are affected by petroleum oils in four ways. The first is inability to fly. Mallards placed in water on which heavy fuel oil floated became incapable of flight for 2 weeks to one month after exposure; their feathers had absorbed approximately 20 ml of oil. Secondly, oil accumulation in feathers causes the birds to preen excessively. Pekin ducks placed in heavy fuel oil slicks devoted so much time to preening that too little feeding time remained and the ducks became weak from starvation. Similar ducks, however, when placed in water with a slick of No. 2 fuel oil, suffered no apparent adverse effects (7).

A flock of 75 ducks, mostly scaup, was almost entirely killed in a few hours by an accidental leak of No. 2 fuel oil from a tank which drained into a small pond where the birds were sleeping and feeding. The leak happened at night, and by morning, most ducks were dead. Deaths were probably due to drowning from loss of buoyancy, a third effect of oil (7). No conclusions were drawn by the author in relation to a dose-response effect.

Ducks died during exposure to SAE 10 lubricating oil flows on the Detroit River, after entering live traps. The birds found dead were normal in weight and showed no evidence of intoxication or disease. Ducks which remained alive were found swimming with their heads barely above water, due to decreased buoyancy. A number of dead ducks had lost all buoyancy and settled to the bottom. Extraction of oil from feathers of some birds revealed that from 0.54 gram to over 4 grams of the lubricating oil was present on specimens. Death probably resulted from becoming waterlogged and drowning (7).

A fourth effect of oil is impairment of the insulating quality of the feathers, leading to decreased tolerance to low temperatures. The authors emphasized that even a nontoxic oil could contribute to the death of waterfowl due to: impaired insulation of oily feathers; loss of buoyancy and drowning; loss of ability to fly and thus increased vulnerability to predators; and excessive preening to the exclusion of feeding and resultant starvation (7).

The waterfowl on the diesel fuel-polluted Puget Sound swallowed oil during preening. Oil swallowing caused severe burns, internal hemorrhages and pneumonia. Cold shock, due to loss of waterproofing or insulation, also contributed to their deaths. The birds were frequently observed waving their heads from side to side as if in a coma (5).

2. EXPERIMENTAL STUDIES

Acute toxicity, effects on yolk and egg survival, and systemic effects of fuel oils and lubricants are discussed in this section.

a. ACUTE TOXICITY

An LD_{50} for technical grade No. 1 diesel fuel oil was >20 ml/kg of body weight for both male and female pen-reared one-year-old mallard ducks. Five ducks were fasted for 16-20 hours prior to administration of gelatin capsules of diesel fuel through a stomach tube. Acute symptoms observed were transient weakness, diarrhea, and regurgitation. This dose was considered to be "far more than any mallard is likely to come into contact with in normal pesticide applications". (Pesticides are mixed with diesel fuel and then aerosolized to spray the desired area) (8).

In ducks which were stressed by exposure to 0 to -10°C and crowding, an LD_{50} of 4 ml/kg of body weight was established for diesel fuel. The oil was administered to 8 Pekin and 9 Mallard ducks by gavage. Non-stressed ducks all survived up to 24 ml/kg of diesel fuel and 20 ml/kg of lubricating oil (9).

b. EFFECT ON EGGS

Egg production in 10-24 week-old Japanese quail, *Coturnix coturnix japonica*, was halted for 6-8 days when the quail were fed capsules containing approximately 3.5 mg/kg of body weight of an aromatic No. 2 fuel oil. However, in comparison to the No. 2 fuel oil, neither medicinal grade mineral oil nor safflower seed oil, in the same dosages, reduced egg production. (The number of quail was not specified). A dose of 3.5 mg/kg of a heavy fuel oil halted egg production in 18 quail for 2 weeks; 0.7 mg/kg had no effect on egg production or on hatchability, when compared with controls fed empty capsules (10).

Yolk abnormalities were observed after feeding 3.5 mg/kg of body weight of No. 2 fuel oil to an unspecified number of quail. Uneven yolk distribution occurred during the first few hours after oil dosing. A less than normal amount of yolk was deposited during the first night, forming a thin layer of yolk which did not stain with dichromate. Yolk spheres in this layer were very small: 10-30 μ in diameter, as compared to normal spheres, 40-120 μ in diameter. The next outer yolk ring was narrow and abnormally dark staining with dichromate. Light-staining yolk was deposited the second night, followed by another dark ring the third morning.

Oil ingestion was often followed by the laying of thin shelled eggs, which cracked easily. Some shells were affected the same day as dosing with oil.

Yolk deposition in two Canadian geese, *Branta canadensis moffitti*, fed 1.5-3.5 mg/kg of body weight of fuel oil revealed a similar light-staining ring, followed by a later deposition of dark yolk.

Chickens, *Gallus gallus domesticus*, administered 3.5 mg/kg of body weight of fuel oil, also showed marked structural changes in yolks of eggs laid. Chickens fed 3 g of the oil (total dose) ceased egg production completely. In some eggs, yolk deposited 2-3 days after dosing with oil was still abnormally dark after staining with dichromate.

The mechanism of action of fuel oils on bird eggs was postulated to be one of absorption of toxic oil components from the digestive tract and transport to the ovary, where yolk deposition occurs, in a manner similar to transport of carotenoids. Oil-influenced disturbances in sodium-potassium metabolism might have influenced yolk formation and embryo survival (10).

c. SYSTEMIC EFFECTS

Ducks will preen about 50% of any polluting oil from their feathers within the first 8 days after exposure, ingesting most of the oil during the process. An "average" oily duck with about 7 g of oil in its feathers would ingest approximately 1.5 g of oil the first day, corresponding to a dose of 2-3 g/kg of body weight per day (9).

With this in mind, the effects of administration of 1-3 ml/kg (0.9-3.1 g/kg*) of fuel oils or lubricating oils by stomach tube to 9 Mallard ducks and 8 Pekin ducks were studied and compared with control ducks.

Lipid pneumonia developed in 24% of the ducks (total number of ducks not specified). A sample of 41 wild oil-killed ducks revealed 25 with pneumonias, of which 4 were diagnosed as lipid pneumonia by histopathological staining with Oil Red O. Oil droplets in peribronchial areas were surrounded by hemorrhagic inflammation, leukocytic and lymphocytic infiltrates, and were frequently walled off by connective tissue scarring. Usually less than half of the lung tissue was affected (9).

Gastrointestinal effects noted included diarrhea which developed 6 hours after fuel oil or diesel fuel dosing and persisted for several days. Lubricating oils were only slightly irritating, and medicinal grade mineral oil produced no gastrointestinal irritation.

Blood changes were also noted. Two to eight days following fuel oil administration, the ducks became anemic. Blood loss from the intestines may have caused the anemia. The oil-killed wild ducks had occult blood in the intestines, as well as severe gastrointestinal irritation.

Liver changes including fatty infiltration and degeneration were found in all oil fed ducks and in some controls. Altered liver function was indicated by abnormal plasma glutamic oxaloacetic transaminase levels and bromosulfophthalein retention times, after administration of 3-12 ml/kg of diesel fuel. In contrast to diesel fuel, lubricating oil had no significant effect on these indices of liver function in doses of 2 ml/kg of body weight (9).

Microscopic examination of the pancreas revealed reduced zymogen granules, indicating reduction in storage of enzyme precursors in the organ. In ducks fed 6 mg/kg of body weight of diesel oil, zymogen granules were absent, and cloudy degeneration of cells and acinar atrophy were reported, although plasma lipase levels were only slightly elevated above control values (9).

Toxic nephrosis, as indicated by elevated non-protein nitrogen blood levels, occurred in 7 ducks after 2 mg/kg of fuel oil and in 11 ducks after the same dose of diesel fuel, but not in 6 ducks fed 2 mg/kg of lubricating oil. There was some cloudy swelling of cells of proximal convoluted tubules and Bowman's capsule in kidneys of oil-fed ducks but not controls. Ducks fed 12-24 ml/kg of body weight of diesel fuel developed tubular cell hydropic degeneration and albumonoid casts in the distal convoluted tubules, indicating a dose dependent effect on the kidneys for diesel fuel (9).

*conversion factor: 1 ml fuel oil = 0.85 g

B. INSECTS

Petroleum oils have been sprayed in California orchards to control a wide variety of insects since the 1880's. Kerosene sprays were effective in killing scale insects in lemon and orange groves, and controlling defoliating caterpillars in apple, peach and prune orchards (11).

The Western Cooperative Oil Spray Project, in 1930, recommended using lubricating oils with viscosities of 100-220 seconds Saybolt in winter crop sprays, and less viscous oils (50-120 sec (Saybolt; 100°C) for summer sprays. Both were effective insecticides and produced comparatively low toxicity in young plants (12) (See Plant section). San Jose scale, orchard leaf-roller, red spider, tree hoppers, codling-moth and aphids were controlled (12).

It was necessary for the oil to directly contact the insect to be effective in killing it (11).

Singh and Sharma (13) found that spraying a 50% emulsion of diesel oil on fruit trees was effective in killing a leaf parasite, *Dendrophthoe falcata*. The emulsion was insecticidal even if washed off by heavy rain 2 hours after spraying. On sunny days, a 35% emulsion was effective, whereas 45-50% was necessary on cloudy days to kill the parasites (13).

Studies on adult insects, as well as on eggs and maggots indicate that petroleum oils have variable effects, depending on the age of the organism, the viscosity of the oil and the dilution of the oil. In most studies, oil concentrations achieved in spraying were not reported.

1. MOSQUITOES

Mosquito control was attempted in a drive-in movie theatre with a 5:1 mixture of No. 2 fuel oil and kerosene. The mixture was aerosolized in fog generators and sprayed around the perimeter of the theatre. When sprayed at dusk, when mosquito infiltration normally was heaviest, a considerable reduction in mosquito density resulted, as determined by pre- and post-spray measurements of mosquito landing rates. The petroleum oil spray did not, however, prevent "mosquito annoyance". (This concept was not defined) (14).

The use of diesel fuel or No. 2 fuel oil fogs protected herds of cattle from mosquitoes, and was economically cheaper than traditional smudge. This brief 1968 communication also indicated that hornfly control was achieved (15).

2. EGGS

A literature review compiled in 1918 indicated that pure kerosene had no effect on eggs of the oyster-shell scale insect, but that it was effective (although no longer recommended) in killing eggs of head lice. Aphid eggs were unaffected by kerosene emulsion sprays containing less than 25% of the oil (16).

By 1939, it was found that the viscosity of petroleum oils was related to ovicidal activity. A series of 28 oils were tested in undiluted form on eggs of the mosquito, *Aedes aegypti* L. Ovicidal activity was minimal in low viscosity oils, increased to a maximum at about 108 sec Saybolt (at 100°C) and then decreased as the viscosity increased. Oils with a viscosity of 45 seconds Saybolt at 100°C (kerosene) were only 20-30% effective, whereas a maximal ovicidal effect of 70% was achieved with oils of 90-135 sec Saybolt viscosities (lubricating oils). SAE 10 motor oil, having a viscosity of 103 sec Saybolt at 100°C, and light spindle oil, with a viscosity of 109 sec Saybolt were 67% and 79% effective, respectively. As the viscosity of lubricating oils became greater, ovicidal effectiveness dropped, reaching 30% with heavy lubricating oils (360 sec Saybolt viscosity) (17).

The mechanism of action of the oils on mosquito eggs was studied by staining the oil with Sudan or Oil Red. The oil did not penetrate the eggs, and did no damage to the chorionic membranes. It was postulated that suffocation, (i.e., oxygen deprivation) occurred when the oils completely coated the eggs, preventing oxygen diffusion (17).

Eggs of the potato beetle, *Leptinotarsa decemlineata* Say, were also sensitive to petroleum oils. Clusters of 20-30 eggs each were dipped into California kerosene, Pennsylvania kerosene and Indiana kerosene fractions. The California kerosene-dipped eggs were examined for percentage hatching. One-day-old eggs generally did not hatch after dipping while eggs 1-2 days old did. Eggs with well developed embryos failed to hatch. Fractions of a Pennsylvania kerosene were tested individually in dipping experiments. The most volatile oil fraction, boiling between 140-187°C, killed only freshly laid eggs, while the 187-234°C fraction killed all eggs (fresh, partially developed and fully developed). Indiana kerosene fractions gave similar results, but the oil was somewhat more toxic to fully developed eggs.

In explaining the results, the authors noted that freshly laid eggs were more permeable to kerosene than partially developed (1-2 day-old) eggs. The low-boiling oil fractions, being more volatile, evaporated from partially developed eggs before hatching, and thus had little effect. The high-boiling fractions, being least volatile, remained on the eggs long enough to kill them, regardless of permeability differences due to age (16).

3. LARVAE

A flowing Alaskan stream containing a large population of 4th-instar blackfly larvae was the site of experiments to test the insecticidal action of kerosene and fuel oil. Rainbow trout and caddis fly larvae were simultaneously exposed to the oils in screened cages placed in sections of the stream, and the oils were applied by a method which maintained parts-per-million concentrations. Detachment of blackfly larvae from the rocks of the streambed was the criterion of effectiveness of the oil. The 24-hour mortality data were obtained by placing larvae, which were previously exposed to the oil for 15 minutes, in screened cages

in the stream. The tests were designed to determine the maximum quantity of oil which could be applied without destroying the trout or caddis fly larvae. Fuel oil in concentrations of 7, 9, or 10 ppm was ineffective in killing blackfly larvae in 24 hours but did cause considerable larval detachment from the rocks. Kerosene was also nonlethal in 24 hours at a concentration of 20 ppm (Neither the toxicity to caddis fly larvae nor trout was discussed). The conclusion reached was that the concentrations of fuel oil and kerosene which were utilized were ineffective in blackfly control (18).

Kerosene was effective in destroying maggots of the latrine fly, *Chrysomyia megacephala*, which, in 1940, was a carrier of many intestinal diseases in China. Pure kerosene killed the larvae by suffocation. The applicability of this method was limited by the fact that the large volume of kerosene needed to completely cover the contents of a latrine was prohibitively costly. In addition, in areas where the latrine contents were to be used as manure fertilizer, kerosene would be undesirable. The best method of extermination of this disease-carrying insect was to pour ample quantities of boiling water upon the surface of the latrines (19).

C. FISH

Fuel oils and lubricating oils have contaminated freshwater and marine environments as a result of tanker accidents and industrial pollution. The results of these incidents include immediate fish kills as well as tainting of the fish with oily flavors or odors as fish incorporate the petroleum hydrocarbons in their flesh. Oil contamination of fisheries has lead to condemnation of catches as unfit for human consumption.

In this section of the report, field observations and laboratory studies are reviewed. As some toxic fuel oil and lubricating oil components in oil slicks are water soluble, most laboratory experiments involve mixing oil and water together before exposing fish to it. The way in which mixing is accomplished is one determinant of the quantity of oil which will dissolve in water, and thus, a determinant of the lethal dose of the oil-water mixture. Another complication which arises in comparing reported toxic concentrations is the fact that the amount of oil added to water has an unpredictable relationship to the amount of oil which dissolves or is otherwise accommodated in the water. In other words, the lethal doses reported as a volume of oil added to water cannot be compared with lethal doses reported as the concentration of dissolved oil in water. A more complete discussion of the behavior of fuel and lubricating oils in water is presented in the chapter on Environmental Fate.

1. FIELD STUDIES

A spill of approximately 19-23,000 liters of No. 2 fuel oil near a southwestern Virginia creek, of which about 13,000 liters entered the creek, caused the death of ducks, watersnakes, and fish. In the first

two days following the accident 33 dead fish were counted. Although no data on number of species had been collected prior to the spill, a reduction in the number of fish in the creek was reported for 44 days. The biological effects of the oil spill upon the creek were thought to be minimal (20).

2. EXPERIMENTAL STUDIES

In the studies reviewed in this section, the expressions LC_{50} and TL_m are used. LC_{50} is a constant value defined as the concentration of test material determined from a plot of concentration vs percent kill which is lethal to 50% of test organisms within a specified exposure period. TL_m is the median tolerance limit, defined as the concentration of test material at which just 50% of test organisms survive 24, 48, or 96 hours (21).

a. FRESHWATER FISH

For the bluegill sunfish, *Lepomis macrochirus*, a reported LC_{50} for kerosene was 3,000 ppm in 24 hours. The 100 test specimens were 5-11 cm long and weighed an average of 5 grams. Kerosene was mixed with water to reach saturation at 20°C; a thin oil film floated on the surface of the bioassay container. Oil was added to the container only at the beginning of the bioassay and its concentration at the end of the study could have been changed due to decomposition, evaporation, or other processes. Both water soluble and insoluble components of kerosene were present and could have influenced toxicity (21).

The acute toxicity of No. 2 fuel oil to Hudson River fish was investigated. Static bioassay tanks were used, and oil was added to test water in mg/l concentrations. Table 1 shows the results. The numbers of fish tested were not stated (22).

b. ESTUARINE AND MARINE FISH

The juvenile American shad, *Alosa sapidissima*, inhabits coastal rivers along the Atlantic shoreline, and is of commercial importance in the United States. Shad averaging 66 mm in length were bioassayed with diesel fuel. A measured amount (mg/l) of the oil was added to circulating aquarium water containing 10 shad per 114 liters of water. Aeration was designed to retain high dissolved oxygen levels of >6.0 mg/l. The 24-hr TL_m was 204 mg/l, the 48-hr TL_m was 167 mg/l. Even in diesel fuel concentrations as low as 84 mg/l, no fish survived for 96 hours (23). For kerosene and No. 2 fuel oil, 24-hr TL_m's of 200 mg/l have also been reported (24). Acute toxic effects including distress and loss of equilibrium, indicating central nervous system disturbances, occurred within 5 hours of exposure to diesel fuel. After an additional 5 hours, death occurred. Control shad did not experience distress or mortality (23).

In similar bioassays utilizing diesel fuel oil with reduced oxygenation of aquarium water, the initial dissolved oxygen content of 6.5 mg/l was reduced at a rate of 1.0 mg/l per 10 hours to 2.5 mg/l by respiratory activity of the fish. Then, 84 mg/l of diesel fuel were added, and the water was circulated. For the duration of testing, oxygen content was

TABLE 1. 24-HR AND 96-HR MEDIAN TOLERANCE LIMITS (TL_m) OF NO. 2 FUEL OIL IN HUDSON RIVER FISH

SPECIES	24-Hr TL_m (mg/1)	96-Hr TL_m (mg/1)
Fundulus diaphanus (banded killifish)	28.5	26.1
Roccus saxatitlis (striped bass)	30.6	22.2
Lepomis gibbosus (pumpkinseed)	42.6	39.2
Roccus americanus (white perch)	41.6	37.2
Anguilla rostrada (American eel)	28.0	31.0
Cyprinus carpio (carp)	52.5	49.1

TL_m - median tolerance limit

Ref: Rehwoldt et al., 1974 (22)

maintained at 2.0-3.0 mg/1. A control with 2.0-3.0 mg/1 oxygen but no oil was run concurrently. Distress and dispersion of fish occurred 120 minutes after the addition of the oil, and mortalities followed in 60-180 minutes. In controls, no deaths occurred for up to 5 hours. No delayed mortality resulted when these control fish were then kept in 6.0 mg/1 oxygenated water for 48 hours.

The conclusion reached was that the low oxygenation of the test water containing diesel fuel increased the toxicity of the oil for juvenile shad. In natural waters, a surface oil slick could retard air-water exchange of oxygen and thereby enhance the toxicity of the spilled oil (23).

Water soluble fractions of No. 2 fuel oil were bioassayed in various species of freshwater, marine and brackish water fish (25). The water soluble oil was prepared by stirring water and oil, in a ratio of 10:1, for 72 hours, allowing for separation, and removing the water phase for dilution and use in experiments. In some trials, the water soluble fraction was exposed to ultraviolet light from a 275 watt sunlamp located 1 meter above the oil during its preparation. Acute continuous-flow bioassay studies were conducted for 96 hours, and LC_{50} values are shown in Table 2.

It can be noted that the ultraviolet irradiated water soluble fraction of No. 2 fuel oil was much more acutely toxic than the non-irradiated water soluble oil fraction, which was almost without lethal effects on the fish species bioassayed.

Natural estuarine conditions were more closely simulated by floating 1 liter of No. 2 fuel oil on top of a tank containing 360 liters of fresh or brackish water with a 3 liter/hour flow rate. Two sun lamps provided ultraviolet radiation. At 24 and 72 hours, and 6 days, water was drawn off from under the oil slick and used for bioassay testing. Results are given in Table 3.

In identical conditions, but without ultraviolet radiation, 90-100% survival occurred in all fish in all oil slick samples (24-hr, 72-hr and 6-day-old oil slicks).

Chemical analysis of the 6-day-old oil slick water which was irradiated revealed 0.75 mg/1 of phenols, 2.7 mg/1 of aromatic hydrocarbons, and 4.3 mg/1 of aliphatic hydrocarbons. In 6-day samples which had not been irradiated with UV light, the concentrations were 0.23 mg/1, 0.35 mg/1 and 1.4 mg/1, respectively. The photochemical alterations which occurred in the water soluble oil fractions exposed to artificial sunlight probably caused the increases in toxicity as compared with non-irradiated water soluble oil fractions (25).

Anderson et al. (26) demonstrated that whole No. 2 fuel oil dispersed in water is less toxic to marine fish than the water soluble elements of that oil. Two methods of mixing oil with seawater were employed. In the first method, a measured volume of No. 2 fuel oil was

TABLE 2. 96-HR MEDIAN LETHAL CONCENTRATIONS (LC_{50}) AND EFFECTS OF IRRADIATED AND NONIRRADIATED DILUTIONS OF THE WATER SOLUBLE FRACTION OF NO. 2 FUEL OIL IN FRESHWATER AND MARINE FISH

SPECIES	96-hr LC_{50} in UV Irradiated Water Soluble Fraction	Effects after 96-hrs in Non-Irradiated Water Soluble Fraction
Fundulus heteroclitis (mummichog: brackish or salt water)	48 vol %	all fish survived at 100 vol %
Cyprinodon varigatus (sheephead minnow: brackish or salt water)	46 vol %	all fish survived at 100 vol %
Ictalurus punctatus (channel catfish: freshwater)	75 vol %	90 % survived at 100 vol %
Lepomis macrochirus (bluegill sunfish: freshwater)	40 vol %	80 % survived at 100 vol %

Ref: Scheier and Gominger, 1976 (25).

TABLE 3. TIME REQUIRED TO REACH 100% MORTALITY IN MARINE AND FRESH-WATER FISH EXPOSED TO WATER REMOVED FROM UNDER A NO. 2 FUEL OIL SLICK 24 HOURS, 72 HOURS, AND 6 DAYS AFTER ULTRAVIOLET IRRADIATION

SPECIES	Time (minutes)		
	24-hr Slick	72-hr Slick	6-Day Slick
F. heteroclitis	295	100	80
C. varigatus	315	110	45
I. punctatus	55	30	25
L. macrochirus	105	125	95

Ref: Scheier and Gominger, 1976 (25).

added to a specific volume of artificial seawater, followed by vigorous shaking for 5 min on a shaker platform. The resulting oil in water dispersion was allowed to separate for 30-60 min, and then the fish were inserted through the oily layer and into the aqueous phase. In the second method, 1 part of No. 2 fuel oil was added to 9 parts of artificial seawater (10% mixture of oil on water) and gently stirred for 20 hours while covered. The two phases were then allowed to separate, and the water phase was siphoned off. The water containing dissolved oil was then diluted with seawater to the desired concentration for the bioassays. The 3 fish species tested were sheephead minnows *(Cyprinodon variegatus)*, silversides *(Minedia beryllina)*, and *Fundulus similus*. At least 10 fish of each species were exposed to each oil concentration utilized. The results were two sets of TL_m values shown in Table 4.

The TL_m oil concentrations for the whole oil represent the quantity of oil added to seawater according to method one. The concentrations for water soluble oil were determined directly by infrared analysis of the aqueous phase.

The concentration of No. 2 fuel oil that dissolved in seawater (10% mixture) was 8.7 mg/l after 20 hours. The relationship between stirring time and dissolved oil, using method two, was described by a sharply increasing oil concentration for the first 10 hours of gentle mixing, followed by a linear increase at a lower slope for the next 19 hours. After 29 hours, bacterial contamination was noted in the aqueous phase and stirring was discontinued. At that point, the concentration of dissolved oil was roughly 10 mg/l.

The concentration of No. 2 fuel oil dispersed in the aqueous phase, when method one was employed, increased linearly when 0.002 to 0.1% of oil were added (shaken vigorously and allowed to settle for 1 hour). When 0.1% of No. 2 fuel oil was added to 20% saline artificial seawater (total volume of 500 ml) the maximum concentration of total petroleum hydrocarbons in the aqueous phase after shaking and separation was 51.8 mg/l. Lower aqueous phase oil concentrations were obtained if more than 0.1% of oil was added, due to coalescence of oil droplets and return to the oil-water interface at a rate which exceeded the increase of oil droplets entering the aqueous phase. The water phase hydrocarbon content of the dispersions ranged from 14-52 mg/l when 0.002-0.1% of oil were added, respectively.

The results of the bioassay in the three fish species indicate that the dissolved oil was more toxic than the whole oil dispersion. The hydrocarbon composition of No. 2 fuel oil water soluble fraction, prior to dilution in method two, included 0.54 mg/l of saturates and 5.74 mg/l of aromatics, with high concentrations of naphthalene and alkylnaphthalenes and low concentrations of C_{12}-C_{24} n-paraffins, indicating that aromatic hydrocarbons were more soluble than paraffins. The oil in water dispersions, on the other hand, had hydrocarbon

TABLE 4. MEDIAN TOLERANCE LIMITS (TL_m) OF NO. 2 FUEL OIL IN MARINE FISH

SPECIES	TL_m (mg/1)[a]					
	Method I - Whole Oil Dispersion[b]			Method II - Water Soluble Fraction[c]		
	24-hr	48-hr	96-hr	24-hr	48-hr	96-hr
C. variegatus	250	200	93	>6.9[d]	>6.9[d]	6.3
M. beryllina	260	125	NA	5.7	5.2	3.9
F. similus	48	36	33	5.6	4.7	3.9

NA = Not Available

[a]Data points analyzed by the graphical method of Litchfield and Wilcoxon

[b]Concentration expressed as the amounts of oil added to the water and does not represent the amount actually present in the aqueous phase

[c]Concentration expressed as total hydrocarbons actually present in the aqueous phase as determined by infrared analysis

[d]Data points could not be analyzed statistically by the Litchfield and Wilcoxon method due to their distribution. These values were computed by the Standard American Public Health Assoc. (A.P.H.A.) methods.

Ref: Anderson et al. (26).

compositions similar to the original No. 2 fuel oil, which was approximately 40% aromatic and 60% paraffinic. When the dispersions were gently aerated for 24 hours, a loss of 80% of the hydrocarbon content occurred. A slightly higher percentage of n-paraffins than aromatic hydrocarbons was lost (26).

D. WORMS

The toxicity of No. 2 fuel oil to soil nematodes is reviewed. Bioassays in marine worms exposed to water soluble components of No. 2 fuel oil are also presented in this section. As mentioned earlier in this report, No. 2 fuel oil is chemically and physically similar to diesel fuel as well as SGF No. 1 fog oil. The term TL_m is defined as the concentration of test material at which just 50% of test organisms survive for a specified period of exposure.

1. SOIL NEMATODES

The toxicity of No. 2 fuel oil or products of its degradation in soil to nematodes was apparent more than one year after its application to various soils (27). The fuel oil was applied at a rate of 2.5 kg/m^2 to 1.7 x 3.0 m level plots of silt loam, sandy loam and black clay loam which had not previously been oil-contaminated. The soils were tilled to a depth of 15 cm, and oil was readily absorbed into the soils. Worms were counted 6 months and 12 months following oil application, and compared with unoiled control soil worm counts. After 6 months, there were 200 nematodes/250 ml of treated soil as compared to 1200 nematodes/250 ml soil in control plots. By 12 months, counts were 1380 and 2880 nematodes/250 ml soil in treated and control plots, respectively.

2. MARINE ANNELIDS

Rossi, Anderson and Ward studied effects of exposing the marine worms, *Neanthes arenaceodentata* and *Capitella capitata*, to solutions of No. 2 fuel oil in seawater (28-31).

Bioassays utilizing young adult worms of both species were performed with No. 2 fuel oil solutions prepared by slowly stirring 9 parts of 32% saline artificial seawater with one part of oil for 20 hr. The aqueous phase which resulted was designated the 100% water soluble fraction of oil (WSF), and was diluted with artificial seawater to the desired concentrations. Ten bioassay flasks, each containing one worm and 50 ml of WSF, were used at each concentration. The flasks were temperature-maintained but not aerated. Resulting TL_m concentrations are given in Table 5.

TABLE 5. 24-, 48-, AND 72-HOUR MEDIAN TOLERANCE LIMITS (TL_m) OF DILUTIONS OF THE WATER SOLUBLE FRACTION OF NO. 2 FUEL OIL IN MARINE WORMS.

SPECIES	TL_m (mg/1)*		
	24-hr	48-hr	96-hr
N. arenaceodentata	> 8.7 (100%)	3.2 (37%)	2.7 (31%)
C. capitata	> 8.7 (100%)	3.5 (42%)	2.5 (56%)

*TL_m values represent mg/1 of the total hydrocarbons calculated from the amount present in the 100% water soluble fraction. Values in parentheses are the equivalent percentages.

Ref: Rossi et al., 1976 (28).

In control trials, no mortality was observed. The concentrations of total hydrocarbons were calculated from the amount present in the 100% WSF, which was analyzed by infrared and gas chromatographic techniques. Chemical analyses of the bioassay solutions revealed initial naphthalene* concentrations of about 2.5 mg/1, but over the course of 24 hours, the level decreased to about 0.5 mg/1, and to 0.18 mg/1 by 96 hours. The naphthalenes were reported to contribute heavily to the toxicity of the fuel oil solutions. Bioassays conducted using 100% WSF which had been previously aerated for 4 hours resulted in a reduction in mortality, which may have been due to the evaporation of these more volatile toxic hydrocarbons from the solution (28).

Bioassays were also performed on the 4, 18, 32, and 40 segment juveniles and 60 segment adult marine worms, *Neanthes arenaceodentata*. The test conditions and preparation of WSF fractions of No. 2 fuel oil were the same as above; 10 animals were used in each bioassay.

The acute toxicity of the water soluble fractions of No. 2 fuel oil increased as worms grew older and larger, with the greatest differences in sensitivity to the oil occurring between the 9-day-olds (4 segments) and the adults; the exception being that gravid females were comparable to 30-day-old juveniles (32 segments) in sensitivity to the oil. Bioassays on immature adult worms failed to show sex related differences in sensitivity (29).

Rossi and Anderson postulated that the observed tolerances of juveniles and gravid females to No. 2 fuel oil are due to the presence of yolk matter, which sequesters the oil hydrocarbons in a manner similar to that for chlorinated hydrocarbons. When the juveniles begin to rely on external food sources at around 20 days of age, the yolk no longer is functional, and toxic hydrocarbons, such as the naphthalenes, can affect other body tissues (29). Results are summarized in Table 6.

*also included methyl- and dimethyl-substituted naphthalenes.

TABLE 6. 24-, 48-, AND 96-HOUR MEDIAN TOLERANCE LIMITS (TL_m) OF DILUTIONS OF THE WATER SOLUBLE FRACTION OF NO. 2 FUEL OIL IN MARINE WORMS BY AGE.

AGE	TL_m (mg/1)*		
	24-hr	48-hr	96-hr
4-segment juveniles	> 8.7 (100%)	> 8.7 (100%)	8.4 (90%)
18-segment juveniles	"	> 8.7 (100%)	5.7 (66%)
32-segment juveniles	"	7.8 (90%)	5.4 (62%)
40-segment juveniles	"	6.2 (71%)	4.0 (46%)
48-segment immature adults	"	3.2 (37%)	2.7 (31%)
60-segment mature males	"	3.0 (35%)	2.6 (30%)
60-segment gravid females	"	5.6 (69%)	4.2 (48%)

*TL_m values represent mg/1 of total hydrocarbons calculated from the amount present in a 100% water soluble fraction. Values in parentheses are the equivalent percentages.

Ref: Rossi et al., 1976 (29).

When male and gravid female *Neanthes arenaceodentata* were exposed for 24 hr to a 25% dilution of the previously described water soluble extract of No. 2 fuel oil, and then placed in clean seawater for varying intervals before sacrifice and determination of petroleum hydrocarbons by ultraviolet spectrometry, naphthalenes were again found to accumulate rapidly in both sexes. Males steadily released the accumulated naphthalenes when placed in clean seawater; by 72 hours, the majority of naphthalenes had been released. Complete depuration (less than 0.1 ppm naphthalenes remaining in body tissues) was evident in 400 hr. Gravid females failed to steadily release the accumulated hydrocarbons in clean seawater. Levels remained high until spawning. Immediately after egg release, tissue naphthalene concentrations in 24 worms were just detectable (0.2 ppm). Zygotes and trochophore larvae contained 18 ppm of total naphthalenes (two hundred were analyzed). In 18-segment juveniles (21 days after spawning) there was 0.4 ppm of naphthalenes. In 32-segment worms (26 days after spawning) naphthalenes were not detected. The high diaromatic hydrocarbon levels in offspring of the oil-exposed gravid female worms were probably the result of compartmentalization of the hydrocarbons into yolk matter and lipid fractions of the polychaete eggs. Transfer of the oil from one generation to the next did not occur; the naphthalenes were released completely by juveniles during utilization of the yolk material during development (30).

In another series of experiments on *Neanthes arenaceodentata*, Rossi and Anderson (31) found that exposures to 30% and 40% WSF concentrations of No. 2 fuel oil (prepared as in their previous work) did not impair the worms' ability to increase hemoglobin content in response to hypoxia. Oxygen levels utilized were 6.9 and 2.0 mg/l of dissolved oxygen. Mortality was measured over an 11-day period, using 20 worms in each test. The 2.0 mg/l dissolved O_2 level in non-oily seawater produced no mortality in 11 days. Survival was greater than 80% in worms exposed to the 40% WSF under conditions of oxygen saturation (6.9 mg/l) for 96 hr. Reducing the oxygen level to 2 mg/l by nitrogen gassing did not alter the toxicity of the 40% WSF solution in the worms. Rates of hemoglobin compensation (Δ µg/mg of hemoglobin) were approximately 4-4.5 for all experiments when oxygen was reduced from 6.9 to 2 mg/l. In a comparable study by Tagatz (23), it was concluded that in the juvenile American shad, lowering the oxygenation of diluted diesel fuel bioassay solutions created an additive toxic effect. In natural waters, where an oil slick might retard air-water exchange of oxygen, the oil toxicity would be enhanced. Rossi and Anderson (31) stated that one reason for the apparent contrast in results between the annelid and the fish was the shad's natural hypoxia tolerance, which was lower than that of the marine worm. (The details of the shad experiments are presented under Fish).

E. MOLLUSKS

Pollution of the oceans with petroleum oils has caused commercially valuable shellfish populations to perish or become oil contaminated and inedible. Death, behavioral changes, neoplastic growths and other pathological findings have been reported in mollusks exposed to fuel-oils in their natural habitats or in the laboratory.

This section presents studies of the effects of petroleum products similar to the fog oils and diesel fuel on snails and bivalues including oysters, scallops, mussels and clams.

1. SCALLOPS

According to a brief news report (32) scallops may be the most sensitive of all mollusks to petroleum oils. Although no experimental methodology was given, it was stated that 12.5 mg/l of No. 2 fuel oil in sea water was lethal to 100% of test scallops within 24 hours. This value may be meaningless in the light of recent data concerning the effect of the method of preparing oil in water mixtures upon the final oil concentration, as well as the fact that static bioassays and continuous flow bioassays yield different toxicity thresholds (26 , 33).

2. CLAMS

Eggs and larvae of the quahaug clam, *Mercenaria* sp., were exposed to No. 2 fuel oil water soluble fractions in order to establish acutely toxic concentrations. The oil was added to 27% saline seawater in a ratio of 1:8 by volume, and the mixture was agitated at 200 rpm on a gyratory shaker for 12 hours. After a 24-hour equilibration interval, the aqueous phase was removed and used either as a concentrate (100%) or in dilutions with seawater to 50%, 25%, 10%, 5% and 1%. Concentrations of oil in the seawater were determined gravimetrically (34).

Shellfish eggs at the second cleavage stage (300 embryos in 10 ml of solution) were exposed for 48 hours to the various dilutions and then stained with neutral red to distinguish living from dead larvae. The 48-hr LC_{50} was 0.43 mg/l. Two-day-old larvae were exposed for 2, 6, or 10 days and the LC_{50} values which resulted were 1.3, 1.3, and 0.53 mg/l of No. 2 fuel oil, respectively.

The growth rate of oil-exposed larvae which survived was slower than that for unexposed organisms. Embryos stopped dividing after 3 hours of exposure to 11.5 mg/l of No. 2 fuel oil in seawater, and structural integrity was lost soon after (34).

Young adult softshell clams, *Mya arenaria* L., were exposed to No. 2 fuel oil in water emulsions in order to study acute mortality as well as morphological abnormalities resulting from the oil exposure. Oil in water emulsions were prepared ultrasonically in 20,000 mg/l emulsions which were then diluted for the bioassays. The No. 2 fuel oil was characterized as 14% aromatic and 86% nonaromatic hydrocarbons. Clams were tested at 40°C and 14°C.

Groups of 15 clams each, with a mean shell length of 25 mm or less, were used in each test. In all trials, the clams exhibited a characteristic response to the oil. At low concentrations (50 mg/l) mucus was excreted through the pedal opening and siphon; higher concentrations caused increased mucus secretions. Muscular contraction became depressed, and decreased irritability and contractility of the siphon were noted. At concentrations above 100 mg/l, the pedal

opening musculature became totally relaxed and adductors lost the ability to contract rapidly. Above 400 mg/l of oil in water, adductor relaxation occurred after 15-20 sec of exposure. The intensity of muscle relaxation varied proportionally with concentration and exposure time. Mucus secretion and muscular narcotization to tactile stimuli were enhanced in trials conducted at 14°C but not at 4°C. There was insufficient mortality in tests at 4°C with as high as 1600 mg/l oil emulsions for 96 hours to calculate LC_{50} values. At 14°C, a 96-hr LC_{50} of 505 mg/l and a 7-day LC_{50} of less than 100 mg/l were reported. This demonstrated the importance of temperature upon lethal threshold response (35).

Using the same methodology, young adult clams were exposed to 10, 50 or 100 mg/l of No. 2 fuel oil in water emulsions under continuous aeration at a temperature of 40°C for 28 days. Exposed clams and unexposed controls were fixed in Davidson's fixative or cold 10% acetate buffered formalin, followed by refrigeration and preparation of sections with special stains for mucosubstances and glycogen.

Anomalies in general morphology most frequent in clams exposed to 100 mg/l of the oil emulsion included: edematous pallial muscle, leukocytosis in pallial blood sinuses, subepithelial bands of leukocytes underlying the mantle, edema and leukocytic infiltration of the anterior adductor muscle, abnormal vacuolation of the style sac, intestine and diverticula, and reduced chromatophilia of the top of the gill filaments. Exposures to 50 mg/l caused less marked morphologic changes; however, diverticula were shrunken with abnormal epithelium, and there was moderate leukocytosis of pallial blood sinuses. Clams exposed to 10 mg/l exhibited reductions in size of diverticula, vacuolated appearance of the diverticula, stomach and intestine, and occasional leukocytosis of pallial blood sinuses.

Visceral sections of both control and exposed clams stained with azure A/eosin B to demonstrate necrotic tissue did not reveal any histologic differences. Mucosubstance staining also revealed no changes in oil-exposed clams except for a decrease in members of mucoid cells along the intestines of a few clams exposed to 100 mg/l and 50 mg/l emulsions. At these exposure levels, glycogen depletion was noted in the cells of the digestive diverticula, intestine, stomach mucosa and gill filament tips. Most clams exposed to 10 mg/l of oil in water emulsions showed almost normal-appearing glycogen deposits, with some vacuolization of diverticular and intestinal cells (36).

From these studies, it was concluded that exposure to No. 2 fuel oil in water emulsions did not promote radical tissue changes in soft shell clams at the short exposure time and oil concentrations used. Although oil in water emulsions ranged from 10-100 mg/l at the start of the experiments, hydrocarbon content had decreased to 0.29-1.52 mg/l during the last 3 weeks of tests (36).

In contrast with the above findings, a high incidence of gonadal tumors was reported in oil-exposed soft shell clams (Laroche, 1972; Barry and Yevich, 1975), and Barry et al. (1971) reported a high incidence of gill and kidney hyperplasias in clams taken from oil polluted waters (36). Barry and Yevich (1975) examined clams which had survived in an oil spill area off the Maine seacoast for 5 months (37). The spill was described as a minimum of 14 metric tons of a mixture of No. 2 fuel oil and JP-5 jet fuel (a kerosene-like oil). Soft clam mortality had exceeded 85% of an estimated 50 million market-size clams. The histopathologic finding in live clams exposed for 5 months was neoplasia, possibly of germ cell origin, in male and female gonadal tissues. Mitotic figures, pleomorphic mononucleated cells and multinucleated giant cells were noted. In some cases, the neoplastic growth completely replaced normal gonadal tissue, invading the interfollicular connective tissue and destroying the normal follicular architecture. Metastases to gills, genital and urinary pores, kidney, pericardium, and red gland were noted. Clams collected from unpolluted areas showed no indications of tumors. The highest incidence of tumors correlated with the major area of the oil spill. Laboratory investigations are presently underway to determine cause-effect relationships involved (37).

3. OYSTERS AND MUSSELS

Experiments on larvae, gametes and developing eggs of *Crassostea* (oysters) and *Mytilus* (mussels) indicate they are sensitive to petroleum oils. No. 1 fuel oil dispersion in a concentration of 1000 mg/l reduced the average number of larvae developing from oyster and mussel eggs from 85 (in control vessels) to 75 within 7 hours. A 6-hr LC_{50} for 7-hr-old larvae was 10,000 mg/l or more of the No. 1 fuel oil dispersion. Observations on immature stages exposed to oil mixed with a dispersant included reduced fertilization capacity of sperm, reductions in swimming activity of larvae and reduced survival rates of eggs and larvae, but whether these effects were due to the petroleum, to the dispersant, or to both was not tested (38).

Bioassays were reported for oysters, *Crassostrea virginica*, exposed to No. 2 fuel oil in seawater (39). The oil dispersions were prepared by mixing 0.001, 0.005, 0.01, 0.05, 0.1, 1.0, 10.0, or 50% oil with artificial seawater for 5 min on a shaker platform at 200 cycles per minute. The authors report, however, that as the amount of oil added was increased, a corresponding rise in total hydrocarbon content in the water phase, did not occur as would be expected. This is attributed to increased droplet coalescence at very high oil concentrations. At 0.1% No. 2 fuel oil, a maximum concentration of 51 mg/l total hydrocarbons was reached, but the addition of more oil gave lower concentrations: 47 mg/l at 1%; 37 mg/l at 10%. Oysters (shell length, 40-90 mm), collected from reefs in Galveston Bay, Texas, were added to the dispersions one hour after mixing; dispersions were gently aerated. Reliable toxicity data were difficult to obtain because oysters ceased pumping, closed their valves, and were able to rapidly depurate the oil-water mixtures. In addition, the dispersions were unstable: hydrocarbon concentrations in the aqueous phase decreased rapidly upon aeration; only 10% of the original concentrations

remaining after 24 hours. Concentrations were also influenced by microorganisms present in the bioassay containers. Percent mortality of oysters after 96 hours of exposure to various concentrations of No. 2 fuel oil dispersions is presented in Table 7. For oysters exposed to 1% dispersions, the first mortalities occurred after 5 days, 50% died after 8 days, and 100% mortality was observed after 14 days (39).

4. SNAILS

Two intertidal gastropods, *Littorina planaxis* and *Littorina scutulata*, abundant species along the southern California coast, were exposed to kerosene characterized as containing 60% n-paraffins in the C_9-C_{16} range. In each experiment 25 animals were placed in pinbowls containing artificial seawater for 24 hours, and then 60 ml of kerosene was added by pouring it over the crawling snails. After 6 more hours, the animals were replaced in bowls of seawater. Mortality was not observed, except in *L. scutulata* held at elevated temperatures (29°C) both with and without kerosene exposure. The behavior of attachment to substrate was observed. The initial time it took for 50% of snails to attach to the substrate after the 6-hr kerosene exposure (TA_{50}) was about 1 hour. The same duration was recorded for unexposed controls. The initial time when 50% of organisms were unattached (TUA_{50}) varied with the species, the location from which it was taken, and the temperature, but in general, rates of attachment fell below 50% after kerosene exposure only slightly more often than in controls. Other behavioral changes were slight. In most cases the snails retracted when exposed to kerosene. At 17°C, the temperature of their natural environment, the kerosene had little effect on crawling activity. There was no obvious correlation between snails previously exposed to petroleum in their natural habitat, and snails from nearly oil-free environments, with respect to effects of kerosene on crawling and attachment rates, as long as the temperatures of their natural environment were maintained (40).

Seawater soluble kerosene fractions were found to alter the attraction of snails to food extracts. Marine prosobranch snails, *Nassarius obsoletus*, were collected and kept without food for 10 days. They were then placed (10 snails per trial) in a plastic Y-chamber with one arm of the Y containing flowing seawater and the other arm containing seawater pre-mixed with either stimulus solutions of food attractant, food attractant plus kerosene solution, kerosene solution alone, or seawater alone. The kerosene solutions were prepared by slowly stirring 25 ml of the oil over 1.5 liters of seawater for 12 hr, followed by separation of the aqueous phase. Analyses showed the solubles to be alkylbenzenes and naphthalenes at calculated concentrations of 610 µg/l of seawater. Extracts of oysters, 0.33 mg/l, and scallops, 3 mg/l, were used for food attractant solutions. Kerosene solutions were used in concentrations of 1 µg/l or 4 µg/l.

There was a reduction in attraction of snails to the food attractant in the presence of kerosene as compared with food attractant alone; this effect was statistically significant ($p<0.05$) with kerosene concentrations of 4 µg/l but not 1 µg/l.

TABLE 7. PERCENT MORTALITY OF OYSTERS EXPOSED TO DISPERSIONS OF NO. 2 FUEL OIL*

% oil added	0.005%	0.01%	0.05%	0.1%	1%	10%	50%
% mortality	10%	10%	10%	50%	40%	50%	20%

*As the amount of oil added was increased, a corresponding rise in total hydrocarbon content in the water phase did not occur. This would explain why the percent mortality was roughly the same from 0.1 to 10%. The decrease in mortality at 50% was attributed to a decreased hydrocarbon content.

Ref: Anderson and Anderson, 1976 (39).

The fact that food finding behavior, which is chemically mediated, was influenced by minute concentrations of kerosene hydrocarbons dissolved in seawater, suggests that other snail behaviors which are dependent upon chemical cues, such as reproduction and orientation, could similarly be disrupted by dissolved petroleum hydrocarbons in µg/l concentrations (41).

F. MARINE CRUSTACEANS

Bioassays are reviewed in which adult shrimp, larvae and mysids were exposed to seawater contaminated by No. 2 fuel oil. Amphipods have been bioassayed with fuel oil and diesel fuel. Copepod survival in diesel fuel, kerosene and No. 2 fuel oil is also reviewed. LD_{50} and LD_{100} values are defined as concentrations of the test substance which are lethal to 50% and 100% of organisms tested within a specified exposure time. LC_{50} was defined in the section entitled "FISH". LD_{50} and LC_{50} data are interchangeable for purposes of this literature review. TL_m is the concentration of test substance in which just 50% of test organisms survive in a given exposure period.

1. SHRIMP AND MYSIDS

The toxicity of a No. 2 fuel oil in grass shrimp *(Palaemonetes pugio)*, mysids *(Mysidopsis almyra)*, and brown shrimp postlarvae *(Penaeus aztecus)*, estuarine crustaceans from the central Texas coast, was studied in two ways. TL_m values for whole oil dispersed in artificial seawater, and for the water soluble fraction of the oil were compared (26).

The oil water dispersions were prepared by adding a specific volume of No. 2 fuel oil to artificial seawater, shaking vigorously for 5 minutes on a shaker platform, allowing a settling period of 30-60 minutes, and inserting the crustaceans through the oily layer into the aqueous layer. The three crustaceans were bioassayed, using at least 10 animals for each concentration tested. Resulting TL_m values, in mg/l of No. 2 fuel oil added, are presented in Table 8.

Water soluble fractions of No. 2 fuel oil were also utilized in bioassays on the same three species. The water soluble oil was prepared by gently stirring one part of oil and nine parts of water for 20 hours, allowing the two phases to separate, and siphoning off the aqueous phase, containing water soluble oil, diluting it and using the solution in the bioassays. The TL_m values, in mg/l of dissolved oil components, are presented in Table 8.

The toxicity of the whole oil dispersion was less than that of the fraction of No. 2 fuel oil which dissolved in artificial seawater. In both bioassays, the immature postlarval shrimp were tolerant to greater concentrations of the oil than the adult crustaceans tested.

The differences in the toxicity between oil in water dispersions and dissolved oil components are due to differences in hydrocarbon content and oil behavior in the artificial seawater. The water soluble fraction of No. 2 fuel oil was richer in aromatic hydrocarbons than the dispersions, which contained the same proportions of aromatic and paraffinic (approximately 60% and 40%) hydrocarbons as the original

TABLE 8. MEDIAN TOLERANCE LIMITS (TL_m) OF DISPERIONS AND SOLUTIONS OF NO. 2 FUEL OIL IN SHRIMP AND MYSIDS

SPECIES	TL_m-Dispersions (mg/l)			TL_m-Solutions (mg/l)		
	24-hr	48-hr	96-hr	24-hr	48-hr	96-hr
Palaemonetes pugio (grass shrimp)	3.8	3.4	3.0	4.4	4.1	3.5
Mysidopsis almyra (mysid)	1.6	1.3	NA	2.6	0.9	NA
Penaeus aztecus (brown shrimp postlarvae)	9.4	9.4	9.4	5.0	5.0	5.0

NA = Not Available

Ref: Anderson et al., 1974 (26)

No. 2 fuel oil (26). A more detailed treatment of this subject follows both the discussion of identical bioassay studies in Fish, and the Environmental Fate section on Dispersion.

Another study reported LC_{50} values for grass shrimp, *Paleomonetes pugio,* exposed to water soluble fractions of No. 2 fuel oil. The solutions were prepared by stirring 10 parts of natural water with one part of this oil for 72 hours, followed by separation of the water phase and dilution. In some cases, oil solutions were prepared under ultraviolet light, by placing a 275 watt sunlamp 1 meter above the oil during stirring. In continuous-flow bioassay studies, 96-hr LC_{50} values for *P. pugio* were 34.4 vol % dilution of the dissolved No. 2 fuel oil solution which had been irradiated, and 100 vol % (undiluted) of the nonirradiated water soluble oil. The grass shrimp were much more sensitive to irradiated than nonirradiated solutions of No. 2 fuel oil (25).

A more accurate simulation of natural estuarine conditions was developed in which 1 liter of No. 2 fuel oil was floated over a tank with 360 liters of brackish water flowing at 3 liters/hour. Two sunlamps provided ultraviolet irradiation. At 24 hr, 72 hr, and 6 days, water was drawn off and utilized in bioassays with *P. pugio*. Nonirradiated solutions prepared in an identical manner were also tested. The resultant mortality data are presented in Table 9.

Analysis of the 6-day old vat water of the UV irradiated oil slick revealed 0.75 mg/l phenols, 2.7 mg/l aromatic hydrocarbons, and 4.3 mg/l aliphatic hydrocarbons. In the 6-day-old nonirradiated oil slick vat water, the concentrations of these chemicals were 0.23 mg/l, 0.35 mg/l, and 1.4 mg/l, respectively. Photochemical alterations which occurred during ultraviolet irradiation of the oil slicks were probably responsible for the observed increase in mortality of the grass shrimp (25). Corresponding results are presented in the section on fish.

Bioassays of dispersions of No. 2 fuel oil, in a continuous flow system, utilized the coon stripe shrimp, *Pandalus danae*. The oil in water dispersions were prepared by vigorous agitation of 2.4 ml/min of No. 2 fuel oil and 15 l/min seawater, followed by removal of the aqueous phase and dilution of it with seawater. Mean waterborne oil concentrations of 0.8 mg/l were lethal to 50% of shrimp in 96 hours (LC_{50}). The 72-hr LC_{50} was 1.3 mg/l. The LC_{50} values in these continuous flow experiments were stated to be more accurate than values reported in static bioassay systems, in which hydrocarbon loss may influence the apparent lethal thresholds (42).

The same authors also studied shrimp mortality when three different methods of No. 2 fuel oil-seawater contact were utilized.

In the first method, designed to eliminate surface turbulence, oil was introduced into 75 liter test chambers below the surface of the water at a point 4 cm from the water entry port. Measured total oil concentrations in tanks sampled 1/2, 1, 3, 6 1/2 and 24 hours after additions of oil were all less than 1 mg/l. Selected samples for soluble components revealed concentrations of 0.01 mg/l for alkylbenzenes and other aromatics as measured by gas chromatography.

TABLE 9. PERCENT SURVIVAL AND SURVIVAL TIME OF GRASS SHRIMP, *PALAEMONETES PUGIO* IN NO. 2 FUEL OIL SLICKS

	% Survival and Survival Time		
	24-hr Slick	48-hr Slick	96-hr Slick
Water from UV-Irradiated No. 2 Fuel Oil Slick	50% - 190 min 0% - 340 min	50% - 60 min 0% - 85 min	50% - 50 min 0% - 70 min
Water from Nonirradiated No. 2 Fuel Oil Slick	100% at 100 vol % for 96 hr.	100% at 100 vol % for 96 hr.	100% at 100 vol % for 96 hr.

Ref: Scheier and Gominger, 1976 (25).

In the second method, No. 2 fuel oil and seawater were introduced into test chambers via static mixers placed 1 cm above the water surface, in order to provide moderate but uniform oil-water mixing prior to delivery. The measured fuel oil concentrations which resulted were 3.16 mg/l one hour following introduction of 5 ml of oil, and 1.57 mg/l 24 hours after addition of 100 ml of fuel oil into the test chamber. Concentrations of individual dissolved fuel oil hydrocarbons were below the lower limits of gas chromatographic detection.

The third method was designed to provide enhanced turbulence and dispersion of oil droplets throughout the experiment. Seawater fell from a height of 20 cm above the tank water surface, while oil was introduced by pouring at the perimeter of the turbulent zone created by the falling water. The oil concentration measured 24 hours after the additional of 100 ml of No. 2 fuel oil was 8.29 mg/l. Soluble oil hydrocarbons were detected in samples (42).

Bioassays on coon stripe shrimp, *Pandalus danae*, 5-8 cm in length, utilized 10 animals in each test chamber. There was no shrimp mortality or behavioral response within 24 hours (2 trials) when the organisms were exposed to the non-turbulent oil-water mixture from method one. Using method two, shrimp mortality did not occur at any concentration in either of 2 trials, but agitation of the shrimp was noted during the first hour of exposure after addition of 25, 50 or 100 ml of No. 2 fuel oil (the highest oil volumes introduced). Six trials were made using the third method. Shrimp mortality occurred after introduction of 50 or 100 ml of oil in every trial, but no deaths were noted among controls or after introduction of 5 ml of the fuel oil.

The degree of dispersion of the oil in oil-seawater mixtures is a determinant of both the oil concentrations reached and the dissolution of water soluble petroleum hydrocarbons. Minimal oil-water disturbance resulted in no detectable fuel oil levels in the water columns. Initial mixing with minimal surface disturbance yielded detectable amounts of the fuel oil in the water column, although the correlation was poor ($r = 0.81$) between the volume of oil introduced and the average oil concentration measured in the water column, and soluble oil was undetectable. Where mixing occurred during introduction of oil, and surface turbulence was maintained throughout the test periods, water column oil concentrations were linearly related ($r = 0.99$) to the volume of oil added, and shrimp mortality correlated well ($r = 0.99$) with the measured total oil levels (33).

Depuration (defined as a purge of aqueous phase fuel from the oil-water system) of No. 2 fuel oil from the system was studied by close time interval sampling after 50-ml oil introductions. A logarithmic curve of depuration occurred when mixing was continuous. Initial oil concentrations were approximately 4 mg/l, falling to 3 mg/l after 5-12 hours, and then to 1 mg/l after 24 hours. Vanderhorst et al. concluded that, "The dependence of water column concentration on the method of contact is so great that data presented in terms of oil-seawater ratios alone preclude meaningful discussion of toxicity" (33); other groups agree (24,26).

2. AMPHIPODS

Bioassays in Baltic amphipods, *Gamarus oceanicus*, employed both adults (6-15 months old; 10-25 mm long) and juveniles (4-6 days old; approximately 1 mm long) which were exposed to mixtures of seawater and No. 1 fuel oil. The oil was mixed with seawater, shaken vigorously for 2 minutes, and allowed to settle for 1 hour; 25 organisms were then exposed to concentrations ranging from 0 to 3000 µl/l, at a constant temperature of 13°C, for 48 hours. Animals were subsequently placed in non-oily seawater for a 72-hour recovery period. The 48-hour LC_{50} values were 173 µl/l of No. 1 fuel oil in seawater for adults and 0.3 µl/l for juveniles, indicating an approximate 600-fold difference in sensitivity between adults and juveniles.

In another experiment, 25 adults were exposed to 5 µl/l of No. 1 fuel oil for 2 months, under conditions of gentle aeration, and a constant temperature of 8.5°C. The solutions, renewed every third day, were prepared as described for the acute bioassay. All animals died by day 50; in the 25 controls, only one death was noted, on day 24. Almost immediately after exposing the adult amphipods to the oil solution, impaired swimming was evident. Later, swimming stopped and the animals crawled on the bottom, becoming sluggish and dying. Renewal of the test medium enhanced the mortality and behavioral disturbances. There was a significantly higher population ($p<0.001$) of organisms in the lighted half of test containers than in the lighted half of control containers. Control animals favored the dark section of their container. Precopulation behavior was abnormal and occurred much less frequently in oil-exposed amphipods than in controls. In control containers, 103 hatched larvae were counted, whereas 7 were counted in test containers. The dead larvae of the oil-exposed amphipods were often stuck to the oil film at the surface of the container (43).

Atlas (44) investigated the effects of diesel fuel oil slicks on the amphipod, *Boeckosimus affinis*. Sixty animals were placed in each test container of 5 liter of water to which 5 ml of this oil was added. In half of the test containers, a nylon mesh screen was placed beneath the oil slick to prevent the amphipods from physically entering the slick. The time required to kill 50% and 100% of the organisms (LD_{50} and LC_{100}, respectively), are reported in Table 10.

TABLE 10. TIME REQUIRED TO REACH LD_{50} and LD_{100} IN AMPHIPODS EXPOSED TO A DIESEL FUEL OIL SLICK

		Time to LD_{50} (Days)	Time to LD_{100} (Days)
Diesel Fuel:	screened	13	15
	unscreened	1	2

Ref: Atlas, 1975 (44).

The obvious conclusion is that amphipods which were not permitted to freely enter the oil slick lived longer than unscreened ones. In a similar procedure, amphipods were exposed to a 4 ml oil slick of a paraffinic fraction or a 2 ml oil slick of an aromatic fraction of fuel oil distilled from Prudhoe crude oil. The results appear in Table 11.

TABLE 11. TIME REQUIRED TO REACH LD_{50} AND LD_{100} IN AMPHIPODS EXPOSED TO AROMATIC OR PARAFFINIC FRACTIONS OF A FUEL OIL SLICK

		Time to LD_{50} (Days)	Time to LD_{100} (Days)
Paraffinic:	screened	10	12
	unscreened	3	4
Aromatic:	screened	15	>23
	unscreened	16	>23

Ref: Atlas, 1975 (44).

Shielding the aromatic oil slick did not alter the time to reach LD_{50}, and no LD_{100} was observed during 23 days of the experiment. The amphipods were noted to veer away from the aromatic oil slick and avoided entering it; they also appeared to be sluggish.

The aromatic fraction of Prudhoe crude oil was much less toxic than either the paraffinic fraction or the diesel fuel. This conclusion is in contrast with many other studies of the toxicity of aromatic components of various petroleum products.

3. COPEPODS

The short-term effects of diesel oil and kerosene on the survival of the tidepool copepod, *Tigriopus californicus* were studied (45). The oils were mixed with seawater using an ultrasonic probe and a magnetic stirrer, giving a temporarily even distribution of oil, at 1 ml/l of seawater. This was poured into petri dishes, and separation was noted after 30 minutes.

Diesel oil in a dispersion of 1.0 ml/l caused total mortality (N = 25) within 3 days. Concentrations of 0.50 ml/l and 0.25 ml/l resulted in total mortality in 4 and 5 days, respectively, while 0.10 ml/l left only 2% of animals alive after 7 days. In contrast, unexposed tidepool copepods showed approximately 50% mortality in 7 days (45).

In experiments with kerosene dispersions at 1.0, 0.50, 0.25, or 0.10 ml/l, there was a steady decrease in survival of animals over 8 days at all concentrations. With the three highest concentrations, less than 15% of animals remained alive after 8 days, while about 70% of control copepods were alive at this time (45) .

Diesel oil was more toxic than kerosene, but this was also related to the evaporation rate of each oil. Diesel oil evaporated more slowly than kerosene, although both evaporated in a linear fashion. At the end of 7 days, 34% of kerosene had evaporated; for diesel oil the value was 14%.

Critical concentrations, which, when exceeded, reduce the survival of this copepod, were postulated to be less than 0.10 ml/l (87 mg/l) for diesel oil, and less than 0.10 ml/l (83 mg/l) for kerosene (45).

Copepod Nauplii, which occur in oceanic and coastal marine zooplankton, were exposed to water soluble fractions (WSF) of No. 2 fuel oil. The solutions were prepared by mixing one part of oil with 8 parts of filtered seawater (30‰ salinity), stirring slowly for 24 hr, and removing the aqueous phase, . The total soluble organic content of the aqueous phase was 18.5-19.5 mg/l. Mortality of coastal zooplankton exposed to 50% dilution of the WSF of No. 2 fuel oil in seawater was generally less than 50% at the end of 48 hours, whereas control mortality was approximately 25%. The zooplankton concentrations utilized were an average of 123 organisms per 5-ml aliquot.

Cyclopoid copepods were less sensitive than calanoid copepods to 50% dilutions of the WSF of No. 2 fuel oil. After 72-hour exposures, almost no calanoids survived, whereas 20% of cyclopoids survived. (In studies with mixed zooplankton species, oceanic zooplankton were more sensitive than coastal zooplankton to the toxic effects of WSF of No. 2 fuel oil) (46).

G. ECHINODERMS

Effects of water soluble fractions (WSF) of No. 2 fuel oil upon sperm, egg permeability, fertilization, cleavage and early development of embryos of marine sand dollars, *Melitta quinquiesperforata* (Leske) were studied (47). Oil was mixed with seawater, 1:8 volume ratio for 24 hr; and then the aqueous layer was drawn off for use and diluted with seawater as required.

A 2% dilution of the WSF of No. 2 fuel oil did not affect fertilization or cleavage of eggs, in comparison with controls. Fertilization membranes became almost indistinguishable in eggs exposed to 10% WSF, and vitelline membranes became indistinguishable with exposures to 20% WSF. In dilutions up to 20% WSF, the number of cells entering first cleavage steadily decreased. The number of second cleavages was reduced in 10% WSF and higher concentrations. Exposures

to the 20% dilution of the WSF resulted in abnormal-appearing cleavage, of which only 2% had proceeded to the 4-cell stage. Living larvae were few and were retarded in development, in comparison with controls. The bottoms of experimental containers were covered with dead eggs, many of which were in various stages of deformed or aborted cleavages. No larvae developed from eggs exposed to a 50% dilution of extract of the No. 2 fuel oil.

Sperm in No. 2 fuel became less motile with increasing concentrations above 4% WSF and with longer exposures. Sperm remained fertile after being placed in 20% fuel oil extract. Sperm were 100% infertile after placement in 50% extract for 30 minutes, although some sperm retained motility.

In conclusion, No. 2 fuel oil in concentrations greater than 4% (0.6 mg/l) water soluble extract notably reduced the number of eggs fertilized, markedly affected the elevation of the fertilization envelope, delayed completion of cleavage stages and development, produced abnormal cleavages, prolonged larval development, reduced the number of larvae produced, and resulted in larval deformities in the sand dollar.

The presence of di- and tricyclic aromatic hydrocarbons may be responsible for the toxic effects. Alkylnaphthalenes, alkyl indans, alkylphenols, alkyl anilines, alkyl indoles, alkyl benzothiophenes and perinaphthenone were detected in concentrations above 0.1 mg/l in the undiluted seawater soluble fraction of No. 2 fuel oil, by gas chromatographic analysis of the extract prepared as described above (47).

H. CORAL

Coral reef communities provide a habitat for other organisms and are important in the overall metabolism of the tropical reef. Four Panamanian coral species, *Pocillopora* cf. *damicornis*, *Pavona gigantea*, *Psammocora (Stephanaria) stellata*, and *Porites furcata*, were exposed to diesel fuel by four different methods (48).

In the first experiment, corals were submerged in the oil for 1 minute, and then placed in seawater to remove the oil. Degrees of tissue death were the same for exposed and unexposed colonies in observations made one week later. In *Pocillopora*, after 13 days the diesel fuel-exposed colonies lost almost all living tissue, while controls lost only about 5% of it. Other species were more resistant to tissue death, showing changes after almost 3 months.

The corals were also placed in bowls, and the diesel fuel was poured over to cover them for 30 minutes. They were then rinsed and placed in seawater. The polyps immediately retracted into coral-lites and did not respond to mechanical stimulation. Tissue rupture and flaking occurred after 18 hours. After 17 days, 70% of *Pocillopora* polyps were dead. Live polyps had open mouths and extruded mesenterial filaments.

In a third experiment, colonies of *Pocillopora*, having 2 distinct branches each, were exposed to the oil on one branch only for 0.5 minutes, while the second branch remained unexposed to oil. After treatment, colonies were again rinsed. The colonies exposed to oil, after 71 days, did not display significant differences between quantity of living tissue left on upper and lower branches ($p>0.05$) or on oily and non-oily branches ($p>0.05$). After 109 days, there was a lower percentage of living tissue left in the oil-exposed branches as compared with controls.

In the final experiments, colonies of the four species were allowed to acclimate in individual bowls, until polyps had expanded. At that time, 1-4 ml of diesel fuel were added to the surface of the water, and behavior and effects were noted.

Mouth opening responses were sustained much longer in oil-exposed organisms than in controls, following an offering of brine shrimp, but in 2 of the 4 species, no ingestion response was observed. The delayed feeding response was notable for 17 days after diesel fuel exposure. Reimer pointed out other studies in which oil exposure led to delayed feeding responses in lobsters and barnacles (Atema and Stern, 1974; and Smith, 1968) (48).

I. PHYTOPLANKTON AND ALGAE

Marine phytoplankton photosynthesis is responsible for the fixation of as much as 80% of the energy utilized by marine ecosystems. Their role as primary producers in the food chain is extremely important. In the presence of No. 2 fuel oil, phytoplankton photosynthesis was investigated.

No. 2 fuel oil was added in a concentration of 5 mg/l to Bedford Basin (Nova Scotia) seawater containing natural phytoplankton communities. After mixing vigorously for 10 minutes and allowing to settle for 30 minutes, the water phase containing the microorganisms was separated from the oil phase. In the water phase, oil concentrations were 19.1-19.8 µg/l. The water phase was inoculated with 2.5-5 µc of radiocarbon source, incubated for 6 hours, and then scintillation counting was undertaken. Based on decreased phytoplankton uptake of the radiocarbon, it was concluded that the oil inhibited photosynthesis in the phytoplankton. The effect was dose dependent (49).

Freshwater phytoplankton are producers in the food chain in the aquatic environment. In an oil polluted canal in London, concentrations of oil ranged from 0 to 7.4 mg/l. Phytoplankton samples from the areas with greater pollution were smaller and less diverse than those at sites where very little oil was present. *Euglena*, an algae which is abundant in oil polluted areas, and *Scenedesmus*, an algae found mostly in cleaner sites, were cultured and grown on diesel fuel or lubricating oil, in concentrations of 0.1, 1.0 or 10.0% by volume, on a shaking platform of an orbital incubator. Diesel fuel blocked the growth of *Scenedesmus* in all concentrations tested; photosynthesis was inhibited by both oils. *Euglena* species showed no significant reductions in population size or growth rate in cultures containing up to 10% lubricating oil or diesel fuel (50).

The growth of microalgae cultures in seawater containing No. 2 fuel oil was studied. For *Skeletonema costatum* (a diatom), 100 µg/l of No. 2 fuel oil was toxic to growth in tightly stoppered flask cultures. When evaporation was permitted (volatile hydrocarbons were driven off), the mixture was toxic at a concentration of 1000 µg/l. The volatile fraction, containing alkylbenzenes and toluenes, was the most biologically active portion of the oil. In *Dunaliella tertiolecta* (a green flagellate) growth was greatly enhanced when cultured in a tightly stoppered flask with the fuel oil in µg/l concentrations but no growth stimulation occurred when volatiles were driven off. Volatile fractions of No. 2 fuel oil induced species-specific stimulation at low concentrations and growth inhibition in both species at high concentrations. The influence of oil on microalgae in seawater can be stimulatory or inhibitory depending on the dispersion rate and volatilization of the oil as well as the phytoplankton species (51).

Growth and photosynthesis were studied in microalgae cultures exposed to water soluble fractions of No. 2 fuel oil (52). The fuel oil was 60% paraffinic and 40% aromatic. Water soluble fractions were prepared by mixing oil with filtered seawater in a ratio of 1:8, shaking for 24 hours, allowing it to separate and removing the aqueous phase. This aqueous phase was diluted to the desired concentration or used undiluted (100%) in the following studies.

A diatom, two green algaes and a dinoflagellate were tested with dilutions of 0.05% to 50% of the water soluble extract. Growth of all microalgae was inhibited either fully or partially but the concentration at which the response occurred varied. The addition of 10% dilutions of seawater containing 15 mg/l of No. 2 fuel oil had no effect on *Dunaliella tertiolecta* or *Chlorella autotrophica* (green algae), but induced 7-, 14-, and 170-hour lag phases in *Thalassiosira pseudonna* (diatom) and *Gymnodinium halli* (dinoflagella), respectively. The C_{16} to C_{20} paraffinic fraction of No. 2 fuel oil (285-350° boiling range), containing 1% pristane and 1% phytane, was the most toxic fraction for *T. pseudonana*.

In contrast to the finding of Dunstan, et al. (51) that high concentration of the volatile fraction of No. 2 fuel oil inhibited growth of phytoplankton species, in this study, the toxicity of No. 2 fuel oil occurred when the less volatile, medium and high boiling point fractions were utilized (52).

In studying photosynthesis in microalgae, water soluble fractions of No. 2 fuel oil were added to algal suspensions 8 minutes before turning on illumination. A rapid and large decrease in O_2 evolution occurred, indicating a decrease in photosyntheses. Inhibitory dilutions of the water soluble oil were 20% for *C. autotrophica* and 12% for the diatom, *T. pseudonana* (52).

The water soluble fraction of No. 2 fuel oil used above was analyzed by gas chromatography and mass spectroscopy. Phenalen-1-one (perinaphthenone) was present in a concentration of 0.2 mg/1. The effect of this compound on growth of microalgae was then studied using liquid cultures at 30°C under continuous illumination and CO_2 aeration. In the green algae, *D. tertrolecta* and *C. autotrophica*, 0.25 mg/1 of phenalen-1-one was lethal under white light, while with a yellow (530 nm) filter, growth was not affected until a concentration of 10 mg/1 was reached. Either the phenalen-1-one or a photochemical product of it was the lethal substance. Incubation of algae with concentrations from 0.50 to 1.0 mg/1 affected photosynthesis only after 6-8 hours, or one generation time. The cells bleached completely in 10-12 hours, indicating complete destruction of the photosynthetic pigment. The estuarine diatom, *Amphora* sp., showed wavelength-dependent growth inhibition at phenalen-1-one concentrations below 5 mg/1; above this level no growth occurred (53).

In another study utilizing water soluble fractions of 4 fuel oils with 55-60% paraffins and 35-40% aromatic hydrocarbons, results were different. Oil and water, in a 1:8 ratio, were gently mixed for 24 hours, the water phase was separated out, and dilutions of it were tested for effects on growth. Green algae and diatoms exhibited growth lags and lower final growth rates in 50% water soluble extra extract growth media of Baytown and Montana oils. New Jersey fuel oil suppressed growth of green algae completely. In diatoms, growth lags were noted but final growth rates were unaffected.

The detrimental effects of the oils may have been due to the alkyl anilines, indoles, and alkylphenols which were present in significant concentrations in the water soluble fractions (54).

J. BACTERIA

Oil may be toxic to ecologically important bacteria involved in the cycling of nutrients in estuarine environments (55). Despite this fact, some bacteria are capable of utilizing petroleum hydrocarbons as a sole source of carbon, and the seeding of oil spills with bacteria to enhance biotransformation has been suggested (56). The dividing line between conditions favoring growth and those causing death or interference of growth is not entirely clear. Biotransformation of fuel oils and lubricants is discussed in the chapter on Environmental Fate.

Kerosene was found to disrupt the attraction of motile marine bacteria to organic matter in seawater. Mixed cultures of bacteria in seawater were added to varying concentrations of kerosene emulsions prepared by sonification. Bacterial motility was adversely affected by kerosene concentrations over 5 mg/ml. In the presence of 3 mg/ml of kerosene, bacteria in seawater were studied with respect to albumin and casein degradation. The results of the 48-hour tests are presented in Table 12.

TABLE 12. BACTERIAL DECOMPOSITION OF ORGANIC MATTER AS INFLUENCED BY KEROSENE

Conditions	Albumin Degradation (%)	Casein Degradation (%)
Without Stirring		
Sterile seawater	5.2	4.3
Seawater + Bacteria	22.0	50.2
Seawater + Bacteria + Kerosene	5.8	7.4
With Stirring		
Sterile seawater	8.8	10.0
Seawater + Bacteria	30.5	48.4
Seawater + Bacteria + Kerosene	39.2	65.5

Ref: Chet and Mitchell, 1976 (57).

The inhibition of decomposition of organic matter was thought to be due to a blockage of chemotaxis of the bacteria to the substrate by the sublethal concentration of added kerosene. This inhibition could be overcome by stirring the medium to promote random contact of the bacteria and substrate. Kerosene did not inhibit bacterial enzymatic activity (57).

Growth of two blue-green algae species, *Agmenellum quadruplicatum* and *Nostoc* sp. was tested with 0.05 to 50% dilutions of the water soluble fractions of No. 2 fuel oil (60% paraffinic and 40% aromatic) in seawater as previously described (52). A 10% dilution of seawater containing 15 mg/l of No. 2 fuel oil induced 7-, 14-, and 170-hour lag phases in *A. quadruplicatum*, and prolonged the doubling time of *Nostoc* sp. from 6 to 9 hours. The C_{15}-C_{18} fraction (270-315°C boiling range) with 1% dimethylnaphthalenes and 1% pristane, the C_{16}-C_{20} fraction (285-350°C), and the C_{17}-C_{24} fraction (300->385°C) were the most toxic for *A. quadruplicatum*.

To study the effects on photosynthesis, water soluble fractions of No. 2 fuel oil were added to algal suspensions 8 minutes before turning on illumination (52). A 50% dilution of the water soluble oil was inhibitory for *A. quadruplicatum* as indicated by a rapid, large decrease in O_2 evolution.

Analysis of a water soluble fraction of No. 2 fuel oil by gas chromatography and mass spectrometry revealed that phenalen-1-one (perinaphthenone) was present in a concentration of 0.2 mg/l (53). The effect of this compound on the growth of blue-green algae, *A. quadruplicatum* and *Coccochloris elabens* was then studied using liquid cultures at 30°C under continuous illumination and CO_2 aeration. Growth of the 2 species was affected only at a concentration as high as 5 mg/l, which was abruptly toxic to growth. This toxicity was independent of the illumination wavelength under which they were tested.

In another study, the effects of water soluble fractions of 4 fuel oils (55-60% paraffins; 35-40% aromatic hydrocarbons) on the growth of *A. quadruplication* and *C. elabens* were investigated (54). Growth of both species was suppressed by a 25% dilution of Baytown and Montana oils. Fifty percent dilutions of New Jersey fuel oil caused long growth lags in both species. Baton Rouge fuel oil caused a lag in growth for *A. quadruplicatum*.

K. PLANTS

Damage to citrus trees, foliage trees and fruit and vegetable crops has resulted from agricultural oil sprays used as insecticides, oil leaks or seepage of petroleum into soils, and oil spills in marine and freshwater environments which have polluted vegetation in sediments and bottoms. The bulk of studies reviewed in this section were undertaken from 20 to 50 years ago, when oil technology was less sophisticated than it is today. The physical and chemical properties of some of the petroleum oils utilized in the research were not specified except in terms of viscosity or geographical origin of the crude oil from which petroleum products were derived.

1. FRUIT TREES

There are many studies of the effects of oils used as agricultural sprays on leaves, fruit and roots and bark of citrus and deciduous trees.

In California orchards, petroleum oils have been used as an insecticide and as a carrier of insecticidal chemicals since the 1880's. Kerosene sprays were first employed to kill scale insects in lemon and orange groves. In deciduous orchards (e.g., apple, peach, prune), petroleum oils help control injurious insects and defoliating caterpillars. At least half of the 250,000 acres of citrus groves in California in 1926 were sprayed annually, consuming 60,000 to 100,000 bbls* of oil a year (11). Crop spraying continues today.

In general, sprays of lubricating oils are less phytotoxic than sprays of fuel oils such as diesel oil and kerosene. Oils rich in aromatic hydrocarbons may be more toxic than the paraffin-rich oils when sprayed on fruit trees (11,58), but for both foliage and nut trees, sprays of aromatic oils were shown to be less damaging than sprays of cycloparaffin-rich oils (59). Other reports indicate that viscosity is a determinant of phytotoxicity (58,60).

*bbls-barrels

Another factor which may influence phytotoxicity of petroleum oil sprays is the type of emulsion employed. There are water-in-oil emulsions which separate into two phases readily, and then there are stable emulsions which separate slowly. Various emulsifiers are used, and oil spray mixtures may contain 1% to 50% or more oil. Where undiluted oils have been tested, though, the results are not dissimilar from studies with the water-in-oil emulsions, so both types of sprays will be considered in this section.

Three-year-old navel and Valencia orange trees were sprayed on one side with a particular oil or oil emulsion, the other unsprayed side serving as a control for comparison. Oil concentrations of 5% were used in most experiments. Tree effects were studied in a 65-day period during the summer. Undiluted kerosene had very little immediate effect when applied to foliage, but severe injuries occurred when it came in contact with the roots. Oils with higher specific gravities than kerosene were found to cause greater injury. Lubricating oil sprays caused little immediate change in leaf appearance, but leaf drop occurred 48 hours following spraying, and continued until the 4th or 5th day. The bearing wood which had been defoliated appeared stunted, new growth was slow to start, and the new foliage was often weak. A thick vigorous growth of sprouts around the base of the defoliated limb indicated a severe disturbance in the sprayed branches. The more viscous the lubricating oil, the slower its effects were manifested (61).

Chronic effects of the lubricating oil sprays included yellowing of foliage a few days after spraying, growth stunting of bearing wood for weeks or months after spraying, sprouting in the center of the tree, and the production of stunted leaves to replace those lost (61). Kerosene sprays increased the severity of these effects, as did aromatic oil sprays.

The Western Cooperative Oil Spray Project, in 1930, provided recommendations for safe oils which would still be effective insecticides. Lubricating oils with viscosities of 100-220 seconds Saybolt were safe when sprayed as emulsions in winter, before bud scales separated. Fuel oils with viscosities of 50-120 seconds Saybolt at 100°C were recommended for summer spraying. Oils which were not rich in aromatic hydrocarbons were preferable for use on growing plants. Summer spraying with viscous lubricating oils caused russeting of fruit and a general reduction in fruit size if applied more than three times in that season (12).

Continuous use of oil sprays did not appear to be detrimental to orange orchards sprayed for 9 consecutive years, or to orchards sprayed annually during dormancy for 8 years. Factors considered in assessing the safety of the spray were the viscosity and concentration of oil in emulsions, temperature, number of sprayings, availability of water and nutrients, abundance of fruit, health and varietal characteristics of trees, and age (12).

Emulsions of 25 different lubricating oils were sprayed on an orchard of 86 apple trees, drenching limbs and twigs, in summer and in winter for 7 years. Injuries were observed to be more marked with 16% and 100% oil emulsion concentrations than with 4% emulsions. Leaf spotting, discoloration, necrosis, and severe premature defoliation commonly preceeded the death of many buds and the bark of attached twigs. Oil sprays also killed flowers of blooming apple trees, and caused discoloration, spotting and cracking of fruit. The magnitude of these effects was "moderate" after sprays of a 4% emulsion of a paraffinic lubricating oil for 3 years. A more aromatic lubricating oil, under the same conditions, severely injured apple trees, causing bud deaths, dwarfed leaves and retarded growth of leaves and flowers (62).

Drought injured apple trees were most susceptible to oil spray damage. Spraying in October, November and December could delay or reduce the development of dormancy in predormant apple trees, increasing the hazard of winter injuries (62).

Citrus trees injuries from spraying with neutral white oils (98% paraffinic lubricating oils) included more or less heavy leaf drop of senile or semi-senile leaves, drop of tree-ripe fruit, inhibiton of normal color in lemons, drop of green Valencia oranges during humid weather and retardation of ripening of fruit (58).

Valencia orange trees were sprayed with an 83% paraffinic fuel oil of a 70 seconds Saybolt viscosity at 100°C in a 1.7% emulsion, at the end of February. Primary oil damage symptoms included general shadowing of old and new foliage and marginal burn on new foliage after several days. The flower petals were spotted brown but blossoms opened normally. At the end of 10 days 39% of the young set fruit on the treated trees were yellow or shed; in untreated trees, 18.3% of fruit showed these symptoms.

The most outstanding effect was the drop of tree-ripe fruit. By the end of April (56 days after spraying) an average count of all fruit on the ground was 52.6 for sprayed trees against 5.4 for unsprayed trees. Fruit remaining on the trees was counted in April and in September, and averaged 51.4 oranges per treated tree and 76.7 per untreated tree.

Secondary botanical effects included a 0.05 cm decrease in fruit diameter over controls: this was probably due to loss of foliage after oil application, although the leaf-to-fruit ratio did not change due to fruit drop.

June growth appeared more rapidly on treated trees, with 21.75% of spring growth terminals on the treated trees having burst buds by June 1st, as compared with only 7.2% in untreated trees.

The same oil was sprayed on orange trees in May. At application time, fruit averaged 3.7 cm in diameter. Mature leaves started dropping 24 hours after treatment and continued to drop for two weeks, possibly because oil penetration was highest in mature leaves. Drop of both spring fruit and foliage was proportional to the oil concentration of the emulsion. Shadowing of leaves and fruit occurred on all sprayed trees, but no foliage burns and only one fruit burn were noted (58).

The untreated trees had an average fruit diameter in August of 6.69 cm, while the diameter of fruit on sprayed trees averaged 6.1 cm. Fruit size was inversely proportional to crop size. In June, counts of 50 spring growth terminals averaged 25% with burst buds on untreated trees, and 70% on sprayed trees. The June bloom crop averaged 3.3 fruits versus 33 fruits for treated and untreated trees, respectively.

Adverse effects in the trees, following oil applications and the resultant loss of large numbers of leaves, included retarded size of immature fruit, reduced numbers of fruit in the succeeding crop with subsequent acceleration in the flush of growth, and finally, killing of wood either due directly to oil penetration or due to an interruption in sap exchange resulting in collapse of wood cells.

Acceleration in appearance of a growth flush when due to decreased or inefficient foliage conditions, signifies a return of the plant to a vegetative state; a compensatory response toward maintaining equilibrium at the expense of fruit production (58).

A highly refined lubricating oil (Nujol; viscosity 200 seconds Saybolt at 100°C) was sprayed on Apple trees four times, with intervals of 2 weeks between each application. One side of each tree was sprayed with a 1% oil-in-water emulsion, the other with a 0.5% emulsion. No leaf injury or excessive fruit drop was noted. The leaf color of sprayed trees became increasingly darker green after the second application, as compared with unsprayed trees. Foliage was thicker and more vigorous on the sprayed trees toward the end of summer. In the fall, sprayed leaves remained attached longer than in unsprayed trees, which was indicative of a prolongation of vegetative growth and higher chlorophyll content. No difference in fruit color between sprayed and unsprayed apple trees was noted (63).

2. FOLIAGE

The delicate foliage of Aspen and sugar maple trees was injured within two days following a summer spraying with 1%, 5%, or 10% strength oils. Leaf scorching or spotting was more severe with a cycloparaffinic distillate fuel oil or a "dormant water oil" (unspecified composition) than with sprays of an aromatic distillate oil or a "summer spray paste oil". The 10% strength of all oils caused injuries, however. Red pine and red cedar trees, when sprayed in the same experiment, were only affected by the oils after 3 months, during autumn, when leaves dropped from injured new branches. Silver and Norway maples, tulip trees and white ash were severely injured by the 10% oil sprays of summer oil paste and the aromatic distillate. Other trees (chestnut, rose, filbert, black walnut, catalpa, ginko, elm, linden, oak, spruce, pine, cherry, pecan, apple and hickory) were less severely harmed. The aromatic oil was safer than the cycloparaffinic distillate for use on foliage (59).

Experiments on summer spraying of citrus, black walnut and wild morning glory, conducted in a humid cool environment, showed lubricating oil to cause leaf yellowing and leaf tissue death. A 10% emulsion of

paraffinic kerosene did not adversely affect morning glory leaves within a 6-day observation period, and the same was true for a 20% emulsion sprayed on walnut trees (61).

Apples trees were sprayed four times with intervals of 2 weeks between each application, with a highly refined, paraffinic lubricating oil emulsion. Analyses of leaves collected in late summer from trees sprayed with 1% oil emulsion revealed 28-47% increases in chlorophyll content compared with unsprayed trees. This increased chlorophyll may have been due to stimulation of chloroplast formation in epidermal leaf cells or to less injury by insects to foliage. A coat of oil on the leaves may have reduced the intensity of light reaching the leaves, thus stimulating chlorophyll accumulation (68).

Leaf injuries which occurred in an apple tree orchard where lubricating oil emulsions (4%-16% oil) were sprayed in summer and winter for 7 years were described. Angular mottling and leaf translucence occurred within a few seconds to hours after spraying. Chlorotic spots appeared within a few hours or days. In mild injuries, translucence and chlorosis sometimes disappeared. Severe injury was characterized by wrinkling, general chlorosis, hypophyllous (spongy parenchyma) browning, silvering and purpling, epiphyllous (palisade parenchyma) purpling, white or tan spots. Translucent or chlorotic spots developed into large brown necrotic areas, causing sprayed leaves to fall sooner than uninjured leaves (62).

Drought injury to apple trees increases their susceptibility to oil spray leaf damage. The decreased water content of droughted leaves may act as a deficient barrier to the spread of oil inside leaves. Oil penetration into leaves is also facilitated by insect injuries. Oxygen from photosynthesis in leaves may contribute to oxidation of oil inside or the surface of the leaf, forming peroxides or oxides. Decomposition of the thin oil film may form acids which are injurious to the leaves (62).

Oil penetration into leaves is greater on the underside of the leaf where the stomates are localized (58). When various oils were placed on the lower sides of living apple leaves, oil entered through the stomates and caused large translucent leaf spots in 1-10 seconds (64). In fruit trees, spraying a 50% oil-in-water emulsion of diesel fuel interfered with the capacity of the stomates to close (13).

Undiluted petroleum oils and undiluted oil sprays were used to study penetration and distribution in living apple leaves. Application of oil to the leaves caused translucence as oil replaced air in intercellular spaces. Oils with low viscosities penetrated leaves faster (1 minute) than heavier oils (up to 30 minutes). Oil spots on leaves were vein-bounded. The oily spots changed very little in size, shape, color or distribution after stabilizing within 2-60 minutes, indicating that evaporation of oil from inside the leaf was negligible with oils ranging in viscosity from 51 to 110 seconds Saybolt at 100°C.

Ten oils with viscosities from 50-108 seconds Saybolt at 100°C penetrated and spread similarly in leaves and twigs. More oil entered from undiluted oil sprays than from emulsions, due to the higher oil concentration of the former. Sectioning of apple leaves 7 days after oil application showed oil in intra- and intercellular locations, including vein and petiole-parenchyma cells and tracheae, and between spongy, palisade, vein and petiole-parenchyma cells (64,65).

Leaf transpiration in Eureka lemon and Bearss lime plants was studied by spraying the citrus with either a paraffinic or a cycloparaffinic oil fraction with comparable boiling ranges of 300-320°C and molecular weights of 306-308. Reduction in transpiraton to one-half of control values occurred the day after spraying with a 1.75% oil-in-water emulsion. But recovery of transpiration was more rapid in plants sprayed with the cycloparaffinic than with the paraffinic fraction. Thirty days after spraying, all plants treated with the cycloparaffinic oil had recovered completely, but the paraffinic oil-sprayed plants did not recover even after 37 days. There were no differences in responses between lemon and lime trees (66).

Inhibition of photosynthesis occurred in banana tree leaves sprayed with a paraffinic kerosene used to control leaf spot fungus (60). Oil treated leaves were cut into discs of 1 cm diameter, which were placed in an illuminated Warberg apparatus using a manometric technique to determine respiration and photosynthesis. Oil applied by helicopter at an 84 $\mu g/cm^2$ concentration on upper leaf surfaces resulted in a 15% reduction in photosynthetic ability which was significantly different ($p = 0.05$) from unsprayed leaves; oxygen evolution was 151.6 μg O_2/30 min. per 5 leaf discs in controls and 129.6 μg O_2/30 min. per 5 leaf discs in treated leaves. Inhibition of photosynthesis may have resulted from interference with gas exchange. The amount of oil applied was directly proportional to the degree of inhibition observed.

In fruit tree leaves treated with a 50% emulsion spray of light diesel fuel (used to control a leaf parasite), respiration was adversely affected. When compared with unsprayed leaves, oiled leaves showed a sharp decrease in O_2 uptake and CO_2 evolution 2 hours after spraying, indicating a large increase in intramolecular respiration. Four hours after treatment, this sharp rise in respiration began to taper; this lasted for 5 hours. By the end of 24 hours the respiratory quotient was still rising slightly and reached 1.6 (normal = 1), indicating that respiration was still increasing (13). High viscosity oils also cause metabolic disturbances in leaves (58).

3. VEGETABLES

Fuel oil No. 2 was applied to level, 1.7 x 3.0 m plots of sandy loam, black clay loam, and silt loam soils at an application rate of 2.5 kg/m^2 and tilled to a depth of 15 cm. Turnips and beans, which were planted following application, grew in sandy loam but not in black clay loam or silt loam. Plants which grew were severely stunted with deformed leaves. Extracts of tops and roots were analyzed for oil content by gas chromatography,

and there were no differences between tops and roots, although characterization of the extracted hydrocarbons was not performed. Phytotoxic effects did not resulve in the 12 months in which the study was conducted (27).

Oil injury to bean and lettuce plants was noted after being sprayed with 10% concentrations of an aromatic fuel oil (the nature of the injury was not discussed) (59).

When undiluted lubricating oil was applied to potato-leaflets, wilting and necrosis occurred. Juvenile leaves were more susceptible than mature leaflets. Lubricating oil sprays in 4-100% concentrations caused black or brown leaf spots, blackening of epiphyllous veins, and large qualtities of oil killed leaves and stems. Purple or white leaf spots were also observed (67).

Kerosene application to potato leaflets caused translucence which abated in 1-24 hours due to evaporation from intercellular leaf spaces. Other effects were chlorosis, browning and killing of petioles of potato leaves (67).

4. AQUATIC PLANTS

Field observations by Blumer et al. (68) after a spill of 600,000 liters of No. 2 fuel oil into Buzzards Bay, Mass., in 1969, included a comment on the fact that bottom plant life had been destroyed as oil contaminated sediment spread along the sea, marsh and river bottoms in the polluted area. In addition, destruction of bottom plants reduced the stability of marshlands and the sea bottom, resulting in erosion and further spread of oil on the sea floor. Oil pollution also may be responsible for the death of eelgrass in coastal waters, although the available literature did not provide in-depth information on this topic.

III. ENVIRONMENTAL FATE

The environmental fate of the petroleum hydrocarbons which constitute fog oils and diesel fuel can be described as an exceedingly complex series of physical, chemical, and biological processes and interactions. Important research has been reported in current literature on a wide variety of topics relating to environmental fate. These will be reviewed under three subheadings: occurrence and dispersion; chemical and biological transformation; and bioaccumulation. The extent to which each of these parameters influences the immediate and ultimate environmental fate of diesel fuel and fog oils depends upon the quantity of oil contamination in a given area, the nature of the environment, and physical and chemical properties of the oil.

The amount of oil contamination at ground level during and following smoke screen operations and the degree of atmospheric dispersion of fog oil screens prior to settling to the ground or water can only be estimated. In aquatic environments, water temperature, salinity and degree of agitation or flow rate of the body of water affect the dispersion, degradation and accumulation of petroleum oils. On land, soil properties, rainfall, temperature, ground water level and grade and meterological conditions must be considered in discussing environmental fate of fog oils, diesel fuel and their screening smokes.

A. OCCURRENCE AND DISPERSION

This section presents factors relating to the contamination of air, soil and water by fog oils and diesel fuel, as well as mechanisms by which water and soil accommodate and disperse these oils. Hypothetical atmospheric and ground level concentrations of fog oil or diesel fuel following one hour of smoke screen production by one M3A3 smoke generator are estimated.

1. SMOKE SCREEN PRODUCTION AND DISPERSION IN AIR

Crude petroleum is found in underground rock formations which range in age from ten to several hundred million years. These oil deposits are formed mainly from remains of microscopic marine flora and fauna buried under accumulations of sediment. Physical and chemical processes such as heat, pressure, catalysis in rock, and bacterial action are responsible for their conversion into crude oil and gas. Crude oil deposits occur throughout the world (39). However, SGF No. 1 and diesel fuel, fuel oil distillates of crude oil, and SGF No. 2, a refined lubricating oil, do not occur naturally in the environment, but are products of oil refineries. Therefore, the only sources of environmental contamination with refined oils are by human intent or accident. This includes: oil spills from pipelines, ocean tankers, oil trucks and other oil transport systems; pollution by petroleum refineries; spraying oils on crops, orchards and livestock for protection against insects and diseases; and domestic and industrial disposal of various petroleum products such as motor and machine lubricating oils.

The U.S. Army has utilized the two fog oils (SGF No. 1 and No. 2) in generators and smoke pots which produce oil smoke screens. Diesel fuel has also been considered for use in producing smoke screens. The smoke generated from any of these three oils consists of dense concentrations of oil microdroplets, forming a fog which effectively obscures the area into which it is directed.

One M3A3 oil smoke generator consumes 95-190 liters of fog oil per hour (average 150 liters) on a continuous operating basis (3). The smoke usually remains aloft for up to 1 hour without coalescence or condensation, and may be carried as far as 10 km (average 5 km) from its point of emergence from the generator, depending on meteorological conditions during smoke production (4). Wind speeds from 8-19 kmph are ideal for smoke screen production. At temperatures above 27°C smoke screens generated by mechanical smoke generators are dissipated more rapidly than they are at lower temperatures. Generators are spaced at specified distances depending upon wind speed and type of terrain: as close as 20 meters apart for open water at a wind speed of 26-32 kmph and as far as 70 meters apart in woods at windspeeds of 1-13 kmph. According to a personal communication (4), the oil smoke emerges from the nozzles of the M3A3 smoke generator in a 3-5 m wide column, and as the smoke moves downwind, spreads in all directions to approximately 100-150 m. At a point approximately 500 m downwind from the generator, the smoke will have risen to an altitude of approximately 100 m. It is possible to calculate a hypothetical atmospheric oil smoke concentration which one M3A3 generator could achieve in one hour by assuming that approximately 150 liters of fog oil are completely vaporized and condensed, without evaporating. If this volume of oil droplets spreads out to occupy an area approximately 100 m wide, 100 m in altitude and 1 km in length, then an atmospheric concentration of oil would average 13 mg/m^3 in a 1 hour operation.

If the data from the Army Field Manual FM3-50 (3) are utilized to calculate atmospheric oil smoke concentrations, again, approximately 150 liters of fog oil per hour are vaporized from one M3A3 generator. If generators are spaced 20 m apart, the target distance is 200 m away, and the smoke rises to treetop level (15 m), then a concentration of smoke of approximately 2000 mg/m^3 for one hour could be present.

The large discrepancy, viz. 13 mg/m^3 vs 2000 mg/m^3, is explainable by the fact that the area into which the fog disperses, before it settles to the ground, depends upon meteorological conditions, such as air currents and prevailing winds, these conditions being extremely variable and unpredictable.

To calculate the fallout from a smoke screen, i.e., the concentration of fog oil which settles to the ground, it is assumed that all of the 150 liters of fog oil from one smoke generator precipitates, ignoring evaporation losses. If it is also assumed that the fog is carried an average of 200 m to 1 km downwind from the generator, and the smoke column spreads out to about 20-150 m, then it is possible to expect fog oil fallout

concentrations in a range of 6 to 60 g/m^2 after one hour of use of one smoke generator. These estimates are based on a hypothetical model. Examples of actual concentrations of the smoke or dimensions of the smoke are not available in the literature.

Once the fog oil or diesel fuel smoke droplets settle to the ground, the physical processes affecting the persistence and transport of these petroleum hydrocarbons include rate of evaporation, dissolution and dispersion in water, movement through soil, adsorption by soil, sediments and other solids, and solubilization by naturally occurring dissolved surfactants. The available literature on these topics comes mainly from studies in the marine environment following real or model spills of oils which are chemically similar to fog oils and diesel fuel. Laboratory studies on oil movement in soil are also reviewed.

2. OIL BEHAVIOR IN WATER

The contamination of bodies of water with petroleum fuels and lubricants such as fog oils and diesel fuel results in an oil slick or film which floats at the water surface. Turbulence within the water can cause emulsions of dispersions of oil droplets. In laboratory experiments by Anderson et al. (26), No. 2 fuel oil in seawater dispersions were produced by vigorous agitation of the oil and water for 5 minutes followed by one hour of separation. Concentrations of oil in the aqueous phase of these dispersions increased linearly between 20 and 1000 mg/l of oil added initially. When 1000 mg/l of No. 2 fuel oil were added to 20‰ saline artificial seawater, the dispersion which resulted after shaking and separation contained 51.8 mg/l of oil in the aqueous phase. A drop in oil content of the aqueous phase dispersions occurred if greater than 1000 mg/l of oil was added, due to coalescence of oil droplets and return to the oil-water interface at a rate which exceeded the increase of oil droplets entering the aqueous phase. The water phase petroleum hydrocarbon content of the dispersions ranged from 14-52 mg/l at concentrations of 20-1000 mg/l of oil added, respectively. Hydrocarbon composition of oil dispersed in the aqueous phase was similar to the composition of the original No. 2 fuel oil: 60% n-paraffins and 40% aromatic hydrocarbons. Gentle aeration of these dispersions for 24 hours caused a loss of 80% of the hydrocarbon content. A slightly higher percentage of n-paraffins than aromatic hydrocarbons was lost.

Vanderhorst et al. (33) showed that surface turbulence determined aqueous phase concentrations of No. 2 fuel oil in seawater. Minimal oil water disturbances resulted in no detectable fuel oil levels in the water column. Initial mixing of water and oil with minimal surface disturbance yielded detectable amounts of fuel oil in the water column. The correlation between the volume of oil introduced and the average oil concentration measured in the water column was poor ($r = 0.81$); no soluble oil component was detected. Where mixing occurred during introduction of oil into water under highly turbulent conditions, and turbulence was maintained throughout a 24 hour period, water column

concentrations of oil were linearly related (r = 0.99) to the volume of oil added. This information was utilized in a study of toxicity of No. 2 fuel oil to coon stripe shrimp. (See Marine Crustacean section).

In addition to the fact that dispersions of oil droplets in the aqueous phase result from turbulence, Anderson et al. (26) found that some of the petroleum hydrocarbons present in No. 2 fuel oil were capable of dissolving in water. Water soluble fractions of oil were prepared by gentle stirring of 1 part of oil in 9 parts of 20‰ saline artificial seawater for 20 hours, and then separating the aqueous phase containing dissolved oil. A maximum concentration of 8.7 mg/l of dissolved No. 2 fuel oil hydrocarbons was reached in that time. Roughly 10 mg/l of oil dissolved in the seawater when 1:9 oil in water mixtures were gently stirred for 29 hours. The aromatic hydrocarbons in No. 2 fuel oil were more water soluble than its paraffins. Whereas the original oil was 60% n-paraffins and 40% aromatic hydrocarbons, the water soluble fraction contained 0.54 mg/l of C_{12}-C_{24} n-paraffins and 5.74 mg/l of aromatics such as alkynaphthalenes.

Nickol et al. (47), in studies on sea urchin eggs exposed to No. 2 fuel oil solutions in seawater, noted the presence of mono- and dicyclic aromatic hydrocarbons such as alkylnaphthalenes, alkyl indans, alkyl phenols, alkyl anilines, alkyl indoles, alkyl benzothiophenes and perinaphthenone in concentrations above 0.1 mg/l. The solutions were prepared by mixing 1:8 oil with water for 24 hours and then drawing off the aqueous phase for determination.

Boylan and Tripp (24) studied water solubility of kerosene in filtered seawater. Slow stirring of 25 ml of kerosene in 1.5 liters of seawater for 12 hours resulted in an aqueous extract containing mostly alkylbenzenes and naphthalenes in concentrations of 160 µg/l. Increasing the turbulence of the system by rapidly stirring for 12 hours resulted in increased levels of non-polar saturated hydrocarbons in the aqueous phase.

The solubility of No. 2 fuel oil in filtered seawater was investigated for solutions prepared by mixing 4 mg of oil with 1 liter of seawater for 15 minutes, allowing it to separate for 1/2 hour, and removing the aqueous phase. Chromatography of the water phase revealed that n-paraffins dissolved less readily than cycloparaffins and aromatic hydrocarbons, as compared with concentrations of these substances in the original oil (70).

Unfiltered seawater was found to dissolve up to 1560 µg/l of No. 2 fuel oil. Filtering removed about 75% of the dissolved oil. This was due to the fact that organic matter present in the unfiltered seawater was capable of solubilizing certain petroleum hydrocarbons (70).

Lysyj and Russell (71) compared diesel fuel and lubricating oil solubilities in distilled water. A mixture of 20 ml of either petroleum product and 180 ml of water was vigorously shaken for 5 minutes and

allowed to equilibrate for 42 days. Samples were taken for analyses at 4 hr, 1, 2, 4, 7, 15, 28 and 42 days. Figure 1 illustrates and compares dissolution of diesel fuel and a lubricating oil identified as SAE20 non-detergent motor oil in distilled water.

Dissolution of petroleum hydrocarbons into the aqueous phase is the result of many interactions, including oxidation and bacterial degradations. The acceleration in dissolution was not evident in the lubricating oil, possibly because of it greater chemical stability or the presence of additives such as antioxidants. These concentrations of aqueous phase hydrocarbons exceeded the "no visual sheen" limit for oil pollution of water, which is roughly 10 ppm (71).

3. EVAPORATION

A No. 2 fuel oil was investigated to determine evaporation rates of oil spills from water at various temperatures (72). At a constant windspeed of 21 km/m, and temperatures of 5, 10, 20 or 30°C in a darkened chamber, oil samples were poured in petri dishes to a thickness of 2 mm, and sampled at various times. A smooth curve of evaporation as a function of the remaining oil at a given temperature was noted. The authors stated that this was a reasonable result since the oil is a multicomponent system and each component would contribute to the total order of the evaporation. The C_9-C_{18} n-alkanes present in the fuel oil were studied in detail, although they represented only 18% of the composition of the oil. Evaporation rate constants and initial alkane concentrations utilized in determination of alkanes present at various time intervals showed that evaporation behavior of the alkanes coincided with that of the total oil.

The first weathering factor acting on a fresh oil spill on water or ice is evaporation. In the above study, oil components with vapor pressures lower than n-octadecane did not evaporate significantly, while those with vapor pressures higher than n-octane evaporated rapidly in an open environment. The evaporation of components with vapor pressures between those of n-octane and n-octadecane seemed to control the short-term aging of the oil (72).

4. DISPERSION AND PERSISTENCE IN SOILS AND SEDIMENTS

Organic matter in marine sediments reduces the uptake and retention of dissolved fuel oil hydrocarbons by the sediments. Bentonite clay was allowed to interact with fuel oil-saline solutions, and the amount of hydrocarbons associated with the settled clay was measured. As the oil solubility increased with increasing temperatures (-1°C to 53°C) less of the compounds became associated with clay particles. Percentage uptake of n-eicosane was 99% at 1°C and 83% at 53°C. For anthracene, the figure was 60% at 25°C. By removing indigenous organic matter from sediment samples from Narragansett Bay, hydrocarbon uptake increased by an average factor of 2.2. Once incorporated into sediments, petroleum hydrocarbons are not quickly released to overlying water; slow escape probably occurs through biological activity and dissolution (73).

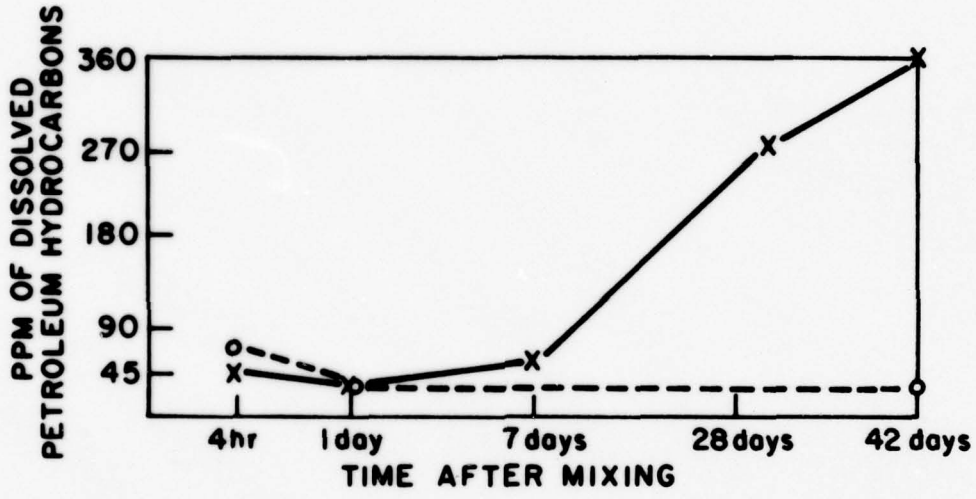

FIGURE 1. SOLUBILITY OF DIESEL FUEL AND LUBRICATING OIL IN DISTILLED WATER

X = diesel fuel; 0 = lubricating oil

Ref: Lysyj and Russel, 1974 (71).

Oil spills in sands and soils have been shown to become immobile after some time. In a study of the oil retention characteristics of beach soil and sand samples which had become polluted following an offshore fuel oil spill in British Columbia, oil was extracted from beach sand samples taken at one, 174 and 368 days after the accident occurred. Gas chromatography and infrared spectroscopy revealed the rapid disappearance of oil hydrocarbons containing less than 12 carbon atoms. Compositional changes included the loss of low molecular weight n-paraffins and oxidation of the fuel oil through the formation of ether and sulfone groups. Up to 4% of the oil was retained in soil samples taken 6 months following the spill, and about 3.5% remained after 1 year of exposure to weathering processes along the shoreline (74).

A spill of 600,000 liters of No. 2 fuel oil into Buzzards Bay, Massachusetts prompted investigations of oil persistence in marsh muds. Surface mud samples were highest in oil hydrocarbon concentrations, one year after the spill, in areas where all vegetation had been killed. In one section there were 6.5 mg of oil per gram of mud at the surface, with an oil penetration depth of 70 cm. The anoxic conditions in the deep marsh muds are poorly conducive to biotransformation; thus oil was predicted to persist at these depths (75,76). Two years after the spill, sediments from the marshes (sand and clay in layers) and harbor (clay with sand; water depth of 1.5 m) still contained detectable levels of petroleum oils. Branched and cyclic hydrocarbons such as isoprenoids (phytane, pristane and C_{18} homologues) and alicyclic and aromatic hydrocarbons (highly substituted benzenes, naphthalenes and tetrahydronaphthalenes) remained prominent in sediments analyzed by mass spectroscopy. The overall rates of depletion of saturated and aromatic hydrocarbons were the same, i.e., the ratio of saturates to aromatics did not change during this period. This was explained to be due to preferred bacterial transformation or dissolution of some oil components within each structure groups. Core samples near the oil spill site showed detectable levels of fuel oil down to a depth of 7.5 cm below the sediment surface. Oil below 2.5 cm was as "fresh" after 2 years as surface oil after 10 months. Penetration into the marshes extended down to at least 60 cm. Bacterial degradation and dissolution were evident at this depth. The author's conclusion that the permeability and aeration of marsh sediments favored biotransformation and dissolution (76) support the earlier statement that anoxic conditions are poorly conducive to biodegradation (77).

The dispersion of petroleum hydrocarbons in soils can contaminate groundwaters and spoil drinking water. Migration of oil in soil, extraction of soluble components from the oil into residual water in the soil, transport of these dissolved components to the groundwater body by rain water or other means, and adsorption of oil components on the soil are four determinants which must be considered in evaluating the risk of water contamination. These factors depend upon the amount and type of oil, nature of the soil, rate of rainfall and the groundwater level and its grade (74,78).

Lau (79) noted that seepage of petroleum oil products in soils was dependent upon capillary flow and adsorptive capacity of the soil which were related to moisture content. Field studies in the upper Rhine area of Germany showed that infiltrated oil was impounded and impregnated at the capillary fringe and did not contact ground water below the soil. It was noted that water leaches out taste and odor producing compounds from oil held in soil pores. The path of petroleum oil migration in two- and three-phase flows in inert granular material, and the characteristics of surface tension along a sloping water table were illustrated in mathematical equations reviewed by the author (79).

Raymond, Hudson and Jamison (27) studied the physical movement of oil from soils treated with No. 2 fuel oil. Pennsylvania Glenville silt loam (pH 5.4), Oklahoma sandy loam (pH 6.3), and Texas black clay loam (pH 7.6) were exposed by applying the oil at a rate of 2.5 kg/m^2 on level 1.7 x 3.0 m plots and tilling to a depth of 15 cm. The oil was readily absorbed into the soils. Physical movement of oil from the treated plot was not noted over one year. There was no visible sheen in runoff or leachate waters from any soils; oil concentrations in water samples were 0.6-14.6 µg/l for untreated control soils and 0.5 to 15.2 µg/l for treated soils.

Van der Waarden et al. (78,80) utilized various soil models and soils to study transfer of oil components to groundwater. A pack of non-adsorbing glass particles was used as a soil model. Oil was injected into a zone of the model with residual water saturation and allowed to disperse freely. Subsequently water was allowed to trickle through the pack. The drain water, containing no free oil, was analyzed for contaminants. Water extractable components of kerosene and fuel oil could be leached out from the oil zone by trickling water at rates determined solely by the partition coefficient of the components and by the water to oil ratio. Oil components which dissolved in water were then transported with the water. In true soils, some adsorption of oil components occurs. In a similar experimental model, natural dune sand was substituted for glass beads. There was a slowing of the extraction of oil hydrocarbons by trickling water, which was attributed to the adsorptive nature of the sand (78). Lime, clay and organic matter in natural soils were also evaluated. In similar experiments as above, limestone packed columns showed negligible adsorption capacity for kerosene and fuel oil, as borne out by comparison with glass particle pack results. With packs of dune sand, glass plus 10% compost (by weight) or glass plus 20% compost, the leaching of hydrocarbons by drain water was delayed. The maximum hydrocarbon concentration in drain water was reduced and the gradual decrease in hydrocarbon concentration with time was no longer exponential, as compared with packs of glass particles (80). It was concluded that soil adsorption reduces the transport of petroleum oils to groundwater by leaching. Low rainfall also increases the persistence of water which contains dissolved oil components; other processes such as degradation and evaporation might thereby be enhanced.

B. TRANSFORMATION

Hydrocarbons in the fog oils, SGF No. 1 and SGF No. 2, and in diesel fuel, are transformed in nature via processes including both photooxidation by ultraviolet light and biodegradation by microorganisms present in terrestrial and aquatic environments.

1. PHOTOLYSIS BY ULTRAVIOLET LIGHT

Photolytic transformation results in oxidation of aromatic hydrocarbons of intermediate and high molecular weights to produce insoluble polycyclic aromatic hydrocarbons (75). Photochemical reactions produce ketones, aldehydes and carboxylic acids of low to medium molecular weight. Radical combinations, condensation of ketones with phenols, and esterification of carboxylic acids may form high molecular weight molecules (81).

Fuel oil irradiation experiments were performed with mercury vapor lamps emitting at 253.7, 312.9, or 365.4 nm. Oil samples received high intensity irradiation at a distance of 4 cm in order to eliminate interference from non-photochemical reactions. Ultraviolet spectrophotometric determinations of the oil before irradiation and again after intervals up to 15 days showed initial absorbance maxima at 228 nm and 260 nm. The maximum originally occurring at 228 nm moved linearly with time, occurring at 200 nm after 15 days. At 214 and 225 nm, absorbance increased with time. At 260 and 300 nm, it decreased with time. Reactants and products were not identified. First order, second order, and second order autocatalytic kinetic models could all be made to agree well with the actual data obtained from the photooxidation experiments. Therefore, no definite conclusion concerning the photochemical reactions occurring in the fuel oil was drawn, although autocatalytic processes were probably involved (81).

A simulated environmental exposure of No. 2 fuel oil to ultraviolet radiation was conducted by Larson et al. (82). The No. 2 fuel oil was characterized as 77% C_{10}-C_{22} n-alkanes and other saturates, and 23% aromatic hydrocarbons. A mercury vapor lamp emitting 30% of its output in the near-UV region (280-400 nm) was positioned 30 cm above the surface of a 15.8 x 25.5 cm tray containing 150 ml of the oil. A second oil tray was kept dark, serving as a reference. Total irradiation of the oil in 24 hours approximated a bright sunny day at 40°N latitude (55 gcal/cm^2/day). Oil temperatures ranged from 21-23°C.

Within 12 hours of the start of irradiation, the oil became visibly turbid, and increased in turbidity for 3 days, after which fine particulate material settled into a resinous film. This precipitate was highly oxidized, and contained alcohols, carbonyl compounds and carboxylic acids. Peroxide formation occurred at a linear rate for 90 hours. Phenolic compounds increased linearly for 165 hours. Acids, including some with phenolic substituents, were produced at a maximal rate for 91 hours. Total carbonyl compounds increased during this time; they were

largely derived from benzylic hydrocarbons. In the control oil sample, no changes in turbidity, acidity or content of peroxides, phenols or total carbonyl compounds were detected.

A proposed mechanism for photooxidation of fluorene and other reactive polycylic compounds involves: conversion of UV light to an excited state and decay; combination with atmospheric oxygen to form hydroperoxy radicals; abstraction of a hydrogen atom to form hydroperoxides; and heterolytic cleavage to form hydroxyalkylbenzenes. Fluorene hydroperoxide could collapse, forming fluorenone, or cleave heterolytically in the presence of Lewis acids to form an electropositive hydroxyl, and hydroxylate electron-rich aromatic hydrocarbons (82). Other hydroperoxides formed after UV irradiation probably include those of tetralin, indan, cumene and their alkyl derivatives.

2. BIOTRANSFORMATION IN SOIL AND WATER

Microorganisms living in soil and aquatic environments, and in the intestinal tract of animals, are capable of growing on various petroleum fuels and lubricants by utilizing the oils as a carbon source. Bacteria, yeasts and fungi have been investigated with respect to oil biotransformation.

There are many reports in the literature which deal with microbial hexadecane breakdown. However, fuel oils and lubricating oils contain many other paraffinic and aromatic hydrocarbons besides hexadecane. Therefore, studies on petroleum oils similar to SGF No. 1 and SGF No. 2 fog oils, and diesel fuel, will be emphasized in this section.

a. SOIL BIOTRANSFORMATION

Garden soil was inoculated into a mineral-salt growth medium plus either fuel oil or lubricating oil. Mixed cultures living in the soil degraded less viscous fuel oils in 3-5 days at room temperature, forming a completely emulsified substrate. More viscous oils (e.g., lubricants) were degraded more slowly, requiring as long as 3 weeks. The growth peak in the mixed cultures occurred mainly between the 3rd to 6th day of fermentation. When mixed cultures from rapidly fermenting oil of a highly aromatic composition were transferred to a paraffinic oil medium (or vice versa) the growth lagged for several days but the cultures became active again in the new oil within one transfer. Ligher molecular weight oils (fuel oils) were more easily attacked than heavier ones (lubricants), as shown by cell counts. The lighter molecular weight oils disappeared from the surface of the medium more rapidly than the heavier oils. Bacterial counts also showed that paraffinic oils were degraded more rapidly than aromatic oils; cycloparaffinic oils supported growth at an intermediate level as compared to the aromatic and paraffinic oils (85).

Manometric studies of oxygen requirements for degradation of various petroleum oils by mixed cultures showed peaks of respiratory activity during the first 5 days of incubation. Oxygen consumption and carbon dioxide liberation varied according to the rate of shaking, temperature

of incubation and the age of the inoculum. Fermentation of heavier oils containing longer-chain molecules did not produce as much CO_2, and gave a much lower RQ than did lighter oils. A large percentage of molecules of light oil attacked were completely oxidized to CO_2, as indicated by the RQ of 0.65. A much less complete oxidation of heavy oils was noted.

A study of 250 representative pure cultures gave no evidence that a particular morphological type or biochemical reaction could be correlated with any type of oil or oil fraction. With the exception of certain pigmented forms, there was no correlation between colony morphology and reactions in the ordinary laboratory media. No organism was limited to attacking one type of oil, but rather attacked many different oils with about equal facility (85).

The fact that viscous lubricating oils disperse poorly in liquid media could account for their low biodegradability relative to the fuel oils. Another explanation is that the bacteria find the large molecules in lubricating oils difficult to attack. In oils of predominantly paraffinic compositions, however, the middle fractions, including kerosenes and fuel oils through medium weight lubricating oils, are most easily biotransformed by bacteria (85).

In terrestrial environments, biotransformation of No.2 fuel oil in soils was investigated by Raymond et al. (27). Level plots of three types of soil (Pennsylvania Glenville silt loam, pH 5.4; Oklahoma sandy loam, pH 6.3; and Texas black clay loam, pH 7.6) which had not previously been exposed to oil pollution were employed. The oil was poured on the 1.7 x 3.0 m plots, at an application rate of 2.5 kg/m^2 and the soils were tilled to a depth of 15 cm to promote aeration. The oil was readily absorbed into the soil. During winter, little transformation of oil was detected. Additions of fertilizer also did not clearly stimulate transformation. After 1 year, average reductions in oil concentration in the soils ranged from 48.5% to 90%. Silica gel fractionation of oil extracted from the soils showed no difference in the rate of transformation between paraffinic hydrocarbons (eluted with heptane) and total oil, but the paraffinic residues, after one year, exceeded in weight any other oil fraction. More polar hydrocarbons degraded more slowly than less polar ones. Transformation rates did not exceed 500 g/m^2 per month. The hydrocarbon utilizing microflora of the soils, mostly aerobic *Nocardia* species and fungi, were plated on hexadecane growth substrate media and then the oil was added, after which large increases in microflora colonies occurred in cultures derived from all three soils. Regardless of soil type, colonies isolated from treated soils contained a very black pigment which was not present in control soil colonies (27).

b. WATER BIOTRANSFORMATION

In the early literature, Lipman and Greenberg (1932) reported the isolation of a coccus or coccobacillus from a 30 m deep oil well. The organism could completely degrade petroleum to carbon dioxide (83). Even earlier, Sohngen (1906) described bacteria and Rahn (1906)

reported fungi which could utilize petroleum hydrocarbons to support their growth (84). Conditions necessary for microbial attack included: the presence of water and mineral salts; a nitrogen source; oxygen and a neutral pH (85).

Pseudomonas and *Corynebacterium* obtained from water, the intestinal tract of domestic animals, and from disease processes in cows and horses were grown in a mineral-salts-hydrocarbon enrichment medium in which the oils were the only carbon and energy source for bacterial growth. The medium (1 liter of distilled water, 0.2 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g of either NH_4NO_3 or $(NH_4)_2\ SO_2$, and 2 drops of concentrated $FeCl_3$) was solidified by 2% agar, pH 7.0-7.2. Kerosene was poured over the surface of the inoculated medium. The oily layer did not interfere sufficiently with oxygen diffusion to affect the growth of aerobic cultures. *Pseudomonas* strains which were able to utilize petroleum hydrocarbons did not produce appreciable quantities of organic acids (as would be indicated by pH changes in the medium). Long chain fatty acids and cycloparaffinic acids are weak and would not affect pH greatly, however. Increased ease of emulsion formation of degraded oil and water was noted. *Pseudomonas pyocyaneus* grown on a kerosene medium did not cause any changes in the kerosene's boiling range until only the last 20% of the distillation volume remained. At this point, a marked expansion in boiling range was noted (average 22°C increase over undegraded kerosene). The increase might have been due to the formation of polymers of unsaturated hydrocarbons during the distillation process. The unsaturation might have resulted from bacterial dehydrogenation of the kerosene hydrocarbons (84).

The respiratory quotient, RQ, (CO_2/O_2) of bacteria grown on various petroleum products varied from 0.50 to 0.70. Hydrocarbons underwent complete oxidation during degradation. No appreciable amounts of water soluble end-products such as acids or alcohols were obtained, and no appreciable pH changes were noted. The possible production of oil soluble acids might have been the cause of increased emulsifiability of the degraded oil. *Pseudomonas* would be capable of oxidizing these fatty acids further. Sterols and other fat soluble substances were not isolated. A species of *Corynebacterium* isolated from an oil field sedimentation pond and grown on petroleum hydrocarbon media produced oil soluble pigments ranging from dark red through orange to yellow. Characterization of the pigments and comparison with carotenes isolated from fresh carrots showed that B-carotene was produced (86).

Mycobacterium cultures produced yellow or orange groups of oil soluble pigments while growing in a mineral-salt medium containing a refined lubricating oil with the viscosity of SGF No. 2 fog oil. *M. laticola* isolated from a crude oil sedimentation pond in an oil field in Kansas was grown in this medium; an opaque, then yellow, band of growth at the oil-water interface formed and became more heavily pigmented as growth continued. Three carotene pigments with absorption maxima similar to pure B-carotene were isolated from the medium. Vitamin A activity was present in two of the three pigments, as determined by rat feeding experiments. A fourth substance isolated from

the water phase after saponification of the bacterial growth medium was a red acidic pigment chemically similar to tetraketo-B-carotene (astacin). Bacterial astacin was similar to lobster astacin in absorbance properties. When *M. laticola* was cultured on nutrient agar, xanthophyll pigments and carotenes but not astacin were produced. Haas and Bushnell suggested that the carotene pigments were oxidized to polyketo-derivatives such as astacin in the petroleum hydrocarbon media (87).

Muddy Creek, an oil-free estuarine marsh near Chesapeake Bay, supports the growth of ecologically important bacteria, yeasts and fungi. Mixed cultures were prepared with 400 ml of creek water and 100 g of sediment, and No. 2 fuel oil, in a concentration of 0.1% V/V (1000 mg/l) was used as an overlay. The stoppered flasks were incubated on a reciprocal shaker, and aliquots were drawn on days 1, 7, 14, 21 and 28, and plated to count the microorganisms. Control cultures were incubated without oil. In comparison with controls, bacterial growth in oil was enhanced as the oil was degraded to support growth (88).

A similar 8-week experiment utilized No. 2 fuel oil (1000 mg/l) overlays in flasks containing Muddy Creek sediment and creek water. Heterotrophic, proteolytic, lipolytic, chitinolytic and cellulolytic bacteria achieved greater growth in oil than controls. But when growth curves were normalized by comparing the percentage of lytic bacteria in oil and control samples, the highest percentage of lytic bacteria was observed in controls. Only the growth of lipolytic bacteria was enhanced by the oil. The concentration of oil which dissolved in the water phase was 50 mg/l. Factors influencing the oil concentration included evaporation of oil from the flasks (7%), adsorption by the sediment (9-20%) and transformation of oil (77%) over the course of the study (88).

Seasonal variation in hydrocarbon oxidation in a number of lakes of varying water quality in northern Wisconsin was studied (89). In petroleum hydrocarbon media, initial lags in oxidation were followed by linear increases in oxygen uptake and CO_2 release. Incubation of samples on a rotary shaker did not influence the oxidation kinetics. Low temperatures prolonged the initial oxidation lag, due to a resultant low microbial population density. Seasonal variation in oxidation rates in natural surface water microbial communities of one lake was followed from mid-March, with the ice break-up, until mid-October. In samples incubated at temperatures corresponding to indigenous water temperatures, oxidation rates were intially low, increasing during spring, maximal in June, decreasing and remaining low until the end of October. The numbers of heterotrophic and oil- and hexadecane-oxidizing bacteria varied over one order of magnitude. The proportion of hydrocarbon utilizing bacteria to heterotrophic bacteria remained low. Low water temperatures slowed oil oxidation and also increased the lag phase. The optimum temperature was 20°C or higher (summer). Below 20°C the low temperature inhibited oxidation. Fall temperatures averaged 12°C and the winter temperature was 0°C.

Nitrogen and phosphorus availability also affected biotransformation. In nutrient limitation experiments, or in samples collected when indigenous nutrient levels were low, addition of nitrogen, as KNO_3, or phosphorus, as KH_2PO_4, alone did not give maximal oil oxidation levels. In the presence of both nutrients, the nutrient in the lower concentration determined the oxidation rate. Half-saturation concentrations for growth rates of natural communities on mineral oil or hexadecane were about 20 µg of P and 50 µg of N per liter of water. Bloom-forming algae compete for N and P, lowering their levels in the epilimnion of eutrophic lakes. In oligotrophic lakes which receive little nutrient input, nutrient limitation would be an important concern in oil biotransformation (89).

After addition of petroleum hydrocarbons to the lake water media, there was a lag phase of at least 20 hours before measurable oxidation occurred, coincident with the development of the bacterial population. During the lag phase oxidizible organic compounds indigenous to the lake water were metabolized. It is possible that enzyme induction was necessary to begin oil biodegradation, or that repression of hydrocarbon-oxidizing enzymes during growth on natural substrates had to be overcome. In water samples aged to remove these compounds prior to addition of oil hydrocarbons, the lag phase was shorter, but not eliminated. Regulatory interference of hydrocarbon metabolism in nature might be significant in the presence of both oil pollution and oxidizable organic compounds (89).

Blumer et al. (75) stated that in the marine environment, microbial attack of No. 2 fuel oil preferentially depletes n-alkanes. Hydrocarbons within the same homologous series are attacked at roughly the same rate. Extensive microbiological transformation of branched alkanes is noted, whereas cycloalkanes and aromatic hydrocarbons are more resistant, disappearing from an oil spill area at a much slower rate. The combination of dissolution in seawater with biotransformation leads to an initial decrease in low-boiling aromatic hydrocarbons, followed by a decrease in saturated hydrocarbons, and a resultant increase in aromatic hydrocarbons relative to the saturates. Eight months following a No. 2 fuel oil spill into Buzzards Bay, Massachusetts, dissolution and transformation had produced a slow but recognizable breakdown of the oil, as demonstrated by chemical analyses. After 2 years, small amounts of n-alkanes were still detectable in oil spill areas. Branched and cyclic alkanes such as phytane, pristane and the C_{18} isoprenoid, and alicyclic and aromatic hydrocarbons remained prominent in the polluted sediments. The overall rate of depletion of saturated and aromatic hydrocarbons was the same; the ratio of saturated hydrocarbons to aromatics did not change in this time. This was thought to be a result of preferred bacterial transformation or dissolution of some components within each hydrocarbon group. In marsh sediments, natural permeability and aeration had permitted biotransformation of the spilled oil at a depth of 60 cm from the surface (77).

Candida lipolytica, a yeast isolated on an oil field, selectively degrades n-alkanes; this process may be applied to refining gas oil by decreasing its n-alkane content and thus decreasing its freezing point.

The gas oil was characterized as 20% paraffinic hydrocarbons from nonane to triacotane. A yeast culture media with 10% gas oil produced a biomass of 2 g of dry weight per ml of volume. A curve representing yeast biomass versus oil freezing point showed a prolonged phase of slow freezing point decrease as the yeast biomass grew. This was followed by a rapid decrease in freezing point. Gas oil concentrations above 10% caused disproportionate changes in yeast biomass and freezing point lowering, due to the facts that the representation of n-alkanes in the oil determines freezing point and the yeast probably did not degrade all n-alkanes simultaneously or at the same rate. Initially, there was a greater decrease in lower molecular weight alkanes (C_{10}-C_{14}) relative to higher molecular weight alkanes $\geq C_{18}$. Medium molecular weight alkanes (C_{15}-C_{17}) were degraded at a constant rate throughout. Further degradation of the oil resulted in increased breakdown of the larger alkanes, which finally affected the freezing point (i.e., deparaffination). Rapid deparaffination only occurs after depletion of n-alkanes smaller than C_{15} (90).

Penicillium sp. were grown in a mineral-salt medium containing 0.5% n-hexadecane by volume, for 3-5 days in a shake culture. Ultrastructural examination revealed mycelia with a central core containing this petroleum hydrocarbon. Mycelia from the inner rind contained many oil inclusions, while the outer margin of the mycelial balls revealed typical fungal ultrastructure. The petroleum inclusions were reportedly similar to those found in yeasts and bacteria grown on media containing paraffinic hydrocarbons (92).

No. 2 fuel oil did not affect the growth of yeast in mixed cultures from sediment of Muddy Creek, an oil-free estuarine marsh near Chesapeake Bay. In the experiment, 100 g of creek sediment were mixed with 400 ml of creek water; 0.1% (1000 mg/l) of No. 2 fuel oil was added to the cultures as an overlay and the flasks were incubated for up to 8 weeks on a reciprocal shaker. Oil dissolved in the water phase to a concentration of 50 mg/l. Enumeration of yeasts grown in the presence or absence of oil revealed no differences in the populations, suggesting that the fuel oil did not support yeast growth (55).

Muddy Creek fungi were grown in mixed cultures derived from 100 g of Creek sediment in 400 ml of creek water to which 0.1% (1000 mg/l) of No. 2 fuel oil was added as an overlay. The flasks were incubated on a reciprocal shaker for up to 8 weeks. The oil dissolved somewhat in the water phase reaching a concentration of 50 mg/l. Fungi, mostly Eurasian milfoil, *Myriophyllum spicatum*, were not affected by oil; growth was no different than in cultures grown without oil. Oil did not enhance growth (55).

Marine fungi may be significant in oil transformation. Mycelia of *Aspergillus, Mucor, Penicillium* and *Cunninghamium* were found growing in the spray zone of rocks above the mean high tide line in an area heavily polluted by stranded fuel oil (92).

C. BIOCONCENTRATION AND BIOACCUMULATION

Bioconcentration of petroleum oils similar to the fog oils, SGF No. 1 and SGF No. 2, and diesel fuel occur in plants, animals and some microorganisms. Oil bioaccumulation in the food chain has been demonstrated in the marine environment following oil spills as well as in terrestrial plants. Oils may be transformed during uptake and storage as well.

Chronic oil pollution contaminates those waters necessary for survival of most marine organisms used for human food. Progressive disappearance of fish and shellfish, plus increases in numbers of oil-tolerant species, have been observed. Remaining organisms of food value to humans may be permanently contaminated with petroleum hydrocarbons that could be hazardous to the health.

1. BIOCONCENTRATION BY AQUATIC ORGANISMS

An accidental spill of approximately 600,000 liters of No. 2 fuel oil into Buzzards Bay, Massachusetts resulted in immediate kills of fish and shellfish, dispersion of oil into nearly marsh muds, and accumulation of oil in many marine species, according to chemical analyses of organisms collected one year after the event (75,76).

Green algae, *Enteromorpha clathrata,* accumulated oil without transforming it, thus constituting an important source of entry of oil into the marsh food chain. Red algae contained much less oil. Higher plant forms *(Spartina* and *Salicornia)* also took up the oil. These plants provide the bulk of food for detritus feeders, and therefore this is another important route of entry of oil into the food chain if oil remains in the plant detritus or associated bacteria of the decaying plant.

Ribbed mussels, *Modiolus demissus,* absorbed oil directly from polluted water by filter feeding and/or by consumption with their particulate diet. The large amount of oil which was incorporated into their tissues showed evidence of biochemical breakdown, in that cyclic and aromatic hydrocarbons were predominant while paraffins were at very low time levels. Blue mussels, *Mytilus edilus,* contained an average of 22 µg of oil hydrocarbons per gram of tissue.

Fish also contained highly transformed oil in their tissues. Two herring gulls, *Larus argentatus,* which were sacrificed (one adult and one juvenile which had fed in the polluted area), had incorporated petroleum hydrocarbons into their fatty muscle tissue and brain. Paraffins predominated in the muscle while aromatic hydrocarbons were contained in the brain tissue. It was concluded that virtually all marsh organisms living in the No. 2 fuel oil polluted area were affected by the oil to the extent that they concentrated or accumulated petroleum hydrocarbons in their fatty tissues (68,76).

In young adult marine annelids, *Neanthes arenaceodentata*, a 4-hr exposure to the water soluble fraction of No. 2 fuel oil, prepared by slowly stirring 9 parts of 32‰ saline artificial seawater with one part of oil for 20 hr, and removing the aqueous phase for use, resulted in the following tissue concentrations of petroleum hydrocarbons (in µg/g of wet weight):

C_4-substituted benzenes	1.9 µg/g
Naphthalene	0.9 µg/g
1-Methylnaphthalene	3.0 µg/g
2-Methylnaphthalene	3.7 µg/g
Dimethylnaphthalenes	5.8 µg/g
Trimethylnaphthalenes	1.5 µg/g
Total aromatics	20.0 µg/g
Total paraffins	5.0 µg/g

The bioconcentration of methyl-substituted naphthalenes in tissues of this marine worm exceeded that of paraffins (28). (The original No. 2 fuel oil was not characterized with respect to percentages of aromatic and paraffinic hydrocarbons).

When these worms were exposed for 24 hr to a 25% dilution of the above-described water soluble extract of No. 2 fuel oil, and then placed in clean seawater for varying intervals before sacrifice and determination of petroleum hydrocarbons by ultraviolet spectrometry, naphthalenes were again found to concentrate rapidly in both sexes. Males steadily released the accumulated naphthalenes when placed in clean seawater; by 72 hours, the majority of the naphthalenes had been released. Complete depuration (less than 0.1 ppm naphthalenes remaining in body tissues) was evident in 400 hr. Gravid females failed to steadily release the accumulated hydrocarbons in clean seawater. Levels remained high until spawning. Immediately after egg release, tissue naphthalene concentrations in 24 worms were just detectable (0.2 ppm). Zygotes and trochophore larvae contained 18 ppm of total naphthalenes· (Two hundred were analyzed). In 18-segment juveniles (21 days after spawning) there was 0.4 ppm of naphthalenes. In 32-segment worms (26 days after spawning) naphthalenes were not detected. The high diaromatic hydrocarbon levels in offspring of the oil-exposed gravid female worms were probably the result of compartmentalization of the hydrocarbons into yolk matter and lipid fractions of the polychaete eggs. Transfer of the oil from one gèneration to the next did not occur; the naphthalenes were released completely by juveniles during utilization of the yolk material in their development (30).

The bay mussel, *Mytilus edulis*, represents a major pathway in the intertidal ecosystem for energy transfer utilizing phytoplankton and debris. The ability of this mussel to concentrate petroleum hydrocarbons was investigated (93). Mussels were exposed to a simulated oil spill environment in a tank with a slick of No. 2 fuel oil (0.55-1.53 nm thickness) for 46 hours. (No oil in water concentrations were provided.) The mussels were then placed in clean seawater. The concentration of petroleum hydrocarbons after one day in the clean seawater was 110 ppm (dry extracted weight) for specimens exposed to the slick, compared with 29 ppm for mussels submerged in water under the slick, and less than 8 ppm in unexposed mollusks. Within 7 days, the residual oil content dropped by 75% (to 50 ppm) and continued to decline. After 35 days in clean seawater the level was still above 8 ppm of petroleum hydrocarbons. N-paraffins extracted from the mussels were chemically similar to those in the No. 2 fuel oil which had been used (93).

In a study of petroleum oil uptake in clams, *Mercenaria mercenaria* concentrated the oil through filter feeding (94). Clams exposed to 3.9 mg /l of No. 2 fuel oil in filtered seawater for 8 hours were then analyzed for petroleum hydrocarbon content by gas chromatography. No attempt was made to differentiate among hydrocarbons on the gill, adsorbed on other membranes or tissues, concentrated in body fluids or incorporated into tissues. The clams took up 1.7 µg of petroleum hydrocarbons per gram of wet weight. When the seawater was charcoal treated to remove dissolved organic matter (DOM) prior to oil addition, the exposed clams took up 12.2 µg/g of wet weight. This indicated a 7-fold increase in oil uptake. A disproportionately large quantity of methyl-substituted naphthalenes was taken up relative to the amount present in the No. 2 fuel oil. Saturated hydrocarbons (paraffins and cycloparaffins) were taken up 17 times more readily by clams feeding in the charcoal treated oily seawater than in the filtered oily seawater: 5.0 µg/g and 0.3 µg/g, respectively. In addition, the alkylnaphthalenes were concentrated twice as readily by clams in charcoal treated oily seawater than in filtered oily seawater: 1.9 µg/g vs 1.0 µg/g. The alkylnaphthalenes comprised an average of 59% of the total concentrated hydrocarbons in the charcoal treated oily seawater. The DOM in seawater solubilizes saturated hydrocarbons and makes them less readily taken up by filter feeding. Therefore, the concentration of saturated hydrocarbons which did occur probably resulted from active uptake rather than simple equlibration across gill membranes. In the complex mixture of alkanes, cycloalkanes and aromatic hydrocarbons in No. 2 fuel oil, aromatic compounds may be co-solubilized within DOM micelles with the saturated hydrocarbons. The aromatics also dissolve in seawater. Other factors influencing the incompletely understood uptake mechanisms for petroleum hydrocarbons were discussed (94).

Once contaminated, shellfish cannot completely cleanse themselves of oil but incorporate it into their lipid pool (75,93). As in fish, the presence or absence of oily smell or flavor is insufficient to indicate oil contamination because even a small fraction of the oil has a pronounced odor. While boiling or frying may remove the odor, the hydrocarbons remain in the fish flesh, and ingestion may put humans at risk (75).

In oysters and scallops in the area of the Buzzards Bay No. 2 fuel oil spill, the petroleum hydrocarbons recovered in their tissues was very low in n-alkanes and low in branched alkanes compared to the original fuel. Cycloparaffins and aromatic hydrocarbons remained in the organisms' lipid pool. Scallops and quahaug clams exposed to the spill as juveniles and upon reaching maturity, contained oil which was low in n-paraffins and low molecular weight hydrocarbons, indicating possible biotransformation of these components prior to storage. One year after these observations were made, oil contamination of shellfish persisted, but the new crop had an oil content with a greater proportion of aromatic hydrocarbons than the earlier crop of shellfish (75).

Fresh and salt water species of fish living or migrating in waters which are oil polluted have been found to incorporate the petroleum hydrocarbons into their flesh. This may change the odor and taste of the fish, making it unfit for human consumption. Chemical analyses of fish flesh have revealed detectable concentrations of petroleum hydrocarbons in fish which did not appear to be tainted.

Early reports of rainbow trout acquiring on oily flavor after exposure to petroleum refinery waste water led Sidhu et al. (1970) to experiments which showed that aquarium mullet acquired the oily taint when placed in water containing 5 mg/l of kerosene for 24 hours. (95,96).

Sea mullet, *Mugil cephalus*, from the South Queensland coast of Australia were found to have a kerosene-like odor and flavor, resulting in condemnation of the catches and limitation of fishing. The taint was most noticeable in May and June, a time coinciding with the mullets' migration from estuaries toward the north for spawning on the Brisbane River, where large catches were made. River water was analyzed by mass spectroscopy and gas chromatography. N-tetradecane, naphthalene, and benzene and naphthalene derivatives were determined. Bottom sediments collected along the Brisbane River included fine grey-black silt with high petroleum hydrocarbon content as well as patches of coarse gravel with low hydrocarbon content. The mullet ingested mud and sand during feeding, as indicated by the gut contents of captured fish. The sediments which were analyzed showed a similar chromatograph to that of the water. Both were similar to commercial kerosene samples.

Sea mullet from different locations were caught and the side fillets were analyzed for petroleum hydrocarbons. Extraction of fish flesh with diethyl ether and steam distillation yielded 24-310 ppm of volatile petroleum hydrocarbons in obviously tainted fish. Gas chromatographic examination indicated a similar hydrocarbon composition to volatiles from river sediments and to commercial kerosene (95). A more detailed investigation was performed, by the same author, on South Queensland and northern New South Wales sea mullet. This time, side fillets were placed in boiling water and the vapors were subsequently assessed for odor intensity. Obviously tainted fish flesh had concentrations of volatile constituents of 82-270 mg/kg. "Untainted" flesh samples were contaminated with lower levels of 11-77 mg/kg of kerosene volatiles (96).

The oil in sediments polluted by kerosene from sewage effluent discharges along the Brisbane River was ingested by the mullet; gill absorption of oil could also have occurred. Petroleum hydrocarbons in the gut were absorbed unchanged, transported through the liver of the fish, unchanged, and deposited in the fishes' fat pool (96).

Eleven days following a diesel fuel spill into a river in Northern Ireland, brown trout *(Salmo trutta* L.) which were caught were contaminated with the oil. Trout were eviscerated, and petroleum hydrocarbons were extracted from the flesh and analyzed by column and gas chromatography, and ultraviolet and fluorescence spectroscopy. The fish samples, prior to cooking, possessed no noticeable foreign odor, but after cooking, a characteristic odor and taste strongly reminiscent of diesel oil was noted, when compared with unpolluted (normal-tasting) fish flesh. The tainted flesh had a strong absorption band in the aromatic hydrocarbon region of the UV spectrum, similar to that for diesel fuel, whereas the non-oil-polluted fish flesh lacked this absorption (275 nm). Fluorescence spectra for the tainted flesh and diesel fuel were also markedly alike. Natural fish oils had dissimilar gas chromatograms from those of petroleum oils, especially in the low molecular weight end of the spectrum. Petroleum hydrocarbons which were isolated from fish included C_{11}-C_{20} paraffins and aromatics, which are normally found in diesel fuel. Concentrations of hydrocarbons in the fish were not reported (97).

The significance of oil biocentration by commercially important fish lies in the fact that humans ingesting these fish will also be ingesting the oils. Although there is no direct evidence for or against toxicity due to chronic ingestion of low levels of petroleum hydrocarbons, polycyclic aromatic hydrocarbons found in lubricating oils and other petroleum products are proven carcinogens and mutagens, as documented in Volume 4 of this series of reports (2).

2. BIOACCUMULATION IN TERRESTRIAL ANIMALS

There is no available literature dealing with bioaccumulation of petroleum hydrocarbons in terrestrial animals. However, the above-mentioned report (2) does contain data on mammalian metabolism of petroleum hydrocarbons. Blumer et al. (68) compared oil products to such persistent poisons as DDT, PCB, and other synthetic compounds. The oil hydrocarbons entering the marine food chain are concentrated in the fatty parts of the organisms, and can be passed from prey to predator, up to and including humans. Oils may also concentrate other fat soluble poisons such as insecticides. The poisons could dissolve in an oil film and reach high concentrations in water. The poisons would then become available to organisms usually not exposed to them, and at concentrations greater than those obtainable in the absence of petroleum (68).

3. BIOCONCENTRATION BY PLANTS

Oil uptake in plants has been demonstrated. In living apple leaves to which undiluted petroleum oils were applied, transluscence resulted as oil replaced air in intercellular spaces. Oils with low viscosities,

similar to fuel oils, penetrated faster (1 minute) than heavier lubricating oils (up to 30 minutes). The oil spots changed very little in size, shape, color or distribution after they stabilized in 2-60 minutes, indicating that evaporation of petroleum hydrocarbons from inside the leaf was negligible. Sectioning of apple leaves 7 days after oil application revealed intra- and intercellular oil deposits in vein and petiole-parenchyma cells and tracheae, and between spongy, palisade, vein and petiole-parenchyma cells (64,65).

In apple leaves and limbs sprayed with a 16% emulsion of a low viscosity oil (50-108 seconds Saybolt at 100°C), oil was found 488 days later in attached stems inside the tracheae, tracheids, pith parenchyma and medullary ray cells. Over a 3-year period, oil moved from old to new wood (65), most likely travelling through intercellular spaces (64).

The same oil was injected into glass tubes inserted into apple limbs and trunks, in quantities of 10-25 ml. Bark became oily within 10-15 cm from the point of entry of the tubes. After 9 to 12 months, oil was found 5-88 cm below the oil injection holes, occurring mainly in the tracheae and sides of annual rings vertically connected with the oil injection holes (65).

Drops of a low viscosity petroleum oil were placed on apples to study penetration and distribution. Oils entered green apples through the lenticels and caused spotting in 5-60 minutes. Parenchymal cells and some tracheae within 1 mm of the epidermis became oily, and most of the oil was intercellular. Oils applied to the stem cavities and calyx basins of ripe apples were absorbed in traces, within 1-4 weeks, and were located in the core cavities and calyx basins, with abundance in inter- and intracellular locations (65).

Many petroleum oils were investigated in potato leaves, turnip leaves, onion and other crops to determine the movement of oils through the plants. Paraffinic, aromatic and cycloparaffinic oils, with viscosities between 51-110 seconds Saybolt at 100°C were tested. Oils placed on living potato leaves penetrated and occupied spaces between mesophyll cells. Stems attached to leaves became oily in 1-5 days after application of either fuel oil or kerosene. Oil movement was mainly extracellular. Oils were abundantly distributed between starch-parenchyma cells, with some deposition inside parenchyma cells and tracheae (64,67).

Kerosene penetrated potato leaves in 0.5 to 10 seconds, but evaporated in 1-24 hours. Lubricating oils, however, penetrated potato leaves in 1 second to 5 minutes, and usually remained in the leaves for the duration of their growth period. Oils passed from potato leaves through stems and into the tubers, via intercellular pathways (67).

Oil distribution occurred similarly in turnip, rutabaga and barley leaves to which fuel oil or kerosene was applied.

Oil placed on cotyledons of seedling cucumbers and squashes caused translucence in 10 seconds, and oil spread to attached stems in 1-14 days. Stem sections revealed oil between cortical parenchyma cells (67).

Oil which was stained with Oil Red O and applied to living turnip leaves caused attached petioles and roots to turn red within 8 days. Oil was distributed between parenchyma cells in the turnip roots (64).

Injection of 1-4 ml of red stained oil into the tip of hollow leaves of onion bulbs with leaves 25 cm long caused reddening of 30-90% of attached roots in 2-10 days. The hollowed leaves acted as oil reservoirs for 3-9 days. Oil was located in sections of roots and bulbs of the onions after this time (64).

IV. SAMPLING AND ANALYSIS

Residues of petroleum products, including both fuels and lubricants in fresh water, ground water, soil, sediments, estuarines, oceans, plants and water-dwelling animals can be examined to identify the type of oil and its quantity. Sampling and analysis of fuels and lubricants in the air and in some biologic media have been reviewed previously in the Problem Definition Studies on Diesel Fuel and Fog Oils (1,2), which are a part of this series of eight reports.

A. EXTRACTION METHODS

Petroleum residues in living material, water and other substances can be extracted by various solvent and procedures.

Oil extractions from fresh water with trichlorotrifluoroethane or carbon tetrachloride were found to be equally effective. Water in oil dispersions were prepared by shaking 5 ml of No. 2 fuel oil in 1.5 liters of tap water for 1 min, allowing the 2 phases to separate for 15 min, and then mixing the bottom layer with an equal volume of tap water. Complete separation of oil was not achieved after fifteen extractions with 25 ml of either solvent. Addition of 5 g of sodium chloride and 5 ml of 50% sulfuric acid to 1 liter of the water in oil dispersion, prior to extraction, permitted the removal of more than 90% of the emulsified No. 2 fuel oil in the first extraction. In seawater, it would be unnecessary to add salt. Trichlorotrifluoroethane is the preferred solvent, because it is essentially nonpoisonous (TLV* 1000 ppm) when compared with carbon tetrachloride (TLV 10 ppm) (98). Three extraction techniques for aqueous phase oil were compared. In the first technique, a reciprocal shaker with a 3-4 cm horizontal stroke and a speed of 150 strokes per minute was used in conjunction with 50 ml of carbon tetrachloride in a 15 minute extraction of a 2 liter petroleum oil sample in a 2.5 liter bottle. Mean recovery was 22% when 3.8 to 188 ppm oil had been added. The second technique utilized a stainless steel impeller in a sample bottle which was immersed in a deep ultrasonic cleaning apparatus for 15 minutes and centrifuged at 2100 ppm, gave a mean recovery of 102% (relative to the first and second techniques) with 3.9 to 4.8 ppm of oil added (99).

In another experiment, oily seawater collected in polyethylene bottles was extracted with either carborn tetrachloride or methylene chloride. Methylene chloride extracted three times as much oil as carbon tetrachloride. The extracted material could be stored for as long as 14 days in darkness at room temperature or at 5°C without decreasing the oil content (100% oil recovery as determined by fluorescence spectroscopy). When the extracts were exposed to laboratory lighting and room temperatures during storage, only 25% oil recovery was obtained after 7 days of storage (100).

It is possible that solvent extraction with methylene chloride or carbon tetrachloride does not remove non-hydrocarbon compounds

*TLV - threshold limit value for occupational exposure

with aromatic chemical structures, such as certain amino acids and humic and fulvic acid present in seawater, because the determination of petroleum hydrocarbons did not show interference by these acids (100).

Prior to spectrophotometry or chromatographic determinations, extraction of petroleum hydrocarbons dissolved in water can also be accomplished with n-heptane (100,102).

Petroleum hydrocarbons from aqueous effluent were separated out and concentrated by adsorption on activated carbon, followed by removal of water and quantitative elution acetone (103).

Steam distillation was used to isolate petroleum hydrocarbons from diesel fuel-contaminated whitefish (104).

Removal of petroleum hydrocarbons from mussels was accomplished by refluxing the flesh with methanol-benzene Soxhlet extractor, prior to chromatographic determination (105).

B. ANALYSIS

Oils of unknown source can be identified by comparing them qualitatively to known oil samples. By most of the following methods, a fuel oil can be distinguished from a lubricating oil of petroleum origin. The application of several techniques may be necessary in matching an oil to its source, due to weathering changes which the oils undergo after they enter the environment (106).

Gas chromatography, infrared spectrometry, fluorescence spectroscopy and ultraviolet fluorescence spectrometry of 20 oil spill samples were compared. All 4 techniques agreed on the type of oil in 45% of cases (N = 9), and at least 3 techniques agreed 73% of the time (N = 14) (107).

Gas chromatography, fluorescence spectroscopy, ultraviolet absorption spectrophotometry, infrared spectrophotometry, laser-Raman spectroscopy, remote sensing and other methods will be examined.

1. GAS CHROMATOGRAPHY

Gas chromatography on high-efficiency packed columns in suitable for rapid identification of traces of petroleum products contaminating natural waters. Concentrations of No. 2 fuel oil, diesel fuel, kerosene and lubricating oils are differentiated by comparing boiling range spread, retention time and relative peak heights (106,108-110). Artificially weathered oils can be correlated with their original source (111).

In biologic samples, such as aquatic fauna, gas chromatography is not particularly sensitive for detecting polycyclic aromatic hydrocarbons (112).

Separation of aromatic and aliphatic hydrocarbons is possible. Disadvantages include interference from polar compounds and other hydrocarbons not originating in the petroleum product, but which are detected

on the chromatogram. If these interfering polar compounds are removed in CCl_4 extracts of the aqueous samples, oxidized oil components and other oil hydrocarbons are also removed, which makes complete quantitation impossible (113).

In analysis of oil-contaminated drinking water, the constituents were identified by gas chromatography of the vapor condensate which collected at the outlet of the apparatus (114).

Gas chromatographic determination was employed after extraction with methanol-benzene followed by column chromatographic separation (silica gel-alumina) n-paraffins from a sample of flesh from oil-polluted mussels. Flesh concentrations of < 5 ppb of n-paraffins containing 14-37 carbon atoms were determined (105).

Computerized gas chromatography-mass spectrometry was used in analyzing petroleum hydrocarbons which were dissolved in estuarine water polluted by No. 2 fuel oil. Water samples were extracted with n-heptane or methylene chloride, and run in didecyl phthalate or OV-25 columns to separate alkylbenzenes or substituted naphthalenes, respectively. All isomers of dimethyl-, diethyl-, methyl-ethyl-, methyl-isopropyl-, trimethyl-, and tetramethylbenzenes were found. All dimethyl-ethylbenzenes, except the 1,3,5-isomer, were detected. Thirty substituted benzenes were identified; 16 more were separated and partially identified. All isomers of monoalkylnaphthlenes (methyl- or ethyl-), four of ten possible dimethylnaphthalene isomers, two of 15 possible trimethylnaphthalenes, seven C_3-substituted naphthalenes, all possible methyltetralins and tetralin (1,2,3,4-tetrahydronaphthalene), ethyl-, dimethyl-, and trimethyltetralins were separated. Also identified were indanes, biphenyls, acenaphthalene, fluorene, anthracene, dibenzofuran and alkyl substituted isomers with 7-15 carbon atoms. Four n-alkanes, with 10-13 carbon atoms, were isolated (101).

2. SPECTROSCOPIC METHODS

These include fluorescence spectroscopy, ultraviolet absorption spectrophotometry (UV), infrared spectrophotometry (IR) and laser-Raman spectroscopy.

IR and UV procedures were more sensitive and accurate than gravimetric procedures (98).

a. FLUORESCENCE SPECTROSCOPY

This method is recommended for seawater containing petroleum residues (100), and can detect the equivalent of 1 µg/l of crude oil in seawater (ppb range) (102). The technique is one of many employed by the U.S. Coast Guard for fingerprinting oil spills (106).

Methylene chloride extracts are evaporated and n-hexane is added prior to excitation at 310 nm. Fluorescence is measured at 374 nm, and compared to samples with known oil concentrations to quantitatively determine the extract (100). Excitation at 260-280 nm also is reported, with fluorescence emission spectra from 330-335 nm (102).

Fluorometric characterization and quantitation, with pyrene as a fluorescence standard, was rapid for screening aquatic fauna for the presence of polycyclic aromatic hydrocarbons (112).

In waste water and lye, dissolved or emulsified petroleum oils in quantities of 10-1,000 mg/l were analyzed. For diesel fuel, there was a double band in the ultraviolet at 328-340 nm and another band near 400 nm, using excitation wavelength of 270 and 345 nm. The method is a adequate for any oil in water, provided no other fluorescent materials are present (e.g., in lye). In the case of lye, detection sensitivity for oil is decreased. By marking the oil with a fluorescent substance (a high molecular weight cyclic compound was suggested) the oil can be detected more readily in lye solutions (115).

Oil components separated from water samples by thin-layer chromatography were detected by fluorescence under ultraviolet light at 365 nm and 254 nm. Iodine staining was an aid in visualizing unsaturated, nonfluorescing compounds. Kerosene, diesel fuel, fuel oils and lubricating oils were fingerprinted (107).

b. ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY

Ultraviolet absorption spectrophotometry is useful in the identification of petroleum products in the marine environment. Comparison of the actual spilled oil with a standard can help identify the source of an oil spill. Only about 0.06 mg of oil is required.

For distillate fuel oils, including diesel fuels, a prominent absorption peak occurs at 224-228 nm and there is a less pronounced peak at 253-256 nm. It is not possible to distinguish between different fuel oils of the same general type (111).

Lubricating oils are distinguished from fuel oils by their weak or absent absorption at wavelengths greater than 250 nm, and a very slightly defined peak around 228 nm. However, spectra for lubricating oils as a group do not differ from each other sufficiently to provide a means of distinguishing between them (111).

c. INFRARED SPECTROPHOTOMETRY

Petroleum oils in effluents and sewage sludges were quantitatively estimated after separation of these oils from vegetable oils by silica gel adsorption and elution with carbon tetrachloride (108).

Oil samples collected from surface waters were analyzed by the method of ratios of infrared absorbance peaks, using 6 wavenumbers. Lubricating oils could be distinguished from other petroleum products such as asphalts. One motor oil, similar to SGF No. 2, had the following ratios of infrared absorbances (116):

$$\frac{720\ cm^{-1}}{1375\ cm^{-1}} \qquad 0.48$$

$$\frac{3050\ cm^{-1}}{2925\ cm^{-1}} \qquad 0.20$$

$$\frac{810\ cm^{-1}}{1375\ cm^{-1}} \qquad 0.06$$

$$\frac{810\ cm^{-1}}{720\ cm^{-1}} \qquad 0.12$$

$$\frac{1600\ cm^{-1}}{1375\ cm^{-1}} \qquad 0.11$$

$$\frac{1600\ cm^{-1}}{720\ cm^{-1}} \qquad 0.24$$

Disadvantages of infrared spectrometry include the following (113):

1. Aromatic hydrocarbons, which dissolve better than aliphatic hydrocarbons in water, can only be partially measured by IR;

2. Calibration is nearly impossible, especially if the origin of the polluting oil is unknown;

3. IR methods measure extinctions of all CH_3- and CH_2-groups, whether they belong to hydrocarbons in the oil or other organic material in the water sample. Interference by polar compounds is also problematic, and can be eliminated only by extraction; and

4. the total concentration of dissolved hydrocarbons is measured; different types of molecules are not distinguishable.

d. LASER-RAMAN SPECTROSCOPY

This method of fingerprinting oil spills is utilized by the U.S. Coast Guard. One problem with laser-Raman spectroscopy is the very intense background of fluorescence of oil which can completely obscure any Raman scattering bands which may be present. The addition of powdered charcoal to a dilute solution of the oil removes the fluorescent compounds. Two ml of oil are added to 4 g of coconut charcoal and diluted with 4 ml of pentane; the solution is stirred for 2 min then

filtered to separate the pentane extract from the charcoal; the pentane is evaporated at room temperature. Treated oil is then placed in a capillary tube and treated with a laser beam for 10 ppm to eliminate any remaining fluorescence before recording the spectrum (106).

The method was effective for kerosenes, No. 2 fuel oils and most lubricating oils which have been investigated. Differences between these three petroleum products were clear. A comparison of three different samples of No. 2 fuel oil revealed similar spectra which differed mainly in the relative peak heights (106).

Charcoal treated oils were drastically decreased in fluorescence, but analyses by gas chromatography and infrared spectroscopy showed only slight differences between treated and untreated oils (106).

3. REMOTE SENSING

The U.S. Coast Guard uses remote sensing techniques to gather signature data for oil in the ultraviolet, visible, infrared and microwave spectra, as well as to assess spread rate and distribution of marine oil slicks. Microwave radiometry, spectroradiometry, radar imagery and multispectral scanning are included (117).

Airborne microwave radiometric measurements of controlled oil spills, using No. 2 diesel fuel in one case, included surface observations and airborne measurements over 3 days or until the slicks dissipated. Oil slicks could be positively identified on the open ocean with microwave radiometry. Significant microwave brightness temperature oil slick signatures were recorded for a wide range of ocean conditions and oil film thicknesses in the range of 1 μ.

Airborne measurements to detect oil spills by reflected sunlight utilized absolute radiometry, differential radiometry and polarimetry. All techniques were based on measurements of the contrast of sunlight reflected or backscattered by oil and water. Maximum contrast was noted between oil and water in ultraviolet and red portions of the spectrum, with minimum contrast in the blue-green region. The highest contrast was observed under overcast sky conditions. No characteristic absorption bands were found which would enable the differentiation of one oil from another. The correlation of oil thickness with radiometric measurements is suggested for further research.

Radar imagery of oil slicks on vertical polarization at frequencies of 428 MHz, 1228 MHz, 4455 MHz and 8910 MHz during low sea state conditions were obtained. Slicks were depicted with sharp boundaries at 1228 MHz and above. Spills were mapped from the initial thickness until the equilibrium thickness of 1 micron or less was reached, and area growth rates were obtained.

Aerial multispectral line scanner data were acquired in 17 spectral bands between wavelengths of 320 to 11,700 nm (ultraviolet through thermal infrared). In the ultraviolet region, diesel fuel was brighter than the water background, with some areas darker than the background, due to thickness variations within one slick. Relative spectral radiance of diesel fuel to water shows no reflectance in the blue-green region, and a slight increase in the infrared, with the rest of the visible spectrum being fairly constant (117).

4. OTHER METHODS

Urea adduction may permit identification of oil in polluted natural water (109).

Oil identification by low temperature luminescence is one technique (of many) used by the U.S. Coast Guard (106).

Trace metal analysis, utilizing the ratio of nickel and vanadium content can also be applied to identify the source of spilled oil (110).

Paper chromatography is an established analytical method for petroleum in sewage sludge and effluents (108).

Estimation of approximately 10 mg/l or greater of kerosene hydrocarbons in aqueous effluents was accomplished by separation and concentration of the hydrocarbons by adsorption on activated carbon. Water was removed, followed by elution of the adsorbed hydrocarbons quantitatively with acetone. Turbidity developed in the eluate with acid-laurynl sulfate reagent in proportion to the original concentration of hydrocarbons in the aqueous solution (103).

V. TECHNICAL SUMMARY

This literature review and analysis covers the environmental effects of fog oils, SGF No. 1 and SGF No. 2, and diesel fuel, which have either been used or considered for use in U.S. Army smoke screening operations. These three oils are petroleum products derived solely from crude oil. Diesel fuel and SGF No. 1 fog oil are in the general classification of distillate fuel oils, while SGF No. 2 is a low viscosity lubricating oil. The oils are vaporized and forced through nozzles of a smoke generator into the atmosphere, where the vapors immediately condense into microdroplets of the oil, producing a fog screen which rises and spreads out as it moves with air currents. The oil droplets eventually settle back to the earth, creating an oily film on plants, bodies of water, personnel and objects which are present in the area. Atmospheric dispersion of oil smokes depends primarily upon meteorological conditions during and after the generation of smoke. Estimates of travel distances of the smoke from its source range from 6.4 to 10 km (4). Actual atmospheric concentrations of fog oil smokes have not been made available. Calculated oil concentrations, based on the operation of one smoke generator, utilizing an average of 150 liters of fog oil per hour, range from 15 mg/m^3 to 2000 mg/m^3, depending upon the degree of dispersion of the fog. Likewise, the ground concentration of oil settling down from the fog are not available. If it is assumed that nearly 100% of the 150 liters of fog oil per hour consumed by the smoke generator actually becomes smoke, and that most of the oil droplets fall back to earth (some may evaporate or remain airborne), then the area under the smoke screen can be utilized to estimate ground concentrations of the oil. A range of 6 g/m^2 to 60 g/m^2 in one hour was calculated.

The environmental consequences of fog oil and/or diesel fuel contamination following smoke screening operations have not been adequately assessed, although oily films, oily odors and minor breathing discomfort have been noted (4). In this report, published literature is reviewed on environmental contamination with petroleum fuel oils and lubricating oils similar in physical and chemical properties to fog oils and diesel fuel. The health consequences of fog oil smoke exposure, and toxicological studies in laboratory and domestic animals exposed to fuels and lubricating oils and their smokes, were previously reviewed in two reports: Volume 1, Occupational Health and Safety Aspects of Diesel Fuel and White Smoke Generated from it (1); and Volume 4, Occupational Health and Safety Aspects of Fog Oils, SGF No. 1 and SGF No. 2 and Smoke Screens Generated from them (2).

The present report, Volume 8, is divided into three parts: toxic effects of fuel oils and lubricating oils in lower animals, microorganisms and plants; environmental fate of these petroleum products; and sampling and analytical techniques applicable to environmental investigations of petroleum contamination of water, soils, flora and fauna.

Results of toxicological studies in waterfowl, insects, aquatic organisms and microorganisms exposed to lubricating oil, diesel fuel and fuel oil are presented in Tables 13-20.

A. TOXICITY IN WATERFOWL

In waterfowl, water pollution by fuel oils and lubricants has caused immediate casualties (5,7), abnormal egg yolk and shell production (6), failure of new eggs to hatch (6), cold shock from loss of buoyancy and drowning (7), loss of ability to fly and thus increased vulnerability to predators (7), and excessive preening to the exclusion of feeding, leading to starvation (7). In experimental studies on waterfowl, listed in Table 13, oral administration of diesel fuel and fuel oil is toxic, although doses are dependent upon the experimental temperature, degree of crowding and stress of the birds (9). An average oily duck with approximately 7 grams of oil in its feathers will preen and ingest approximately 1.5 g of oil in one day, corresponding to a dose of 2-3 g/kg of body weight (9). This level of dosing produced oil pneumonia, gastrointestinal disorders including diarrhea and occult blood loss, anemia, altered liver function, reduction in pancreatic zymogen stores and toxic nephrosis in experimental ducks and in wild killed ducks which were oil-exposed in their natural environment (9).

B. TOXICITY IN INSECTS

Petroleum oil sprays have been used as insecticides in California fruit orchards since the 1880's, and are effective against scale insects, defoliating caterpillars, red spider, tree hoppers, codling-moth and aphids (11,12). Leaf parasite control was achieved with a 35% diesel fuel in water emulsion spray on sunny days, but a 50% strength diesel fuel emulsion was required to kill the parasite on cloudy days (13). Diesel fuel and No. 2 fuel oil sprays were reported to protect cattle from mosquitoes (15). Pure kerosene was effective treatment for head lice in the early 1900's (16). It worked by killing the eggs. Fuel and lubricating oils were up to 70% ovicidal to mosquito eggs, oxygen deprivation of oil-covered eggs causing their death (17). Kerosene distilled from California, Pennsylvania and Indiana crude oils were toxic to potato beetle eggs. Freshly laid eggs were more permeable to the kerosenes than 1-2 day old eggs, and were more easily killed (16). Control of Alaskan 4th instar blackfly larva could not be achieved by 7-10 ppm concentrations of fuel oil in stream waters; these concentrations did not damage fish or caddis fly larvae coexisting with the blackfly larvae. Kerosene, in a concentration of 20 ppm, was also ineffective (18). The pouring of kerosene over the content of latrines suffocated and killed maggots of the disease carrying latrine fly, but boiling water was an equally effective larvacide (19). Toxic effects of oils in insects are summarized in Table 14.

C. TOXICITY IN AQUATIC ORGANISMS

Toxic levels of fuel oils and lubricants (similar to fog oils and diesel fuel) in fish and other aquatic organisms are difficult to assess due to the variables associated with mixing oil and water. Dispersions, solutions and suspensions are possible in natural waters polluted with oil, depending upon the degree of agitation of the water, amount of oil,

TABLE 13. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN WATERFOWL

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Waterfowl	field studies in fuel oil-polluted environments	inability to fly; preening to the exclusion of feeding; loss of buoyancy and drowning; loss of insulation against cold.	Hunt and Ewing, 1953 (7)
Waterfowl	field studies in diesel-fuel polluted area	oil swallowing caused burns, pneumonia, internal hemorrhages, comatose behavior	Chia, 1971 (5)
1-year-old Mallard ducks	intragastric administration of gelatin capsules containing >20 mg/kg b.w. of diesel fuel	LD_{50}; weakness, diarrhea, regurgitation.	Tucker and Crabtree, 1970 (8)
Pekin and Mallard ducks	intragastric administration of 4 ml/kg b.w. of diesel fuel under stress conditions of crowding and 0 to -10°C.	LD_{50}	Hartung and Hunt, 1966 (9)
Ducks	intragastric administration of 24 ml/kg b.w. of diesel fuel	no mortalities	"
Ducks	intragastric administration of 20 ml/kg b.w. of lubricating oil	no mortalities	"
Quail	oral administration of capsules containing 3.5 mg/kg b.w. of No. 2 fuel oil	egg production halted for 6-8 days; egg yolk deposition abnormalities	Grau, 1977 (10)

LD_{50} - median lethal dose

b.w. - body weight

TABLE 13. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN WATERFOWL (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Mallard and Pekin ducks	intragastric administration of 1-3 ml/kg b.w. of fuel oil or lubricating oil	oil pneumonia, diarrhea, anemia, occult blood in intestines, toxic nephrosis	Hartung and Hunt, 1966 (9)
Ducks	intragastric administration of 3-12 ml/kg b.w. of diesel fuel	liver function alterations, reduced pancreatic zymogen, toxic nephrosis, lipid pneumonia, diarrhea, occult blood in intestines, anemia	"

TABLE 14. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN INSECTS

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Mosquito eggs	undiluted lubricating oil to coat eggs	ovicidal to 75% of eggs by oxygen deprivation	Powers and Headlee, 1939 (17)
Potato Beetle eggs	egg clusters dipped into fuel oil	age-dependent ovicidal effects	"
4th Instar Blackfly Larvae	15 min exposure to 10 mg/l fuel oil or 20 mg/l kerosene added to stream water.	larval detachment from rocks; no mortalities	Gjulin, 1949 (18)
Latrine fly maggots	undiluted kerosene poured over latrine contents	death by suffocation	Jettmar, 1940 (19)

water temperature, sunlight, presence of organic matter in the water which adsorbs oil hydrocarbons, and degree of biotransformation which occurs. When a toxic concentration is given in the literature, the method of mixing the oil and water must be considered in order for the information to be of value. Oil concentrations may have been measured analytically, calculated, or given as an amount of oil added to water, making it difficult to compare various concentration and establish meaningful toxicity data. Therefore, the data in Table 15 for fish species and other aquatic organisms are difficult to interpret.

Nevertheless, it can be stated that petroleum fuels and lubricants are toxic in fish when added to water in sufficient concentration (22-24). One factor which influences oil toxicity is oxygenation of the water. An oil film or slick may retard gas exchange, lowering the oxygen content of the body of water. It was noted that distress and nervous system depression in juvenile American shad occurred 120 min after addition of 84 mg/l of diesel fuel to water containing 2-3 mg/l oxygen, whereas the toxicity was manifested only after 5 hours of exposure to this amount of diesel fuel in water with 6.5 mg/l dissolved oxygen (normal level). Shad exposed to the low oxygen levels in non-oily water exhibited no adverse effects within 48 hours. Low oxygenation of water increased the toxicity of diesel fuel in shad (23), but not in marine worms (31).

Oils dissolve in water in a variable manner (24,26,33,36,39,42), depending upon time, temperature, evaporation and transformation occurring during the course of the studies being undertaken. Water soluble fractions (WSF) of No. 2 fuel oil, and other oils, are prepared by slowly stirring 10% oil in water mixtures for 1 to 3 days or more, allowing the phases to separate, and utilizing only the water phase containing dissolved petroleum hydrocarbons. If undiluted, it is termed 100% WSF; dilutions are, however, usually made. Some investigators have analytically quantitated the concentrations of various petroleum hydrocarbon types in 100% WSF, but, again, the concentrations are time-dependent and subject to change due to evaporative losses, transformation and concentration by test organisms. Aromatic hydrocarbons such as naphthalenes are more soluble than paraffins (n-alkanes), but various investigators disagree on the relative toxicity of aromatic versus aliphatic hydrocarbons present in WSF of fuel oils.

Irradiation with ultraviolet light during the preparation of WSF of fuel oils causes it to be much more toxic to fish than that prepared without ultraviolet radiation (25). Whereas undiluted WSF of No. 2 fuel oil had no effect on sheephead minnows exposed to it for 96 hours, a 96-hr LC_{50} was obtained with a 49% dilution of WSF irradiated prior to the bioassays.

Oil slicks also dissolved to some degree in water. One liter of No. 2 fuel oil was floated on top of 360 liters of water flowing at a 3 l/hr rate. After 6 days, water was taken from under the slick and analyzed: there were 0.23 mg/l phenols, 0.35 mg/l aromatic hydrocarbons and 1.4 mg/l aliphatic hydrocarbons. Fish exposed to the water from the 6-day old oil slick survived in 90-100% of cases. When the oil slick experiment was conducted under ultraviolet irradiation, there were 0.75 mg/l

TABLE 15. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN FISH

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Bluegill sunfish	exposure to 3000 mg/l kerosene added to water	24-hr LC_{50}	Turnbull et al., 1954 (21)
Banded killifish	exposure to 28.5 mg/l No. 2 fuel oil added to water	24-hr TL_m	Rehwoldt et al., 1974 (22)
	exposure to 26.1 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
Striped bass	exposure to 30.6 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
	exposure to 22.2 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
Pumpkinseed	exposure to 42.6 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
"	exposure to 39.2 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
White perch	exposure to 41.6 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
"	exposure to 37.2 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
American eel	exposure to 28.0 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
"	exposure to 31.0 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
Carp	exposure to 52.5 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
"	exposure to 49.1 mg/l No. 2 fuel oil added to water	96-hr TL_m	"

LC_{50} - median lethal concentration

TL_m - median tolerance limit

TABLE 15. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN FISH (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Juvenile American Shad	exposure to 204 mg/l diesel fuel added to water	24-hr TL_m; loss of equilibrium, and other central nervous system disturbances preceeded death	Tagatz, 1961 (23)
"	exposure to 167 mg/l diesel fuel added to water		"
	exposure to 200 mg/l kerosene added to water	24-hr TL_m	Boylan and Tripp, 1971 (24)
	exposure to 200 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
Mummichog	exposure to undiluted soluble fraction of 10% mixture of No. 2 fuel oil in water	no mortalities in 96 hr	Scheier and Gominger, 1976 (25)
Sheephead Minnow	exposure to same	no mortalities in 96 hr	"
Channel catfish	exposure to same	10% mortality in 96 hr	"
Bluegill sunfish	exposure to same	20% mortality in 96 hr	"
Mummichog	exposure to 48 vol % dilution of soluble fraction of 10% mixture of No. 2 fuel oil in water previously UV irradiated for 72 hr	96-hr LC_{50}	"
Sheephead Minnow	exposure to 46 vol % of same	96-hr LC_{50}	"
Channel Catfish	exposure to 75 vol % of same	96-hr LC_{50}	"
Bluegill Sunfish	exposure to 40 vol % of same	96-hr LC_{50}	"

TABLE 15. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN FISH (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Sheephead Minnow	exposure to dispersion of 250 mg/l No. 2 fuel oil added to water	24-hr TL_m	Anderson et al., 1974 (26)
"	exposure to dispersion of 93 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
Silverside	exposure to dispersion of 260 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
"	exposure to dispersion of 125 mg/l No. 2 fuel oil added to water	48-hr TL_m	"
Sheephead Minnow	exposure to >6.9 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 6.3 mg/l No. No. 2 fuel oil dissolved in water	96-hr TL_m	"
Silverside	exposure to 5.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 3.9 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"

phenols, 2.7 mg/l aromatic hydrocarbons and 4.3 mg/l of aliphatic hydrocarbons in the water below the slick after 6 days, and the water proved lethal to 100% of fish tested in 25 to 95 minutes of exposure (25).

WSF of No. 2 fuel oil was more toxic to fish than oil droplets dispersed in water by vigorous agitation. This was due to the following: aromatic hydrocarbons were more concentrated in WSF than in dispersions, which contained almost equal amounts of aromatic and paraffinic hydrocarbons; and the dispersion was unstable in water, showing a loss of 80% of the oil from the water phase in a 24-hr period of gentle aeration (26). The differences in toxicity of the WSF and the dispersion of No. 2 fuel oil in silversides and sheephead minnows are presented in Table 15.

Results of marine annelid bioassays with WSF of No. 2 fuel oil are presented in Table 16. The aromatic hydrocarbons which dissolved in the WSF, consisting mainly of methyl and dimethylnaphthalenes, were considered responsible for toxicity of the WSF. Initial concentrations of 2.5 mg/l were measured, but by 96 hours, the naphthalenes level was 0.18 mg/l, demonstrating the instability of WSF of fuel oils utilized in static bioassays (28).

Age differences in tolerance of marine worms to WSF of No. 2 fuel oil (29) as reported in Table 16, were greatest between 9-day-old, 4-segment worms and adults, with the exception that gravid females and 30-day-old, 32-segment worms were of comparable sensitivity, due to the presence of yolk matter in these juveniles and gravid females. The yolk sequestered petroleum hydrocarbons, rendering them less toxic. Once yolk matter was no longer functional, the petroleum hydrocarbons could then affect body tissues (29).

Whereas reduced oxygen levels in water augmented the toxicity of diesel fuel to exposed shad, this effect did not occur in marine annelids. Exposures of *Neanthes* to 30%-40% WSF of No. 2 fuel oil did not impair their ability to increase hemoglobin content in response to hypoxia at dissolved oxygen levels of 2.0 mg/l. Exposure to low oxygen levels alone for 11 days produced no mortalities, and exposure to 40% WSF alone for 96 hours caused <20% mortality. The combination of low oxygenation of water and presence of soluble petroleum hydrocarbons derived from 40% WSF did not increase mortality in marine worms (31).

The toxic thresholds for mollusks, including scallops, clams, oysters, mussels and snails, exposed to fuel oils mixed with water are presented in Table 17, with the same reservations as in other bioassays with oil-water mixtures. Young adult soft shell clams which were exposed to 50-500 mg/l of No. 2 fuel oil emulsions (concentration determined gravimetrically) exhibited a characteristic, dose-dependent response. Mucus was secreted excessively through the pedal opening and siphon, adductor muscle contraction was depressed, and decreased irritability and contractility of the siphon were noted. Mucus secretion and muscular narcotization were further

TABLE 16. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN WORMS

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Soil Nematodes	No. 2 fuel oil applied to 1.7 x 3.0 m plots of soil; application rate 2.5 kg/m^2	nemacidal after 6 and 12 months	Raymond et al., 1976 (27)
Marine annelids			
Neanthes arenaceodentata	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	Rossi et al., 1976 (28)
"	exposure to 3.2 mg/l No. 2 fuel oil dissolved in water	48-hr TL_m	"
"	exposure to 2.7 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
Capitella capitata	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 3.5 mg/l No. 2 fuel oil dissolved in water	48-hr TL_m	"
"	exposure to 2.3 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
Neanthes arenaceodentata:			
4-segment juveniles	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	Rossi and Anderson, 1976 (29)
"	exposure to 8.4 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
18-segment juveniles	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 5.7 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"

TL_m - median tolerance limit

TABLE 16. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN WORMS (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
32-segment juveniles	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	Rossi and Anderson, 1976 (29)
"	exposure to 5.4 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
40-segment juveniles	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 2.7 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
48-segment immature adults	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 2.7 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
60-segment mature males	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 2.6 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
60-segment gravid females	exposure to >8.7 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 4.2 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"

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TABLE 17. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN MOLLUSKS

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Scallops	exposure to 12.5 mg/l No. 2 fuel oil in water	100% mortality in 24 hr	Tarzwell, 1971 (32)
Quahaug clam eggs 2nd-cleavage stage	exposure to 0.43 mg/l No. 2 fuel oil dissolved in water	48-hr LC_{50}	Byrne and Calder, 1977 (34)
2-Day old Quahaug larvae	exposure to 1.3 mg/l No. 2 fuel oil dissolved in water	2-Day LC_{50}	"
"	exposure to 0.53 mg/l No. 2 fuel oil dissolved in water	10-Day LC_{50}	"
Young adult soft shell clams	exposure to 505 mg/l No. 2 fuel oil in water emulsion	96-hr LC_{50}; excessive mucus secretion; muscular narcotization.	Stainken, 1976 (35)
"	exposure to <100 mg/l No. 2 fuel oil in water emulsion	7-day LC_{50}; excessive mucus secretion; muscular narcotization.	"
Soft shell clams	5 months exposure to No. 2 fuel oil and jet fuel polluted waters	male and female gonadal tissue invasive neoplasms possibly of germ cell origin; metastases to gills, genital and urinary pores, kidney, pericardium and red gland.	Barry and Yevich, 1975 (37)
Soft shell clams	taken from oil-polluted waters	gill and kidney hyperplasia	Stainken, 1976 (36)
7-hr old Larvae of Oysters & Mussels	exposure to 10,000 mg/l No. 1 fuel oil in water dispersion	6-hr LC_{50}	Renzoni, 1973 (38)
Oyster & Mussel eggs	exposure to 1,000 mg/l No. 2 fuel oil in water dispersion	reduced numbers of larvae developed	"

LC_{50} - median lethal concentration

TABLE 17. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN MOLLUSKS (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Oysters	exposure to dispersion of 0.1% or 10% No. 2 fuel oil added to water	50% mortality in 4 days	Anderson and Anderson, 1976 (39)
"	exposure to dispersion of 1% No. 2 fuel oil added to water	50% mortality in 8 days; 100% mortality in 14 days	"
Snails *Littorina sp.*	60 ml of kerosene poured over animals	slight effect on crawling activity and attachment rates	Hadley, 1977 (40)
Nassarius obsoletus	exposure to 1 to 4 µg/l of kerosene dissolved in water	interference with chemically-mediated food-finding behavior	Jacobsen and Boylan, 1973 (41)

enhanced in clams exposed at 14°C than at 40°C. Increased temperature also influenced mortality: at 40°C with dispersions of petroleum hydrocarbons in water as high as 1600 mg/l there was insufficient mortality in 96 hours to calculate an LC_{50}. At 14°C, the 96-hr LC_{50} was 505 mg/l, and the 7-day LC_{50} was less than 100 mg/l (35). Morphological abnormalities in clams exposed to 50-100 mg/l of No. 2 fuel oil hydrocarbons in water emulsions included pallial muscle edema, leukocytosis of pallial blood sinuses, mantle subepithelium and anterior adductor muscle, abnormal vacuolization of the style sac, intestine and diverticula, and reduced gill chromatophilia. Decreased numbers of mucoid cells along the intestines, and glycogen depletion of intestinal, diverticular and mucosal epithelium and gill filament tips were noted. These changes were not considered "radical" (36).

In contrast, soft shell clams exposed for 5 months to an environment polluted by a spill of No. 2 fuel oil and JP-5 jet fuel (a kerosene-like petroleum oil) were found to develop invasive, obliterative neoplasms of the male and female gonads. The tumor mass was hypothesized to be of germ cell origin. Metastases to gills, genital and urinary pores, kidney, pericardium and red gland were common. Clams collected from unpolluted waters showed no indications of tumors (37).

In oysters and mussels, No. 1 fuel oil reduced fertilization capacity of sperm, swimming activity of larvae and survival rates of eggs and larvae in dispersions of 1000 mg/l (38). Oysters cease pumping and close their valves when exposed to dispersions of No. 2 fuel oil in seawater, and reliable toxicity data are therefore difficult to obtain (39). Bioassay results are presented in Table 17.

Kerosene poured over snails in pinbowls did not influence mortality, and only slightly affected behaviors such as attachment and crawling, as long as temperatures closely approximated those of their natural habitat. At elevated temperatures (29°C), mortality was observed both with and without kerosene exposure, and behavioral responses under the influence of kerosene were significantly abnormal in comparison with unexposed controls (40).

Kerosene concentrations of 4 µg/l in seawater significantly ($p<0.05$) disrupted the attraction of marine prosobranch snails to food stimuli. The effect was due to interference with the chemically-mediated food-finding behavior. It is possible that other behaviors which are dependent upon chemical cues, such as reproduction and orientation, could likewise be disrupted by dissolved petroleum hydrocarbons in low concentrations (41).

Toxic levels of dispersions and solutions of No. 2 fuel oil in water to grass shrimp, mysids, brown shrimp postlarvae and coon stripe shrimp are presented in Table 18. It can be noted that immature postlarval shrimp were tolerant to greater concentrations of this oil than the adult crustaceans tested (26). No. 2 fuel oil solutions prepared under ultraviolet light were more toxic to grass shrimp than non-irradiated solutions, the results being similar to the previously reviewed fish study conducted by the same authors (25).

TABLE 18. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN MARINE CRUSTACEANS

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Grass shrimp	exposure to dispersion of 3.8 mg/l No. 2 fuel oil added to water	24-hr TL_m	Anderson et al., 1974 (26)
"	exposure to dispersion of 3.0 mg/l No. 2 fuel oil added to water	96-hr TL_m	"
Mysid	exposure to dispersion of 1.6 mg/l No. 2 fuel oil added to water	24-hr TL_m	"
"	exposure to disperion of 1.3 mg/l No. 2 fuel oil added to water	48-hr TL_m	"
Brown shrimp Postlarvae	exposure to dispersion of 9.4 mg/l No. 2 fuel oil added to water	24-hr and 96-hr TL_m	"
Grass shrimp	exposure to 4.4 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 3.5 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
Mysid	exposure to 2.6 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 0.9 mg/l No. 2 fuel oil dissolved in water	48-hr TL_m	"
Brown shrimp postlarvae	exposure to 5.0 mg/l No. 2 fuel oil dissolved in water	24-hr TL_m	"
"	exposure to 4.9 mg/l No. 2 fuel oil dissolved in water	96-hr TL_m	"
Grass shrimp	exposure to undiluted soluble fraction of 10% mixtures of No. 2 fuel oil in water	96-hr LC_{50}	Scheier and Gominger, 1976 (25)
"	exposure to 34.4 vol % dilution of soluble fraction of 10% mixture of No. 2 fuel oil in water previously UV irradiated for 72 hr.	96-hr LC_{50}	"

LC_{50} - median lethal concentration; TL_m - median tolerance limit

TABLE 18. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN MARINE CRUSTACEANS (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Grass shrimp	exposure to water under 6-day-old No. 2 fuel oil slick prepared from 1 part oil and 360 parts water and UV irradiated for 6 days	50-min TL_m	Scheier and Gominger, 1976 (25)
"	exposure to same, but not irradiated during preparation	100% survival after 96 hr	"
Coon stripe shrimp	exposure to 0.8 mg/l of No. 2 fuel oil dissolved in water	96-hr LC_{50}	Vanderhorst et al., 1976 (42)
"	exposure to 1.3 mg/l of No. 2 fuel oil dissolved in water	72-hr LC_{50}	"
Amphipod *Gammarus oceanicus* Adults	exposure to dispersion of 173 µl/l of No. 1 fuel oil in water	48-hr LC_{50}	Linden, 1976 (43)
4-6 day old juveniles	exposure to dispersion of 0.3 µl/l of No. 1 fuel oil in water	48-hr LC_{50}	"
Adults	exposure to solution of 5 µl/l of No. 1 fuel oil in water	100% mortality in 50 days; impaired swimming; loss of photophobia; reduced sexual activity; reduced numbers of offspring	"
Amphipod *Boeckosimus affinis*	exposure to 1.0 ml/l of diesel fuel oil slick on water	24-hr LC_{50}	Atlas, 1975 (44)
"	exposure to 0.8 ml/l of paraffinic fuel oil slick on water	72-hr LC_{50}	"
"	exposure to 0.4 ml/l of aromatic fuel oil slick on water	16-day LC_{50}	"

TABLE 18. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN MARINE CRUSTACEANS (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Copepod *Tigropus californicus*	exposure to 1.0 ml/l of diesel fuel dispersed in water	100% mortality in 72 hr	Barnett and Kontogiannis, 1975 (45)
"	exposure to 0.50 ml/l of diesel fuel dispersed in water	100% mortality in 96 hr	"
"	exposure to <0.10 ml/l (87 mg/l) of diesel fuel in water	reduced survival critical concentration	"
"	exposure to 1.0 ml/l of kerosene mixed with water	85% mortality in 8 days	"
"	exposure to <0.10 ml/l (83 mg/l) of kerosene in water	reduced survival critical concentration	"
Cyclopoid Copepod Nauplii	exposure to 50% dilution of soluble fraction of 10.1% mixture of No. 2 fuel oil in water	20% survival in 72 hr	Lee and Nicol, 1977 (46)
Calanoid Copepod Nauplii	exposure to 50% dilution of soluble fraction of 10.1% mixture of No. 2 fuel oil in water	100% survival in 72 hr	"

Baltic amphipods, *Gammarus oceanicus*, which were exposed to dispersions of No. 1 fuel oil in seawater for 48 hr, exhibited a 600-fold difference in sensitivity between adults and juveniles (43) as noted in Table 18. Adult amphipods which were exposed to 5 µl/l of No. 1 fuel oil in water exhibited impaired swimming almost immediately, followed by cessation of swimming, sluggish crawling along the bottom of the test container, and death of all organisms in 50 days. Although controls preferred the darkened portion of their test containers, the oil exposed amphipods were counted in significantly higher numbers ($p<0.001$) in the lighted half of the containers. Abnormal precopulation behavior, decreased numbers of live larvae and sticking of dead larvae to the oil film at the surface of the container were noted.

In studies on copepod Nauplii exposed to WSF of No. 2 fuel oil, it was found that cyclopoid copepods were less sensitive than calanoid copepods to 50% WSF. In mixed zooplankton, oceanic species were more sensitive than coastal zooplankton to the WSF of No. 2 fuel oil (46). Results of bioassays are presented in Table 18.

In starfish exposed to WSF of No. 2 fuel oil, development of fertilized eggs was affected, resulting in abnormal elevation of the fertilization envelope, delayed completion of cleavage stages, abnormal cleavages, prolonged larval development, reduced numbers of larvae produced, and larval deformity. No larvae developed from fertilized eggs exposed to 50% WSF. Sperm motility was decreased and sperm were infertile after 30 minutes of exposure to 50% WSF (47). Table 19 summarizes this data.

In Panamanian coral species, exposure to undiluted diesel fuel by pouring it over the coral, or submerging the coral in it for 1 minute, produced delayed tissue death, retraction of polyps and lack of response to mechanical stimulation, and delayed feeding responses (48). Table 19 presents the data for coral exposure to diesel fuel.

D. EFFECTS IN ALGAE AND PHYTOPLANKTON

The effects of petroleum products on growth and photosynthesis of microalgae and phytoplankton are summarized in Table 20. In one study (51) it was demonstrated that the volatile compounds of No. 2 fuel oil were both inhibitory and stimulatory to phytoplankton growth depending on the concentration. But another investigation in algal species showed the less volatile, high boiling hydrocarbons of No. 2 fuel oil to be the toxic substances present in media on which the algae were grown (52). Phenalen-1-one, which was present in a concentration of 0.2 mg/l in WSF of No. 2 fuel oil, was abruptly toxic to growth of blue-green algae in concentrations of 5 mg/l or higher. In green algae, the toxicity of phenalen-1-one was greater when cultures were concomitantly exposed to white light than to yellow light (53). Further data are presented in Table 20.

TABLE 19. SUMMARY OF TOXICITY OF FUEL AND LUBRICATING OILS IN ECHINODERMS AND CORALS

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Sand dollar eggs	exposure to 20% dilution of soluble fraction of 10.1% mixture of No. 2 fuel oil in water	abnormal fertilization, abnormal cleavage, reduced number of living larvae, deformed larvae.	Nicol et al., 1977 (47)
Sand dollar eggs and sperm	exposure to 50% dilution of soluble fraction of 10.1% mixture of No. 2 fuel oil in water	no larvae developed from eggs; sperm 100% infertile after 30 min. exposure	Reimer, 1975 (48)
Coral *Pocillopora* sp.	submerged for 1 minute in diesel fuel	loss of almost all living tissue in 13 days	"
"	diesel fuel poured over coral to cover for 30 min	70% death of polyps in 17 days	"
Pocillopora, Pavona, Porites, Psammocora	1-4 ml of diesel fuel added to water with organisms	sustained mouth-opening response to food; no ingestion response in 50% of organisms; delayed feeding for 17 days after exposure.	"

TABLE 20. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN ALGAE, PHYTOPLANKTON, AND BACTERIA

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Natural marine phytoplankton communities	exposure to 19.1 to 19.8 µg/l No. 2 fuel oil dissolved in water	inhibition of photosynthesis	Gordon and Prouse, 1973 (49)
Fresh water Phytoplankton (Algae): *Euglena*	cultured and grown on 0.1% diesel fuel medium or on 10% lubricating oil medium	no reduction in population size or growth rate	Dennington et al., 1975 (50)
Scenedesmus	cultured and grown on 0.1% diesel fuel medium	no growth; inhibition of photosynthesis	"
"	cultured and grown on 0.1% lubricating oil medium	inhibition of photosynthesis	"
Skeletonema (diatom)	stoppered flask culture with 100 µg/l of No. 2 fuel oil medium	no growth	Dunstan et al., 1975 (51)
"	unstoppered flask culture with 1000 µg/l of No. 2 fuel oil medium	no growth	"
Dunaliella (green flagellate)	flask culture with µg/l quantities of No. 2 fuel oil medium	enhanced growth	"
"	cultured on 10% dilution of seawater with 15 mg/l No. 2 fuel oil	no effect on growth	Pulich et al., 1974 (52)
Chlorella (green)	cultured on 10% dilution of seawater with 15 mg/l No. 2 fuel oil	no effect on growth	"
Agmenellum (blue-green)	cultured on 10% dilution of seawater with 15 mg/l No. 2 fuel oil	7-hr lag phase	"
Thalassiosira (diatom)	cultured on 10% dilution of seawater with 15 mg/l No. 2 fuel oil	14-hr lag phase	"

TABLE 20. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN ALGAE, PHYTOPLANKTON, AND BACTERIA (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Gymnodinium (dinoflagellate)	cultured on 10% dilution of seawater with 15 mg/l No. 2 fuel oil	170-hr lag phase	Pulich et al., 1974 (52)
Nostoc (blue-green)	cultured on 10% dilution of seawater with 15 mg/l No. 2 fuel oil	prolongation of doubling time from 6 to 9 hours	"
Agmenellum (blue-green)	suspension in 50% dilution of soluble fraction of 10.1% No. 2 fuel oil in water mixture	inhibited photosynthesis	"
Chlorella (green)	suspension in 20% dilution of soluble fraction of 10.1% No. 2 fuel oil in water mixture	inhibited photosynthesis	"
Thalassiosira (diatom)	suspension in 12% dilution of soluble fraction of 10.1% No. 2 fuel oil in water mixture	inhibited photosynthesis	"
Coccochloris (blue-green)	in 25% dilution of soluble fraction of 10.1% Baytown or Montana fuel oils mixed with water	no growth	Winters et al., 1976 (52)
Agmenellum (blue-green)	in 25% dilution of soluble fraction of 10.1% Baytown or Montana fuel oils mixed with water	no growth	"
Dunaliella (green)	suspension in 50% dilution of soluble fraction of 10.1% New Jersey fuel oil mixed with water	no growth	"

TABLE 20. SUMMARY OF TOXICITY OF FUEL OILS AND LUBRICATING OILS IN ALGAE, PHYTOPLANKTON, AND BACTERIA (CONT.)

SPECIES	EXPERIMENTAL PROTOCOL	RESULTS	REFERENCE
Chlorella (green)	suspension in 50% dilution of soluble fraction of 10.1% New Jersey fuel oil mixed with water	no growth	Winters et al., 1976 (52)
Mixed culture of motile marine bacteria	kerosene emulsion ≥5 µg/l in seawater	motility adversely affected	Chet and Mitchell, 1976 (57)
"	kerosene emulsion 3 µg/l in seawater	blockage of chemically-mediated attraction to organic matter in water	"

E. EFFECTS IN BACTERIA

Petroleum fuels and lubricants are toxic to some bacteria, while others are able to utilize the oils as carbon and energy sources. Motile marine bacteria exposed to 3 µg/l of kerosene were found to decompose organic matter at a much lower rate than bacteria not exposed to the oil. Kerosene did not inhibit bacterial enzymatic activity, but blocked the chemically mediated attraction of the bacteria to the substrate (57).

F. EFFECTS IN PLANTS

Although there are no available studies on effects of fog oils or smokes on plants, similar oils have been sprayed on vegetable and fruit crops for insect and pest control. Additional information has been obtained from laboratory studies of exposure to plants, fruit, leaves and vegetables to various petroleum lubricating and fuel oils.

Crop spraying with kerosene in deciduous orchards and citrus groves helps control various insect pests (11). Lubricating oils are less phytotoxic than kerosene, according to some authors (11,58), while others evaluate phytotoxicity of a given oil based on viscosity (12, 60), paraffinic vs aromatic hydrocarbon content (59) or specific gravity (61). In trees sprayed with petroleum lubricating oils, leaf drop, stunted growth of bearing wood, weak new foliage, yellowing of leaves (61), death of buds and bark of attached twigs, discoloration, spotting and cracking of apples (62), dropping of tree ripe citrus fruit, inhibition of normal color in lemons, retarded ripening of fruit and decreased fruit size (58) have been reported, although the amount of oil causing these effects is reported only as that quantity which drenches limbs and twigs with the sprayed oil (62).

Injuries to Aspen and sugar maple tree foliage was reported two days following sprays with fuel oils. Cycloparaffinic oils caused more severe leaf scorching or spotting than aromatic fuel oil, but 10% strength (diluted in water) oils of both types caused injuries (59).

Increased chlorophyll content of leaves occurred following spray application of lubricating oil on apple trees 4 times in 8 weeks. The oil coating might have reduced the intensity of light reaching leaf surfaces, thus stimulating chloroplasts and chlorophyll accumulation (63).

Drought injury may increase the sensitivity of apple trees to phytotoxic effects of petroleum oil sprays. Water normally acts as a barrier to the spread of oil inside leaves (62). Oil penetration is greater on the underside of most leaves, due to the presence of stomates in this location. Diesel fuel sprays, in 50% strength, interfered with stomate closing ability (13). Oil which penetrates living apple leaves replaces air in intercellular spaces. Fuel oils penetrate faster than lubricating oils, and once inside a leaf, evaporation is negligible. The oils spread from inside the leaf to the twigs (64-65),

Reduced transpiration in lemon and lime plants sprayed with 1.75% paraffinic or cycloparaffinic fuel oils was noted one day after spraying. The cycloparaffinic oil sprayed plants recovered in 30 days, while the injury after the paraffinic oil spray was still detected 37 days after spraying (66).

Banana leaf photosynthesis was reduced 15% by a kerosene spray from a helicopter at 84 $\mu g/cm^2$ to the upper leaf surfaces (60). A 50% emulsion spray of diesel fuel increased leaf respiration in fruit trees 2 hours after spraying, indicating metabolic disturbance in O_2 uptake and CO_2 evolution. The effect lasted for more than 24 hours (13).

Vegetable crops did not grow in black clay loam or silt loam soils treated with 2.5 kg/m^2 of No. 2 fuel oil and tilled to a depth of 15 cm. Turnips and beans which grew in similarly treated sandy loam plots were severely stunted with deformed leaves. The phytotoxicity of the soil lasted longer than 1 year (27). Oil injury to beans and lettuce plants was noted after spraying them with 10% aromatic fuel oil (59), and wilting and necrosis occurred after applications of undiluted lubricating oil to potato leaflets (67). Brown and black leaf spots and blackening of epiphyllous veins were found. Kerosene applied to potato leaflets caused temporary translucence, which abated in 24 hours as the kerosene evaporated from intercellular leaf spaces (67).

In the marine and marsh environment, No. 2 fuel oil pollution spread out along the bottom sediments, destroying bottom plants in the process (68). Oil pollution may be responsible for the death of eelgrass in coastal waters in America and Europe.

G. ENVIRONMENTAL FATE

An extremely complex series of interactions among physical, chemical and biological processes determines the immediate and ultimate fate of fuel oils and lubricating oils which contaminate terrestrial and aquatic environments and the resident flora and fauna.

As it is almost impossible to calculate the amount of oil which is left behind after smoke screening operations, the data presented in this section are based primarily on studies of fuel oil spills in marine environments, which have received considerable attention in the past 5-10 years.

1. OCCURRENCE AND DISPERSION

Fuel and lubricating oils do not occur in nature; they are products of petroleum refineries and are introduced into the environment solely by human intent or error, viz. accidental oil spills, crop spraying and smoke screening utilizing fog oil. Atmospheric dispersion of smoke screens generated from fog oils, and consisting of oil microdroplets, is dependent upon meteorological conditions such as wind speed, air currents, humidity, precipitation, and air temperature, among others (3). Most of the oil droplets eventually settle to the ground, although some evaporation may occur. Once on the ground, physical processes which

affect the transport or persistence of fog oils and diesel fuel include evaporation, dissolution in water, movement through soil, adsorption by soil and organic matter, and solubilization by naturally occurring dissolved surfactants (26,47,70,72-74).

The solubility of fuel oil hydrocarbons in water depends upon the degree of agitation, water temperature, and volume of oil and presence of organic matter in the water. Slow agitation produces solutions whereas rapid agitation produces unstable dispersions of oil droplets in water (26). The aromatic hydrocarbons are more soluble than the paraffinic hydrocarbons of fuel oils and lubricants. Mono- and dicyclic aromatic hydrocarbons, such as alkylnaphthalenes, alkyl indans, alkyl phenols, alkyl anilines, alkyl indoles, alkyl benzothiophenes and perinaphthenone, are found in concentrations above 0.1 mg/l after gentle stirring of 1:8 No. 2 fuel oil:water mixtures for 24 hours (47).

Under highly turbulent conditions the volume of No. 2 fuel oil added to water could be linearly correlated ($r = 0.99$) to the water concentration of oil which resulted (33).

Mixtures of kerosene in water (25 ml of kerosene/1.5 liter seawater) prepared by slow stirring were analyzed; the water soluble portion contained mostly alkylbenzenes and naphthalenes in concentrations of 160 µg/l. More turbulent preparation of the mixture resulted in higher levels of non-polar saturated hydrocarbons in the aqueous phase (24).

Unfiltered seawater containing dissolved organic matter which solubilizes certain petroleum hydrocarbons, could dissolve up to 1560 µg/l of fuel oil. Filtering removed about 75% of dissolved oil (70).

The solubility of diesel fuel and lubricating oil in distilled water differed; biotransformation increased the fuel's solubility to 360 mg/l in 42 days. For lubricating oil, the maximal solubility was 45 mg/l in 42 days (71).

Evaporation of oil films in the aquatic environment was studied utilizing a constant windspeed of 21 km/hr, temperatures of 5-30°C, and absence of light. A smooth curve of evaporation resulted from 3 mm thick oil samples in petri dishes, which had been expected for the complex hydrocarbon mixture of No. 2 fuel oil. Evaporation of C_9 to C_{18} n-alkanes present in the fuel oil was similar to evaporation of the whole oil. The evaporation of components with vapor pressures between those of n-octane and n-octadecane seemed to control the evaporation rate (72).

Organic matter in marine sediments incorporates petroleum hydrocarbons, and releases them slowly to overlying water via biological oxidation and dissolution (73).

Petroleum fuels and lubricants released on beach sand or soil are retained, although evaporation of hydrocarbons with less than 12 carbon atoms occurs, and there are compositional changes due to oxidation of fuel to form ether and sulfone groups. Up to 4% of oil was retained in soil samples taken 6 months after an offshore fuel oil spill; 3.5% remained after 1 year (74).

The dispersion of No. 2 fuel oil from an offshore oil spill resulted in pollution of marsh muds and river bottoms in the area. Oil which had penetrated to a depth of 7.5 cm below the surface of sediments persisted at least 2 years, gradually spreading along bottoms. Branched and cyclic hydrocarbons such as isoprenoids and highly substituted benzenes, naphthalenes and tetrahydronaphthalenes remained prominent in sediments, although overall depletion rates of saturated and aromatic hydrocarbons were the same, due to preferred bacterial degradation or dissolution of some compounds within each structure group (75-77).

In soils, dispersion of petroleum hydrocarbons can contaminate ground waters and spoil drinking water. The movement of oil in soil depends upon extraction of soluble components into residual water in soil, transport to groundwater by rain, and adsorption of oil by soil components (4-78). The adsorptive capacity of the soil is related to moisture content. Studies showed that oil which infiltrated soil near the site of an oil field was compounded and impregnated at the capillary fringe and did not contact ground water below the soil. Water could leach out taste and odor-producing compounds of the oil held in soil pores (79).

Physical movement of No. 2 fuel oil in soil plots treated with 2.5 kg/m^2 of the oil, and tilled to a depth of 15 cm, did not occur during a one year period of observation. No visible sheen in runoff or leachate waters were noted, and oil concentrations in water did not exceed those in water sampled at unpolluted locations (27).

Sand, lime, clay and organic matter adsorb petroleum hydrocarbons and prevent or reduce transport to ground water by leaching. Rainfall, bacterial degradation and evaporation influence the fate of petroleum in soils (80).

2. TRANSFORMATION

Petroleum fuels and lubricants are transformed by photolysis as well as by microorganisms present in terrestrial and aquatic environments.

Photochemical reactions result in oxidation of intermediate and high molecular weight aromatic petroleum hydrocarbons to insoluble polycyclic molecules (75). Ketones, aldehydes and carboxylic acids of low to medium molecular weight are formed. Radical combinations, condensation of ketones with phenols, and esterification between alcohols and carboxylic acids may form high molecular weight compounds (81).

Ultraviolet spectrophotometric determinations of fuel oil prior to, during, and after ultraviolet irradiation for up to 15 days showed changes in absorbance maxima from 228 nm to 200 nm, as well as increased absorbance at 214 and 225 nm, and decreased absorbance at 260 and 300 nm, with time. Autocatalytic reaction kinetics were possibly involved (81).

One proposed mechanism for photooxidation of reactive polycyclic aromatic compounds, such as fluorene, involved excitation and decay, combination with atmospheric oxygen to form hydroperoxy radicals, formation of hydroperoxides via hydrogen atom abstraction, and heterolytic cleavage to form hydroxyalkylbenzenes. Hydroperoxides of tetralin, indan, cumene and their alkyl derivatives are probably formed (82).

Bacteria, yeast and fungi transform petroleum hydrocarbons. Complete and partial oxidation results in compounds such as long chain fatty acids and cycloparaffinic acids, dehydrogenations and formation of polymers of unsaturated hydrocarbons, carotene pigment derivatives, and carbon dioxide (83-87).

Studies on bacterial growth in mixed aquatic cultures containing No. 2 fuel oil showed that lipolytic bacterial growth is enhanced (55). Temperature and availability of nitrogen and phosphorus govern oil biotransformation rates in aquatic environments (89). In the marine environment, microbial attack preferentially depletes n-alkanes, but homologous hydrocarbons are attacked at roughly the same rate. Cycloalkanes and aromatic hydrocarbons are more resistant to transformation. Two years after an offshore No. 2 fuel oil spill, small amounts of n-alkanes were still detectable while branched and cyclic alkanes, and aromatic and alicyclic hydrocarbons remained prominent in polluted sediments. Bacterial transformation was evident at marsh depths of 60 cm from the surface (75,77).

In soils treated with No. 2 fuel oil at a rate of 2.5 kg/m^2, and tilled to 15 cm to promote aeration, there were no differences in transformation rates between paraffinic hydrocarbons and total oil, but more polar hydrocarbons degraded slower than less polar ones. Rates of transformation did not exceed 500 g/m^2 per month. The hydrocarbon-utilizing microflora in the soil were mostly aerobic *Nocardia* sp. and fungi (27).

Yeasts, bacteria and fungi grown on media containing petroleum hydrocarbons developed oil inclusions. In *Penicillum* sp., mycelia contained a central core of oil in the inner rind of mycelial balls (92).

3. BIOCENTRATION AND BIOACCUMULATION

Plants, animals and some microorganisms take up and store petroleum hydrocarbons of fuel oils and lubricants. In marine oil spill investigations, No. 2 fuel oil was concentrated in algae, molluscs, and fish and accumulated by herring gulls living or feeding in the polluted area. Since algae provide the bulk of food for detritus feeders, they represent an important route of entry into the food chain (68,75,77).

Marine annelids exposed to water soluble components of No. 2 fuel oil concentrated methyl-substituted naphthalenes in excess of paraffinic hydrocarbons. When placed in clean seawater following 24-hr exposures to the water soluble No. 2 fuel oil, males steadily released most of the accumulated naphthalenes over 72 hours, and complete depuration (less than 0.1 ppm naphthalenes remaining in body tissues) was evident in 400 hr. Gravid females stored the naphthalenes in yolk substance, and only after spawning did the females release the oil. Zygotes and trochophore larvae contained 18 ppm of oil compartmentalized in their yolk stores, which were depeleted by 26 days after spawning. Thus permanent transfer of oil from one generation to the next did not occur (30).

In mussels, concentration of up to 110 ppm of No. 2 fuel oil hydrocarbons occurred after 46 hours of exposure. When placed in non-oily seawater, residual oil dropped to 50 ppm in 7 days, but after 35 days, 8 ppm of oil still remained in mussel tissues. N-paraffins extracted from tissues were chemically similar to those of the No. 2 fuel oil which was utilized (93).

Clams took up 1.7 µg of No. 2 fuel oil hydrocarbons per gram of wet body weight during exposure to 3.9 mg/l of the oil for 8 hours. A disproportionately large quantity of methyl-substituted naphthalenes was concentrated in relation to their quantity in the oil. Filter feeding, adsorption on gill membranes, and active uptake are possible mechanisms involved in bioconcentration of the oil (94).

Oysters and scallops contaminated by No. 2 fuel oil pollution showed evidence of transformation of alkanes, but cycloparaffins and aromatic hydrocarbons were stored unchanged in the organisms' lipid pools (75).

In fish, petroleum hydrocarbons are bioconcentrated in fatty tissue and flesh. Mullet accumulated kerosene as a result of feeding on polluted silt, mud and sand. Polluted fish with up to 77 mg/kg of petroleum hydrocarbons in their flesh do not smell or taste oily, whereas fish containing above 80 mg/kg are obviously "tainted" (95,96). Oil is taken up by gill absorption as well as ingestion, transported unchanged through the liver of the fish, and deposited in the fat pools of the fishes' bodies (96).

Humans ingesting fish or shellfish which are oil contaminated, will also be ingesting these oils; health hazards associated with bioaccumulation of fuel oils and lubricating oils were previously reviewed (2).

In plants sprayed with petroleum oils, uptake through leaf stomates is reported, and oil has been found inter- and intracellularly in leaves, stems and twigs (64,65). Lubricating oils placed on potato leaves migrated into stems and into tubers within 1-5 days of application (67). Within 8 days, turnip roots also accumulated petroleum oils which were sprayed on leaves (64).

VI. ENVIRONMENTAL IMPACT ASSESSMENT

Utilization of fog oils SGF No. 1 and SGF No. 2, and possibly diesel fuel, in smoke pots and generators which produce oil smokes, leads to atmospheric dispersion of the oil microdroplets which comprise the smoke, and deposition onto vegetation, soil and bodies of water in the smoke screening areas. In Section IIIA, Occurrence and Dispersion in the Environment, it was stated that measurements are unavailable of either atmospheric oil smoke concentrations or oil contamination at ground level following the settling of the oil droplets into a film on vegetation, soil and bodies of water. Therefore, data on the average amount of fog oil consumed by one M3A3 oil smoke generator in one hour, and on the spread and travel of the resultant smoke screen downwind from its source, were used to estimate possible atmospheric and ground levels of oil contamination which could result from one hour of smoke screen production. The figures reported in that section were:

average consumption of fog oil by one M3A3	150 liters/hr
average time before smoke settles to ground	1 hour
average travel of smoke downwind from generator	1 to 10 kilometers
average spread of smoke screen	20 to 100 meters
average altitude of smoke screen	15 to 100 meters
calculated average atmospheric oil smoke (droplet) concentration	13 to 2000 mg/m^3 per hour
calculated average ground level oil film concentration	6 to 60 g/m^2 per hour

These figures are purely speculative estimates and do not represent actual field concentrations, which can only be determined by direct measurements. Section VIII of this report, entitled Recommendations, deals specifically with the types of measurements necessary to assess environmental contamination levels. These calculations are for only one M3A3 smoke generator operating for one hour. In actual smoke screening operations, generators or smoke pots are placed in rows of about 6 each, spaced from 20 to 70 m apart to screen a wide target area downwind from the generators. Operations may continue for hours, days, or months in wartime, e.g., Anzio, Italy during World War II. Thus, accumulation of fog oil would greatly exceed 60 g/m^2 at ground level. In addition to the fact that more than one generator is utilized for more than one hour, other considerations which were ignored in the estimations include meteorological factors such as wind and air currents, precipitation and air temperature, nature of the terrain, e.g. dense forest vs. seacoast, and loss of oil from incomplete conversion to fog and evaporation of oil droplets prior to settling.

The chemical compositions of the fog oils and diesel fuel, as discussed in depth in two other reports in this series, Volume 1 (1) and Volume 4 (2), are essentially complex mixtures of aromatic and aliphatic

hydrocarbons containing anywhere from 9 to 25 carbon atoms each for diesel fuel and SGF No. 1 (fuel oils), and from 20-50 carbon atoms in SGF No. 2 (lubricating oil). Aliphatic compounds include straight and branched alkanes and cyclic alkanes, while aromatics include alkyl substituted benzenes, naphthalenes and three and four-ring compounds, collectively referred to as polycyclic aromatic hydrocarbons, including the carcinogenic benzopyrenes. Both fog oils and diesel fuel are products of crude petroleum.

The behavior of the various petroleum hydrocarbons in these three oils determines their toxicity and environmental fate.

The toxicity of oil smokes to humans and other mammals inhaling them was previously reviewed (1,2). In industry, the recommended maximum allowable concentration of oil smoke in workroom air is 5 mg/m^3. Above this level, the smoke is visible and annoying, but inhalation toxicity has not been well documented. Chronic skin exposures to some industrial oils have caused dermatitis and excessive cancers of the skin and possibly the digestive and respiratory systems, although unidentified constitutional factors may have influenced the cancers. Ingestion of petroleum hydrocarbons by drinking the oils results in gastrointestinal tract trauma, lipid pneumonia and, occasionally, death. The effects of ingestion of fish, shellfish or other flora and fauna which have accumulated petroleum hydrocarbons are not reported in the available literature. However, tainted fish and shellfish crops are usually condemned as unfit for human consumption, due to the presence of polycyclic aromatic hydrocarbons in the edible parts.

A. IMPACT IN AQUATIC ECOSYSTEMS

In aquatic environments, oil forms a film on the surface of bodies of water, and some of the petroleum hydrocarbons, mostly the aromatic naphthalenes, dissolve in water. Tainted fish and shellfish result when oil is concentrated in their flesh. Wind, waves and bottom sediment movement spread oils in the marine environment, destroying bottom plants and animals, reducing the stability of the seabottom and causing erosion and further spread of oil. Bacterial transformation and photo-oxidation of oils contaminating aquatic environments lead to a more rapid depletion of the aliphatic hydrocarbons than the polycyclic aromatic compounds, which can form tar-like deposits in bottom sediments. In these environments, the oil hydrocarbons are persistent poisons, resembling DDT, PCB and other synthetic materials in their longevity. The hydrocarbons enter the marine food chain and are concentrated in the fatty parts of the organisms. They can then be passed from prey to predator, where they may become a hazard to marine life as well as humans (68).

Oil may concentrate other fat soluble poisons in the environment, such as insecticides. Once dissolved in an oil film, these poisons may reach concentrations many times higher than expected in waters. The poisons then become available to organisms not usually exposed, leading to eventual accumulation, with petroleum hydrocarbons in the food chain (68).

Fog oil films on bodies of water near smoke screening areas would be subjected to evaporation, biotransformation, dissolution in water and bioconcentration in aquatic organisms. Assessment of the degree of oil contamination by smoke screens, and the short-term and long-term ramifications of this contamination require the implementation of appropriate field studies.

B. IMPACT IN TERRESTRIAL ECOSYSTEMS

Petroleum oil sprays on fruit and citrus orchards are insecticidal to some species, and are moderately phytotoxic. Leaf spotting, premature fall of leaves, bark injuries, root injuries, stunting, reduced fruit yield, premature fruit drop and oil penetration into fruit are reported. (58,61,62). Oil application to leaves of turnips, onions and other vegetables results in oil contamination of their edible roots (64,67). Oil sprays also caused leaf scorching and spotting in foliage trees (59), and inhibited photosynthesis in banana tree leaves when sprayed at a concentration of 84 $\mu g/cm^2$ on leaf surfaces (60).

In soils, one application of 2.5 kg/m^2 of fuel oil was nemacidal even after 12 months. Growth of turnips and beans planted in these soils was severely stunted with deformed leaves. The oil did not leach out of the soils with runoff water. Oil biotransformation rates in soil were approximately 500 g/m^2 per month (27).

Ground level fog oil or diesel fuel concentrations resulting from one hour of smoke screening, in an estimated theoretical range of 6-60 g/m^2, could be expected to damage plants, accumulate in soils and create other potentially harmful situations, but field studies around heavily used smoke screening areas are necessary to assess short-term and long-term consequences of the oil contamination.

VII. RECOMMENDATIONS

Recommendations for further study of the environmental impact of fuel oil (SGF No. 1) diesel fuel and lubricating oil (SGF No. 2) smoke screens include the following:

1. Measurement of atmospheric concentrations of the particulate (droplet) oil smoke screen at specific distances from the source of the smoke (e.g. smoke generator or smoke pot) and at various times after the generation of a specific volume of smoke.

2. Estimation by appropriate models to arrive at ground concentrations of these oils at various sites in relation to the source of the oil smoke generator, and in relation to the volume of smoke generated.

3. Estimation by appropriate models to arrive at the concentrations of petroleum hydrocarbons dissolving in waters contaminated by oil smoke fallout in areas heavily used for smoke screening operations.

4. Estimation by appropriate models to arrive at soil concentrations of petroleum hydrocarbons in areas heavily used for smoke screening operations.

5. Other studies to evaluate transport, distribution, biotransformation, bioconcentration and bioaccumulation of the fog oils and/or diesel fuel by water, soils, sediments and organisms contaminated as a result of long-term smoke screening operations.

6. Matching of these water, sediment and soil concentrations of fog oils and/or diesel fuel to known toxic effects on flora and fauna in the environment of the smoke screening operations.

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APPENDIX

Information Sources Examined

Computer Searchable Data Bases

1 - National Technical Information Services (searched on Oct. 5, 1977)

2 - Toxline/Toxback (searched on September 20, 1977)

3 - Chemical Condensates (searched on September 27, 1977)

4 - ISI SCISEARCH (searched on Oct. 5, 1977)

5 - Cancerline (searched on October 4, 1977)

6 - NIOSH Technical Information Center file (received on Oct. 11, 1977)

7 - Defense Documentation Center (received on September 25, 1977)

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