ISOLATION AND CHEMICAL CHARACTERIZATION OF PETROLEUM REFINERY WASTEWATER FRACTIONS ACUTELY LETHAL TO *DAPHNIA MAGNA*

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PREFACE

This study is concerned with the isolation and identification of the most toxic portion of petroleum refinery wastewaters. It is hoped that this study will aid the goal of decreasing the discharge of toxic wastewaters and thus contribute to a healthier aquatic environment.

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CHAPTER I

INTRODUCTION

Amendments to the Federal Water Pollution Control Act established federal programs to make the waterways of the United States fishable and swimmable by 1983 and to achieve zero discharge of pollutants by 1985. In addition to these constitutional definitions, the scope of the law has been defined by court action. A settlement agreement in 1976 in response to four suits brought against the Environmental Protection Agency (EPA) by various environmental groups resulted in a "consent decree" in which EPA agreed to develop and promulgate effluent guideline limitations for 65 compounds or types of compounds. The "consent decree" provided that 21 industries including the petroleum refining industry be addressed by those effluent limitations. The result of that court action has been the establishment of effluent standards which must include the toxicity of the effluent to aquatic organisms (Greenwood 1979). These guidelines particularly impact petroleum refineries and petrochemical operations.

Petroleum refineries and petrochemical plants must treat and dispose of huge volumes of toxic wastewater from a variety of sources. Quality and quantity of wastewater as well as the toxic portion of that wastewater may fluctuate significantly within a plant as well as among refineries (Matthews 1978). Each wastewater contains a complex and

somewhat unique mixture of refractory organic pollutants which have toxic effects on aquatic organisms and persist in the environment (Zeitoun 1979).

Current treatment processes were not specifically designed to eliminate toxicity and precludes EPA's goal of zero discharge. It is necessary to design new treatment systems that will allow removal of all toxic elements. The development of such a system requires a knowledge of the physical and chemical characteristics of the toxic components.

The objective of this project is to determine if fractionation of a complex wastewater followed by Daphnia magna toxicity testing is an effective method for isolating the acutely toxic components.

Secondary objectives are:

1) to determine if the acutely toxic components are organic or inorganic in nature and,
2) to determine if the acutely toxic components could be isolated on the basis of physical and chemical properties such as volatility or aqueous solubility in acidic and alkaline solutions.

CHAPTER II

LITERATURE REVIEW

Petroleum Refinery Wastewaters

Toxicity

Little extensive information exists on the toxicity of refinery wastewaters to aquatic organisms, but fragmentary toxicity data has been generated in a variety of ways (Appendix A). Graham (1968) found 48 h TLm (median tolerance level) values that ranged from 4-70% (volume of effluent/volume of dilution water) for fathead minnows (Pimephales promelas) exposed to petroleum refinery wastewaters. The fish were also adversely affected by extended exposure to subacute concentrations of effluents with initially low acute toxicity. Effluent components responsible for the chronic effects were not determined. The 24 h TL_m for bluegill sunfish (Lepomis macrochirus) exposed to composite refinery effluents was 62%, 55%, and 21% (Turnbull 1954). A "safe" concentration for bluegill was estimated to be 6% of the refinery effluent. The toxicity of refinery effluents to redear sunfish (Lepomis microlophus) expressed as the 24 h median lethal concentration (LC50) ranged from 18-100% effluent (Matthews and Myers 1976). The toxic effects decreased with increased wastewater treatment.

An assay of petroleum refinery effluents with rainbow trout (Salmo

gairdneri), tropical flagfish (Jordanella floridae), and Daphnia pulex demonstrated the chronic toxicity of the effluents (Sprague 1978). Growth of rainbow trout was severely affected at 30% effluent with a threshold judged to be near 10%. The gill purge rate (cough response) of rainbow trout increased linearly between 25 and 50% effluent. A "safe" concentration which would not significantly affect reproduction of Daphnia was estimated to be 0.52% effluent. The concentration for chronic lethality of Daphnia was 6.4%. Daphnia pulex were 2.5 times more sensitive to refinery effluents than rainbow trout.

Population, community, and behavioral responses have indicated that refinery effluents may cause chronic or cumulative effects. Several studies conducted at Oklahoma State University document those effects. A study of Tendipedidae in oil refinery effluent holding ponds indicated that population fluctuations were related to effluent toxicity (Tubb 1965). One species was more resistant to the toxic effects of the effluent than were the others . Species diversity of benthic macroinvertebrate assemblages has been used in comparing the effectiveness of different wastewater treatments of a refinery effluent (Burks 1977). Effluent passed through biological treatment-dual media filtration was toxic to the benthic macroinvertebrates. That toxicity was eliminated by adding an activated carbon filtration system after the dual-media filtration. Fathead minnows exposed to a continuous flow of the effluents yielded toxicity results similar to the macro-invertebrates. Behavioral assays have indicated that refinery effluents may affect the behavior of fish. Biologically treated refinery wastewaters caused a decrease in agonistic displays of orange-spotted sunfish (Lepomis humilis) (Petersen 1979). Those

population, community, and behavioral effects indicate that subacute exposure to the effluents can be damaging to the stream community.

Adequately treated petroleum refinery effluents may not cause acutely toxic effects. A Canadian refinery wastewater,tested three times within 18 months,demonstrated no acute toxicity to fish (Tertipis 1974).

Composition

The complexity of refinery wastewaters has hindered the identification of the components responsible for the toxicity. Any component present in crude oil, generated by petroleum processing, or added to treat process water may be present in the wastewater. In spite of this complexity numerous inorganic compounds and metals as well as hundreds of organic compounds have been identified in refinery effluents. The most comprehensive study of refinery effluents was conducted by the American Petroleum Institute (API) and the EPA. They determined the concentrations of 129 inorganic and organic "priority pollutants" (pollutants representative of the most potentially dangerous environmental contaminants) in several refinery effluents to serve as baseline data to aid in the regulation of the refining industry (Radian 1978). The API data revealed the presence of 36 organics and 13 elemental "priority pollutants" from six refineries. Concentrations ranged from <1 to 60 ug/l for organics and <1 to 1100 ug/l for elemental pollutants. The EPA identified 18 organic and 12 elemental "priority pollutants" in 17 refineries. The concentrations ranged from <1 to 2000 ug/l for organics and <1 to 1000 ug/l for the elements. The API identified 15 volatile compounds, 13 polynuclear

aromatic hydrocarbons, four phthalate esters, and seven phenols.

Since the complete chemical analysis of refinery effluents can be overwhelming, many investigators analyze only a certain fraction of the effluent such as volatiles, sulfides, heavy metals, or polynuclear aromatic hydrocarbons (Appendix B). Two of the more extensive studies include Raphaelian (1978) who identified 304 organic compounds in the neutral fraction of a dissolved air floatation unit (DAF) (a pressurized aeration tank used to remove oil in the wastewater treatment system of most refineries) and Burlingame (1976) who identified seven aromatic and five non-aromatic types of compounds present in refinery effluents (Appendix C and D).

Correlations of Refinery Wastewater Components and Toxicity to Fish

Most of the components identified in petroleum refinery wastewaters are acutely toxic to aquatic organisms. Toxicity has been determined through bioassays of individual compounds and by correlating the toxicity of effluents with chemical data. Matthews (1976) exposed redear sunfish to refinery effluents and process streams. Correlations between toxicity and chemical data indicated that ammonia, sulfides, cyanides, and phenolic compounds alone or in combination were major contributors to toxicity. Three refineries produced samples more toxic than chemical data predicted which indicated the presence of toxic components other than those measured. Orange-spotted sunfish (Lepomis humilis) bioassays revealed that the most toxic of several refinery effluents had the highest chromium and zinc concentrations, total organic carbon values, and conductivity values (Petersen 1979).

Mattson (1976) determined 96 h LC50 values for juvenile fathead minnows exposed to five different classes of compounds often detected in refinery wastewaters (Appendix E). Pentachlorophenol had a 96 h LC50 of 600 ug/l and was the most toxic of the compounds tested. Phenolic compounds, di-n-butyl phthalate, and bis-(2-ethylhexyl) phthalate, common contaminants of refinery effluents are acutely toxic to rainbow trout (DeGraeve 1980, Hrudey 1976).

Daphnia

Considerations for Use of Daphnia

Many investigators have used Daphnia to test the toxicity of various materials. Several ecological and laboratory advantages exist for using Daphnia instead of other organisms. Daphnia and other zooplankters are an important link in the aquatic food chain and are relatively sensitive to toxicants. Anderson (1950) compared the toxicity of metals to Daphnia and fish, concluding that Daphnia were more susceptible to toxic cations than fish. Daphnia are also more sensitive than rainbow trout to refinery effluents (Sprague 1978).

The laboratory advantages of using Daphnia magna have been reviewed by Anderson (1944). Daphnia are small (5 mm) and cultures can be maintained in a small area. They have a relatively short life span and mature rapidly. Those characteristics facilitate chronic bioassay studies. Daphnia produce young in about 1 week and may release 20 or more offspring every 2 to 3 days. The rapid production of large numbers of offspring provides many organisms. Normal Daphnia

reproduction is parthenogenic, assuring minimal genetic variation among test organisms.

Temperature has a strong influence on the survival of Daphnia magna (Warren 1899). The mean lifespan for Daphnia magna varied from 25 days at 28°C, 42 days at 18°C, 88 days at 10°C and 108 days at 8°C. The cladoceran (Daphnia) heartbeat increases with an increase in temperature until the organism nears death (Seiwell 1930) and is important since the life span is shortened or lengthened as the average metabolic rate is increased or decreased (MacArthur 1929).

Light, pH, and age as well as the condition of the culture may affect the response of Daphnia to toxicants. Adult Daphnia can survive a pH range of 5.4 to 9.5 (Klugh 1926) or 6 to 9.5 (Anderson 1946). The optimal pH range is 8.1 to 8.5 (MacArthur 1929). Light can affect reproduction as well as sensitivity to toxicants (Buikema 1973). Lower light intensities stimulate reproduction, reduce metabolic stress, affect filtration rate, and enhance the assimilative capacity and energy budget of Daphnia pulex (Buikema 1980). Daphnia magna may be more sensitive to some toxicants during ecdysis, but develop increased resistance to some materials with age (Breukelman 1932, Anderson 1980). This was demonstrated with amyl alcohol, DDT, and most other substances tested by Anderson (1980). Chromium also shows increased toxicity during ecdysis (Lee 1976). Daphnia from a stressed culture may be less sensitive to toxicants than Daphnia from an unstressed culture. During periods of stress, Daphnia produce large numbers of ephippia, sexual eggs (Pennak 1979). A simulated refinery effluent was more toxic to cultures without ephippia than to cultures with ephippia (Lee 1976).

Toxicity of Chemicals to Daphnia

Many inorganic compounds and elements are toxic to Daphnia. Anderson performed numerous studies on the effects of inorganics on Daphnia cultured in Lake Erie water. Analysis of the toxicity of 38 sodium salts to Daphnia magna indicated that sodium chromate was the most toxic (Anderson 1946). Sodium dichromate, sodium cyanide, and sodium iodide were toxic and sodium bromide was the least toxic. The threshold for immobilization ranged from less than 0.31 to 8200 mg/1. The toxicity thresholds have been determined for various substances found in industrial wastes (Anderson 1944). Copper salts, chromates, and potassium permanganate were the most toxic compounds. The threshold concentration for immobilization was less than 0.63 mg/1 for those compounds. The most toxic compounds tested in another study were cadmium chloride, mercuric chloride, and silver nitrate (Anderson 1950). The threshold concentration was less than 0.006 mg/1 for those three compounds. The 24 h and 48 h LC50's were determined for eight inorganic "priority pollutants" for Daphnia magna (Appendix F), (LeBlanc 1980). The 24 h LC50's ranged from 0.0015 mg/1 for silver to >530 mg/1 for antimony.

Certain heavy metals have a chronic effect on Daphnia magna. Three week exposures to determine effects upon reproductive impairment demonstrated that cadmium, mercury, cobalt, copper, lead, nickel, and zinc were toxic at concentrations less than 0.1 mg/1 (Biesinger 1972). Metal toxicity was positively correlated with the solubility of the metal sulfides, indicating that the metals might combine with sulfhydral groups on enzymes. Such combinations could affect the

solubility and catalytic activity of the enzymes.

Inorganic compounds in combination may be more toxic to Daphnia than single compound bioassays indicate. Freeman (1953) tested sodium bisulfite, sodium carbonate, sodium chromate, sodium silicate, and sodium sulfate individually, in pairs, and in triplets. The most toxic were the combinations of three compounds with the single compounds the least toxic.

Anthropogenic and natural organic compounds may be toxic to Daphnia. Pesticides and herbicides have been shown to be toxic to Daphnia. The 32 hour LC50 for Daphnia exposed to DDT is less than 0.001 mg/l (Anderson 1945). The 26 hour LC50 was 0.0044 mg/l (Frear 1967). Within 24 hours, Daphnia magna accumulate DDT 16,000 to 23,000 fold from dilute suspension in water (Crosby 1971). DDT uptake is principally through the carapace and not by ingestion. Frear and Boyd (1967) tested the toxicity of 30 pesticides to Daphnia magna. The 26 hour LC50 was less than 0.06 mg/l for all pesticides. Analysis of 37 herbicides determined that a dichloronapthoquinone was the most toxic and that Silvex, 2,4-D, and a dinitrotriflurotoluidine were also toxic (Sanders 1970). The 48 hour TL50 for those four herbicides was less than 1 mg/l. Sodium anthraquinone-a-sulfonate was the most toxic of 10 sodium sulfonates tested (Freeman 1953). Toxicity threshold determinations for Daphnia magna indicated that the more complex the ring structure and the more ring substitutions, the greater the toxicity of the sulfonates.

DeGraeve (1980) found several phenolic compounds to be toxic to Daphnia pulicaria. Hydroquinone had a 48 hour LC50 of 0.162 mg/l. That was 100 times more toxic than the other phenols tested. Acute

toxicity tests of 78 organic "priority pollutants" to Daphnia magna indicated that chlorinated phenols were more toxic than chlorinated benzenes, chlorinated ethanes and nitrated phenols (Appendix F) (LeBlanc 1980). Acrolein (LC50 = 0.083 mg/l) was the most toxic organic tested.

Loeb (1909) studied the narcotizing effects of various alcohols on Daphnia. The minimal concentrations to cause anesthesia were methanol (38.4 g/l), ethanol (27.6 g/l), propanol (7.2 g/l), and amyl alcohol (2.96 g/l). The threshold concentration for immobilization of Daphnia was 32,000 mg/l for methanol and 18,400 mg/l for ethanol (Anderson 1944).

Petroleum and petroleum wastes can be toxic to Daphnia. Oil emulsifiers proved to be more toxic than crude oil but a crude oil and emulsifier mixture was more toxic than the emulsifier alone (Dowden 1962) . The addition of the emulsifier allowed closer contact between the oil and the organism. Volatile organic compounds appeared to be the agents in petroleum refinery wastewater which were toxic to Daphnia (Dorris 1972). Some of those compounds have been identified as methyl, dimethyl, and ethyl phenols.

Analytical Methodology

Chemical analyses

Established procedures exist for analyzing metals in water (EPA 1974, 1979). The concentration of most metals can be determined by digesting the sample with nitric acid and analysis on an atomic absorption (AA) spectrophotometer. Atomization of the sample in the

AA can be done with a graphite furnace or an oxygen-acetylene flame. Mercury is analyzed by the cold vapor technique. A chemical reaction in the sample releases ground state mercury into a purge gas which passes through the AA.

The EPA method for analyzing semi-volatile organics in water involves a liquid-liquid extraction procedure (EPA 1974, Federal Register 1979). The sample is extracted at basic (pH >11) and then acidic (pH <2) conditions with methylene chloride to produce two fractions. The extracts are condensed to 1 ml and analyzed by gas chromatography.

Volatile organic compounds are determined with a purge and trap technique (Bellar 1974, Federal Register 1979). The water sample is purged with an inert gas at room temperature. The gas strips volatile organics from the sample and carries them into a trap packed with Tenax© and silica gel adsorbents. The volatile organics are trapped by the adsorbents. The trap is heated and the organics are purged into a gas chromatograph.

A cooperative survey by the EPA and the American Petroleum Institute (API) used the EPA methods to analyze the wastewaters of 17 petroleum refineries (Radian 1978). Special analytical studies were conducted by the API including spiking experiments, inter-laboratory comparisons, and analysis of sample blanks. Results from those investigations indicated that recoveries and precision were extremely variable. The API concluded that currently applied EPA sampling and analytical protocol was inadequate for the quantitation, and in some cases, identification at the low ug/l level.

Raphaelian (1978) used EPA methodology for analysis of organics in a refinery wastewater treatment system. A major problem encountered was emulsion formation requiring emulsion breaking and phase separation by various techniques. The trace concentrations of most components and the complexity of the sample prevented the complete characterization of the sample.

Other techniques have been used in analyzing organics when the EPA procedures were not appropriate. Continuous flow extraction techniques, gentle liquid-liquid extraction methods which do not require vigorous sample agitation, can be used to prevent emulsion problems. High resolution mass spectrometry combined with capillary column gas chromatography has been used to overcome problems associated with the characterization of trace organics in a petroleum refinery wastewater sample (Burlingame 1977). Detailed analysis of the high resolution mass spectral data can reveal the identity of components not chromatographically resolved. ^{13}C Fourier transformed NMR spectra have been used in a further attempt to characterize the samples. Organic environmental contaminants have also been analyzed by gas chromatography with electron capture detection and flame ionization detection. High pressure liquid chromatography has been used with ultraviolet and fluorescent detectors for the analysis of organics (Saxena 1980, Preston 1979, Katz 1980, Giam 1980).

EPA procedures for analyzing volatile organics have been modified in various ways by environmental chemists. Several different polymer adsorbents have been used in place of Tenax© and silica gel (Murray 1977, Keith 1979). Some chemists have chosen to heat or stir the sample while purging (Lingg 1977, Murray 1977). The configuration of the

purge and trap device as well as the time of purging have been varied in an attempt to increase the sensitivity (Murray 1977, Lingg 1977, Keith 1979). Qualitative and quantitative analysis of polar volatile organics, which are not retained by the polymeric adsorbents has been done with a recirculating steam distillation technique (Peters 1979). The volatile organics concentrated by this method are injected directly into a gas chromatograph.

Chemical Class Fractionation

Fractionation of complex organic mixtures into chemical classes is an important step in identifying components and in screening for biological activity of the components. Some degree of chemical class fractionation prior to a bioassay is usually required because toxic effects of the entire sample often prevent the analysis of the effect of interest. An iterative process of fractionation followed by bioassay, subfractionation, and further bioassay is useful in identifying bioactive constituents.

Numerous separation techniques and combinations of techniques have been used for fractionating complex mixtures. Ion exchange and adsorption chromatography are two such techniques. A portion of the sample is placed at the top of a chromatographic column and eluted with various solvents. Each solvent should elute a chemically distinct fraction. Alumina and silica gel are two adsorbents commonly used to separate hydrocarbons into aliphatic and aromatic classes. Snyder (1961) reviewed compound class separation over these two adsorbents and reported that silica gel is superior in the separation of aliphatics from olefins and aromatics while alumina provides sharper

fractionation of aromatics. Saturates can be cleanly separated from aromatics by pentane elution from alumina. Alumina is also useful in separating nitrogen and oxygen compounds from other fractions.

Alumina adsorption chromatography allowed lake sediment extracts to be analyzed for aromatic hydrocarbons (Strosher 1975). Alumina was able to separate the aromatics from aliphatic hydrocarbons, porphyrins, chlorins, and carotenoids which were also present.

Sulfoxides have been separated from petroleum by cation exchange chromatography (Okuno 1967). Fifty milliliters each of n-pentane, benzene, methanol, and 10% isopropylamine in methanol were successively used as eluents. The methanol fraction was chemically analyzed for sulfoxides. Cation and anion exchange chromatography were used in characterizing refractory organic compounds present in coal conversion streams (Pitt 1979). The sample was separated on a heated, high pressure anion exchange column with an ammonium-acetic acid buffer gradient as the eluent. Fractions were collected and applied to a cation exchange column for further separation. The resulting fractions were analyzed by GC/MS.

Gel permeation chromatography has been useful in fractionating complex environmental samples. Gels are normally assumed to separate compounds on the basis of molecular weights. Urano (1980) measured the characteristics of Sephadex G-15 and G-25 in the separation of 46 soluble organic pollutants. His data indicates that for many low molecular weight compounds, the chemical structure is more important than weight in determining separation. Gel chromatography can also be used for lipophilic-hydrophilic partitioning (Jones 1977). A fractionation procedure using Sephadex LH-20 gel provides a gentle

preparative scale, chemical class separation for a shale-derived crude oil and a coal-derived oil. The gel eventually separates the sample into hydrophilic, polymeric, hydrogen bonding, aliphatic, one and two ring aromatic, and polynuclear aromatic fractions.

Lumpkin (1964) isolated a trinuclear aromatic fraction from a coker gas oil. The separation steps included the use of distillation, silica gel, thermal diffusion, and alumina gel. After isolation of the trinuclear fraction it was analyzed by high resolution mass spectrometry.

Sediments have been analyzed for polycyclic aromatic hydrocarbons by a fractionation technique (Giger 1974). The sediments were soxhlet, extracted and chromatographed on copper, Sephadex LH-20, silica gel, and alumina. The fraction of interest was complexed with trinitrofluorenone and rechromatographed on alumina. Seven fractions were collected from the alumina column and analyzed by UV-visible spectrophotometry and mass spectrometry.

Nitrogen bases in petroleum and petroleum products have been characterized by isolation and separation techniques (Jewell 1965, McKay 1976, and Jewell 1972). Paper electrophoresis, thin layer chromatography, the Hinsberg reaction, and ferric chloride on clay were used in addition to separation techniques previously mentioned.

A 19-step procedure has been developed for analyzing the extractable priority organics in municipal wastewater sludge (DeWalle 1979). The sample is extracted at acid and base pH. The acid portion is fractionated by gel chromatography, florisil separation, and cesium silicate separation. Various fractions produced are then extracted with ether or methylene chloride and analyzed by GC/MS.

Fractionation and Bioassays

The concern that cigarette smoke is carcinogenic has prompted studies to determine the identity of the bioactive compounds (Wynder 1957, Bock 1958, Swain 1969, Severson 1976, Snook 1977, Mizusaki 1977, Lee 1976, and Severson 1980). The cigarette smoke condensate (CSC) has been fractionated and tested for bioactivity in several different ways. CSC is initially partitioned into organic solvents at varying pH. The resulting fractions are further separated by gel and ion exchange chromatography. Mouseback testing, rabbitback testing, and the Ames test have been used to test the mutagenicity and carcinogenicity of the various fractions. Compounds present in the samples have been identified by gas chromatography, high pressure liquid chromatography, NMR, fluorescent and UV analysis, and GC/MS. Results from those studies indicate that the most biologically active fraction contains polynuclear aromatic hydrocarbons of three rings and greater. Chemical analyses indicate the presence of over 200 polynuclear aromatic hydrocarbons.

Organic wastewater concentrates from six treatment plants were tested for mutagenicity using the Ames bioassay test (Rappaport 1979). Concentrates were prepared by passing 4-8 l of wastewater through a mixture of XAD-2 and XAD-7 resin. The concentrates were extracted at acidic and basic pH and then back extracted at the opposite pH. The mutagenic activity was primarily in the basic and neutral fractions.

A similar fractionation and bioassay procedure has been used for identifying organic compounds in a mutagenic extract of a surface drinking water (Coleman 1980). The water was concentrated by reverse

osmosis, partitioned into several fractions, and tested for mutagenicity with the Ames test. The mutagenic fraction was partitioned by gel chromatography, liquid-liquid extractions, and analyzed by GC/MS. Polynuclear aromatic hydrocarbons and polychlorinated biphenyls were the predominant compounds identified in the mutagenic fraction.

Chemical Class Fractionation and Bioassay Studies In the Energy Industry

Guerin (1978) reviewed the use of chemical class fractionation and bioassay in analyzing complex environmental mixtures. The review determined two areas of concern which must be considered in this type of analysis: (1) the relevance of the material applied to the bioassay and (2) the compatibility of the material with the bioassay. Chemical relevance is achieved when the bioassay is dosed with a material whose chemical composition mimics that which reaches the natural point of impact. Difficulties with compatibility occur when the material being tested contains constituents which interfere with the test organisms ability to respond to the effect of interest. Liquid-liquid extractions and gel chromatography were both found viable for the bio-testing of coal and shale-derived oils (Guerin 1978).

A separation procedure that had been used for fractionating cigarette smoke condensate was applied to coal liquefication products (Rubin 1976). The Ames test demonstrated high mutagenic activity in the neutral fractions and in the ether-soluble base fraction. Further studies on these samples indicated that alkaline constituents were the major contributors to the mutagenic effect (Guerin 1980). High

resolution chromatographic and mass spectroscopic analysis showed the causative agents to be polycyclic aromatic primary amines.

Tabata (1961) used a fractionation procedure and Artemia salina bioassays to determine the most toxic components of a gas liquor. The main toxic components were in the cyanide-sulfide fraction and the phenol fraction. The toxicity of the gas liquor could be reduced 10 fold through extraction of the phenol fraction with benzene, followed by boiling the residual solution.

Dorris (1972, 1974) developed a procedure for isolating a toxic fraction from oil refinery effluents. A flash evaporation technique produced the most significant results. The volatile fraction produced by the flash evaporator was much more toxic to fathead minnows and Daphnia magna than the original effluent or the non-volatile fraction. Some compounds in the volatile fraction were identified by GC/MS as phenols and normal hydrocarbons. Heavy metals were not present in acutely toxic concentrations.

CHAPTER III

METHODS

Introduction

Wastewater was collected in amber colored glass containers from the final effluent of a petroleum refinery, transported on ice to the laboratory, and stored in the dark at 4° C. The sample was used within a few days of collection to minimize changes in the chemical characteristics of the water.

Steam distillation, cation exchange, anion exchange, solvent extraction, column chromatography, and carbon adsorption were used to produce separate fractions from the wastewater. Each fraction, including the raw wastewater, was tested with Daphnia bioassays to determine relative toxicity. The most toxic fraction was analyzed chemically, fractionated further, tested for toxicity, and again analyzed. The chemical characteristics of the toxic fraction determined how the second fraction was produced.

Steam Stripping

A falling film evaporator was used to steam strip the wastewater to provide a volatile and a nonvolatile fraction . Wastewater dripped through a 122 cm by 5 cm glass column partially filled with glass marbles. Steam produced from wastewater passed up the column, stripped volatile compounds from the falling wastewater, entered a cold water condenser, and was collected in a round bottom flask. A non-volatile

fraction was collected from the bottom of the column. The rate at which the wastewater dripped was monitored and adjusted to maximize the toxicity difference among the fractions.

Cation and Anion Exchange

Wastewater samples were passed separately through a cation and anion exchange column to produce two fractions. The cation column should remove all or most of the positively charged ions from the wastewater and the anion column should remove the negatively charged ions and permit evaluation of the relative contribution of these ions to toxic effects of the wastewater. The ion exchange resin was packed between plugs of glass wool in a 30 by 1.5 cm glass column. The wastewater passed slowly upward through the column by a siphon action and collected at a rate of about 20 ml/min.

Carbon Adsorption

A column of activated carbon, ICI-Hydrodarco©, was used to remove many non-polar organic compounds from the wastewater. The column was packed with granular activated carbon and used in the same manner as the ion exchange columns.

Solvent Extraction

Solvent extraction of the volatile fraction was used to generate a base-neutral residue and an acid residue. One or 2 liters of the volatile fraction was extracted at pH $\geq$ 11 with 100 ml methylene chloride. The pH of the volatile sample was then adjusted to $\leq$ 2 and extracted with another 100 ml methylene chloride. The extraction at

pH $\geq$ 11 concentrated basic and neutral organic compounds in the solvent. Organic acids were concentrated in the solvent by extraction at pH $\leq$ 2. The solvent of each fraction was removed by air drying. The remaining residue was dissolved in 2 ml methanol and added to *Daphnia* culture water in an amount equal to that extracted. Those samples were then tested for acute toxicity.

The base-neutral residue was further separated by silica gel column chromatography into aliphatic, aromatic, and semipolar fractions. The base-neutral residue in 2 ml methanol was placed at the top of a silica gel-hexane column instead of being added to culture water. The residue was eluted from the column in three steps with 50 ml each of hexane, benzene, and methanol, which removed aliphatic, aromatic, and semipolar compounds, respectively. The solvents were removed by air drying and the resulting residues were dissolved in 2 ml methanol and placed in the appropriate volume of culture water. This procedure allowed separate toxicity testing for volatile, base-neutral aromatics, aliphatics, and semipolar compounds.

Daphnia Bioassays

Neonate *Daphnia magna* (Strauss) served as the bioassay test organism. *D. magna* were cultured in aged, dechlorinated tap water and fed a suspension of powdered alfalfa and trout chow. The organisms were kept in a constant temperature chamber at 20°C and exposed to a 16h photoperiod (0700 - 2300).

Static bioassays were structured to provide the LT50 (time necessary for a solution to kill 50% of the exposed organisms) for each fraction. Tests were performed in quadruplicate with six *D. magna* per

100 ml glass container. The neonates were placed one at a time into each of the randomly arranged test solutions. Twenty-four D. magna in four containers of culture water served as controls. Neonates were observed constantly for the first hour after their addition to the test solutions. They were also observed at 2, 4, 8, 24, 48, 72, and 96 hours. Some experiments were terminated when treatment controls died or when the experiment could produce no further useful data. At each observation the number of immotile D. magna in each container were recorded. An organism was considered immotile if it showed no viable movement even after a test organism was placed in the water column. Although a nonmotile organism may have been alive, it was considered dead since it probably would die if not placed in a nontoxic solution (APHA 1981). At the conclusion of the bioassay, the results of the 24 D. magna exposed to each fraction were pooled and the percent immotile calculated. The bioassay was considered invalid if more than 10% of the controls died.

Organic Extractable Compounds

An aliquot of the sample was extracted with methylene chloride, dried over sodium sulfate, concentrated, and analyzed by capillary column, gas chromatography-mass spectrometry (GC/MS). Two liters of sample, with pH adjusted above 11, was extracted in a 3 liter separatory funnel with three 60 ml portions of methylene chloride in succession. The methylene chloride was dried over sodium sulfate and concentrated to about 200 ul. One microliter of the concentrate was injected into a Hewlett Packard 5992B gas chromatograph/mass spectrometer

to separate the components and generate a mass spectrum for each compound . Many of the compounds present were identified by comparing the generated spectra with reference spectra. The Library of Mass Spectral Data (Cornu and Massot 1966) and the NIH-EPA mass spectral data base contain reference spectra which were used to identify unknown compounds. After the basic sample had been extracted and the methylene chloride removed, the pH of the sample was adjusted to <2. This acidic sample was extracted and carried through the same procedure as the basic sample.

Volatile Organics

Volatile organic materials were analyzed by a purge and trap method followed by desorption into a GC/MS (Bellar and Lichtenberg 1974). Nitrogen was bubbled through 50 ml of sample contained in a purging chamber. The procedure transfers the volatile organics from the aqueous phase to the gaseous phase which then passes through a sorbent bed designed to trap the non-polar compounds. Once purging was complete, the trap was rapidly heated and flushed with helium to desorb the components into a GC/MS. The desorbed components were identified by comparing their spectra with mass spectral patterns of known compounds.

Ammonia

An Orion model 407A specific ion meter with ammonia electrode was used to measure the ammonia concentration. The log scale of the meter was calibrated with 1, 10, and 100 mg/l ammonia. The pH of the sample was adjusted to 11 with 10 molar sodium hydroxide and the ammonia

concentration was read directly from the meter.

pH

The hydrogen ion concentration was measured with an Instrument Lab, Inc., model 165 pH meter. The pH probe was calibrated against standard buffers at 4.0 and 9.0 pH units.

Total Organic Carbon (TOC)

A Beckman 915, two channel carbon analyzer was used to determine the total carbon and the inorganic carbon present in the sample. The total organic carbon was measured by high temperature oxidation of the organic matter and infrared analysis for CO_2. Inorganic carbonates were decomposed with phosphoric acid at a much lower temperature and analyzed for CO_2. The difference between total and inorganic carbon was the total organic carbon.

Dissolved Oxygen, Conductivity

Dissolved oxygen concentrations during bioassays was monitered with a 51B YSI oxygen meter. Conductivity of the sample was measured with a YSI conductivity meter.

Hardness

Hardness as calcium carbonate was determined by computation from the concentrations of hardness-producing cations. The concentration of each hardness-producing cation was multiplied by the proper factor to obtain the equivalent calcium carbonate concentrations which were then totaled . To obtain the $CaCO_3$ equivalent, the concentration found was

multiplied by 2.497 and 4.116 for the Ca and Mg cations respectively. (APHA 1981):

Heavy Metals Analysis

The concentrations of 10 metals in the samples were determined with a Perkin-Elmer 5000 atomic absorption spectrophotometer (AA). Sodium, potassium, calcium, magnesium, iron, zinc, and manganese concentrations were measured by sample digestion and atomization with an air:acetylene flame. Lead, chromium, and cadmium were analyzed by furnace atomization with the AA.

Sulfide

Lead acetate paper was used to detect the presence of sulfide at concentrations greater than 1 mg/l. In an acidic medium the sulfide ion combines with lead to form a black precipitate on the paper.

Cyanide

The chloramine-T procedure was used to detect the presence of cyanide at concentrations greater than 50 ug/l. Chloramine-T forms CNCI when exposed to cyanide. CNCI forms a red-blue color when mixed with a pyridine-barbituric acid reagent.

Statistical Analysis

The LT50's were calculated with the Statistical Analysis System (SAS) PROBIT procedure (Helwig 1979). Ninety-five percent fiducial intervals were calculated for each sample that had at least two partial kills. The TTEST procedure was used to determine significant differences among the data.

CHAPTER IV

RESULTS AND DISCUSSION

Steam Stripping

In order to compare the relative toxicity of volatile and non-volatile components, a petroleum refinery wastewater was stripped with steam to produce a volatile and a non-volatile fraction. Steam stripping and subsequent toxicity testing was performed 12 times in the course of the study. In nine of the 12 bioassays, the LT50 of the volatile fraction was less than 8 h, while the LT50 of the original wastewater was greater than 30 h (Table I). The volatile fraction was significantly more acutely toxic than the non-volatile fraction or the original wastewater ($p = 0.0001$ for both). The acute toxicity of the non-volatile sample was less than the original wastewater and indicated that steam stripping actually removed acutely toxic components from the original wastewater. Apparently, the majority of the acutely toxic components were removed from the wastewater by steam stripping and concentrated within the volatile fraction.

Chemical characteristics of the volatile and non-volatile fractions were different. The volatile fraction had low TOC values and no detectable heavy metals, but ammonia concentrations significantly greater than the original wastewater ($p = 0.042$). In contrast, the non-volatile fraction contained heavy metals, high TOC values, but no detectable ammonia (Appendix H, I, and J). Comparison of GC/MS scans

of solvent extracts from the two fractions revealed the presence of comparatively high levels of low molecular weight organic compounds in the volatile fraction, but not in the non-volatile fraction.

TABLE I

DAPHNIA LT50's (h) FOR THE ORIGINAL WASTEWATER, VOLATILE FRACTION, AND THE NON-VOLATILE FRACTION

Sample	Original wastewater	Volatile	Non-volatile	Duration of test (h)
1	4	0.5	NM*	48
4	56.3 (49.4-63.2)†	5.6	60 (53.3-66.7)	96
5	NM	5.6	--	96
7	19.2 (8.5-29.2)	7.0 (5.6-8.4)	NM	96
16	NM	6.6	NM	96
20	NM	0.2	NM	48
25	NM	40.0	NM	144
28	--	17.0	--	96
29	105.9 (89.8-122.0)	21.5 (19.4-23.6)	NM	161
30	NM	3.0	NM	28
31	NM	2.2	NM	96
33	NM	3.3	NM	117

* NM = no mortality.
† 95% fiducial intervals were calculated when there were at least 2 partial kills.

The contrast in chemical characteristics of the two fractions permit certain inferences to be reached concerning the chemicals responsible for the acutely lethal effects. The toxic effects of the volatile fraction were due to either low molecular weight volatile organic compounds or volatile inorganics such as ammonia, hydrogen sulfide, or hydrogen cyanide. It was concluded that metals were not a major contributor to the acute toxicity since metals were not detected in the toxic volatile fraction but were in the non-toxic, non-volatile fraction. Qualitative analysis of hydrogen cyanide indicated that it was below acutely toxic concentrations (Environmental Protection Agency 1976, Thurston et al. 1979). Therefore, most of the acutely lethal effects of the refinery wastewater were suspected to be due to either low molecular weight volatile organics, ammonia, or hydrogen sulfide, either individually or collectively.

Activated Carbon

Since volatile organics or inorganics were suspected to be the toxic components, activated carbon filtration was selected as the next treatment to aid in further characterizing the acutely lethal constituents in the wastewater. Activated carbon filtration selectively adsorbs non-polar chemicals from aqueous soultion. Therefore, the volatile organics would not pass through the carbon but polar chemicals (e.g. ammonia, hydrogen sulfide, and metals) would.

Treatment of highly toxic steam volatile fractions with activated carbon resulted in a sharp decrease in acutely lethal effects (Table II). In three experiments, separate steam volatile fractions were filtered with activated carbon. The steam volatile fraction and the carbon

filtered fraction were tested for relative toxicity, and in each instance Daphnia survival time increased after activated carbon filtration.

As expected, results of chemical analyses indicated that activated carbon treatment of the steam volatile fraction removed most of the non-polar organic compounds but not the inorganics (Appendix K, J, and H). The decrease in the concentration of organic compounds was demonstrated by a 78 % decrease in TOC after carbon filtration. Comparison of the results from GC/MS analysis before and after carbon filtration also indicated the removal of organic components (Appendix K). The carbon filtered fraction contained essentially the same concentration of ammonia and metals as the volatile fraction before filtration (Appendix J and H).

TABLE II

DAPHNIA LT50's (h) FOR STEAM VOLATILE SAMPLES BEFORE AND AFTER ACTIVATED CARBON ADSORPTION

Sample	LT50 Before Carbon Adsorption	LT50 After Carbon Adsorption
16	7.0	226.0 (132.4-323.6)†
28	19.0 (17.5-20.5)	65.0 (49.5-80.5)
29	21.5 (19.4-23.6)	>42*

† 95% fiducial intervals were calculated when there were at least 2 partial kills.

* Ten percent mortality had occurred when the test was terminated at 42 h. That was insufficient mortality for an LT50 calculation to be made.

The comparison of the chemical characteristics of the toxic volatile fraction and the non-toxic filtered fraction led to certain conclusions concerning the components responsible for the acute toxicity. The presence of non-polar organic compounds in the toxic volatile fraction and their absence from the non-toxic carbon filtered fraction indicated that these compounds were responsible for the acutely lethal effects. Since the activated carbon removed the toxicity but did not affect the concentration of metals, ammonia, or hydrogen sulfide, it was inferred that those components were not major contributors to the acutely lethal effects of the wastewater.

The use of steam stripping and activated carbon in sequence led to the conclusion that low molecular weight, steam volatile, non-polar, organic compounds were the probable cause of the acute toxicity of the wastewater.

Cation Exchange

In two tests, cation exchange was the separation technique used to ascertain if the acutely toxic components of the steam volatile fraction were positively ionized. The resulting cation exchanged fraction and the steam volatile fraction were chemically analyzed and assayed for toxicity. The median survival time (LT 50) of the Daphnia increased from 5.6 h to 17 h and from 5.5 h to 11 h after passage of the steam volatile fractions through cation exchange resin. Since ammonia was the most toxic positive ion present in the volatile fraction, any decrease in toxicity could be attributed to its removal. However, this treatment did not remove as much of the acute toxicity as adsorption by activated carbon. Consequently, non-polar organics

appeared to contribute more to the acute lethality of the wastewater than ammonia.

Solvent Extraction

Since most of the acute toxicity of the refinery wastewater had been isolated in the class of steam volatile organic compounds, solvent extraction was used to determine if the organics were basic, neutral, or acidic. Aliquots of highly toxic steam volatile fractions were further fractionated into base-neutral and acidic components by methylene chloride extraction at pH >11 and pH <2. Results of repeated testing demonstrated that the base-neutral components were significantly more toxic than the acidic components ($p = 0.064$). The relative toxicities of the acid and base-neutral fractions were determined for three separate samples. The LT50's were 27.2, 69.5, and 44.9 h for the base-neutral fractions and 69.5, 110.2, and >146 h for the acid fractions (Table III).

Based upon the previous results, the base-neutral residue of the steam volatile fraction was separated by silica gel column chromatography into aliphatic, aromatic, and semipolar compounds to facilitate further characterization of the toxic components. In a preliminary screening test, the aliphatic fraction (LT50 = 41 h) was more toxic than the aromatic (LT50 = 60.3) fraction, but in the two more extensive bioassays the aromatic fraction was much more toxic (Table III). The second comparison gave an LT50 of 82.8 h for the aromatic fraction but produced less than 10 % mortality in 96 h for the aliphatic fraction, and the third comparison gave LT50's of 43.6 and 146.4 h for the aromatic and aliphatic fractions respectively.

The aromatic fraction was significantly more toxic than the semipolar fraction ($p = 0.056$).

TABLE III

DAPHNIA LT50's (h) FOR THE FRACTIONS SEPARATED BY SILICA GEL CHROMATOGRAPHY OF THE STEAM VOLATILE PORTION

Sample	Acid	Base-neutral	Aliphatic	Aromatic	Semi-polar
20	69.5 (63.1-75.1)†	27.2 (25.2-29.4)	----	----	----
25	110.2 (95.8-124.6)	69.5 (63.9-75.1)	----	----	----
29	>146	44.9	----	----	----
30	----	----	41	60.3 (51.9-68.6)	123.6 (108.8-138.4)
31	>96*	----	>96*	82.8 (74.9-90.7)	>96*
33	49.3 (43.9-54.7)	----	146.4 (115.6-177.2)	43.6 (39.6-47.6)	161.2 (123.4-199.0)

† 95% fiducial intervals were calculated when there were at least two partial kills.

* Less than 10 % mortality at 96 h.

Treatment Combinations

The use of different fractionation techniques in succession proved to be an effective procedure for isolating the components responsible for the

acutely lethal effects of the refinery wastewater. In one experiment the original wastewater was steam stripped to produce a volatile and a non-volatile fraction (Figure 1). The volatile fraction was acutely toxic (LT50 = 2.2 h); whereas, neither the original wastewater nor the non-volatile fraction produced any acutely lethal effects. The volatile fraction was then separated into base-neutral and acid components by solvent extraction. Since the resulting acid fraction produced no acute toxicity and previous tests revealed the base neutral fraction to be acutely toxic, that fraction was treated with silica gel column chromatography to isolate the aliphatic, aromatic, and semipolar fractions. Daphnia bioassays indicated that the aromatic fraction was more toxic than the aliphatic or semipolar fractions (Table III). The entire experiment was repeated with a different refinery wastewater and provided similar results, leading to the conclusion that the acutely lethal effects of the refinery wastewater were produced by steam-volatile, base-neutral, aromatic compounds.

Chemical Characterization of Toxic Fractions

Cation exchange treatment suggested that ammonia might be an important contributor to the acutely lethal effects of some of the wastewater samples. Ammonia was concentrated to high levels in the volatile fraction and in combination with high pH, was probably responsible for the acute toxicity in two experiments. The relationship between toxicity and ammonia was investigated in two different tests (Tables IV and V). The toxicity of a volatile sample with a high ammonia level was tested at acidic, basic, and neutral ph (Table IV). The un-ionized ammonia concentration ranged from 0.1 mg/l at acidic pH

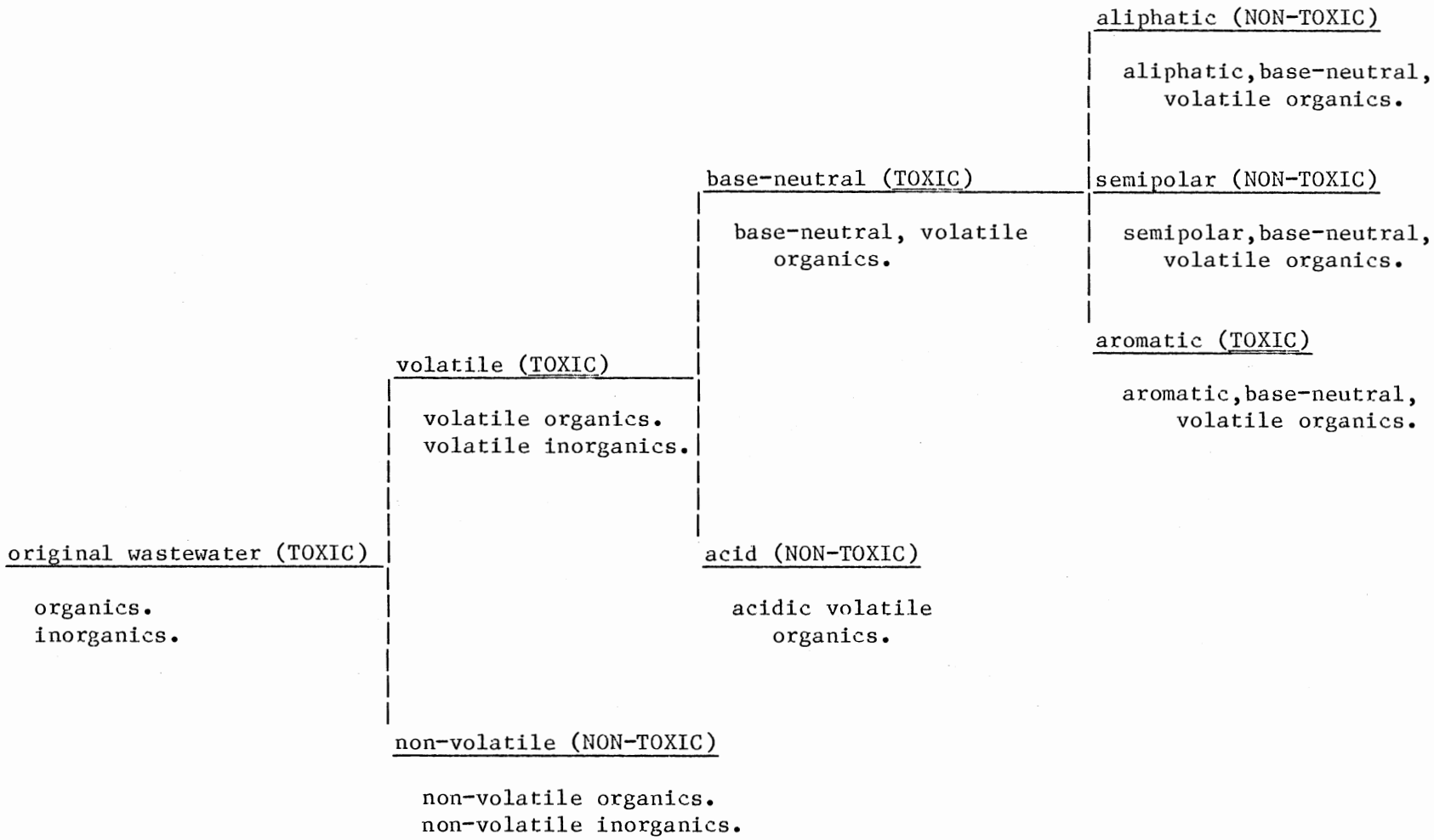


Figure 1. Fractionation scheme and chemical characterization of the toxic and non-toxic fractions produced from petroleum refinery wastewater.

to 22.4 mg/l at basic pH. (The percent un-ionized ammonia increases tenfold with every pH unit increase). Ammonia, which is more toxic when un-ionized, was responsible for the acute toxicity at basic and neutral pH but not at acidic pH. A second test was designed to determine if the organics were accountable for the acutely toxic effects at acidic pH. A volatile sample was split into two aliquots, one of which was passed through activated carbon to remove organics. Both aliquots were then tested for acute toxicity at acidic, neutral, and basic pH. The carbon filtered and the non-filtered, acid samples had an un-ionized ammonia concentration of 0.13 mg/l but the LT50's were 65 and 19 h, respectively. The acute toxicity of the carbon filtered, acid fraction was much less than the acute toxicity of the non-filtered, acid fraction (Table V). A greater reduction occurred in acutely toxic effects due to removal of organic contaminants than by decreasing the concentration of un-ionized ammonia. Therefore, although high concentrations of ammonia in combination with basic pH did contribute to the acute toxicity of some samples, organic components were the major toxic agents.

GC/MS analysis resulted in identifying 53 organic compounds in one or more volatile fractions (Appendix L). Analysis of the original wastewater and non-volatile fractions indicated that all organic compounds were below detectable limits (2 -10 ug/l) of the GC/MS. Published data on the acute toxicity to Daphnia was available for only six of the 53 compounds. The published values were 300 to 150,000 times the concentrations found in this study (Appendix M). Since the 48 h LC50's for those six compounds were much greater than the concentrations found in this study, the acute toxicity was not due to the individual effects of those six compounds.

TABLE IV

COMPARISON OF *DAPHNIA* LT50 (h) AND CONCENTRATION OF UN-IONIZED AMMONIA AT VARIOUS pH'S

Sample	pH	Un-ionized NH_3-N mg/l	LT50
Volatile	6.50	0.10	11.5 (75.-15.5)†
Volatile	6.95	0.27	15.3
Volatile	7.40	0.74	11.4
Volatile	7.95	2.64	2.6
Volatile	8.50	8.80	2.6
Volatile	9.00	22.40	2.6

† Fiducial intervals were calculated when there were at least two partial kills.

TABLE V

LT50 VALUES FOR UN-IONIZED AMMONIA CONCENTRATIONS WITH AND WITHOUT ORGANICS PRESENT

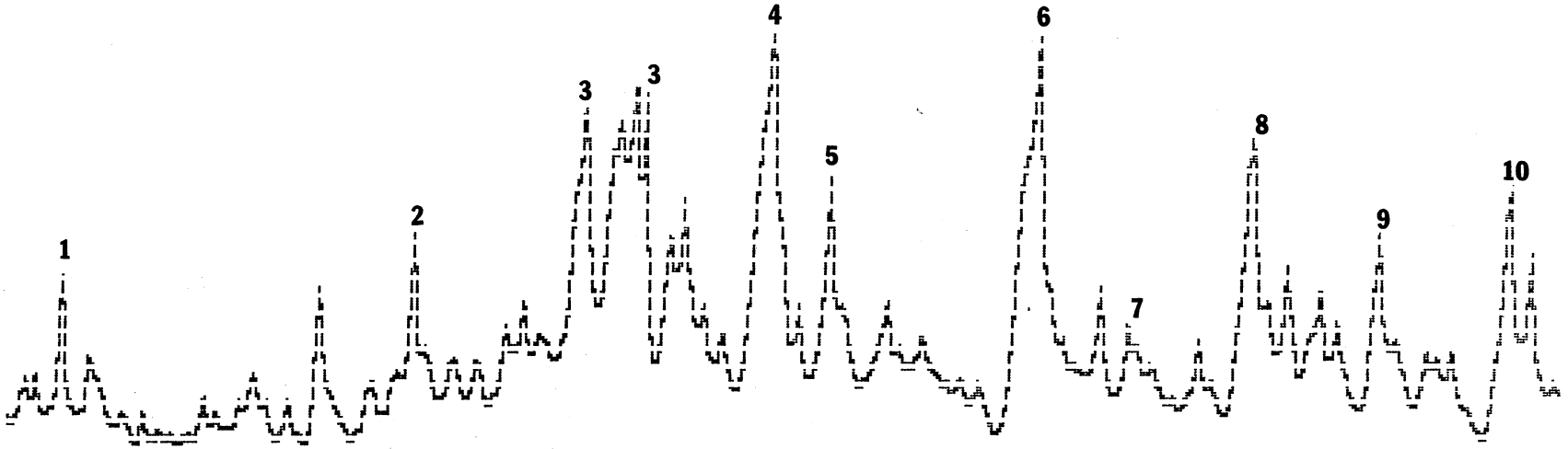
Sample	Organics	pH	Un-ionized NH_3-N	LT50
Volatile	absent	6.6	0.13	65.0 (49.5-80.5)†
Volatile	absent	7.5	1.06	12.0 (9-15)
Volatile	absent	8.5	9.68	2.1
Volatile	present	6.6	0.13	19.0 (17.5-20.5)
Volatile	present	7.5	1.06	9.8
Volatile	present	8.5	9.68	1.0

† Fiducial intervals were calculated when there were at least two partial kills.

Eleven compounds (polycyclic aromatic hydrocarbons, PAH's) were identified in the steam volatile, base-neutral, aromatic fraction (Figure 2). Those eleven compounds accounted for 28 % of the total peak area of the chromatogram, 13 of the 15 largest peaks, and had a combined concentration of 1100 ug/l (Figure 2).

Thirty of the 50 peaks present could not be identified but the fractionation scheme used to generate the aromatic fraction characterizes those compounds as steam volatile, basic or neutral, and aromatic. Some of the unidentified peaks were not completely separated and produced mass spectrum which represented more than one compound and prevented identification. Lack of separation of individual compounds on the 30 m fused silica capillary column (SE54 phase) indicates the presence of several isomers with similar physical and chemical properties. Other unidentified peaks produced what appeared to be single compound spectra, but the spectra were not present in the NIH-EPA Mass Spectral Search System data base. The unidentified spectra appeared to be from compounds similar to those identified (PAH's). Some were probably heterocyclic compounds containing nitrogen, oxygen, or sulfur atoms while others appeared to be hydroxylated, polycyclic aromatic hydrocarbons. Close examination of the unidentified spectra further revealed that the compounds were non-halogenated and had molecular weights of 180 to 300.

The eleven identified compounds were polycyclic aromatic hydrocarbons, a class of compounds not usually considered acutely toxic at the ug/l level. Although PAH's have been demonstrated to be chronically toxic, the concentration of individual compounds identified in this study do not appear to be high enough to account for the observed toxic effects. The eleven compounds and the unidentified compounds could be acting in an



1. Dihydromethyphenyllbenzofuran
2. Benzofluorene
3. Methyl benzofluorene
4. Chrysene/benzanthrene
5. C_2-benzofluroene
6. Methyl(chrysene/benzanthracene)
7. C_3-benzofluorene
8. C_2-(chrysene/benzanthrene)
9. Benzopyrene/benzofluoranthene
10. Methyl (benzopyrene/benzofluoranthene)

inject temp.	20° C
inject time	1.5 min.
flush time	0.5 min.
ramp 1	20° C/min.
temp. 1	180° C
ramp 2	4° C/min.
temp. 2	300° C
final hold	20 min.
column	J & W DB-5, 30 m

Figure 2. The major portion of the GC/MS chromatogram from the steam volatile, base-neutral, aromatic fraction.

additive or synergistic fashion to produce the acute toxicity.

The compounds in the aromatic fraction are persistent through the wastewater treatment system. They are in low ug/l concentrations upon entering the treatment system . A recent study indicated that most of the PAH's in the refinery wastewater came from the catalytic cracking unit, crude desalting unit, and the barometric condenser (Burks and Wagner 1982). That study indicated that activated carbon or activated sludge treatment at the process unit would remove the PAH's. Unpublished research (Reece) indicated that the extreme toxicity of the catalytic cracker process wastewater could be substantially reduced by activated carbon treatment (LT50 before treatment = 0.004 h; LT50 after treatment = 9.5 h). A study at a different refinery demonstrated that activated carbon treatment of the final wastewater could remove chronic toxicity (Burks 1979).

A refinery wanting to reduce the acute toxicity of their final wastewater could use the following procedure:

1) Fractionate the toxic wastewater and test the portions produced for relative toxicity to identify the toxic fraction.

2) Chemically analyze the toxic fraction.

3) Identify the wastewater streams within the plant which contribute the majority of those toxic components.

4) Design an intensive treatment system to remove those toxic components at the point of generation.

CHAPTER V

SUMMARY AND CONCLUSIONS

Fractionation and toxicity testing proved to be effective in isolating the acutely toxic components in petroleum refinery wastewater. Steam stripping led to the determination that the acutely toxic components were steam volatile, and treatment of the volatile fraction with activated carbon revealed that non-polar, organic compounds were major contributors to the acute toxicity. Although cation exchange treatment indicated that ammonia was the acutely toxic agent in two experiments, the results supported previous conclusions that steam volatile, non-polar organics were the most important toxicants. Solvent extraction and silica gel column chromatography split the volatile fraction into four subfractions, the most toxic being the aromatic. Those results identified the steam volatile, non-polar, base-neutral, aromatic fraction as the subfraction containing the acutely toxic components. Phenol, hydrogen sulfide, and hydrogen cyanide were eliminated as major contributors to the acute toxicity.

Eleven specific organic compounds (polycyclic aromatic hydrocarbons) accounting for 28 % of the total peak area of the chromatogram were identified in the steam volatile, non-polar, base-neutral, aromatic fraction. Although not specifically identified, other components of that fraction could be characterized as steam volatile, basic or

neutral, aromatic, non-halogenated, and with a molecular weight of 180 to 300. Some of the unidentified compounds may have been heterocyclic aromatics or hydroxylated forms of polycyclic aromatic hydrocarbons.

1. Fractionation of a complex wastewater followed by Daphnia toxicity testing is an effective method for characterizing the acutely toxic components.
2. Steam volatile, base-neutral, aromatic compounds were the major contributors to the acutely lethal effects on Daphnia exhibited by the refinery wastewater. Those compounds were further characterized as polycyclic aromatic hydrocarbons with molecular weights of 180 to 300.
3. Those compounds and the unidentified compounds could be acting in an additive or synergistic fashion to cause the acute toxicity, since the identified compounds were not individually responsible for the acute toxicity.
4. Ammonia, in combination with an elevated pH, was the causative agent for additional acutely lethal effects measured in two of the wastewater samples.
5. The toxic compounds persist through the wastewater treatment system but could possibly be isolated within the refinery process units and treated intensively at that point.

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APPENDIX A

THE TOXICITY OF REFINERY WASTEWATERS

Organism	Effect	Time or % Effluent	Reference
Daphnia pulex	48 h LC 50	76% *	Sprague, 1978
Daphnia pulex	72 h LC 50	52% *	Sprague, 1978
Fathead minnow	LT 50	20 days	Burks, 1977
Fathead minnow	LT 50	12 days	Burks, 1977
Fathead minnow	LT 50	25 days	Burks, 1977
Fathead minnow	LT 50	0.48 days	Burks, 1977
Fathead minnow	LT 50	13 days	Burks, 1977
Fathead minnow	LT 50	28 days	Burks, 1977
Bluegill sunfish	24 h TLm	55%	Turnbull, 1954
Bluegill sunfish	24 h TLm	65%	Turnbull, 1954
Fathead minnow	96 h TLm	6.5 to 16.5%	Graham, 1968
Redear sunfish	24 h TL 50	0.04 to 100%	Matthews, 1976

* Average of nine refinery effluents.

APPENDIX B

COMPOUNDS AND ELEMENTS IDENTIFIED IN REFINERY WASTEWATERS

Component	Reference
Chloromethane	1
Methylene chloride	1
Dichloroethylene	1
Dichloroethane	1
Chloroform	1
Trichloroethane	1
Carbon tetrachloride	1
Bromodichloromethane	1
Dichloropropane	1
Trichloroethylene	1
Benzene	1
Toluene	1
Ethylbenzene	1,4
Naphthalene	1,3
Acenaphthylene	1
Acenaphthene	1,3
Fluorene	1,3
Phenanthrene	1,3
Anthracene	1,3
Diethyl phthalate	1
Fluoranthene	1,3
Pyrene	1,3
Di-n-butyl phthalate	1
Chrysene	1
Benz(a)anthracene	1,3
Bis-2-ehtylhexyl phthalate	1
Benz(a)pyrene	1,3
Benzo[b/k]fluranthene	1,3
Benzo[ghi]perylene	1,3
Dimethylphenol	1
Phenol	1
Chlorocresol	1
Dinitrocresol	1
Tetrachloroethane	1
Dimethyl phthalate	1
Xylyl disulfide	2
Mesityl xylyl disulfide	2
Mesityl disulfide	2
Phenyl disulfide	2
Tolyl phenyl disulfide	2
Metenyl disulfide	2
Ditolyl sulfide	2
Dixylyl sulfide	2
Ethyl phenyl disulfide	2
Butyl phenyl disulfide	2
Indeno[1,2,3-cd]pyrene	3
Dimethyl sulfide	4

Component	Reference
Methyl ethyl sulfide	4
Methyl thiabutane	4
Thiapentane	4
Thiaheptane	4
Ethyl benzene	4
Methyl biphenyl	4
Diphenyl benzene	4
Methyl chrysene	4
Methyl pyrene	4
C_2 - pyrene	4
Dichlorobenzophenone	4
Dichlorobenzene	4
Methyl indene	4

Component	Reference	Concentration ug/l
Zinc	1	10 - 700
Chromium	1	1 - 1100
Copper	1	3 - 180
Lead	1	2 - 58
Beryllium	1	0.2 - 2.1
Antimony	1	1 - 370
Thallium	1	3 - 12
Nickel	1	0.9 - 82
Arsenic	1	2 - 900
Selenium	1	3 - 74
Silver	1	0.8 - 170
Cadmium	1	0.8 - 16
Mercury	1	0.5 - 6

(1) Radian 1978
(2) Arthur D. Little 1967
(3) Katz 1980
(4) Reece, unpublished data

APPENDIX C

ORGANICS IDENTIFIED IN A DAF UNIT

(Modified from Raphaelian and Harrison 1978)

	Relative Conc. in DAF Neutral Fraction	Presence(+) Absence(-) (FC effluent)	Presence(+) Absence(-) (MMF/AC effluent)
Chloroform	high	+	+
1,1,1-trichloroethane	high	+	+
benzene	medium	+	+
carbon tetrachloride	high	+	+
cyclohexene	high	+	+
toluene	high	+	+
ethyl benzene	low	+	+
p-xylene	high	+	+
m-xylene	high	+	+
o-xylene	medium	+	+
n-nonane	low	+	-
i-propyl benzene	trace	+	-
n-propyl benzene	low	+	-
m-ethyl toluene	medium	+	-
p-ethyl toluene	medium	+	-
1,3,5-trimethyl benzene	low	+	-
o-ethyl toluene	low	+	T
C_3-phenanthrene/anthracene (6)	trace	NM/T	-
pyrene	low	+	-
n-heneicosane	medium	+	+
$C_{17}H_{12}$ PNA (6)	trace	NM/T	-
n-docosane	medium	+	+
$C_{18}H_{14}$ PNA (3)	trace	+/T	-
chrysene	trace	+	-

	Relative Conc. in DAF Neutral Fraction	Presence(+) Absence(-) (FC effluent)	Presence(+) Absence(-) (MMF/AC effluent)
1,2,4-trimethyl benzene	high	+	+
cycloalkane	trace/medium	T/+	-
i-butyl benzene	trace	+	-
s-butyl benzene	trace	+	-
n-decane	medium	+	-
1,2,3-trimethyl benzene	medium	+	+
m-isopropyl toluene	trace	T	-
o-isopropyl toluene	trace	-	-
p-isopropyl toluene	trace	-	-
indan	medium	+	+
indene	trace	+	-
m-diethyl benzene	low	T	-
m-n-propyl toluene	low	+	-
p-m-propyl toluene	low	+	-
n-butyl benzene	trace	T	-
1,3-dimethyl-5-ethyl benzene	low	+	-
o-n-propyl toluene	low	+	-
1,4-dimethyl-2-ethyl benzene	low	NM	NM
ethyl styrene	low	-	-
1,3-dimethyl-4-ethyl benzene	low	+	-
ethyl styrene	medium	+	-
1,2-benzanthracene	low	+	-
n-tetracosane	low	+	+

	Relative Conc. in DAF Neutral Fraction	Presence(+) Absence(-) (FC effluent)	Presence(+) Absence(-) (MMF/AC effluent)
1,2-dimethyl-4-ethyl benzene	low	+	-
1,3-dimethyl-2-ethyl benzene	low	-	-
1,2-dimethyl-3-ethyl benzene	low	T	-
C_5-benzene	trace	NM	NM
1,2,4,5-tetramethyl benzene	low	+	-
1,2,3,5-tetramethyl benzene	medium	+	+
n-undecane	high	+	-
2-methyl indan	medium	T	-
1-methyl indan	medium	+	-
1,2,3,4-tetramethyl benzene	medium	+	-
tetralin	low	-	-
naphthalene	high	+	+
C_6-benzene (16)	trace	+	-
n-dodecane	high	+	+
ethyl indan	low	-	-
C_{13}-alkane	high	+	-
dimethyl indan (3)	medium	T	T
methyl tetralin	medium	T	-
C_3-indan	trace	-	-
methyl benzothiophene (4)	low	+	-
methyl ethyl indan	trace	-	-
N-pentacosane	low	+	NM
phthalate (2)	medium/high	+	+

	Relative Conc. in DAF Neutral Fraction	Presence(+) Absence(-) (FC effluent)	Presence(+) Absence(-) (MMF/AC effluent)
2-methyl naphthalene	high	+	+
trimethyl indan (3)	trace	-	-
C_4-indan/C_3-tetralin (7)	trace	+/-	-
dimethyl tetralin	low	T	-
n-tridecane	high	+	+
biphenyl	low	+	T
dimethyl benzothiophene (5)	trace	+	-
ethyl benzothiophene (2)	trace	+	-
ethyl naphthalene	medium	+	
dimethyl naphthalene (6)	high	+	+
C_{14}-alkane (2)	high	+	+
n-tetradecane	high	+	+
acenaphthene	trace	+	-
methyl biphenyl (2)	low	+	-
C_3-naphthalene (14)	low to high	+	+
C_{15}-alkane	high	+	+
n-pentadecane	high	+	+
fluorene	low	NM	NM
C_2-biphenyl (4)	trace	NM	-
methyl acenaphthene (3)	low	NM/+	-
n-hexadecane	high	+	+
C_3-biphenyl (5)	trace	NM	-
methyl fluorene (3)	low	+	-/T
C_2-acenaphthene (5)	low	NM	-

	Relative Conc. in DAF Neutral Fraction	Presence(+) Absence(-) (FC effluent)	Presence(+) Absence(-) (MMF/AC effluent)
n-heptadecane	high	+	+
dibenzothiophene	low	T	-
pristane	high	+	-
anthracene/phenanthrene	high	+	+
C_2-fluorene (7)	low	NM	-
n-octadecane	high	+	+
methyl dibenzothiophene (2)	low	NM/+	-
phytane	medium	+	+
methyl phenanthrene (3)	medium	NM	T
2-methyl anthracene	low	+	T
1-methyl anthracene	low	+	T
C_3 fluorene (2)	trace	+	T/-
n-nonadecane	high	+	+
C_2-dibenzothiophene	trace	-	-
C_2-phenanthrene/anthracene (8)	trace/low	-/+	-
fluoranthrene	trace	NM	-
C_2-phenanthracene/anthracene	trace	NM	-
n-eicosane	high	+	+

T = trace
NM = not measurable due to interferences
Numbers in parentheses refer to number of isomers detected
DAF = Dissolved Air Floatation
FC = Fluid Catalytic Cracker
MMF/AC = Mixed Media Filter/Activated Carbon

APPENDIX D

PARTIAL LIST OF COMPOUNDS IDENTIFIED IN EXTRACTS FROM OIL REFINERY WASTEWATER (Modified from Burlingame *et al.*, 1976)

Compound Type	Formula	Present in No. of Scans	Fraction Phenolic	Fraction Acidic
Anisole	C_7H_8O	1	x	x
Methyl Anisole	$C_8H_{10}O$	1	x	
C_2 Anisole	$C_9H_{12}O$	1	x	
Methyl Benzoate	$C_8H_8O_2$	1	x	x
Saturated Methyl esters	$C_nH_{2n}O_2$	28		x
Saturated Ethyl esters	$C_nH_{2n}O_2$	6		x
Olefinic Ethyl esters	$C_nH_{2n-2}O_2$	1		x
Saturated Propyl esters	$C_nH_{2n}O_2$	1		x
Cyclic Alkyl Methyl esters	$C_nH_{2n-2}O_2$	4		x
Alkyl-sub. Methyl Benzoates	$C_nH_{2n-8}O_2$	16		x
Phenylalkyl Methyl esters	$C_nH_{2n-8}O_2$	5		x
Alkyl-sub. Naphthenic Methyl esters	$C_nH_{2n-8}O_2$	8		x
Indenic Methyl esters	$C_nH_{2n-12}O_2$	1		x
Sulfur-sub. Aromatic Methyl esters	$C_nH_{2n-6}O_2S$	3		x
Alkyl-sub. Methyl sulfides	$C_nH_{2n-6}S$	1		x

Compound Type	Formula	Sample Location		
		K-1-N	K-2-N	K-3-N
n-alkanes	C_nH_{2n+2}	n = 11-33	n = 12-33	n = 15
branched alkanes	C_nH_{2n+2}	series		n. d.
mono-saturated or mono-cyclic alkanes	C_nH_{2n}	n = 11-28	n. d.	n. d.
alkyl benzenes	C_nH_{2n-6}	n = 9 (3) n = 10 (6) *n = 11	n = 9 n = 10(yes)	n = 8 (2) n = 9 (3) n = 10 (3)
naphthalenes	C_nH_{2n-12}	*n = 10-13 (several)	n = 10-14	n = 12-14 (yes)
phenanthrenes or anthracenes	C_nH_{2n-18}	n = 14-19	n = 14-19 **n = 17	n = 15-17 trace
pyrene or fluoroanthrene	C_nH_{2n-22}	n = 16 minor	n = 16 minor	n. d.
alkyl biphenyls	C_nH_{2n-14}	n = 13 significant n = 14	n. d.	n = 12 n = 13 trace
methyl indan	$C_{10}H_{12}$	trace	n. d.	n. d.
alkylated phenols	$C_nH_{2n-6}O$	n = 7-12 *n = 8 & 9		n = 7-13
Thiocyclanes	$C_nH_{2n}S$	n = 6 n = 8	n = 8-11	n = 6-11 (several)
benzothiophenes	$C_nH_{2n-10}S$	n = 8-12	n = 8-11	n = 9 n = 10 (few)

* major constituent of extract
** relatively abundant
Numbers in parentheses indicate number of isomers detected

APPENDIX E

TOXICITY OF VARIOUS ORGANIC COMPONENTS FOUND IN REFINERY WASTEWATER TO JUVENILE FATHEAD MINNOWS (MATTSON 1976)

Compound	96 h LC50 mg/l
Pentachlorophenol	0.6
Cyclohexane	93
Indan	14
Methyl naphthalene	9
Xylene	42
Furfural	32
P-cresol	19
Phenol	32
3,4-xylenol	14

APPENDIX F

THE ACUTE TOXICITY OF PRIOITY POLLUTANTS

TO *DAPHNIA MAGNA* (LEBLANC 1980)

Test Substance	LC50 (mg/l) 24-hour	LC50 (mg/l) 48-hour	No discernible effect conc. (mg/l)
chlorobenzene	140	86	10
1,2-dichlorobenzene	2.4	2.4	0.36
1,4-dichlorobenzene	42	11	0.68
1,2,4-trichlorobenzene	110	50	<2.4
1,2,3,5-tetrachlorobenzene	18	9.7	<1.1
1,2,4,5-tetrachlorobenzene	>530	>530	320
pentachlorobenzene	17	5.3	1.3
1,2-dichlorethane	250	220	<68
1,1,1-trichlorethane	>530	>530	530
1,1,2-trichlorethane	19	18	1.0
1,1,2,2-tetrachloroethane	18	9.3	<1.7
1,1,1,2-tetrachloroethane	27	24	<10
pentachloroethane	63	63	46
hexachloroethane	26	8.1	0.28
2-chlorophenol	>22	2.6	1.0
4-chlorophenol	8.8	4.1	1.1
2,4-dichlorophenol	>10	2.6	0.46
2,4,5-trichlorophenol	3.8	2.7	0.78
2,4,6-trichlorophenol	15	6.0	<0.41
2,3,4,6-tetrachlorophenol	>1.0	0.29	0.010
2,3,5,6-tetrachlorophenol	2.5	0.57	0.010
pentachlorophenol	1.5	0.68	0.32
4-chloro-6-methylphenol	1.9	0.29	0.028
2,4-dichloro-6-methylphenol	>1.7	0.43	0.078
4-nitrophenol	24	22	13
2,4-dinitrophenol	4.5	4.1	3.1
2,4,6-trinitrophenol	>220	85	<28
2,4-dinitro-6-methylphenol	4.3	3.1	1.5
1,2-diphenylhydrazine	8.1	4.1	0.41
ethylbenzene	77	75	6.8
fluoranthene	1300	320	<8.8
4-bromophenyl phenyl ether	0.46	0.36	<0.046
isophorone	430	120	15
naphthalene	17	8.6	0.60
nitrobenzene	24	27	0.46
nitrosodiphenylamine	>46	7.8	1.0
phenol	29	12	2.2
selenium	0.66	0.43	0.22
tetrachloroethylene	18	18	10
thallium	3.6	2.2	1.7
toluene	31	310	28
trichloroethylene	22	18	2.2
beryllium	1.9	1.0	0.25
bis(2-ethylhexyl)phthalate	>68	11	1.1
silver	0.0015	0.0015	0.0011
barium	>530	410	68

Test Substance	LC50 (mg/l) 24-hour	LC50 (mg/l) 48-hour	No discernible effect conc. (mg/l)
bromine	1.5	1.0	0.46
camphene	46	22	<13
p-cymene	9.4	6.5	<4.6
n-decane	23	18	1.3
butylbenzylphthalate	>460	92	<36
diethylphthalate	52	52	10
dimethylphthalate	150	33	<1.7
bromoform	56	46	<7.8
dichloromethane	310	220	68
1,1-dichloropropane	30	23	<6.8
1,2-dichloropropane	99	52	<22
1,3-dichloropropane	490	280	68
1,3-dichloropropene	7.2	6.2	0.41
1,1-dichloroethylene	98	79	<2.4
1,2-dichloroethylene (trans)	230	220	<110
1-chloronaphthalene	>3.6 <10	1.6	<0.17
octachloronaphthalene	>530	>530	530
acenaphthene	>280	41	0.60
acrolein	0.23	0.083	0.034
acrylonitrile	13	7.6	0.78
antimony	>530	>530	530
benzene	250	200	<13
carbon tetrachloride	35	35	7.7
bis(2-chloroethyl)ether	340	240	<7.8
chloroform	29	29	<7.8
2,4-dimethylphenol	8.3	2.1	1.0
2,3-dinitrotoluene	>2.8	0.66	<0.046
diethanolamine	170	55	<24
n-dibutyl ether	32	26	4.6
diphenyl ether	1.4	0.67	0.41
n-docosane	>530	>530	<68
sodium fluoride	680	340	110
methylethylketone	>520	>520	<70
a-pinene	68	41	8.8
styrene	27	23	<6.8
biphenyl	27	4.7	<2.2
dibenzofuran	7.5	1.7	0.28

APPENDIX G

LT50's (h) FOR DAPHNIA MAGNA EXPOSED TO VARIOUS FRACTIONS OF OIL REFINERY WASTEWATERS

Sample	*Vol	Orig	N-vol	Orig A.C.	Vol A.C.	Orig Cation	Vol Cation	Vol B-N	Vol Acid	Ali	Aro	Sp
1	0.5	4	0 @ 48									
4	5.6	56.3	60	0 @ 96								
5	5.6						17					
6		0 @ 97				30						
7	7.0	19.2	0 @ 96				11					
8		33				18.5						
16	7.0	0 @ 96	0 @ 96		226							
20	0.23	<10 48	<10 48					27.2	69.5			
25	40.0	0 @ 144	0 @ 144					69.5	110.2			
27,30	3.0	0@ 28	0 @ 28							41	60.3	123.6

Sample	*Vol	Orig	N-vol	Orig A.C.	Vol A.C.	Orig Cation	Vol Cation	Vol B-N	Vol Acid	Ali	Aro	Sp
28	19.0				65							
29	21.5	98	0 @ 161		<10 42			44.9	>146			
31	2.2	0 @ 96	0 @ 96						0 @ 96	<10 96	82.8	0 @ 96
33	3.3	0 @ 117	0 @ 117						49.3	146.4	43.6	161.2

*Vol = Volatile
Orig = original wastewater
A.C. = Activated carbon filtered
B-N = Base-neutral extract
Acid = Acid extract
N-vol = non-volatile
Ali = Aliphatic
Aro = Aromatic
SP = Semipolar
Mixed = Mixed ion exchange

APPENDIX H

CONCENTRATION OF METALS IN THE VARIOUS FRACTIONS (mg/l) OF PETROLEUM REFINERY WASTEWATERS

Fraction	Sample	Na	Ca	Mg	K	Fe	Pb	Zn	Cu	Cr	Cd
Original	6	470.0	92.8	15.4	32.7	0.184	<0.005	0.088	0.009	0.052	<0.005
Original Cation	6	637.6	0.3	0.09	0.4	0.120	<0.005	0.012	0.012	0.050	<0.005
Original	8	764.3	95.9	14.9	91.81	0.22	<0.005	0.020	<0.005	0.045	<0.005
Original Cation	8	882.3	0.4	<0.5	4.2	0.19	<0.005	<0.01	<0.005	0.022	<0.005
Culture	8	23.7	60.6	13.9	6.3	<0.10	<0.005	<0.01	<0.005	<0.010	<0.005
Volatile	16	<0.5		<0.5	<0.5	<0.05	<0.001	<0.01	<0.01	<0.01	<0.005
Original	16	290	66.2	10.8	12.3	1.38	0.006	0.05	0.018	0.076	<0.005
Non-volatile	16	197.7	49.3	7.6	9.5	0.08	0.187	0.02	<0.01	0.03	<0.005
Carbon	16	<0.5	0.52	<0.5	<0.5	<0.05	<0.001	0.07	<0.01	<0.01	<0.005
Cation	16	105.8	0.12	<0.5	<0.5	<0.05	<0.001	<0.01	<0.01	<0.01	<0.005
Culture	16	23.6	45.5	13.8	6.9	<0.05	<0.001	<0.01	<0.01	<0.01	<0.005

APPENDIX I

TOTAL ORGANIC CARBON (mg/l) FOR THE DIFFERENT FRACTIONS ISOLATED FROM OIL REFINERY WASTEWATERS

Sample	*Vol	Orig	N-vol	Orig A.C.	Vol A.C.	Orig Cation	Vol Cation	B-N	Acid
04	16.3	48.4	34.7	<1					
07	13.4	54.7	37.1				10.6		
08		50.8				45.4			
15	22.1				7.2		11.4		
20	6.4	44.4	29.2					937.5	1033
25	3.43	30.1	16.2						
28	31.3				4				
29	11.0	40.5	28.6						
31	<1	32.5	15.5						
33	7.2								

* See Appendix G

APPENDIX J

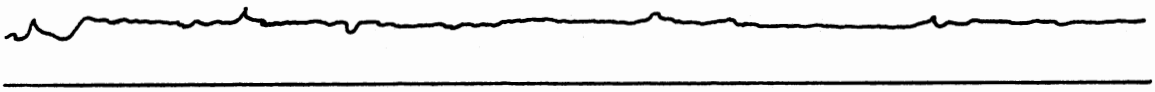
TOTAL AND UN-IONIZED AMMONIA CONCENTRATIONS (mg/l) IN THE DIFFERENT FRACTIONS ISOLATED FROM OIL REFINERY WASTEWATERS

Sample	*Vol	Orig	N-vol	Orig A.C.	Vol A.C.	Orig Cation	Vol Cation	B-N	Acid
4 Total	54	9.2	<1	9.5					
Un-ionized	.43	.04	<.01	.04					
5 Total	54						<1		
Un-ionized	.32						<.01		
6 Total		6.3				<1			
Un-ionized		<.01				<.01			
7 Total	1.7	<1					<1		
Un-ionized	.01	<.01					<.01		
8 Total	5.9	5.9				2.6			
Un-ionized	<.01	<0.1				<.01			
16 Total	62.9	16.7	2.5		55.8		<1		
Un-ionized	.38	.25	.02		.33		<.01		
20 Total	140	11.1	<1					<1	<1
Un-ionized	5.18	.03	<.01					<.01	<.01
25 Total	42	2.7	<1					<1	<1
Un-ionized	.50	.03	<.01					<.01	<.01
27 Total	80								
Un-ionized	2.96								
28 Total	88				88				
Un-ionized	0.13				0.13				

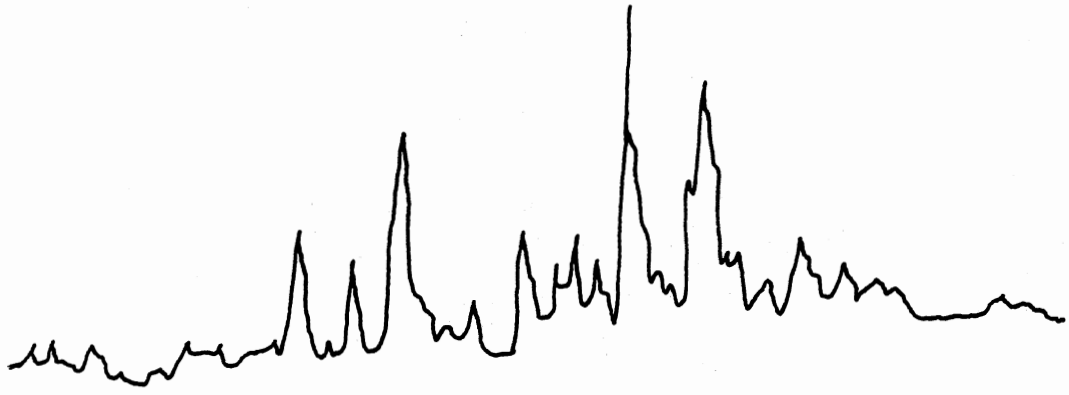
Sample	*Vol	Orig	N-vol	Orig A.C.	Vol A.C.	Orig Cation	Vol Cation	B-N	Acid
29 Total	84				84				
Un-ionized	.50				.31				
31 Total	77								
Un-ionized	.46								

APPENDIX K

CHROMATOGRAPHIC TRACE PRODUCED BY GC/MS BEFORE AND AFTER CARBON ADSORPTION



GC/MS total ion current profile of a volatile fraction after activated carbon filtration.



GC/MS total ion current profile of a volatile fraction before activated carbon filtration.

APPENDIX L

ORGANIC COMPOUNDS IDENTIFIED IN THE VARIOUS FRACTIONS (mg/l)

Compound	7 Vol	13 Vol	14 Vol	15 Vol	20 Vol	29 B-N	29 Acid	31 B-N	31 Acid	33 ARO
Hexadecane	<2									
Heptadecane	<2									
Pristane						<2				
C_2-Hexadiene	<2					<2				
Diethyleneglycol monoethylether	<2									
bix-2-ethoxy ethyl ether										
methyl pentanol						40	<2			
methyl pentanone						<2				
methyl laurate						<2				
methyl myristate									<5	
methyl palmitate									<5	
fatty acid, methyl ester	10								<5	
Dimethyl sulfide		2-5							<5	
Diethyl sulfide		2-5								
methylethyl suflide		2-5								
methyl thiabutane		2-5								
thiapentane		2-5								
thiaheptane		2-5								
Dithiabutane			<2	<2						
Trimethyl pyridine						<2				
Indole								100		
methylthiacycothiapentane				<2						
methylthiacyclothiahexane				<2						
Toluene	<2							<5		
Xylene	<2									
Cresol	<2									
ethyl benzene		2-5								
Trimethyl benzene			<2							
C_4-benzene			<2							
butyl hydroxy toluene					<5					
methyl indene	<5									

Compound	7 Vol	13 Vol	14 Vol	15 Vol	20 Vol	29 B-N	29 Acid	31 B-N	31 Acid	33 ARO
Naphthalene	<5									
methyl thiophene			<2							
Dimethyl Quinoline							45			
methyl biphenyl	<5									
butyl benzoic acid						4				
Dihydroxymehtylphenylbenzofuran										18.8
Pyrene/fluoranthene							100			
methyl(pyrene/fluoranthene)	<5									
C_2-(pyrene/fluoranthene)	<5				<5					200.0
Dichlorobenzophenone	<5									
Benzofluorene						10				518.2
methyl benzofluorene					<5	53				235.9
C_2-benzofluorene						13				<5
C_3-benzofluorene						25				<5
chrysene/benzanthracene	<5									56.1
methyl(chrysene/benzanthracene)	<5				<5					37.5
C_2(chrysene/benzanthracene)	<5				<5					7.5
benzopyrene/benzofluoranthene	<10									<10
methyl(benzpyrene/ benzofluoranthene)										<10
Diphenyl benzene	<5									

Vol = Volatile fraction
B-N = Volatile base-neutral fraction
Acid = Volatile acid fraction
ARO = Volatile, Base-neutral, aromatic fraction

APPENDIX M

COMPARISON OF CONCENTRATIONS (mg/l) OF COMPOUNDS IDENTIFIED IN THIS STUDY WITH PUBLISHED *DAPHNIA* LC50's

Compound	Concentration in this study	48 h LC50	Reference
cresol	<.002	22.7	DeGraeve, 1980
ethyl benzene	<.005	75.0	LeBlanc, 1980
fluoranthene	0.100	32.0	LeBlanc, 1980
naphthalene	<.005	8.6	LeBlanc, 1980
toluene	<.002	310.0	LeBlanc, 1980
cymene	<.002	6.5	LeBlanc, 1980

VITA 2

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Doctor of Philosophy

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