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Fractionation of Extractable Organics in Urban Runoff for Toxicity Identification

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Civil Engineering

by

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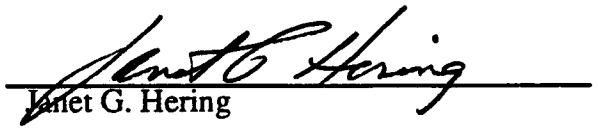

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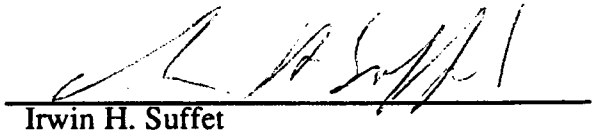

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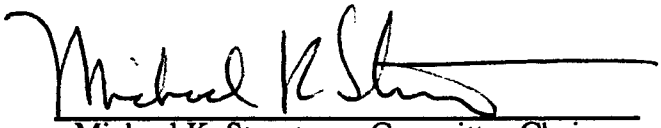

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ABSTRACT OF THE DISSERTATION

Fractionation of Extractable Organics

in Urban Runoff for Toxicity Identification

by

Sim Lin Lau

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Professor Michael K. Stenstrom, Chair

This dissertation addresses toxicity in urban runoff and methods to identify and reduce it. A initial study of the toxicity in dry weather runoff indicated that modest amounts of toxicity are present, which can generally be reduced to below detection limits by a 10 fold dilution with seawater. The dry weather study revealed shortcomings of the toxicity identification technique, which prompted the development of better techniques to measure soluble oil and grease, and to divide the extract into meaningful fractions for toxicity evaluation.

An alternative analytical method using commercially available C18 solid phase extraction (SPE) columns was developed for soluble oil and grease analysis. The method has advantages over the conventional liquid-liquid extraction method such as less solvent usage, more reproducible results and higher recovery of semi-volatile compounds.

An additional toxicity-based fractionation was also developed which further divides the C18 SPE extract into a single aliphatic and three aromatic fractions. The proposed fractionation utilized a commercially available silica gel column and an elution scheme consisting of hexane and methylene chloride-hexane mixtures. Good separation of

hydrocarbons was observed from the recovery studies. In addition, it was not affected by the sample matrix, and the solvent-exchange procedure only slightly reduced the mass of semi-volatile (< 10%). Sea urchin fertilization tests were conducted on the oil and grease fractions of synthetic samples. The technique was modestly successful but further research is still required.

A bench-scale feasibility study of an oil sorbent system to remove oil and grease from the runoff samples was also performed. The bench-scale study which involved three adsorption tests: batch adsorption tests, micro-column and continuous flow adsorption/filtration studies, using four commercially available oil sorbents. The sorbents removed greater than 50% of the oil and grease from spiked samples. A pilot-scale study of this oil sorbent system is needed to develop a prototype design which includes considerations such as flow rate and maintenance requirements.

1.0 INTRODUCTION

The Clean Water Act (CWA) Amendments of 1972 prohibited discharges of toxic contaminants to the waters (e.g., lakes, rivers, oceans, etc.) of the United States. Under this amended CWA, the National Pollutant Discharge Elimination System (NPDES) was established whereby pollutants, which meet the effluent limits imposed by the EPA in conjunction with the state water quality standards, can only be discharged with this permit. At the early stage of the implementation of the CWA, efforts to improve water quality under the NPDES program focused primarily on reducing pollutants from point source discharges of industrial and municipal wastewaters. Pollution by non-point sources has not been aggressively managed by the EPA and state agencies, and stormwater and urban runoff pollution are still a significant problem in the environment.

Stormwater discharge is considered a non-point source and its impacts were not well studied until early 1980s. Past efforts to address stormwater discharges in the NPDES program had been limited to certain industrial categories. The EPA Nationwide Urban Runoff Program (NURP) from 1979 to 1983 (EPA, 1983), and other stormwater studies (e.g., Eganhouse and Kaplan, 1981; Eganhouse *et al.*, 1981; Hoffman *et al.*, 1982; Hoffman *et al.*, 1984; Fam *et al.*, 1987; Latimer *et al.*, 1990) showed that stormwater pollution was contributing to the poor quality of receiving waters. Efforts such as prevention and treatment are required to reduce pollutant inputs to the storm drain system in order avoid further contamination of receiving waters.

Thus in 1987, Congress amended the CWA by introducing new provisions to address additional sources of water pollution and advance the effort to control stormwater pollution. The 1987 amendments to the CWA are commonly known as the "Water Quality

Act of 1987", whereby a comprehensive stormwater program was established to control urban and industrial stormwater runoff pollution.

One of the main objectives of the NPDES program is to prevent toxic discharges into receiving waters. Toxicity is a useful parameter to detect potential effects on receiving waters from the mixture of toxic pollutants in the stormwaters (which include industrial, municipal, and stormwater runoff). Within the thousands of pollutants found in the stormwater discharges, only a subset cause toxicity. Therefore the EPA developed a toxicity-based method to separate the toxic and nontoxic components using the response of one or more aquatic organisms. If toxicity is detected in the sample, it is the responsibility of the NPDES permittee to isolate and identify the possible sources of toxicity by conducting toxicity reduction evaluations (TRE) or toxicity identification evaluations (TIE). The TIE approach uses three phase approach: Phase I, toxicity characterization (EPA, 1988); Phase II, toxicity identification (EPA, 1989a); and Phase III, toxicity confirmation (EPA, 1989b). Once the identity of the toxicant(s) have been confirmed, the NPDES permittee must develop a stormwater pollution prevention plan (SWPPP) which includes best management practices (BMPs) that can be used to prevent, reduce, or eliminate the discharges of the toxic pollutants from entering into receiving waters.

One of the objectives of this study is to develop TIE procedures to isolate the probable toxic pollutant(s) in stormwater runoff. The target pollutants in this study are the total extractable "soluble" organics (such as oil and grease), and solid phase extraction (SPE) columns are used to fractionate these organics into different homogeneous groups. Short-term chronic toxicity testing which utilizes marine organisms was performed on these fractions so that toxic fraction(s) were identified. Next gas chromatography/mass spectrometry analysis was performed on the toxic fraction(s), to try to identify the toxic compound(s). Finally, a feasibility study of a sorbent treatment system to remove oil and grease was studied.

This dissertation is divided into five chapters. Chapter 2 is an overview of previous research on stormwater urban runoff. Chapter 3 discusses the toxicity of dry weather urban runoff. A portion of this chapter was presented in the 4th IAWQ Asian Regional Conference on Water Conservation and Pollution Control (at Jakarta, Indonesia). Chapter 4 describes the method development process for oil and grease analysis. This chapter has been accepted for publication in the *Water Environment Research.* Chapter 5 includes a method development for a toxicity-based fractionation of oil and grease. A portion of this chapter was presented at the Water Environment Federation 68th Annual Conference (at Miami, Florida). A feasibility study of an oil sorbent system in oil and grease removal from stormwater runoff is presented in Chapter 6. This chapter was also presented at the Water Environment Federation 68th Annual Conference and will be submitted to *Water Environment Research* for publication after obtaining additional data. Finally, the conclusions of all studies are presented in Chapter 7, along with recommendations for future work.

2.0 TOXICITY ASSESSMENT OF URBAN RUNOFF

Contaminated stormwater runoff contains a complex mixture of many hundreds or even thousands of potential toxic organic compounds. Many standard analytical methods (e.g., GC/MS) are incapable of detecting many of these contaminants at such a low concentrations. This limitation is due to selection and efficiency of solvent extraction techniques, analyte volatility and thermal stability, detector specificity and sensitivity, and analytical interferences and artifacts (EPA, 1988). In addition, among the hundreds or even thousands of the contaminants present in the runoff samples, only a subset may cause toxicity to human and aquatic life. Therefore, to simplify the analytical problems (through reduction of complexity of the sample) and reduce costs, a toxicity-based approach is generally used to determine and partially identify the toxicants. This toxicity approach involves a fractionation procedure where contaminants are separated into different fractions. General description on this toxicity-based fractionation, typical method or mode of separations, the types of organic contaminant present in the urban stormwater runoff, and treatment methods used to control these contaminants are summarized in this Chapter.

2.1 Water Quality of Urban Stormwater Runoff

Stormwater discharge is considered a non-point source and its impacts were not well studied until the early 1980s. Past efforts to address stormwater discharges in the NPDES program had been limited to certain industrial categories. The EPA Nationwide Urban Runoff Program (NURP) from 1979 to 1983 (EPA, 1983), and other stormwater

studies (e.g., Eganhouse and Kaplan, 1981; Hoffman *et al.*, 1982; Hoffman *et al.*, 1984; Fam *et al.*, 1987; Latimer *et al.*, 1990) showed that stormwater pollution was contributing to the poor water quality of receiving waters.

Stenstrom and Strecker (1993a) had also assembled the water quality data of stormwater runoff for major storm drains in Los Angeles, CA, during both wet weather and dry weather flow. The period of observation for the various data sets ranged from 1967 to 1990. The parameters of interests in that study include conventional water quality parameters (e.g., COD, BOD, TSS, etc.), trace metals (e.g., total cadmium, copper, lead, and zinc), microbiological contaminants (e.g., fecal coliform and fecal streptococci), and organics (e.g., oil and grease, total organic carbon, etc.). Based on the collected data, an IBM compatible database - SMBURD (Santa Monica Bay Urban Runoff Database) - was developed and allows users to easily extract water quality data for several major Los Angeles storm drains.

Recently, a detailed data collection of the storm water quality over the past 25 years had been conducted by Makepeace *et al.* (1995). Unlike the data collected by Stenstrom and Strecker (1993), Makepeace *et al.* (1995) extracted the data based on an extensive literature review of available research studies on the urban stormwater runoff. Instead of the overall water quality parameters such as BOD and TSS, the authors focused on work that presented specific physical, chemical and biological parameters. Table 2.1 lists the possible contaminants that may cause major concern to either human or the aquatic life. The listed contaminants are those with upper concentrations which are ten times the regulated maximum allowable concentration (MAC) for water quality guidelines or aquatic life guidelines.

Table 2.1 is only a partial list of the parameters collected by Makepeace *et al.* (1995). The data assembled by Makepeace *et al.* (1995) show inadequate information

Table 2.1 List of possible contaminants that may cause major concern to the human and aquatic life (adapted from Makepeace *et al.*, 1995).

Type	Contaminants	Concentration range* (mg/L)
Physical	Total solids	76 -36,200
	Total suspended solids	1 - 36,200
Inorganic chemicals	Aluminum	0.1 - 16.0
	Beryllium	0.001 - 0.049
	Cadmium	0.00005 - 13.73
	Chloride	0.30 - 25,000
	Chromium	0.001 - 2.30
	Copper	0.00006 - 1.41
	Iron	0.08 - 440.0
	Lead	0.00057 - 26.0
	Mercury	0.00005 - 0.067
	Nitrogen (all forms)	0.07 - 16.0
	Silver	0.0002 - 0.014
	Zinc	0.0007 - 22.0
Other chemical parameters	Dissolved oxygen	0 - 14.0
Organic chemicals	Polychlorinated biphenyl	2.7×10^{-5} - 1.1×10^{-3}
	Benzo(a)pyrene	2.5×10^{-6} - 1×10^{-2}
	Tetrachloroethylene	0.00045 - 0.043
	Bis(2-ethylhexyl)phthalate	0.007 - 0.039
	γ-BHC	5.2×10^{-5} - 1.1×10^{-2}
	Chlordane	0.0001 - 0.010
Microbiology	Fecal coliform	0.2 - 1.9×10^{6}
	Fecal streptococci	3 - 1.4×10^{6}
	Enterococci	1.2×10^{2} - 3.4×10^{5}

Note: * Related references to the obtained concentration range can be found in Makepeace *et al.* (1995).

about specific organic compounds. This may be due to the lack of advance analytical protocol where isolation and separation of the complex organic mixtures in the stormwater runoff can be performed in order to identify the toxic compound(s). An alternate approach to measuring and quantifying all organic compounds in urban runoff us to first characterize them with respect to importance. A toxicity-based fractionation procedure could be used to identify the potential toxic components of the organics. Additional chemical analyses could be used to identify the toxic fraction(s).

2.2 Toxicity-Based Fractionation

The objective of toxicity-based fractionation is to separate those compounds that are causing toxicity from those that are not causing toxicity before conducting the chemical and in-depth toxicity analysis. In the toxicity-based approach a toxic sample is fractionated and the toxicity of each fraction is determined (Lukasewycz and Durhan, 1992). The test organism used in the toxicity test is the "detector" of the compounds that are causing toxicity in the sample. The sensitivity of the test organism to the artifactual toxicity (caused by extraction procedures, solvents, trace contaminants acquired in the laboratory procedures, etc.) and trace concentration of the toxicants, imposes limits on the chemical methods and materials that can be used to carry out the fractionation. Solvents and sorbents used for chemical separations can impart artifactual toxicity to samples and sample fractions. Therefore, it is important to select the right isolation and fractionation method to minimize artifactual toxicity. When artifactual toxicity is added, "tracking" of toxicity is invalidated. Figure 2.1 shows a simple example of a toxicity-based fractionation adapted from the scheme used by Burkhard *et al.* (1991).

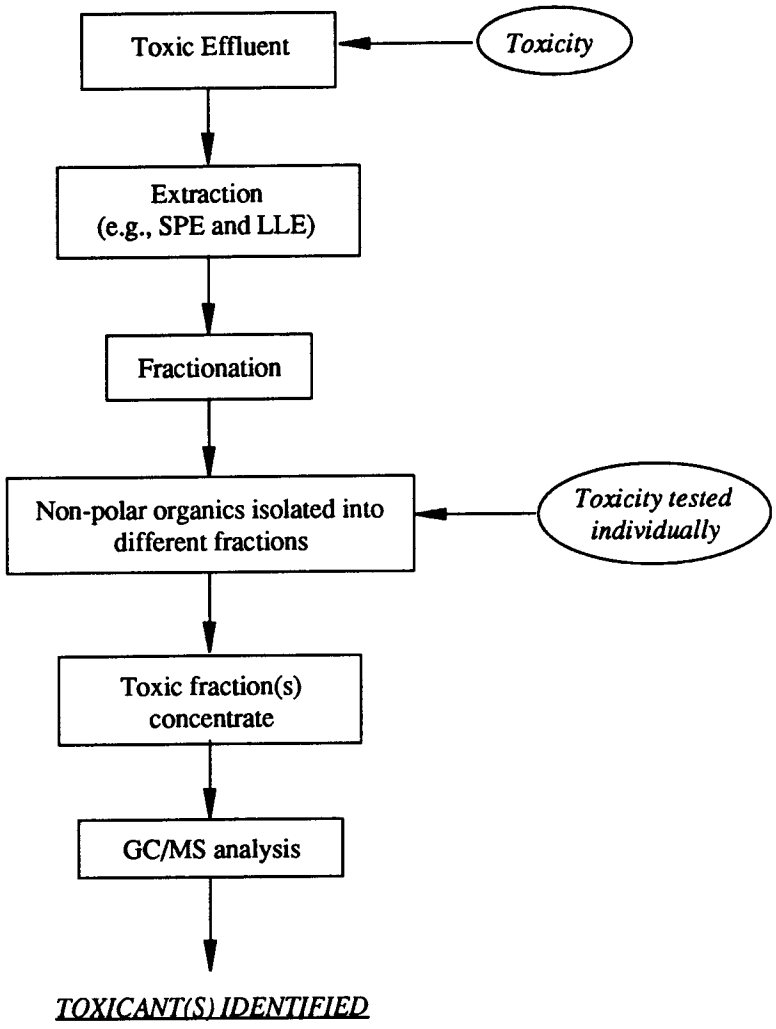


Figure 2.1 Schematic diagram of a simple toxicity-based fractionation for the isolation and identification of non-polar organic toxicants.

The strategies for identifying the non-polar toxicant in aqueous environmental samples (such as industrial wastewaters) using toxicity-based fractionation has been discussed in detailed by Lukasewycz and Durhan (1992). The first step of the toxicity-based fractionation is the isolation of non-polar organic compounds from the aqueous sample matrix. Two commonly used methods to isolate non-polar organics from aqueous

samples are liquid-liquid extraction (LLE) using a water-immiscible solvent such as methylene chloride or hexane, and solid phase extraction (SPE) using a sorbent such as activated carbon, XAD resins, and octadecyl-bonded silica. Table 2.2 shows examples of research that has used either LLE or SPE methods for the isolation and fractionation of non-polar organics in the aqueous environmental samples. Most of the earlier studies used LLE to isolate the non-polar organics from the aqueous samples (or particulate matter); the obtained LLE extracts were then fractionated into different groups using classical chromatography method, i.e., the SPE method. Current and recent researchers are trying to eliminate using LLE for the isolation step. Instead, a single method, i.e., SPE, is used to isolate and fractionate the non-polar organics from the aqueous samples at the same time. Examples of using this single step method include Burkhard *et al.* (1990), Durhan *et al.* (1993) and Lau *et al.* (1994).

Liquid-liquid Extraction

Liquid-liquid extraction (LLE) is one of the most versatile methods of isolating organic compounds from aqueous samples and is a well documented method (Voke and Suffet, 1979; Suffet and Malaiyandi, 1987). In this method, the organic compounds are partitioned between the solvent and aqueous phases, and concentration is achieved by solvent reduction through evaporation. Despite the extensive experience with this method to isolate organic compounds, there are several important disadvantages in using LLE, i.e., poor or inconsistent recovery, emulsion formation, usage of large solvent volume, long analysis time, and loss of volatile and semi-volatile compounds during evaporation of the solvent.

In addition to the above mentioned disadvantages, the LLE method is also not very suitable for the toxicity-based fractionation of non-polar organic compounds. The undiluted organic solvents used in the LLE, such as methylene chloride and hexane, are

mostly toxic to aquatic organisms. Moreover, these solvents are also immiscible with water. Before sample extracts dissolved in these solvents can be tested for toxicity, they

Table 2.2 Examples of research using LLE and SPE methods for isolation and fractionation of non-polar organics.

Example	Sample	Isolation method	Fractionation method
Eganhouse and Kaplan (1981)	stormwater runoff	LLE - hexane - methylene chloride	SPE - silica gel
Hoffman *et al.* (1982) and (1984)	urban runoff	LLE - methylene chloride (dissolved) - methanolic KOH, followed by petroleum ether (particulate)	SPE - silica gel
Fam *et al.* (1987)	urban runoff	LLE - methylene chloride	SPE - silica gel
Latimer *et al.* (1990)	urban runoff	LLE - methanolic KOH, followed by petroleum ether (particulate) - methylene chloride (dissolved)	SPE - silica gel
Bomboi and Hernandez (1991)	urban runoff	LLE - methylene chloride (dissolved) - 2:1 of methylene chloride/methanol (particulate)	SPE - florisil
Burkhard *et al.* (1991)	municipal and industrial wastewaters	SPE - Octadecylsiloxane (C18)	SPE - C18
Durhan *et al.* (1993)	sediment pore water	SPE - C18	SPE -C18
Stenstrom *et al.* (1994)	stormwater runoff	SPE - C18	SPE -C18

must be first solvent-exchanged to a suitable solvent that is both miscible with water and also non-toxic to the testing organisms. Without the solvent-exchange procedure, large dilution is generally needed that may cause the sample toxicant in the extract be diluted to below toxic concentrations, and thus preventing its detection.

Further discussion on the fractionation using LLE method can be found in Well *et al.* (1990) and Lukasewycz and Durhan (1992). In addition to an extensive review of this method, both papers also listed examples of studies which utilized LLE to fractionate the organic compounds from the municipal and industrial wastewater.

Solid Phase Extraction

The solid phase extraction (SPE) method, also known as liquid-solid extraction, has been widely used to remove organic compounds from water (Chladek and Marano, 1984; Wells and Michael, 1987; Junk and Richard, 1988; Wells *et al.*, 1990). The growing popularity of the SPE application is due to the commercial availability of pre-packed sorbents in disposable columns or cartridges and the development of stable, covalently bonded sorbents. Solid phase extraction isolates organic compounds by utilizing the principles of liquid chromatography (LC), in which the organic compounds are partitioned out of the aqueous sample onto the solid phase. Compounds adsorbed to the solid phase are then recovered from the solid phase by elution with a suitable solvent. Sample concentration is achieved by eluting with the smallest possible volume that will result in good compound recoveries. The literature on the SPE method has been reviewed in detail by McDowall *et al.* (1986) and Liska *et al.* (1989).

The major obstacle when using the SPE procedure is the need for method development since efficiency and precision depend upon the type of analyte, sample matrix, type of sorbents, and elution solvent. General method development for the SPE procedure

has been discussed in detail by Chladek and Marano (1984), McDowall *et al.* (1986) and Wells and Michael (1987).

Wells *et al.* (1990) have also discussed in detail the application of SPE in toxicity-based fractionation of industrial wastewater effluents. The types of sorbents used in the toxicity-based fractionation schemes include activated carbon, XAD resins and octadecyl (C18) bonded silica. Among these sorbents, C18 SPE is a good choice for isolating organic compounds from aqueous samples in toxicity-based fractionations (EPA, 1988, 1989a; Burkhard *et al.*, 1991; Stenstrom *et al.*, 1994). C18 SPE does not contribute artifactual toxicity to the sample or sample fractions, and therefore does not interfere with toxicity tracking. In addition, non-polar compounds with a significantly wide log K_{ow} range can be recovered with methanol elution allowing for toxicity tracking (Durhan *et al.*, 1990; Burkhard *et al.*, 1991). Finally, C18-bonded silica is available in convenient-to-use columns, cartridges, and disks.

Mount and Anderson-Carnahan (EPA, 1988 and 1989a) and Burkhard *et al.* (1991) have described in detail a fractionation method in which non-polar organic compounds are isolated and fractionated using C18 SPE and a methanol-water elution scheme. Using the methanol-water solutions to generate fractions of non-polar compounds from C18-bonded silica reduces the artifactual toxicity caused by the solvents. The loss of volatile compounds through the solvent evaporation step can also be reduced. However, poor recoveries of highly hydrophobic organics (log $K_{ow} > 5$) such as chrysene and benzo(a)pyrene using the methanol-water elution scheme have been observed. Therefore, an alternative elution scheme developed by Durhan *et al.* (1993) and Lau and Stenstrom (1993) can be used to recover these toxicants from the sorbent with a combination of methanol-water and methanol-methylene chloride as the elution solvents. The procedure described by Durhan *et al.* (1993) involved the usage of 100% methylene chloride as an

elution solvent that necessitates a solvent-exchange of the collected C18 SPE fractions to methanol so that toxicity tests can be performed. Lau and Stenstrom (1993), however, used an elution scheme in which only a 50% (v/v) of methylene chloride (in methanol) was used to elute those highly hydrophobic organic compounds. A solvent-exchange procedure was not needed for the subsequent toxicity tests as artifactual toxicity to their testing organisms was found not to be introduced by this solvent mixture.

2.3 Total Extractable Organics in Urban Runoff

The total extractable organic compounds in the stormwater runoff include the non-polar organic compounds that can be extracted either through LLE or SPE. One of the early studies of the composition of the total extractable organics in stormwater runoff samples is the work by Eganhouse and Kaplan (1981). In that study, Eganhouse and Kaplan (1981) characterized the extractable organic matter in urban stormwater runoff of Los Angeles River into five groups, i.e., total hydrocarbons, fatty acids, ketones, polar and non-elutable polar compounds. For both dissolved and particulate samples, a majority of the total extractable organics (~ 15% and ~ 60% for the dissolved and particulate samples, respectively) was associated with the total hydrocarbons. In the dissolved samples, ~ 88% of the total hydrocarbons were aliphatic hydrocarbons. After comprehensive characterization of the organic extracts, the hydrocarbons were found to be mainly from the anthropogenic sources, especially from petroleum hydrocarbon origins. Eganhouse and Kaplan (1981) concluded that the presence of petroleum in runoff probably results from incomplete fuel combustion and vehicular losses of lubricating oils. Only minor constituents of the hydrocarbons were of biogenic sources such as the higher plant waxes.

Similar to the work of Eganhouse and Kaplan (1981), Bomboi and Hernandez (1991) also conducted a hydrocarbon characterization analysis on the extractable organic matter for the runoff from Madrid, India. Their findings were very similar to those obtained by Eganhouse and Kaplan (1981). The main contribution of the hydrocarbon loads is from the anthropogenic sources such as vehicular exhaust, which are distinguished by the presence of petroleum residues in the form of aliphatic and aromatic hydrocarbons. Polyaromatic hydrocarbons of carcinogenic potential (ranging from fluoranthene to benzo[g,h,i]perylene) have also been associated with incomplete combustion in automobile exhaust. Natural hydrocarbons derived from higher vascular plants were also present in residential and landscaped areas.

Hoffman *et al.* (1984) studied the sources of PAHs to coastal waters of Narragansett Bay, Rhode Island. They found that the PAHs load from urban runoff was higher from highway and industrial land uses in comparison to commercial and residential areas. Hoffman *et al.* (1982) and Latimer *et al.* (1990) also studied the sources of petroleum hydrocarbons in urban runoff. Hoffman *et al.* (1982) found that the petroleum hydrocarbons were largely associated with particulates, where 83% to 93% of the total hydrocarbons were from the particulate fraction. Latimer *et al.* (1990) also found the hydrocarbon content in urban runoff originated primarily from used crankcase oil. The majority of this oil probably came from: (1) oil drops within the driving lanes on the road surfaces or deposits in parking areas, and/or (2) direct dumping of waste crankcase oil into storm drains.

Fam *et al.* (1987) studied the hydrocarbons in runoff from the 15 watersheds in the San Francisco Bay area. Motor oil and diesel fuel were also found to be the major anthropogenic sources of hydrocarbons. Higher aliphatic hydrocarbons emissions were detected in the high commercial/industrial commercial areas than in non-commercial areas. Aromatic hydrocarbons were present at much lower concentrations than the aliphatics. A

slight increase in urbanization can cause significant increase the amount of extractable organics in the stormwater runoff.

The category of oil and grease corresponds to the total extractable organics, as *Standard Methods* (1992) defines oil and grease as "any material recovered as a substance soluble in the solvent". Oil and grease include hydrocarbons, vegetable oil, animal fats, waxes, soaps, greases, etc. One of the early discussions of oil and grease contamination in urban runoff can be found in Stenstrom *et al.* (1982). In another study by Stenstrom *et al.* (1984), it was also observed that the oil and grease concentrations in the urban stormwaters were found to be highly dependent upon land-use. The concentration of oil and grease ranged from 4.1 mg/L in runoff from residential areas to 15.3 mg/L in runoff from parking lots. Qualitative analysis of the oil and grease by gas chromatography showed that the extractable oil and grease most resembled used automobile crankcase oil.

Vazquez-Duhalt (1989) reviewed the environmental impact of used motor oil. In addition to the production and fate of used motor oil in the environment, the author also analyzed the mutagenic and carcinogenic effects of used motor oil to humans and aquatic organisms. Due to the harmful effects of certain components of the used motor oil (e.g., PAHs), measures to control and prevent the discharge of used motor oil to receiving waters (such as storm drains, rivers and oceans) are needed.

2.4 Measures and Controls

As mentioned above, the total extractable organics in the stormwater runoff consists of a wide variety of different organic compounds. By utilizing an appropriate toxicity-based fractionation, the toxic component(s) of the extractable organics can be determined and then further identified through gas chromatography-mass spectrometry. Once the

identity of the toxicant(s) have been confirmed, the NPDES permittee can develop a stormwater pollution prevention plan (SWPPP) which include controls, measures and best management practices (BMPs) that prevent or eliminate the discharges of the toxic pollutants from entering receiving waters. Best management practices (BMPs) include a wide range of management practices that can be used to prevent or reduce the pollution of stormwater runoff.

The EPA emphasizes the implementation of pollution prevention measures and BMPs that reduce possible pollutant discharges from the source. Source reduction measures include education, preventive maintenance, chemical substitution, spill prevention, good housekeeping, training, and proper materials management. Stenstrom and Strecker (1993b) reviewed general purpose BMPs.

Best management practices specific for oil and grease control were discussed in detail by Stenstrom *et al.* (1982) and Silverman *et al.* (1986). Eight control measures were identified as offering the best potential for reducing oil and grease loading from urban areas:

1. oil and grease recycling;
2. incorporating leak inspections into vehicle inspection programs
3. surface cleaning (in the parking areas and commercial streets);
4. porous pavements (e.g., in the parking areas)
5. wetlands;
6. greenbelts (e.g., in parking areas);
7. adsorbents in storm drain inlets (in the parking areas and commercial streets), and
8. dispersion devices

Some of these measures (measures 3 - 7) are not widely used to prevent stormwater pollution due to lack of experimental validation. Comprehensive pilot studies are needed before cost effectiveness of these control measures are determined. Very few studies have been performed to evaluate the ability of the previously mentioned BMPs to remove toxicity from urban runoff.

The study conducted by Pitt *et al.* (1995) is an excellent example of developing the treatment methods to control the toxic pollutants in urban stormwater runoff prior discharging to the receiving waters. Pitt *et al.* (1995) divided their study into two phases. During the first phase of the study, the authors investigated the typical toxicant concentrations in stormwater, the origins of these toxicants, and storm and land-use factors that influenced these toxicant concentrations. Then, the control of stormwater toxicants was studied using several bench-scale conventional treatment methods such as settling, flotation, screening and filtering, photodegradation, and aeration. In addition to the determination of type of toxic pollutants, Pitt *et al.* (1995) also used the Microtox® toxicity-screening procedure to monitor the changes of toxicity of their stormwater samples before and after the treatment process.

The approach used by Pitt *et al.* (1995) is considered a rational approach for developing appropriate control and treatment measures by which toxic pollutants in stormwater runoff can be prevented from being discharged into the receiving waters. Each treatment measure should be validated by bench- and pilot-scale studies before it can be implemented.

3.0 TOXICITY OF DRY WEATHER URBAN RUNOFF

ABSTRACT

Four storm drains representing different types of land-use and hydraulics were sampled over an extended dry weather season. Samples were taken for routine water quality analysis as well as short-term, chronic toxicity analysis. The water quality parameters for samples collected from the four drains approximated secondary treated wastewater effluents for many parameters and were somewhat higher for other parameters, such as COD, TSS and turbidity. Varying amounts of toxicity were found in all four drains. The most toxic drain had the least flow rate, and it is suspected that the higher toxicities are associated with stagnant drain water and lack of dilution from flushing, which occurred with the other drains. One storm drain was analyzed intensively to ascertain the source of the toxicity but results were inconclusive. In three different samplings, toxicity appeared to have different origins. In one case the observed toxicity was consistent with the presence of organic chemicals and in another case with the presence of toxic metals. On another occasion the toxicity disappeared after 24 hours, which is consistent with the presence of an oxidizing agent, such as residual chlorine from disinfection. Toxicity was generally measurable in samples that contained more than 10% and less than 50% storm drain effluent. This suggests that a 10 fold dilution would reduce the toxicity below the detection limits used in this analysis.

3.1 Introduction

This study is one of many studies sponsored by the Santa Monica Bay Restoration Project to ascertain the status of contaminants inputs to the Bay. The eventual goal of these studies is the development of a comprehensive action plan to restore and maintain the quality of Santa Monica Bay.

Toxicity studies (chronic and acute) have become increasing important in the assessment of the biological impacts of urban runoff and thus to the development of corrective or preventative actions to protect receiving waters from the potential contaminants. Like other types of effluents (e.g., municipal or industrial wastewater effluents, hazardous wastes, etc.), urban runoff can contain thousands of chemicals (organics or inorganics), but usually only a few chemicals are responsible for any observable toxicity (Burkhard *et al.*, 1991). In order to identify the toxic components in the urban runoff, it is important to separate the toxicants from the non-toxic components using a stepwise process (which includes physical and chemical manipulation of the samples) designed to identify oxidative compounds, EDTA chelatable, filtratable, volatile and non-volatile organic compounds.

3.2 Experimental Procedures

Sampling Location

The selection of storm drains as sampling locations of this study were based on the types of land-use, location and ease of sampling. Four storm drains in the Santa Monica Watershed were selected for sampling: Pico-Kenter, Ashland Avenue, Ballona Creek at Inglewood and Sepulveda Channel at Ballona Creek (the first two storm drains were named

with reference to their neighboring streets). Figure 3.1 shows the location of these four storm drains.

Sampling Procedures

Samples were bailed from the storm drains using a stainless steel bucket. Morning and afternoon grab samples were collected into a 2-L or 4-L glass bottles, composited, and stored in ice chests with blue-ice packs during transportation from the sampling locations to the laboratory. Samples were collected from the middle of the open channel from Ballona Creek and Sepulveda Channel. At Pico-Kenter, they were collected from the wet well installed to divert low flow to the sanitary sewer. At Ashland Avenue samples were withdrawn from an open access hole on Neilson Avenue in Santa Monica. All samples were stored in a refrigerator at 4°C until the time of analysis. The time between sample collection and analysis was within the holding times recommended by the US EPA (1983).

Materials

Chemicals. Analytical or better grade chemicals and HPLC grade organic solvents (e.g., methanol and methylene chloride) were used for the chemical analyses and solid phase extraction. All these materials were obtained from Fisher Scientific (Tustin, CA).

SPE columns. The 1000 mg C18 columns used for the solid phase extraction were obtained from Burdick and Jackson (Muskegon, MI).

Conventional Chemical Analysis

Conventional water quality analyses (see Table 3.1) were performed on the collected storm drain samples. All the parameters, except uv-absorbance, used unfiltered samples and analyzed according to the *Standard Methods* (1992) procedures. Samples for

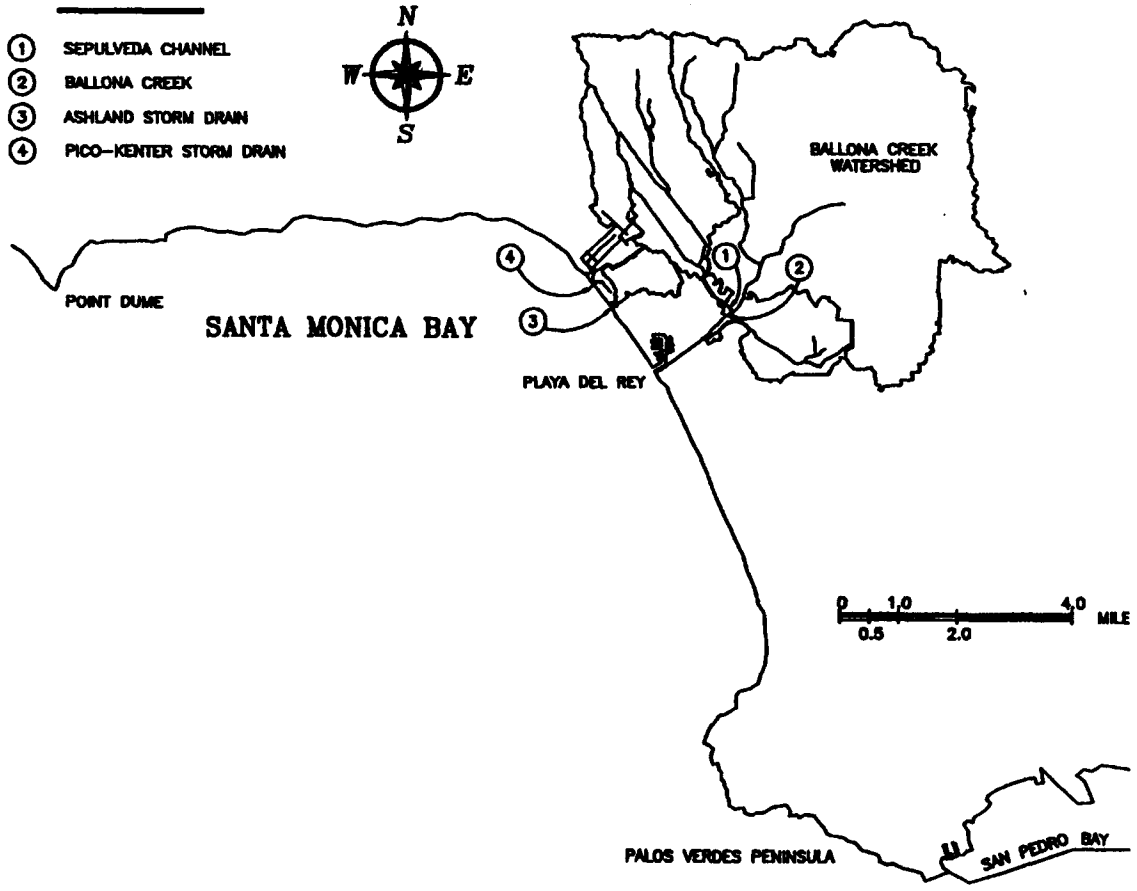


Figure 3.1 Sampling locations of four selected storm drains.

Table 3.1 Conventional water quality parameters measured in the study.

Water quality Parameter	Standard Method No.[1]	Preservation[2]	Holding time[2]
Field analysis			
Dissolved Oxygen - Probe DO Temperature % Salinity	4500-OG		
Laboratory analysis			
Alkalinity	2320.B	Cool, 4°C	14 days
Hardness	2340.C	Acidify with HNO_3 to pH < 2	6 months
Ammonia	4500-NH_3.F	Cool, 4°C; acidify with H_2SO_4 to pH < 2	28 days
Nitrite	4500-NO_2.B	Cool, 4°C	48 hours
Total Dissolved Solids (TDS)	2540.C	Cool, 4°C	7 days
Total Suspended Solids (TSS)	2540.D	Cool, 4°C	7days
Volatile Suspended Solids (VSS)	2540.E	Cool, 4°C	7 days
Chemical Oxygen Demand (COD)	5220.B	Cool, 4°C; acidify with H_2SO_4 to pH < 2	28 days
Dissolved Organic Carbon (DOC)		Cool, 4°C; acidify with H_3PO_4 to pH < 2	28 days
uv absorbance (λ = 254 nm)	HP 8452A[3]	Cool, 4°C	
Conductivity	2130.B	Cool, 4°C	28 days
pH	pH probe	None	Analyzed immediately
Turbidity	2130.B	Cool, 4°C	48 hours

Note: [1] *Standard Methods* (1992); [2] US EPA (1983); [3] Hewlett-Packard HP 8452A Diode Array Spectrophotometer

uv-absorbance analysis were filtered with 1 μm glass fiber filter (Whatman GF/B) and measured at a wavelength of 254 nm, using Hewlett-Packard HP 8452A Diode Array Spectrophotometer. The measured uv-absorbance is a qualitative measure of the amount of organic carbon in the samples.

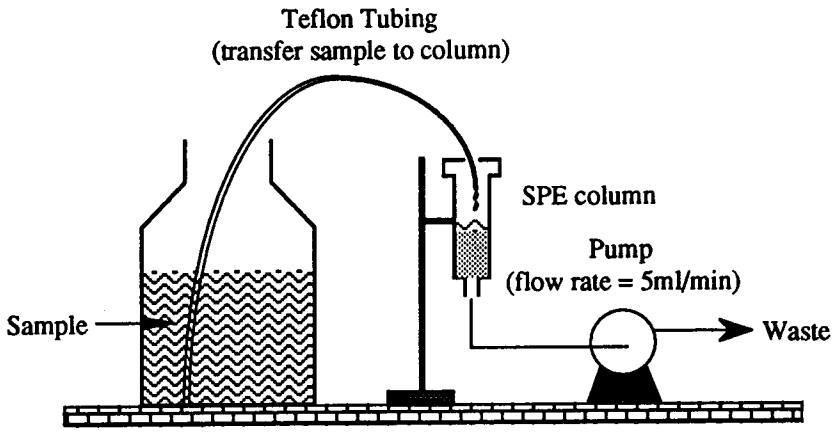
Velocity Measurement

The velocities of the flow across Ballona Creek and Sepulveda Channel were measured during each sampling period, using a Marsh McBirney velocity meter at approximately five foot intervals across the channel. The water depth was also recorded at the same time and location. The obtained data were used to calculate the average flow rate through the channel.

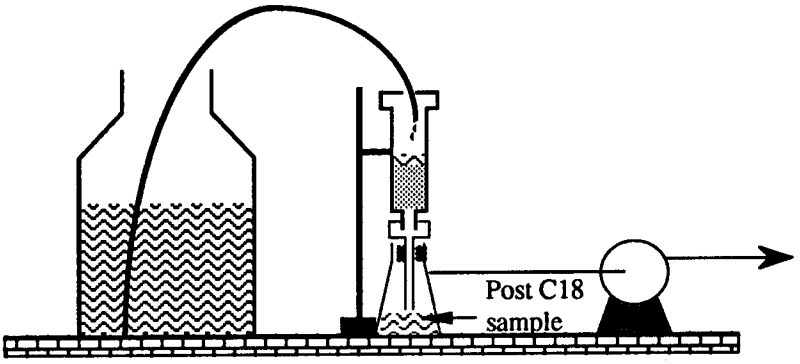
Solid Phase Extraction Procedures

Filter blank. A 1 μm glass fiber filter (Whatman GF/B) was prepared by first acid washing with 10% nitric acid and then rinsing thoroughly with deionized water. Next, approximately 200 ml of deionized water was passed through the filter, and the last 30 - 50 ml of filtrate were collected for the filter toxicity blank. The storm drain sample was then filtered using the same filter. Figure 3.2 shows the schematic diagram of the C18 SPE set-up.

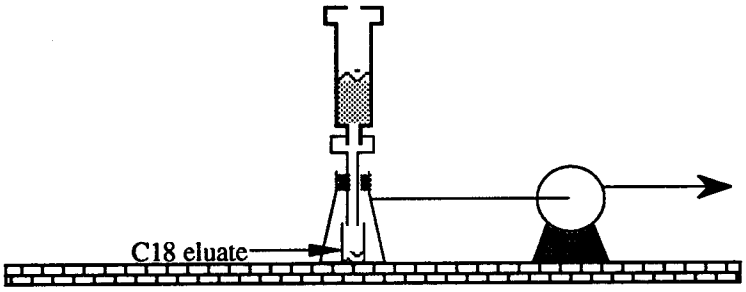
Column blank. The 1000 mg C18 SPE column was conditioned by pumping (Masterflex® peristaltic pump) 25 ml of HPLC grade methanol through the column at a flow rate of 5 ml/min. Before the sorbent dried, approximately 50 ml of deionized water were pumped through the column. The last 25 - 30 ml deionized water that were passed



(a) Introducing sample to the SPE column



(b) Collecting post C18 sample



(c) Collecting SPE eluates

Figure 3.2 Schematic diagram of the C18 solid phase extraction set-up.

through the column were collected at the end of the column for a column blank toxicity test. Pumping continued until no water emerged from the column.

Elution blank. Three elution blanks were collected from the prepared column by pumping 2 x 1.0 ml of each of the following solvents: 50% (v/v) methanol in water, 100% methanol, and 50% (v/v) methylene chloride in methanol, through the column. The eluates were collected in a clean glass vial as the SPE elution blanks. The column was allowed to dry between each elution.

SPE fractionation. The same 1000 mg C18 SPE column was again conditioned with 25 ml of methanol and 25 ml of deionized water. Before the sorbent dried, 1000 ml of filtered storm drain sample were pumped through the column at a rate of 5 ml/min. The sorbent was not allowed to dry while the 1-L sample passed through the column to maintain the interaction between the sorbent and analytes. A 30 ml sample of the post C18 column effluent was collected after 500 ml of the sample passed through the column. The sorbent was dried by continuing pumping after the entire 1000 ml sample passed through the column. Then 2 x 1.0 ml of 50% (v/v) methanol in water, 100% methanol, and 50% (v/v) methylene chloride in methanol were eluted sequentially through the column. Each fraction was collected into clean glass vials. The column was allowed to dry prior addition of each elution solvent mixture.

Toxicity testing was performed on the filtered sample, post C18 sample, the SPE eluates, and all blanks (i.e., filter blank, column blank and elution blank).

Toxicity Procedures

Three marine test methods described in the California Ocean Plan (SWRCB, 1990) were used in this study: the sea urchin fertilization test, red abalone embryo development test, and giant kelp germination/germ tube growth test. Storm drain samples were refrigerated in a sealed 4-L glass bottles until the day of testing (no more than 2 days).

Samples were thoroughly mixed before a 2.5-L subsample was removed and filtered through 1 μm glass fiber filter (Whatman GF/B).

The toxicity tests were conducted in two phases: Phase 1 - Relative toxicity of the storm drains and Phase 2 - Examination of toxic components. The toxicity tests were performed in at the Southern California Coastal Water Research Project's (SCCWRP) laboratory in Long Beach. Seawater dilutions of each sample were prepared by adding appropriate amounts of seawater and brine solutions to create the desired dilutions and maintained a salinity of 32 - 35 mg/g. The dilution of the collected storm drain sample produced the required concentrations of storm drain sample for the toxicity tests, and test organisms were added to each sample within three hours of dilution. The concentrations of storm drain sample used in the toxicity tests were expressed in percentage of storm drain sample used in the dilutions. For example, a concentration of 56% corresponds to a diluted sample consisting of 56% (v/v) of storm drain sample and 44% of dilution water. The number of concentrations and replicates of the samples used in the toxicity tests are shown in Table 3.2.

Table 3.2 Number of dilutions and replicates of each toxicity test.

Phase	No. locations	No. dilutions	Concentration (% v/v)	No. replicates
1	4	5	5.6, 10, 18, 32, 56	3
	3	4	5.6, 12, 25, 56	3
2	1	3 for blanks	12, 25, 56	2
		2 for SPE eluates	0.1, 0.2	2

Sea urchin fertilization test. The sea urchin or echinoderm fertilization test was conducted according to methods described by Dinnel *et al.* (1987). Purple sea urchins

Strongylocentrotus purpuratus were collected from the intertidal in northern Santa Monica Bay and held at SCCWRP until used in the tests. Ten ml of each sample dilution were added to replicate glass tubes and equilibrated to 15°C in a water bath. Sea urchins were then induced to spawn through injections of potassium chloride. The gametes were collected and diluted with seawater to produce stock solutions of the density recommended by the protocol (i.e., 5.6 x 10^6 sperms and 2.2 x 10^3 eggs per ml). The test was conducted by adding sperm to each test tube. After 60 minutes of sperm exposure, eggs were added to each tube for a 20 minutes of fertilization period. The sample was then preserved for microscopic examination. Toxic effects were indicated by a reduction in the percentage of fertilized eggs from that observed in a control sample (seawater, brine and distilled water).

Abalone development test. The abalone development test, using embryos of the red abalone *Haliotus rufescens*, was conducted according to methods described by Anderson *et al.* (1990). Sexually mature abalone were obtained from a commercial aquaculture facility and held at SCCWRP until used in the tests. Two hundred ml of each sample dilution were added to replicate 250 ml glass beakers and placed in a 15°C water bath. Abalone were induced to spawn by exposure to a hydrogen peroxide solution. The eggs were then fertilized, diluted to the appropriate density (300 per ml), and added to the exposure beakers. The developing embryos were exposed for 48 hours and preserved for microscopic examination. Toxic effects were indicated by an increased incidence of larvae with abnormally developed shells.

Giant kelp test. Tests with giant kelp were also conducted according to the procedures described by Anderson *et al.* (1990). Kelp blades containing reproductive spores (sporophyll) were obtained from offshore, uncontaminated kelp beds located near Santa Barbara and used within 24 hours. The toxicity test was conducted in 250 ml

beakers containing 200 ml of the sample dilution. A glass microscopic slide was placed on the bottom of each beaker to provide a surface for settlement of the kelp spores. Zoospore release from the sporophyll blades was induced by desiccation followed by immersion in seawater. The density (7.5×10^5 spores per ml) of suspension of the released spores was adjusted and the appropriate number of spores was added to each beaker. The spores were exposed to the sample dilutions for 48 hours at 15°C and a controlled light level (50 $\mu E^{-2} sec^{-1}$). During this period of 48 hours, the spores germinated and formed gametophyte plants. The slides were then removed from each beaker and preserved for microscopic examination. Two endpoints were assessed: percentage spore germination and gametophyte length. Toxic effects were indicated by reductions in germination and gametophyte length, relative to a control group.

EDTA and Sodium Thiosulfate Additions Toxicity Tests

EDTA and sodium thiosulfate addition tests described by EPA (1992) were conducted during the second phase of the toxicity test. The unfiltered storm drain samples with EDTA or sodium thiosulfate were analyzed for toxicity using the sea urchin fertilization test.

EDTA addition test. A stock solution of EDTA was prepared and added into 30 ml unfiltered storm drain samples. The final concentrations of EDTA in samples were 3, 8, and 30 mg/L. Three different concentrations, 12%, 25% and 56% (v/v) of storm drain sample, were prepared from these EDTA-added samples and used for toxicity test.

Sodium thiosulfate addition test. A stock solution of sodium thiosulfate was prepared and added into 30 ml of unfiltered storm drain samples. The final concentrations of sodium thiosulfate in the samples were 10 and 25 mg/L. Similar to the EDTA addition

test, three concentrations, 12%, 25% and 56% (v/v) of storm drain sample, were prepared and used for toxicity test.

3.3 Results and Discussion

Summary of Water Quality Data

Samplings were conducted between April 1992 and January 1993. The number of samples collected from each storm drain during this period are given in Table 3.3. The number of samples collected varied from location to location due to factors such as the condition of flow, and salinity of the samples. For example, no sample was collected from the storm drain at Ashland Avenue on several occasions due to seawater intrusion into the storm drain. More samples were taken from the Ballona Creek since it was selected as the storm drain for the second phase of the toxicity testings.

Conventional water quality parameters (Table 3.1) of the collected samples were analyzed according to the *Standard Methods* (1992). The average and standard deviation of each analyzed parameter are given in Table 3.3. From Table 3.3, it is observed that the water quality of the storm drain at Ashland Avenue is usually worse than the other three storm drains. This poor water quality may be due to the storm drain condition. The Ashland Avenue storm drain is stagnant during low flow periods, due to perhaps because of sand plugging its mouth (the Ashland Avenue drain, unlike Pico-Kenter drain, terminates at the surf line). During high tides, sea water may enter the drain, which was detected by high conductivity and total dissolved and total dissolved solids (TDS) concentration. Ashland Avenue is the only drain that has a tidal interaction (the sampling station on Ballona Creek is above the point of tidal interaction).

Table 3.3 Summary of water quality data (average and standard deviation) for all the selected storm drains.

Parameter	Pico-Kenter	Ashland Avenue	Ballona Creek	Sepulveda Channel
No. of sampling	10	7	10	9
Alk (mg/L as $CaCO_3$)	266 ± 36	316 ± 64	233 ± 40	176 ± 49
Hardness (mg/L as $CaCO_3$)	287 ± 90	1290 ± 1122	675 ± 349	1513 ± 792
Conductivity (µmho/cm)	1795 ± 927	7560 ± 6702	2052 ± 919	4852 ± 1411
TDS (mg/L)	1050 ± 510	4618 ± 4323	1445 ± 795	3346 ± 930
TSS (mg/L)	49 ± 55	365 ± 475	47 ± 65	24 ± 32
VSS (mg/L)	21 ± 25	86 ± 101	9 ± 9	9 ± 6
COD (mg/L)	66 ± 35	249 ± 61	41 ± 18	70 ± 16
DOC (mg/L)	31 ± 32	46 ± 18	28 ± 33	29 ± 27
Turbidity (NTU)	15.5 ± 13	145.4 ± 208.2	23.3 ± 43.9	7.3 ± 12.2
DO (mg/L)*	7.0 ± 1.3	3.3 ± 2.6	13.7 ± 1.1	14.5 ± 0.5
pH	8 ± 0	7.6 ± 0	8.6 ± 0.5	8.7 ± 0.3
uv-absorbance	0.407 ± 0.102	0.870 ± 0.339	0.172 ± 0.051	0.173 ± 0.053
Ammonia (mg/L as NH_3-N)	0.18 ± 0.22	0.84 ± 0.96	0.28 ± 0.33	0.22 ± 0.49
Nitrite (mg/L as NO_2-N)	0.10 ± 0.05	0.12 ± 0.18	0.10 ± 0.08	0.16 ± 0.15

Note: * Parameter measued in the field.

Table 3.3 also shows that samples from the Sepulveda Channel had high TDS and hardness. The high TDS concentration results from ion exchange regeneration waters released by NPDES permit to this storm drain. The dissolved oxygen (DO) concentrations in Ballona Creek and Sepulveda Channel were often greater than the saturation concentration because of photosynthesis; both drains are open channels and had abundant algae during the sampling. The algae were attached to the surfaces of the drain in films or strings. Free floating algae were generally not observed, which probably resulted because the rapidly flowing stormwater drain had insufficient hydraulic retention to allow free floating algae to grow. The stormwater had no color tint (either green or brown) to suggest the presence of large concentrations of free floating algae.

At various sampling times, the water quality of some of the storm drains was comparable or worse than typical secondary effluents. Table 3.4 shows the selected water quality comparison between the storm drain samples and typical secondary effluent. The secondary effluent parameters are typical of those plants which discharge into the storm drains in Los Angeles County. These discharges are regulated more strictly than other plants, due to the possibility of human contact in the open drain channels and infiltration ground water basins. The results show that the chemical oxygen demand (COD) of water samples from Ashland Avenue is much greater than the value of typical secondary effluents prior discharge to the receiving waters. Ashland Avenue storm drain is a completely enclosed drain and had no observable algae; all of the COD is probably from storm drain inputs. A similar observation was made on the total suspended solids (TSS) of the analyzed storm drain samples.

Hardness Interferences

According to the *Standard Methods* (1992), the presence of certain metallic ions

Table 3.4 Comparison of water quality of storm drain samples and typical secondary effluent.

Parameter	Storm drain				Secondary Effluent
	Pico-Kenter	Ashland Avenue	Ballona Creek	Sepulveda Channel	
COD (mg/L)	72	249	41	70	~ 50 - 100
TSS (mg/L)	49	365	47	24	< 30
Turbidity (NTU)	15.5	145.5	23.3	7.3	< 2.2
DO (mg/L)	7	3.3	13.7	14.5	> 2
pH	8	7.6	8.6	8.7	~ 6 - 9
Ammonia (mg/L as NH_3-N)	0.18	0.84	0.28	0.22	< 2

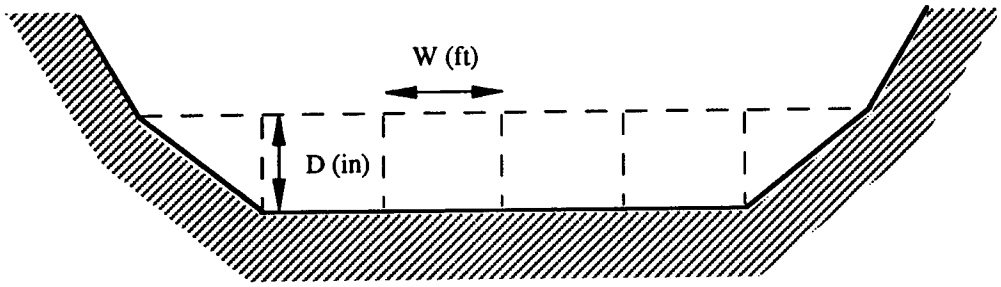
such as aluminum, cadmium, copper and lead may interfere the hardness test. Indistinct end-point or stochiometric consumption of EDTA may occur. False, high indications of total hardness may be obtained. This type of interference can be eliminated by adding certain inhibitors (i.e., sodium sulfide nonahydrate or sodium cyanide) as suggested by *Standard Methods*. It was observed that the total hardness of some samples from the Ashland Avenue, Ballona Creek and Sepulveda Channel were lower after addition of sodium sulfide nonahydrate. For example, the afternoon grab sample from Ballona Creek which was collected on December 14, 1992 had a total hardness of 1750 mg/L as $CaCO_3$ without the addition of inhibitor. The total hardness of the same sample decreased to 1180 mg/L as $CaCO_3$ (~ 33% decrease) after adding the inhibitor. This indicates the presence of interfering ions such as aluminum, cadmium, copper or lead in those samples. Metals concentrations were not measured in this study; therefore it is not known if sufficient metal concentrations were present to cause the interferences. There are NPDES-permitted cooling tower and ion-exchanger regenerant discharges into both Ballona Creek and Sepulveda Channel, which may increase aluminum and copper concentrations.

Mass Emission

The velocity and depth of water in the Ballona Creek and Sepulveda Channel were measured during sampling. Figure 3.3 shows the cross-section of Ballona Creek and Sepulveda Channel. The velocity and water depth measurements were used to calculate the flow rate of the water passed through the storm drain, using the following Equation (3.1):

$$\text{Flow rate } \left(\frac{ft^3}{s}\right) = \text{Area } (ft^2) \times \text{Velocity } \left(\frac{ft}{s}\right) \qquad (3.1)$$

Ballona Creek



Sepulveda Channel

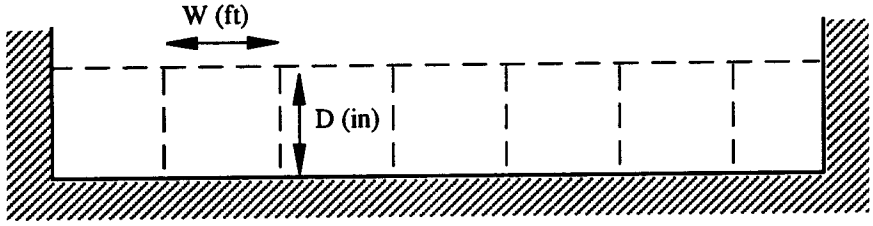


Figure 3.3 Cross-section of Ballona Creek and Sepulveda Channel.

The area of Ballona Creek (except the first and last 5 ft) and Sepulveda Channel was determined as follows:

$$\text{Area (ft}^2\text{)} = \text{width (ft)} \times \text{depth (ft)} \qquad (3.2)$$

For the Ballona Creek, the areas of the first and last 5 ft sections were determined as follows:

$$\text{Area } (ft^2) = \frac{1}{2} \text{ x width (ft) x depth (ft)} \qquad (3.3)$$

Table 3.5 shows the flow rates of the Ballona Creek and Sepulveda Channel. It is observed that Ballona Creek has greater flow rates than Sepulveda Channel. The average flow rate during the sampling of Ballona Creek and Sepulveda Channel are 3.37 ft^3/s and 0.85 ft/s, respectively. The calculated flow rate at Ballona Creek and Sepulveda Channel were then used to determine the annual mass emission of pollutants from dry weather flow into the Santa Monica Bay using the following equation:

$$\text{Mass emission } (\frac{kg}{yr}) = \text{Concentration}(\frac{kg}{m^3}) \text{ x Flow rate } (\frac{m^3}{yr}) \qquad (3.4)$$

The calculated average dry weather mass emission of selected pollutants (i.e., TDS, TSS, COD, NH_3-N and NO_2-N) from Ballona Creek and Sepulveda Channel are given in Table 3.6. The obtained results show that the mass emission of these selected pollutants from Ballona Creek are greater than those from Sepulveda Channel. No estimates are given for Pico-Kenter and Ashland Avenue storm drains as the flow rates were not determined in these two drains. The flow at Ashland Avenue was mostly stagnant during the dry season, suggesting that few pollutants from this storm drain were discharged into the Bay on a routine basis. It is assumed that the stagnant water was "blown out" from the drain from time to time due to the release of the sand plug at the surf line; however no blow outs were observed during testing. The dry weather flow from the Pico-Kenter storm drain during the period of the study was discharged to the Hyperion treatment plant.

The flow rates reported in Table 3.5 vary from those indicated by the gauging stations on Ballona Creek. A review of the procedure and the gauging station data found

no error in reporting or calculating flow rates. One possible source of error is a difference in calibration - the gauging station might be calibrated for wet weather flow.

Table 3.5 Flow rate measured at various sampling periods for Ballona Creek and Sepulveda Channel.

Sampling date	Flow rate (ft^3/sec)	
	Ballona Creek	Sepulveda Channel
7/7/92 pm	2.80	0.92
7/27/95 am	3.04	2.14
7/27/92 pm	3.07	1.83
8/24/92 am	1.90	0.60
8/24/92 pm	2.52	0.58
9/8/92 am	3.29	0.61
9/8/92 pm	2.98	0.63
9/29/92 am	3.66	0.61
9/29/92 pm	2.50	0.84
10/12/92 am	2.55	0.81
10/12/92 pm	2.53	0.90
11/2/92 am	2.48	0.56
11/2/92 pm	2.83	0.65
11/23/92 am	2.66	-
11/23/92 pm	2.18	-
12/10/92 am	2.85	0.96
12/10/92 pm	3.35	0.59
12/14/92 am	2.86	-
12/14/92 pm	3.05	-
Average	**3.37**	**0.85**

Table 3.6 Average mass emission of selected pollutants from Ballona Creek and Sepulveda Channel.

	Location	
	Ballona Creek	Sepulveda Channel
Average flow rate (m^3/hr)	391.52	90.01
Mass emission (kg/yr)		
TDS	3.44×10^6	2.41×10^6
TSS	18.5×10^4	1.97×10^4
COD	13.4×10^4	5.55×10^4
NH_3-N	724	158
NO_2-N	275	104

Other Observations

The appearance of the drain water varied from drain-to-drain. The open channel drains (Ballona Creek and Sepulveda Channel) were usually clear in appearance except for algae. Strings and rafts of algae were routinely observed in these drains. The color was usually green but occasionally they were "sandy" colored. Pico-Kenter frequently appeared highly colored from high turbidity. The color was often light orange or tan, which suggest the presence of clays in the suspended solids. Water from Ashland Avenue always appeared black or dark gray and frequently had odor.

Phase 1 Toxicity Testing - Relative Toxicity of Storm Drains

The objectives of this phase of toxicity were to determine the most toxic storm drain among the four selected storm drains, and the most sensitive test organism among the three test species. Four samplings were performed in this phase, i.e., August 24, September 8, September 29 and October 12, 1992. Sampling was performed at two locations on August 24 (i.e., Pico-Kenter and Ashland Avenue) and September 8, 1992 (i.e., Ballona Creek and Sepulveda Channel). It was necessary to sample drains in pairs because only two set

of storm drains could be analyzed by SCCWRP at a time. The storm drain with the least toxicity from this first sampling was excluded from the subsequent toxicity tests. Appendix B include the raw toxicity data generated from this study.

For each toxicity test, except the kelp germ tube test, the percentage response of the organisms at each tested concentration/dilution of the collected storm drain samples was calculated as follows:

$$\text{Dose - response} = \frac{\text{Number of normal organisms in the sample}}{\text{Total number of organisms in the sample}} \times 100 \qquad (3.5)$$

For the kelp germ tube test, the mean length of the kelp germ tube was measured instead. These dose-response results were then plotted versus the various concentration of the samples [expressed in % (v/v) of storm drain sample] used in the toxicity tests. Figures 3.4 - 3.7 show examples of dose-response plots for abalone, sea urchin, giant kelp germination and germ tube length tests (for samples collected on August 24 and September 8, 1992).

From the dose-response data, EC50 values, i.e., the effective concentration that caused 50% toxic effect on the test organisms, were calculated. The obtained EC50 values are used as the indicator of relative toxicity; lower EC50 values indicate greater toxicity. Table 3.7 shows the EC50 values obtained from the first toxicity testing for the collected samples of the four selected storm drains. The Ashland sample showed significant toxicity in three toxicity tests, except the kelp germ tube test. Both Pico-Kenter and Ballona Creek samples showed toxicity in one toxicity test, i.e., abalone and urchin tests, respectively. The EC50 value of both abalone and germ growth tests for Ashland sample were lower than Pico-Kenter and Ballona Creek samples (EC50 values greater than 56% indicate that no or very little toxicity was present for the condition tested).

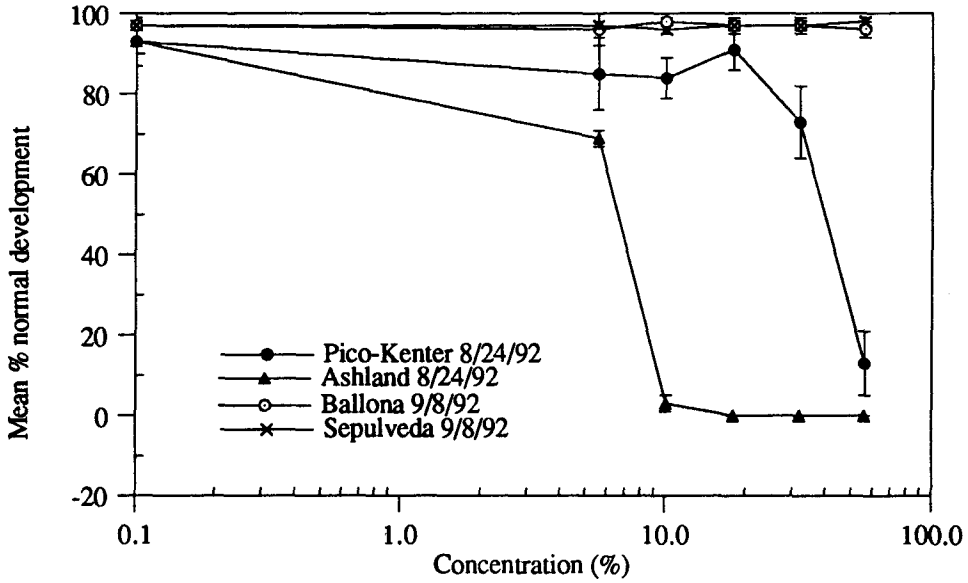


Figure 3.4 Example of dose-response plot for abalone development test. Control values are those plotted at a concentration of 0.1%.

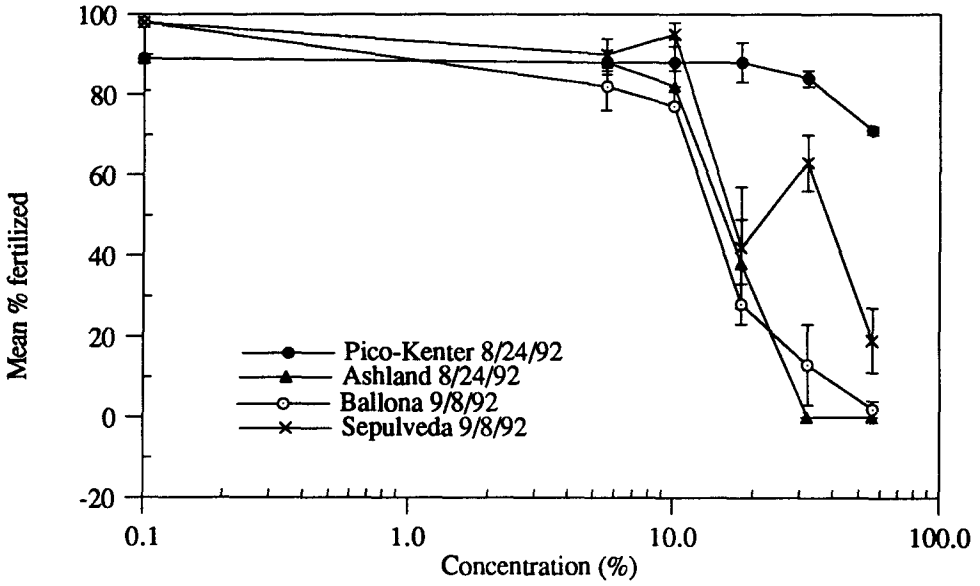


Figure 3.5 Example of dose-response plot for sea urchin fertilization test. Control values are those plotted at a concentration of 0.1%.

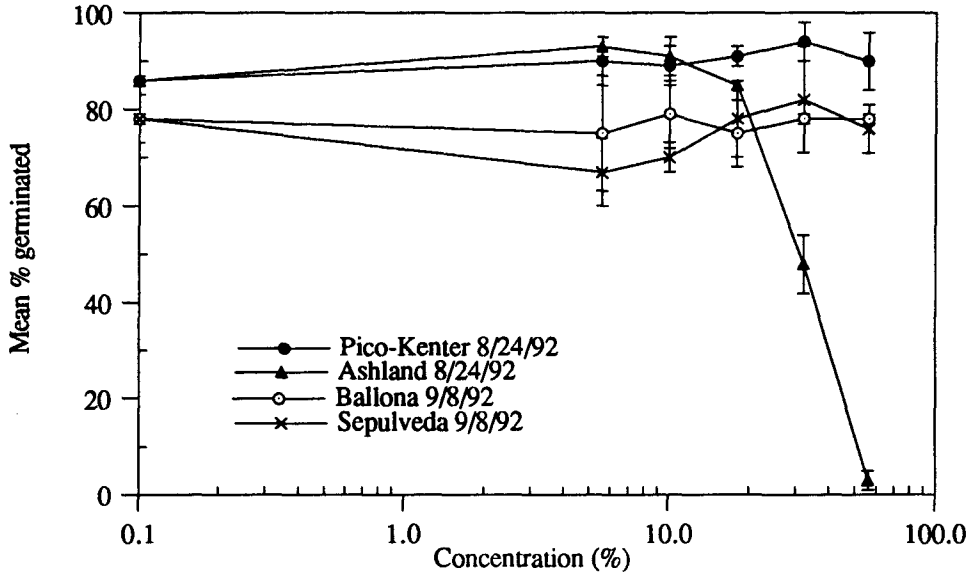


Figure 3.6 Example of dose-response plot for giant kelp germination test. Control values are those plotted at a concentration of 0.1%.

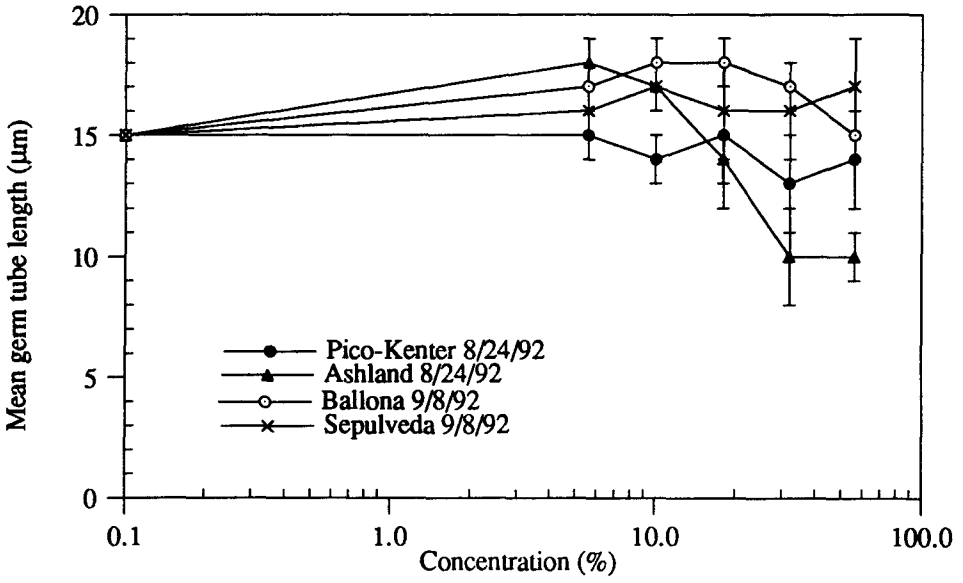


Figure 3.7 Example of dose-response plot for germ tube length test. Control values are those plotted at a concentration of 0.1%.

In addition to EC50, another parameter was also obtained from the toxicity data, i.e., NOEC - the highest concentration not statistically different from controls. When both NOEC and EC50 values of a sample are 56%, they indicate that no toxicity is present in the sample. Little toxicity is said to be present in the sample when its NOEC value is < 56%, and the EC50 of the sample is > 56%. Table 3.7 shows an example of each observation. For the Ashland sample collected on August 24, EC50 value of kelp germ tube test is > 56%; however, the NOEC value of the same test is 18%. This result indicates that toxicity was present in the Ashland sample even though the EC50 value could not be determined.

The Sepulveda Channel sample collected on the September 8 had very little toxicity. The EC50 values were greater than 56% (see Table 3.7) and NOEC values for all toxicity tests, except urchin test, were greater than 56%. An inconsistent pattern of toxicity was found in the urchin test and thus EC50 value could not be determined (see Figure 3.5). Based upon these data, Sepulveda Channel was, therefore, excluded from subsequent toxicity tests.

The second and third samplings of the Phase 1 toxicity tests were conducted on the September 29 and October 12, 1992 where only three storm drains were sampled. Instead of five concentrations, only four concentrations (% in v/v) of the collected storm drain sample were used in the toxicity tests, i.e., 5.6%, 12%, 25% and 56%. Three replicates of each sample were conducted for each toxicity test. The EC50 values calculated from the dose-response data for the toxicity tests are shown in Table 3.7.

For samples collected on September 29, the EC50 of the abalone and kelp tests was not determined due to technical difficulties which prevented the measurement of toxicity. The EC50 could only be determined on the urchin test. Table 3.7 shows that Ashland Avenue samples had the lowest EC50 value, e.g., 14%, among the three storm drains tested. Very little toxicity was present in the samples collected from Pico-Kenter and

Table 3.7 NOEC and EC50 values for storm drain samples during the Phase 1 of toxicity testing.

Location	Sampling Date	NOEC				EC50			
		Abalone Develop-ment	Kelp		Urchin Fertiliza-tion	Abalone Develop-ment	Kelp		Urchin Fertiliza-tion
			Germ.	Length			Germ.	Length	
Pico-Kenter	Aug. 24 '92	18	≥ 56	≥ 56	≥ 56	42	> 56	> 56	> 56
	Sept. 29 '92	nd	nd	nd	≥ 56	nd	nd	nd	> 56
	Oct. 12 '92	12	≥ 56	25	25	21	> 56	> 56	41
Ashland Avenue	Aug. 24 '92	< 5.6	18	18	10	6.8	32	> 56	17
	Sept. 29 '92	nd	nd	nd	5.6	nd	nd	nd	14
	Oct. 12 '92	5.6	5.6	5.6	< 5.6	10	22	50	< 5.6
Ballona Creek	Sept. 8 '92	≥ 56	≥ 56	≥ 56	< 5.6	> 56	> 56	> 56	14
	Sept. 29 '92	nd	nd	nd	12*	nd	nd	nd	> 56
	Oct. 12 '92	≥56	≥ 56	≥ 56	≥ 56	> 56	> 56	> 56	> 56
Sepulveda Channel	Sept. 8 '92	≥ 56	≥ 56	≥ 56	10	> 56	> 56	> 56	nt

Note: All values are in % (v/v) of the storm drain samples. NOEC = the highest concentration not statistically different from controls; EC50 = effective concentration to cause 50% toxic effect; nd = not determined as technical difficulties prevented measurement of toxicity; nt = toxicity found but data not amenable to testing for EC50 (see Figure 3.5); * = NOEC can also be stated as ≥ 56 since 56% concentration was not significantly different from respective brine control. A NOEC of 12 is felt to be more appropriate since 25% conentration was significantly toxic and 56% brine control was toxic, making accuracy of 56% effluent results more questionable.

Ballona Creek on September 29, as shown by the EC50 of > 56%. However, the NOEC of the Ballona Creek and Pico-Kenter samples were found to be 12% and ≥ 56%, respectively, in the urchin test. Therefore, Ballona Creek sample collected on September 29 was more toxic than Pico-Kenter sample in the urchin test.

For samples collected on October 12, toxicity was detected in all four toxicity tests for the Ashland Avenue sample. The Pico-Kenter storm drain was tested toxic in all toxicity tests, except the kelp germination test (where both EC50 and NOEC were > 56%). There was very little toxicity detected in all toxicity tests for the Ballona Creek samples. The NOEC and EC50 values obtained from the toxicity tests for samples collected on October 12 are shown in Table 3.7.

Relative toxicity. By using the obtained EC50 values of second and third sampling of Phase 1, the relative toxicity of Pico-Kenter, Ashland Avenue and Ballona Creek storm drains were assigned 3 for the most toxic to 1 for the least toxic storm drain for each toxicity test. For example, for samples collected on October 12, Ashland was the most toxic to the abalone test, followed by Pico-Kenter and Ballona Creek. Therefore, 3 was assigned to Ashland Avenue, 2 to Pico-Kenter and 1 to Ballona Creek. By using the same procedures, similar ranking were also assigned to all samples for all four toxicity tests and the results are shown in Tables 3.8 and 3.9. Table 3.8 shows the relative site toxicity ranks by species whereas Table 3.9 shows the relative rank test sensitivity to storm drain samples. The NOEC was used to assign rank for samples where toxicity was present but the EC50 could not be determined. For example, for kelp germination/growth tests, EC50 values of Ballona Creek and Pico-Kenter samples collected on October 12 were > 56%. However, the NOEC value of Pico-Kenter was 25% whereas NOEC value of Ballona

Creek was > 56%. Therefore, Ballona Creek sample (Oct. 12) was ranked the least toxic (1) in the kelp germination/growth test.

Table 3.8 Relative site toxicity ranks by species (3 = most toxic, 1 = least toxic).

Location	Relative toxicity 8/24 or 9/8/92	9/29/92	10/12/92	Sum of ranks
Abalone development				
Ashland	3	nd	3	6
Ballona	1	nd	1	2
Pico-Kenter	2	nd	2	4
Kelp germination/growth				
Ashland	3	nd	3	6
Ballona	1.5	nd	1*	2.5
Pico-Kenter	1.5	nd	2*	3.5
Sea urchin fertilization				
Ashland	2.5	3	3	8.5
Ballona	2.5	2	1	5.5
Pico-Kenter	1	1	2	4

Note: nd = technical difficulties prevented measurement of toxicity; these two locations were ranked based on their NOEC values as EC50 values could not be determined.

Table 3.9 Relative rank test sensitivity to storm drain effluents. Rank assignments made on the basis of EC50 values (3 = most sensitive test, 1 = least sensitive).

Species		Relative sensitivity 8/24 or 9/8/92	10/12/92	Sum of ranks
Ashland Avenue				
	Abalone	3	2	5
	Kelp	1	1	2
	Sea urchin	2	3	5
Pico-Kenter				
	Abalone	3	3	6
	Kelp	1.5	1	2.5
	Sea urchin	1.5	2	3.5
Ballona Creek				
	Abalone	1.5	2	3.5
	Kelp	1.5	2	3.5
	Sea urchin	3	2	5

The Ashland Avenue storm drain was usually the most toxic to each test species and consistently produced the greatest toxicity in all tests conducted. No clear distinction between the relative toxicity of the Ballona Creek and Pico-Kenter storm drains was observed. The abalone test was more sensitive to Pico-Kenter samples, with kelp test being the least sensitive. Ballona Creek samples produced the greatest toxic effects on sea urchin sperm while the abalone and kelp tests were unaffected by samples from this storm drain.

Phase 2 Toxicity Testing - Examination of Toxic Components

The objective of this phase of toxicity testing was to determine the types of contaminants (e.g., organics or metals) that caused the toxicity in the selected storm drain. Based on the toxicity results from Phase 1, the Ballona Creek storm drain and the sea urchin fertilization test were selected for this phase. Even though the relative toxicity of this location is not as great as Ashland Avenue, the annual input of runoff from Ballona Creek to Santa Monica Bay is much greater than the other storm drains, which means the mass emission from Ballona Creek will be much larger.

Three samplings were performed during this phase, i.e., November 23 and December 14, 1992 and January 19, 1993. The sampling procedures were slightly different than previous samplings. Grab samples from morning and afternoon were collected separately. Preliminary toxicity tests were performed on these two grab samples in order to determine which grab sample had a higher level of toxicity. Solid phase extraction (SPE) was then performed on the grab sample which exhibited greater toxicity. Samples collected from the extraction (e.g., SPE eluates, post C18, column blanks, etc.) were tested for toxicity. These tests were performed after the first rainfall of the 1992-93 water year, which occurred in late October. In order to ensure that only dry weather flow

was collected during this phase, the storm drain flow was monitored to insure that it returned to dry weather flow rates prior to sampling.

SPE eluates. The first sampling of this phase was conducted on November 23, 1992. Preliminary toxicity results on the morning and afternoon grab samples showed that the afternoon sample produced toxic effects at concentrations ≥ 25%. Therefore, SPE procedures were used to fractionate the afternoon grab sample of Ballona Creek. Prior to the SPE procedures, the pH of the sample was adjusted to pH 3 and 11 by using 1N hydrochloric acid and sodium hydroxide, respectively. The pH of deionized water used to prepare the filter and column blanks for the pH 3 and pH 11 samples was also adjusted prior to the extraction. During the SPE, two 30 ml samples of post C18 column effluents (i.e., after 25 ml and 950 ml of the sample passed through the column) were collected from each SPE column. After the whole sample passed through the column and dried, 2 x 1.0 ml volume of six solvent mixtures were used to elute the sorbed organics from the C18 column. The solvent mixtures used were 80%, 90% (v/v) of methanol in water, 100% methanol, 10%, 20% and 50% (v/v) of methylene chloride in methanol.

Initially sea urchin tests were conducted on the filter blanks, column blanks and post C18 column effluents. Three concentrations were used, i.e., 12%, 25% and 56% (v/v) of sample [e.g., 12% (v/v) of filter blank]. The results show that the pH_0 (initial pH) filter and column blanks were highly toxic. Filter blank toxicity was also found at pH 3 and less at pH 11. Post C18 column effluent at pH_0 was not toxic. This may be due to the toxicity present in the deionized water used in the sample preparation in UCLA. The percentage fertilization of UCLA's deionized water was only 38% at the concentration of 56% (v/v). In addition, a repeat of the baseline toxicity test with the Ballona Creek afternoon sample stored at SCCWRP showed a reduction of toxicity. Therefore, it was

decided that no further toxicity should be performed on the other samples, such as the SPE eluates, in order to save costs.

Two additional samples from Ballona Creek were collected on December 14, 1992 and January 19, 1993. The afternoon grab sample for December 14 and morning grab sample of January 19 were selected for the SPE. Unlike the first sampling, the pH of the samples was not manipulated as US EPA had later reported that major pH adjustment tests were not needed to characterize the toxicity of the sample (EPA, 1992). The number of elution solvents were also reduced from six fractions to only three fractions. The elution solvent system used to fractionate the sorbed organics from the SPE column was as follows: 2 x 1.0 ml volume of 50% (v/v) of methanol in water, 100% of methanol and 50% (v/v) of methylene chloride in methanol.

For the three SPE eluates, two concentrations were used for the sea urchin tests: 0.1% and 0.2%. These two concentrations were corresponded to 50% and 100% (v/v) of storm drain sample, including the 500 fold increase obtained through the SPE procedures (the concentration factor of 500 times was obtained based on a sample volume of 1000 ml and elution volume of 2 ml). Table 3.10 shows the percentage fertilization of the SPE eluates (which were also normalized for blank response), post C18 effluents and the filtrates (pre-C18) of the Ballona Creek samples collected during this phase. The results show that the 100% methanol fraction was the most toxic among the three eluates for both sampling periods. Little or no toxicity was present in both 50% methanol and 50% methylene chloride fractions. The results suggest that most of the toxicants were present in the 100% methanol fraction for both sampling periods.

Table 3.10 also shows that the toxicity results of pre- and post C18 samples at the concentrations tested. For the December 14 afternoon sample, a decrease in toxicity was observed in the post C18 sample showed greater percentage fertilization (76%) than the

Table 3.10 Sea urchin fertilization results of C18 solid phase extraction samples.

Sampling date and grab sample analyzed	Filtrate (pre-C18)			SPE eluates: 50% MeOH		100% MeOH		50% $MeCl_2$		Post C18		
	12%	25%	56%	0.1%	0.2%	0.1%	0.2%	0.1%	0.2%	12%	25%	56%
Dec. 14 '92 pm												
Blank				n/a	100	n/a	96	98	76			
Ballona sample	86	66	15	100	100	90	7	98	48	-	92	76
Normalized				100	100	94	7	100	63			
Jan. 19 '93 am												
Blank				81	82	82	74	82	88			
Ballona sample	50	30	16	72	87	43	42	76	77	72	43	20
Normalized				89	100	52	56	93	88			

Note: MeOH = methanol; $MeCl_2$ = methylene chloride; all values are expressed in mean % fertilization (duplicate) and only SPE eluates' values are normalized; n/a = not measured.

untreated (pre-C18) sample (which only has 15% fertilization). This observation suggests that the C18 column removed toxicity and organic toxicants were most likely present in the sample. For the January 19 morning sample, no reduction of toxicity was observed in the post C18 sample and only moderate toxicity was observed in the 100% methanol fraction. Normally this result would suggest the presence of non-organics (e.g., metals), which are not removed by the C18 column; however, in this case it is not conclusive due to poor fertilization in the column blank. The percentage fertilization of column blank and post C18 sample at 56% (v/v) concentration were 11% and 20%, respectively. The presence of metals and other toxicants such as oxidative compounds in the samples can be confirmed by the EDTA and sodium thiosulfate addition tests.

EDTA and sodium thiosulfate addition tests. The objective of EDTA addition test is to detect toxicity caused by certain cationic metals. Non-toxic complexes will be formed after EDTA addition to the collected storm drain samples. Loss of toxicity with EDTA addition suggests that cationic metals are causing toxicity. The sodium thiosulfate addition test can detect toxicity caused by oxidative compounds (such as chlorine) and other compounds (such as copper and manganese). Toxicity from bromine, iodine, ozone, and chlorine dioxide is also reduced by the addition of sodium thiosulfate (EPA, 1992). The toxicity results of EDTA and of sodium thiosulfate addition tests are shown in Table 3.11. For the sample collected on December 14 sample, sodium thiosulfate reduced toxicity while EDTA only partially reduced the toxicity. This indicates that oxidative compounds may have caused toxicity in the December 14 sample. Reverse results were obtained for the sample collected on January 19, 1993; high percentage fertilization was observed in the samples with added EDTA whereas low percentage fertilization was found in sodium thiosulfate addition test. These results show that EDTA completely removed the toxicity of the January 19 sample while sodium thiosulfate had no effect on the sample toxicity.

Therefore, cationic metals may be present in the January 19 sample and thus causing the toxicity.

Table 3.11 Sea urchin fertilization results of the EDTA and sodium thiosulfate addition tests.

Sampling date	Pre-treated sample	EDTA addition			Thiosulfate addition	
		3 mg/L	8 mg/L	30 mg/L	10 mg/L	25 mg/L
Dec. 14 '92	15	44	12	-	99	98
Jan. 19 '93	16	92	96	92	10	12

Note: All values are the mean value of % fertilization of sea urchin at a concentration of 56% (v/v) storm drain sample.

The toxicity results obtained from the Phase 2 were variable and not conclusive due to the small number of samples tested. For example, for the December 14 sample, toxicity was eliminated in the thiosulfate addition test and partially removed by C18 column. It is not clear what might cause this type of toxicity, but an organic oxidant is possible; it would be reduced by the thiosulfate and through adsorption onto the C18 column. More toxicity tests should be performed in order to examine this variability.

3.4 Conclusions

Water quality of the selected four storm drains varied during the sampling periods from April 1992 to January 1993. It was found that some of the observed water quality parameters were often comparable or worse than the typical secondary wastewater effluents. These results suggest that it may be as important to control dry weather urban runoff to Santa Monica Bay as it is to control secondary effluents, even though the volume

of dry weather flow is only 5 to 10% of the dry weather secondary effluent flow. Dry weather flow for most storm drains discharging into Santa Monica Bay occurs at the beach or surf line. Secondary effluents are all discharged into deep ocean outfalls.

Short-term chronic toxicity tests also show that significant toxicity was present in the selected storm drains. Probable sources of the toxicity ranged from non-organics (e.g., metals and oxidizing compounds) to organic contaminants. More samplings are needed to determine the variability of the toxicity. Further work to identify the toxic components through quantitative chemical analysis such as GC/MS is also needed.

ABSTRACT

Conventional oil and grease analysis which involves liquid-liquid extraction (LLE) has many disadvantages which include poor reproducibility, emulsion formation, large solvent usage and loss of volatile and semi-volatile compounds during evaporation of the solvent. Therefore, an alternative method using octadecyl siloxane (C18) solid phase extraction (SPE) columns was developed in order to overcome these analytical problems. The amount of the solvent was reduced and more reproducible results were obtained using this C18 SPE method. The time required for analysis is approximately the same for both methods. Higher recovery of semi-volatile compounds was also obtained. The proposed C18 SPE method was also found to be comparable to other commercial SPE columns and disks. The proposed procedure was designed to analyze the soluble oil and grease in the stormwater runoff samples.

4.1 Introduction

According to *Standard Methods* (1992), oil and grease are defined as "any material or substance that is soluble in the solvent". In the context of *Standard Methods* definition, the solvent implies non-polar organic solvents such as methylene chloride, hexane and chlorofluorocarbon (CFC). Oil and grease do not measure the presence of any specific compound, but is an important analytical procedure for environmental samples. The conventional liquid-liquid extraction (LLE) procedure for oil and grease analysis is plagued by various analytical problems such as poor or inconsistent recovery, emulsion formation, usage of large solvent volume, and loss of volatile and semi-volatile compounds during evaporation of the solvent. Stenstrom *et al.* (1986) reviewed the development of oil and grease analytical procedures and their disadvantages. An alternative method for the oil and grease analysis is needed to overcome these analytical problems, and more importantly, to avoid or reduce the use of solvents that may be greenhouse or smog forming gases. Solid phase extraction (SPE) is one candidate procedure and has been used extensively over the past 20 years for sample preparation in the analysis of semi- and non-volatile organic compounds for both environmental samples and for drugs in the pharmaceutical industry. The advantages of using SPE are reduced analysis time, cost, labor and elimination of emulsion formation problem. Solvent usage is also reduce.

The development of disposable columns with pre-packed bonded silica adsorbents in recent years has encouraged the usage of solid phase extraction for environmental and pharmaceutical applications. The most commonly used silica bonded adsorbents include octadecyl (C18), octyl (C8), ethyl (C2), cyclohexyl (CH), diol (OH) and cyanopropyl (CN). Two major uses of the SPE method are sample cleanup and concentration. Sample cleanup is required when impurities in the sample matrix interfere with analyte measurement in the analytical method of choice, such as gas chromatography. Increasing

the concentration of analyte is important when the sample is too dilute for direct measurement.

The major obstacle when using the SPE procedure is the need for method development since efficiency and precision depend upon the type of analyte, sample matrix, type of sorbents, and elution solvent. General method development for the SPE procedure has been discussed in detail by Chladek and Marano (1984), McDowall *et al.* (1986) and Wells and Michael (1987). A trial-and-error approach is generally used during these method developments.

Recently, Analytichem, a division of Varian, developed the EnvirElut™ Oil and Grease column for analysis of oil and grease. 3M (St. Paul, MN) also developed a specific type of SPE disk, Empore™ extraction disk, for oil and grease analysis. Both of these proprietary methods (EnvirElut™ and Empore™) have the reported advantage of reduced solvent usage, and may have some of the other advantages over liquid-liquid extraction (LLE) [e.g., prevention of emulsion formation and shorter analysis time] (Well *et al.*, 1995; Nguyen *et al.*, 1992). Unfortunately not all the details about the sorbent comnposition of these proprietary methods are published. This research was initiated to develop an SPE procedure using commercially available and characterized materials that have the aforementioned advantages. The goals of this method development are to: (1) reduce solvent volume, (2) provide more reproducible results, (3) improve recovery of semi-volatile compounds, and (4) reduce analysis time. The recovery of oil and grease in synthetically spiked samples and environmentally contaminated samples were studied.

4.2 Experimental Procedures

Instrumentation

A Sartorius Model 1712MP8 (Brinkmann Instrument Co., Westbury, NY) analytical balance was used for the gravimetric analysis of the recoverable oil and grease. A Masterflex® peristaltic pump (Cole-Parmer, Niles, IL) was used for the solid phase extraction procedures.

Materials and chemicals

The SPE columns used in this study were 1000 mg size Mega Bond Elut™ columns [i.e., ethyl (C2), octyl (C8) and octadecyl (C18) siloxane bonded to silica columns] obtained from Analytichem (Harbor City, CA). Reagent grade methylene chloride, n-hexane, isopropanol and concentrated hydrochloric acid used in the SPE procedures were obtained from Fisher Scientific (Tustin, CA).

Sample Preparation

Automobile crankcase oil was used to prepare the working standard solutions for the oil and grease analysis in this study. A stock solution of motor oil was prepared by mixing a known amount of motor oil in 100 ml deionized water using a wrist action shaker (Burrell Scientific, Pittsburgh, PA). This sample was used to simulate the oil and grease found in urban runoff (stormwater) since vehicle crankcase emissions are known to be large contributors to stormwater pollution (Stenstrom *et al.*, 1984).

Solid Phase Extraction Procedures

Figure 4.1 shows the setup of the solid phase extraction. The 1000 mg C18 column was first conditioned with 5 ml isopropanol, followed by 5 ml deionized water. A 500 ml sample was treated by adding 25 ml isopropanol and 1 ml concentrated HCl acid. The sample was then passed through the column at a flow rate of 5 ml/min. To remove oil and grease from the wall of sample container, 5 ml of isopropanol were added into the empty sample container and used to rinse the wall of the container. One hundred mls of deionized water containing 0.1% concentrated HCl were then added to the same empty container and the mixture was passed through the column as before. The column was then dried for approximately 25 minutes under vacuum (~ 44.5 cm Hg).

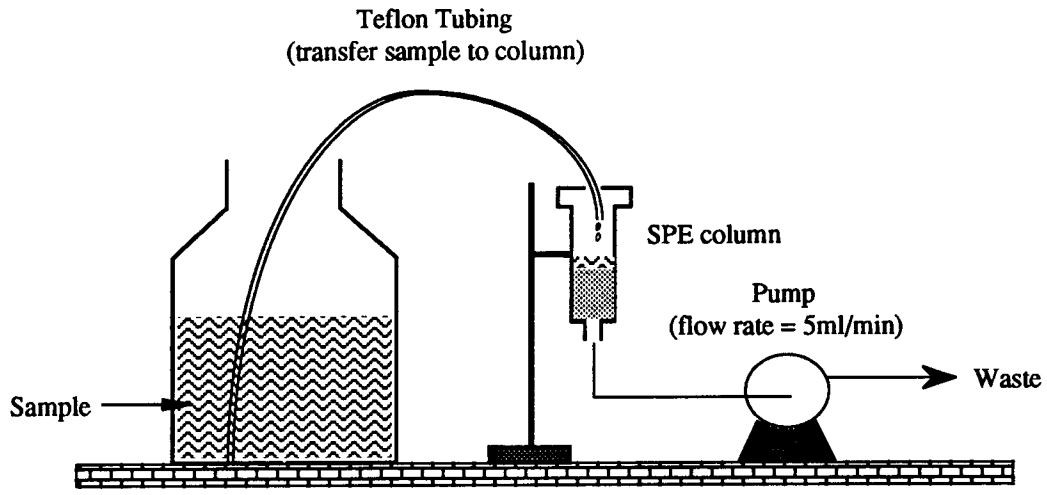


Figure 4.1 Schematic diagram of C18 SPE set-up for oil and grease analysis.

A tared collection tube was placed under the column after it was dried. The column was eluted with 3 ml of methylene chloride, and followed by 2 ml of hexane. Each elution

fraction in the collection tube was evaporated to dryness at approximately 55°C under a slow stream of nitrogen gas. The tube was then weighed to determine the mass of oil and grease eluted from the C18 column. The concentration of recoverable oil and grease was determined as follows:

$$\text{Concentration (mg/L)} = \frac{\text{mass of oil and grease eluted (mg)}}{\text{sample volume (L)}} \qquad (4.1)$$

The mass of oil and grease eluted in Equation (4.1) is the combined mass of oil and grease eluted in the methylene chloride and hexane fractions. Percentage recovery of oil and grease was then determined by comparing the obtained concentration with the expected concentration of used motor oil in the initial samples.

Liquid-liquid extraction procedures

This liquid-liquid extraction (LLE) procedure described in the *Standard Methods* (Method 5520B) was used in this study. The extracting solvent used in the LLE was methylene chloride. Methylene chloride is frequently used in research applications instead of Freon® 113 because of the desire to minimize freon usage as well as to maximize recovery. The sample volume used in the LLE was 500 ml instead of 1000 ml as suggested by the *Standard Methods*. The sample was acidified to pH 2 or lower using concentrated HCl and then transferred to a separatory funnel. The sample container was rinsed with 15 ml methylene chloride and then added into the separatory funnel. After shaking the funnel vigorously for approximately 2 minutes, the funnel was left to stand for 5 to 10 minutes until stable layers were formed. The methylene chloride layer was then drained through a funnel which contained a solvent-moistened filter paper into a clean,

tared distilling flask. If an emulsion preventing the formation of a clear solvent layer formed, 1 g of sodium sulfate was added to the filter paper cone. The sample was then extracted twice more with 15 ml methylene chloride. The extracts were combined and the filter paper was washed with an additional 5 - 10 ml of methylene chloride. The solvent was then evaporated at approximately 55°C under a slow stream of nitrogen gas. The dried flask was then cooled in a desiccator for 30 minutes and then weighed. A total of 45 ml methylene chloride was used in this procedure. The percentage recovery obtained from the LLE were then compared with those obtained from the modified C18 SPE procedures.

Matrix Interference study

One liter of filtered stormwater runoff samples (1 μm glass fiber filter paper) were used in this study. A known amount of used motor oil solution was spiked into the stormwater sample along with 50 ml of IPA and 1 ml concentrated HCl. The previously described C18 SPE procedures were used to analyze the oil and grease content in the spiked sample. Recovery was calculated on the basis of the spiked oil and grease and the background oil and grease concentration, which was also measured using the measured SPE. In general the background oil and grease concentration averaged 1.2 mg/L.

4.3 Results and Discussion

The experimental program evaluated all the major parameters affecting the SPE procedure, except sample flow rate. The effects of C2, C8 and C18 sorbents, sample volume, isopropyl alcohol volume, and oil and grease concentration were all evaluated. Finally a comparison with LLE and other SPE methods was made.

Sorbents

The first step in developing an SPE method is the selection of an appropriate sorbent that will extract oil and grease most efficiently. The recovery of oil and grease using three different sorbents, C2, C8 and C18, were studied and results are shown in Table 4.1. Among these three sorbents, C18 exhibited the best recovery of oil and grease, with an average percentage recovery of 89%. Table 4.1 also shows the confidence interval at $\alpha = 0.10$ for the percentage recoveries. The extraction efficiency of C2 and C8 columns are not significantly different. However, the extraction efficiency of the C18 column was significantly better than both C2 and C8 columns, and showed reduced variability in recovery. Thus, C18 column was used for the subsequent extraction of oil and grease.

Table 4.1 Comparison of percentage recovery of oil and grease using different sorbent.

Sorbent (1000 mg)	Avg ± SD*	CI ($\alpha = 0.10$)
C2	81 ± 8	76 - 86
C8	84 ± 4	81 - 87
C18	89 ± 2	88 - 90

*Based on 8 replicate extractions of 500 ml sample with prior addition of 25 ml isopropanol to the sample. Avg = average percentage recovery; SD = standard deviation; CI = confidence interval.

Elution Volume Effect

Methylene chloride and hexane have been widely used in the extraction of non-polar compounds. Preliminary C18 SPE studies had demonstrated the efficiencies of these two solvents in eluting oil and grease from the C18 column. In addition to methylene chloride, hexane was also used to elute the oil and grease from the C18 sorbent, and preliminary studies showed that high molecular weight hydrocarbons (such as C30 and C33

hydrocarbons) would only be desorbed from the C18 sorbent by hexane. After the appropriate elution solvents were selected, the effect of elution volume was studied so that the optimum elution volume could be determined. The optimum volume is the minimum volume which will elute all the adsorbed organics. Figure 4.2 shows the obtained percentage recovery of the oil and grease at four different elution volumes. The results show no improvement of extraction efficiencies after the addition of the third elution volume (E3). Therefore, the selected elution volumes used for the C18 SPE is as follows: 3.0 ml of methylene chloride and 2.0 ml of hexane (i.e., E4). The last elution step (i.e., the second 1.0 ml of hexane) was used to insure that all sorbed material is eluted.

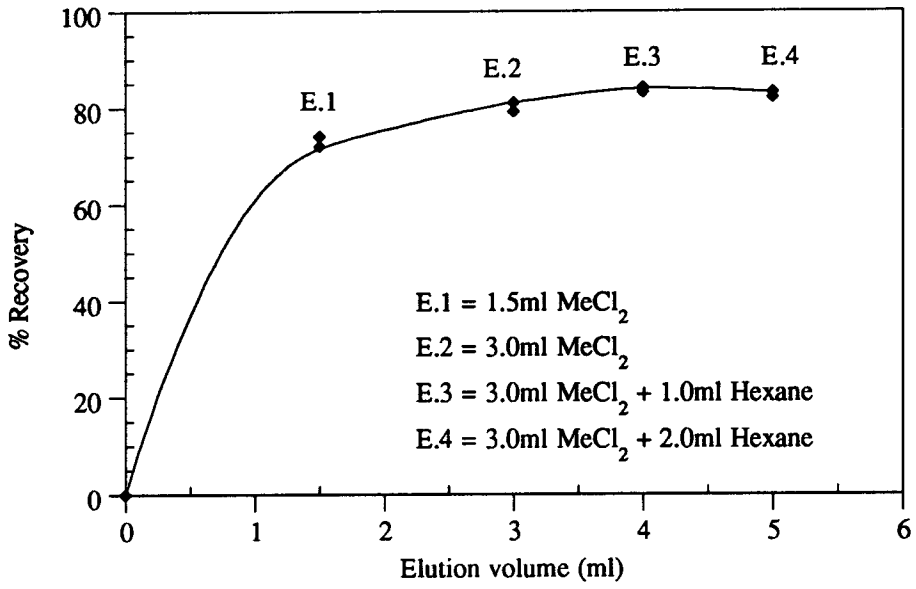


Figure 4.2 Elution volume effect on the percentage recovery of oil and grease.

Used motor oil was used to prepare the standard oil and grease solution in this study. To account for the less than 100% recovery of C18 column, one has to look at the

composition of the used motor oil. The used motor is composed of different groups of compounds which include the aliphatic (such as alkanes and cycloalkanes of 1-6 rings) and aromatic hydrocarbons, polar compounds, and heavy metals (Vazquez-Duhalt, 1989). The following components of the used motor oil probably account for the partial recovery:

1. polar fractions and heavy metals that are not adsorbed to the C18 sorbent;
2. asphaltenes that are adsorbed to the C18 sorbent and may not be elutable;
3. loss of semi-volatile compounds during the evaporation of C18 eluates.

Isopropanol Volume Effect

Sample pretreatment, such as the addition of an appropriate organic solvent is known to improve the efficiency of extraction. By adding the solvent into the sample prior to extraction, the solubility of the least soluble compounds can be increased and physical losses in the sample container minimized. In addition, the solvent also promotes the interaction between C18 bonded phase with the water sample and thus helps to maintain the equilibrium between the solid and liquid phase (Chladek and Marano, 1984; McDowall *et al.*, 1986). The solvent used in this sample pretreatment step is usually the same as the solvent use to condition the SPE column. Therefore, isopropanol (IPA) was added into the sample prior to passing it through the C18 column. Table 4.2 shows the average percentage recovery of oil and grease using three different IPA volumes.

Isopropanol volumes of 10, 25, and 50 ml were added to 500 ml samples that were subsequently analyzed using the SPE procedure. The recovery using 25 ml of IPA was significantly better ($\alpha = 0.10$) compared to the recovery when using 10 ml and 50 ml IPA volumes. Therefore, at least 5% (v/v) concentration of IPA is needed in order to achieve desirable recovery of oil and grease; less than 5% (v/v) of IPA may not be sufficient in promoting the desired interaction between the sorbent with oil and grease compounds in the

aqueous sample. Using more than 5% (v/v) of IPA reduced recovery, and may have caused the breakthrough of the oil and grease compounds from the C18 sorbent.

Table 4.2 Isopropanol volume effect on the percentage recovery of oil and grease.

IPA volume (ml)	IPA (% v/v)	Avg ± SD*	CI (α = 0.10)
10	2	80 ± 3	77 - 83
25	5	89 ± 2	87 - 91
50	10	82 ± 1	81 - 83

*Based on 3 replicate extractions using 1000 mg C18 column and 500 ml sample volume. IPA = isopropanol; avg = average percentage recovery; SD = standard deviation; CI = confidence interval.

Oil and grease adsorbs to glass and plastic, and for this reason Teflon is generally required to handle any water sample for oil and grease analysis. This usually imposes additional cost and it is often not possible to use Teflon for all applications. In order to overcome the adsorption problem of oil and grease to the wall of the glass sample container, a small volume of IPA (i.e., 5 ml) was added to the sample container after the whole sample had passed through the C18 column. The sample container was then swirled in a circular motion with the added IPA. Then 100 ml deionized water was added into the same sample container and mixed well with IPA. The IPA and deionized water mixture were then passed through the C18 column. The additional IPA at the end of sample extraction redissolved the oil and grease material from the glass wall of sample container, thus improving the recoveries of extraction. Under similar extraction conditions, it was found that the percentage recovery of the oil and grease, without addition of IPA in the final washing step, was below 60%, suggesting that the final IPA wash increased recovery by 20 - 25%. The greater the concentration of the oil and grease, the more important this washing step becomes.

The use of IPA to improve recovery and reduce oil and grease retention on glassware introduce questions about waste production. Introducing large amounts of solvents into wastewaters from laboratories, especially a production laboratory where large numbers of analyses are performed, is undesirable. Fortunately IPA is not a listed hazardous air pollutant (Kao, 1994). It is easily biodegradable and has less smog (ozone) formation potential than many other solvents, such as hexane (Carter, 1994). Its short life-time in the atmosphere is also low enough to prevent it from becoming a green house gas.

Sample Volume Effect

The mass of oil and grease adsorbed on the C18 SPE column is dependent on the volume of the sample used for the extraction: the greater the sample volume used, the greater the mass of oil and grease transferred to the sorbent. Figure 4.3 shows the percentage recovery of the oil and grease from five different sample volumes. Each sample volume had similar oil and grease concentration. The results show that the recovery of oil and grease remains almost unchanged when the volume of the sample increased from 500 ml to 1500 ml. It was observed that breakthrough occurs when more than 1500 ml sample passed through the 1000 mg C18 sorbent, as indicated by the decrease of the percentage recovery from approximately 90% at 1500 ml to 79% at 2000 ml. The adsorption capacity (q) of the 1000 mg C18 column was determined as follows:

$$q\ (mg/g) = \frac{\text{mass of oil and grease eluted (mg)}}{\text{mass of C18 sorbent (g)}} \qquad (4.2)$$

The maximum capacity of the 1000 mg C18 SPE column was found to be approximately 27 mg/g, which is approximately 2.7% of the mass sorbent. The obtained maximum capacity of a 1000 mg C18 sorbent for the oil and grease is within the range

suggested by the Majors (1986) and Van Horne (1990), i.e., 1% - 5% of the sorbent mass. The extraction efficiency decreases when the maximum capacity of the sorbent has been exceeded, allowing material to pass through the column. The extraction efficiency was not analyzed statistically (i.e., t-test) as only duplicate samples were performed on each sample volume.

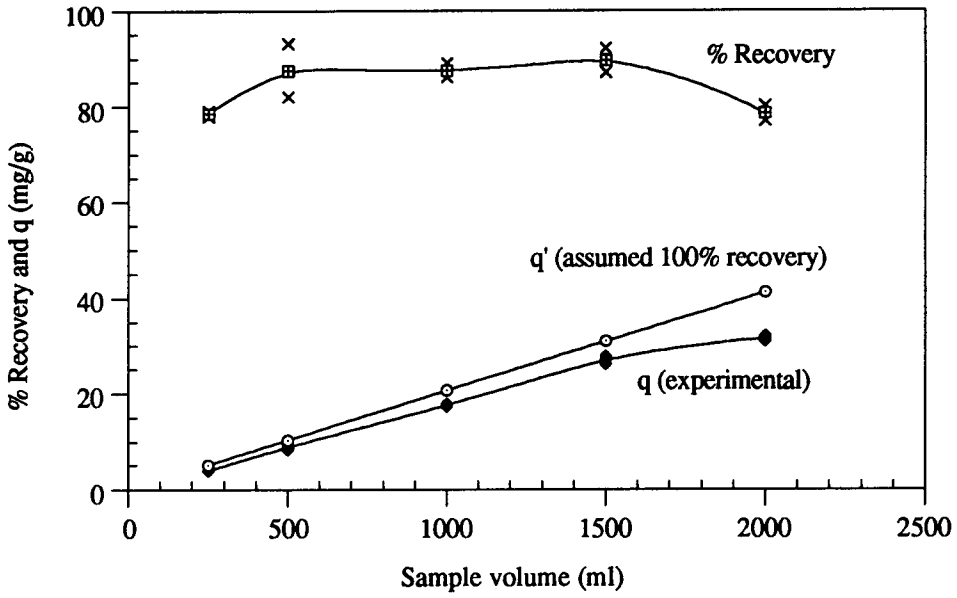


Figure 4.3 Sample volume effect on the percentage recovery of oil and grease.

Based on the results shown in Figure 4.3, it is concluded that a minimum sample volume of 500 ml is needed for the oil and grease analysis using the developed C18 SPE method. This volume is appropriate for the range of oil and grease typically found in environmental samples (1 - 50 mg/L). This volume at this concentration will provide a q ranging from 0.5 - 25 mg/g. A sample volume of less than 500 ml may cause inefficiency

of extraction that will lead to false low oil and grease results (as shown by the low recovery of oil and grease at 250 ml sample volume in Figure 4.3). Similarly, large sample volume such as 2000 ml should be avoided as it may cause the breakthrough of oil and grease compounds from the C18 column. Figure 4.3 also shows that 500 - 1500 ml is the range of sample volume that is suitable for a 1000 mg size C18 SPE column. The sample size must be adjusted as a function of the expected concentration.

Matrix Interference Study

In addition to the deionized water, a known amount of motor oil solution was also spiked into environmental samples which were collected from a storm drain. In order to avoid the clogging of the C18 column, the environmental samples were filtered with a 1 μm glass fiber filter paper prior addition of the known oil and grease solution. The concentration of oil and grease present in this environmental sample was 1.2 mg/L (average), and the amount of used motor oil spiked into the environmental samples was 18 mg/L. The recovery of the environmental sample was based upon the total oil and grease concentration (sample spiked components).

The C18 SPE conditions used were 1000 mg C18 column, 500 ml of sample volume, 5% of isopropanol for sample pretreatment and the E4 elution scheme. The average percentage recovery of oil and grease from these environmental spiked samples was then compared with those obtained from the synthetically spiked samples (Table 4.3). The obtained results show that similar percentage recovery of oil and grease in the environmental spiked samples were almost the same as those obtained from the synthetic samples. There was no significance difference, at the confidence level of α =0.10, between these two types of samples. This shows that the developed C18 SPE procedure can be used in environmental samples with a complex mixture of compounds.

Table 4.3 Comparison of percentage recovery of synthetic and environmental spiked samples.

Sample	n	Avg ± SD	CI ($\alpha = 0.10$)
Synthetic spiked sample	8	89 ± 2	88 - 90
Environmental spiked sample	10	88 ± 4	86 - 90

* n = number of samples; avg = average percentage recovery; SD = standard deviation; CI = confidence interval.

Comparison of C18 SPE with LLE

The extraction efficiency of the C18 SPE was compared with the conventional liquid-liquid extraction for the oil and grease analysis. The obtained results, as shown in Table 4.4, show that the extraction efficiency of C18 SPE is greater than LLE. The average percentage recovery of oil and grease is 85% and 76% for C18 SPE and LLE, respectively. The extraction efficiency of the C18 SPE is also significantly different than the LLE at the confidence interval of $\alpha = 0.10$. This shows that the developed C18 SPE procedure is a good candidate for replacing LLE for oil and grease analysis.

Table 4.4 Comparison of percentage recovery of C18 SPE and LLE.

Analytical method	n	Avg ± SD	CI ($\alpha = 0.10$)
C18 SPE	4	85 ± 2	84 - 86
LLE	4	76 ± 4	73 - 79
C18 SPE/LLE	4	1.12 ± 0.04	-

Note: n = number of samples; avg = average percentage recovery; SD = standard deviation; CI = confidence interval.

In addition to the improved and more consistent recovery of oil and grease, the C18 SPE was also able to recover more volatile components of oil and grease than the conventional LLE. This should be anticipated since there is much less solvent to evaporate. The C18 SPE and LLE extracts obtained from an aqueous sample spiked with crude oil were analyzed with GC-FID. Unlike used motor oil, crude oil contains many low molecular weight hydrocarbons. The obtained chromatograms of the LLE and SPE extracts are shown in Figures 4.5 and 4.6, respectively. From Figure 4.4, it was observed that there was no peaks detected between the retention time of 7.5 min and 13 min whereas numerous peaks were detected in the SPE extract (Figure 4.6). The most volatile compounds are not recovered by either method (see Figure 4.7). This shows that some of the semi-volatile components of the oil and grease were lost during the LLE process.

Several stormwater runoff samples were also analyzed for oil and grease using the above mentioned C18 SPE and LLE methods and the results are shown in Table 4.5. The oil and grease results using LLE method was found to be lower than those obtained from the C18 SPE method. These results support the results presented earlier in this paper that show the SPE procedure is capable of accurately quantifying oil and grease in a complex mixture, such as normally found in environmental samples. Table 4.5 also shows the ratio of oil and grease concentrations measured by the C18 SPE and LLE. The ratio ranged from 1.06 - 2.29, which indicates the SPE method recovered more oil and grease than the LLE method. These results are consistent with the findings of Wells *et al.* (1995). The ratios in their study, as measured by the EnvirElut™ Oil and Grease column and manually shaken separatory funnel LLE, were ranged from 0.73 - 2.0.

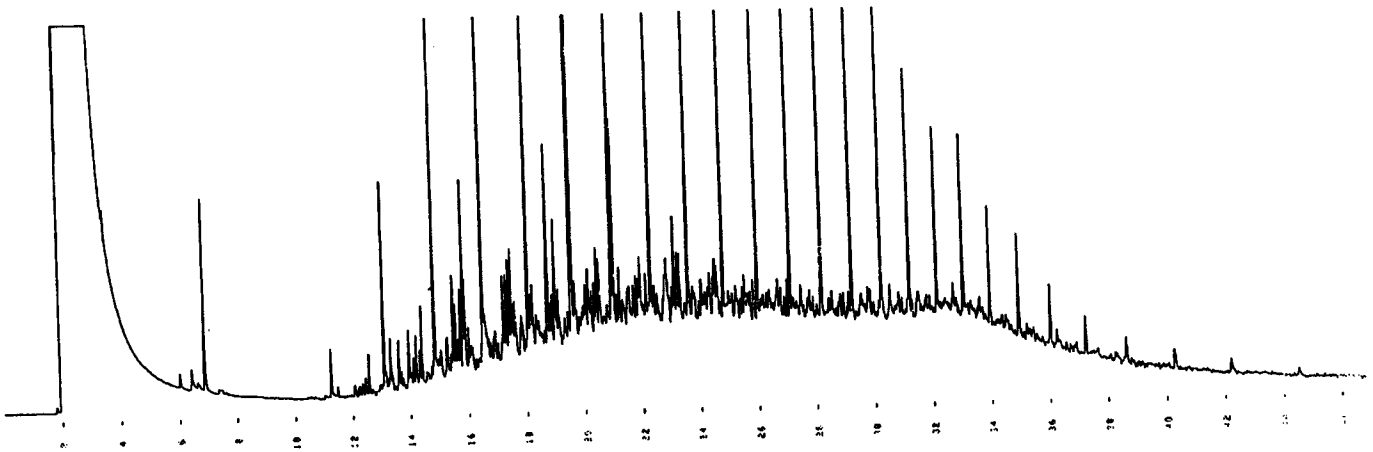


Figure 4.4 Gas chromatogram of LLE extract of a crude oil sample (3x dilution).

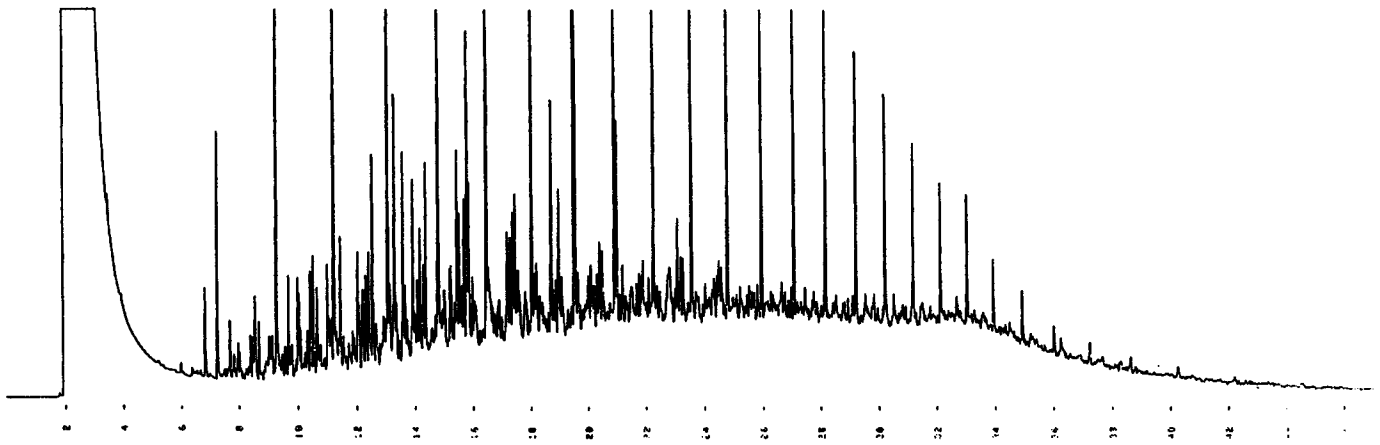


Figure 4.5 Gas chromatogram of C18 SPE extract of a crude oil sample (3x dilution).

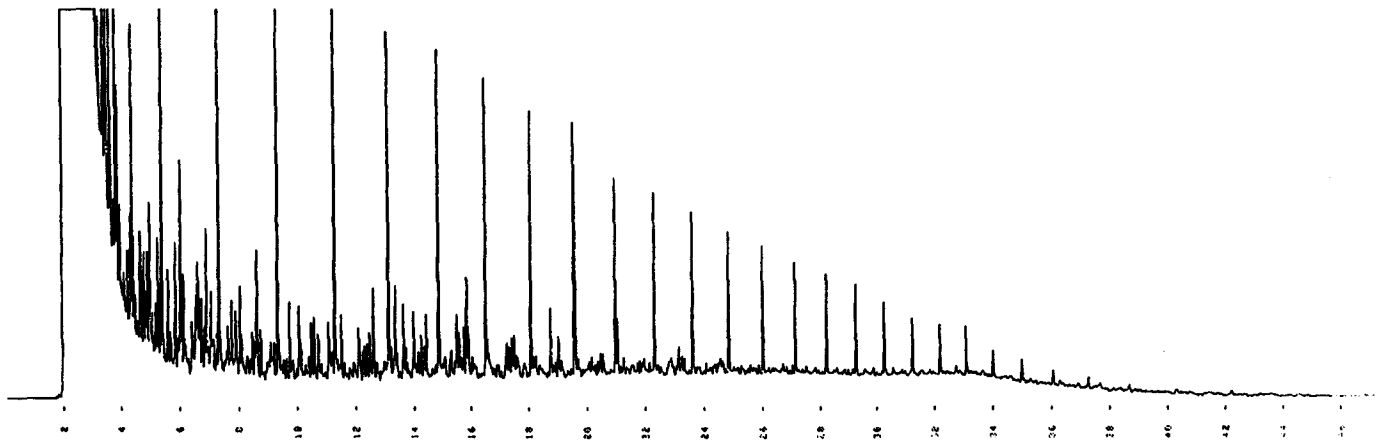


Figure 4.6 Gas chromatogram of an unextracted crude oil sample (10x dilution).

Table 4.5 Oil and grease results of several stormwater runoff samples using C18 SPE and LLE methods.

Type of sample	Oil and grease concentration (mg/L)		Ratio of
	C18 SPE	LLE	C18 SPE/LLE
Storm drain sample	2.43	1.96	1.24
Storm drain sample	8.63	7.23	1.19
Storm drain sample	30.11	24.61	1.22
Runoff from a parking facility	17.17	16.19	1.06
Runoff from a parking facility	13.98	8.39	1.67
Runoff from a parking facility	9.31	4.07	2.29

* only duplicate samples were analyzed.

Comparison to Commercially Available Procedures

The extraction efficiency of the C18 SPE was also compared with those obtained from using the EnvirElut™ Oil and Grease column (Varian) and Empore™ Oil and Grease disk (3M). The extraction procedures recommended by the manufacturer were used for these two methods. Table 4.6 shows the percentage recoveries of oil and grease using the proposed C18 SPE procedure as well as two commercial procedures. Used motor oil solution in deionized water was used in all extractions.

Preliminary studies showed low recoveries of oil and grease using the procedures recommended by the manufacturer for the EnvirElut™ Oil and Grease column, where an average of 71% of oil and grease was recovered. When 5% (v/v) of IPA was used instead of the 1% (v/v) as suggested by the manufacturer, a dramatic improvement of the percentage recovery from 71% to 89% was observed (see Table 4.6). Based on four replicate extractions, it was found that there is no significant difference (at $\alpha = 0.10$)

between the C18 SPE column and the EnvirElut™ Oil and Grease column with 5% (v/v) IPA for oil and grease analysis. The proposed C18 SPE procedure is comparable to EnvirElut™ Oil and Grease column for the oil and grease analysis. The EnvirElut™ Oil and Grease columns are approximately twice as expensive as the C18 SPE columns. The EnvirElut™ Oil and Grease procedure also uses 10 ml more solvent than the proposed C18 SPE method.

Table 4.6 Percentage recoveries of various SPE methods.

Method	n	Avg ± SD	CI (α = 0.10)
C18 SPE	4	88 ± 2	86 – 90
EnvirElut™ Oil and Grease (1% IPA)	4	71 ± 3	69 – 73
EnvirElut™ Oil and Grease (5% IPA)	4	89 ± 5	85 – 93
Empore™ Disk	14	74 ± 6	71 – 77

Note: n = number of samples; avg = average percentage recovery; SD = standard deviation; CI = confidence interval.

Based on the procedures recommended by the manufacturer, preliminary studies of Empore™ disk recovered less than 70% of oil and grease. The percentage recovery of oil and grease improved only slightly after 5% (v/v) of IPA was added into the sample prior to extraction. Sample flow rate through the disk was also varied in an attempt to enhance recovery; unfortunately recovery was the same at reduced flow rate. The average percentage recovery of oil and grease was 74%, which was significantly lower than the recoveries of both the C18 SPE and EnvirElut™ Oil and Grease columns.

Dissolved Oil and Grease vs Total Oil and Grease

Oil and grease is often separated into two classes: "free" and "dissolved". Free oil and grease refer to the oil and grease floating on the surface of the water or adsorbed to the container walls. Dissolved oil and grease refer to that portion which is truly dissolved, and colloidal particles which are so small that they cannot be removed by floatation or sedimentation. The previously described analysis concentrated mainly on the total extractable oil and grease, i.e., the combination of free and dissolved oil and grease. In order to show that the proposed C18 SPE procedure is capable of detecting both free and total oil and grease, a modified protocol was developed. A sample was prepared in the normal way except that it was allow to sit, undisturbed for 24 hours. In this way the free oil and grease floated to the surface or adsorbed to the container walls. The Teflon tubing used to transfer the solution from the sample container to the C18 column was submerged half way below the surface of the sample. Isopropanol was not added into the sample prior introduction to the C18 column. Only the half sample was pumped through the SPE column. In this way no oil and grease that was adsorbed to the container walls or floating on the liquid surface was analyzed. The obtained eluate was used to calculate the concentration of "dissolved oil and grease" using Equation (4.1). A range of total extractable oil and grease concentration from 6 mg/L to 320 mg/L was studied. Figure 4.7 shows the relationship between the total extractable and dissolved oil and grease. The results show that as the total extractable oil and grease concentration increases, the dissolved oil and grease concentration also increases. However, at approximately 220 mg/L of total extractable oil and grease, the dissolved oil and grease concentration saturates and remains almost unchanged. This information suggests that all containers and tubing, not made of Teflon, which contact the sample during the analysis, should be washed with solvent to avoid sample bias by adsorption. Using this technique with the proposed C18 procedure recovers total oil and grease with approximately 90% recovery.

This procedure may require that the sample be filtered prior to analysis. Fine suspended solids may clog the SPE column. The conventional LLE procedure does not require filtration prior to analysis. The suspended solids will be partially extracted in the LLE procedure. When the oil and grease concentration adsorbed to suspended solids is desired, the soxhlet extraction (*Standard Methods*, 1992) is recommended. The LLE procedure may not completely extract the suspended solids, and the SPE procedure may suffer from clogging columns.

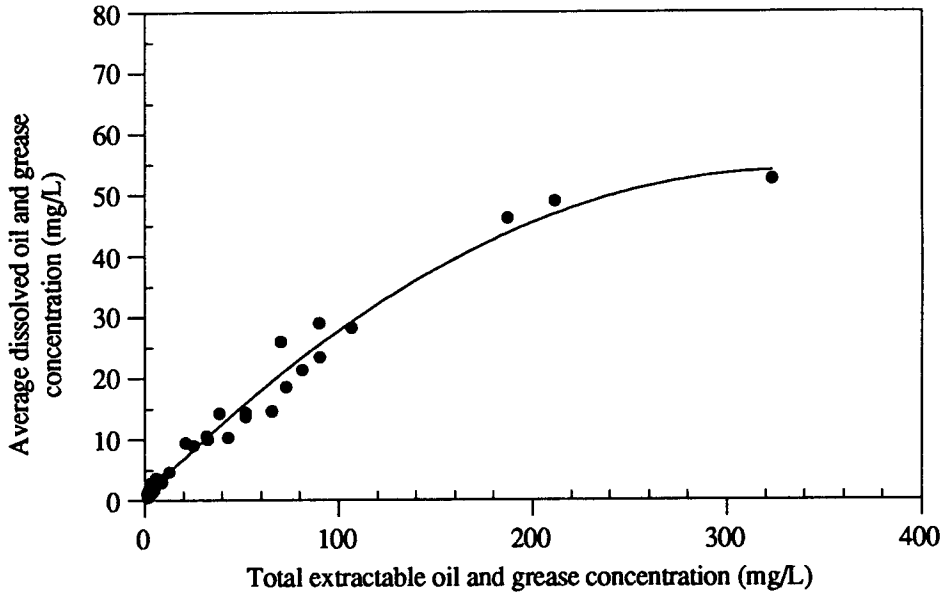


Figure 4.7 Correlation of total extractable oil and grease and dissolved oil and grease.

4.4 Conclusions

The C18 SPE procedures developed in this study shown excellent potential for oil and grease analysis. Greater recovery of oil and grease was observed using the C18 SPE

procedures as compared to the conventional liquid-liquid extraction and its efficiency is also comparable to those obtained from the EnvirElut™ Oil and Grease column. The loss of some semi- volatile components of the oil and grease can also be prevented when the SPE procedure is used. The volume of solvent was reduced and more reproducible results were obtained using the C18 SPE method as compared to liquid-liquid extraction. The C18 SPE procedures require an average of 2 hours per 500 ml sample analysis, which is approximately the same as liquid-liquid extraction. The length of analysis time might be reduced using a higher flow rate (i.e., > 5 ml/min). Analysis in parallel using multiple head pumps will reduce the analysis time for multiple samples.

The proposed procedure was designed to analyze the soluble oil and grease in the stormwater runoff samples. When using the proposed procedure it is recommended that the effects of several variables (such as sample volume and isopropanol volume) be considered. For example, 25 ml isopropanol was optimal in this research, but may be different for different sample types (e.g., high ionic strength samples or industrial wastewater). The proposed procedure also has advantages when fractionation or analysis of the extracted oil and grease is required. The reduced analyte volume means that the extracts are more concentrated, which facilitates analysis using gas or liquid chromatography or gas chromatography/mass spectrometry.

ABSTRACT

Fractionation of oil and grease can help to better define the nature and potential environmental significance of oil and grease pollution by separating the toxic and unharmful components. A toxicity-based fractionation for oil and grease was developed in this study. With commercial pre-packed silica gel SPE column and an elution scheme consisting of hexane and hexane-methylene chloride it was possible to separate the hydrocarbons of oil and grease into four different fractions: (1) aliphatics, (2) 1- and 2-ring aromatics, (3) 3- and 4-ring aromatics, and (4) more than 4-ring aromatics. The proposed toxicity-based fractionation was also not affected by the sample matrix, and the solvent-exchange procedure only slightly reduced the mass of semi-volatile compounds (< 10%). Sea urchin fertilization tests were conducted on the oil and grease fractions of synthetic samples and a consistent toxicity pattern was observed in the first two fractions. Fraction (1) exhibited toxic effects to sea urchin whereas no toxicity was detected in fraction (2). Fractions (3) and (4) did not show consistent toxicity results. One test showed no toxicity in either fraction whereas in another test, toxicity was detected in both fractions. The interaction between the toxic compounds and the test organisms require further investigations. The solubility of toxic compounds and effective concentration available to the organisms also require further investigations. However, the proposed toxicity-based fractionation of oil and grease is still considered to be viable for identifying the toxic fraction(s) of oil and grease qualitatively.

5.1 Introduction

Oil and grease includes a broad range of organic compounds such as hydrocarbons, vegetable oil, animal fats, waxes, soaps, greases, etc. It is usually difficult to identify the types of organic compound present in the oil and grease pollutants without further clean-up of the sample through chromatographic separation or fractionation. Fractionation is a method of separating a sample mixture into several fractions based on its chemical properties, such as polarity and pK_a (Lukasewycz and Durhan, 1992). In addition to the ease of identification, fractionation of oil and grease also can help to define more completely the nature and potential environmental significance of oil and grease pollution. Toxicity identification and best management practices may be developed based upon the significance of the fractions.

Solid phase extraction (SPE) is one of the most common methods used for fractionation. Table 5.1 shows some examples of fractionation procedures (e.g., type of column, solvent mixtures, etc.) using the SPE mode. Silica gel is the most commonly used sorbent for the fractionation of hydrocarbons. Two commonly used solvents for the elution of aliphatic hydrocarbons are n-pentane and hexane, due to the close similarity of their chemical characteristics with those of aliphatic hydrocarbons. For eluting the aromatic fractions, the elution scheme is less straight-forward than the aliphatic fraction. More polar solvents than n-pentane or hexane (such as benzene and methylene chloride) are required, and the amount of this solvent is manipulated so that the aromatics can be separated according to their structure or number of rings . For example, Bundt *et al.* (1991) used 5% (v/v) of methylene chloride in n-pentane for the elution of mono-aromatics and 60% (v/v) of methylene chloride for other greater than 2-ring aromatics. The fractionation procedures

described in Table 5.1 were used as the basis to develop the toxicity-based fractionation procedure for the oil and grease components in the stormwater runoff.

Table 5.1 Examples of fractionation procedures used by other researchers.

Work	Column	Sample type	Solvent or solvent mixtures	
			Aliphatics	Aromatics
Wang *et al.* (1994)	silica gel	light crude oil	n-pentane	50% (v/v) benzene in pentane for all the aromatics.
Bundt *et al.* (1991)	silica gel	diesel fuel	n-pentane	5%, 10% and 60% (v/v) $MeCl_2$ in n-pentane for 1, 2, and > 2 rings aromatics, respectively.
Bomboi and Hernandez (1991)	florisil	runoff sample	hexane	50% (v/v) $MeCl_2$ in hexane for all aromatics.
Theobald (1988)	silica gel	crude oil and product oil	hexane	10% and 20% (v/v) $MeCl_2$ in hexane for 1-3, and 3-6 rings aromatics, respectively.
Fam *et al.* (1987)	silica gel	runoff samples	n-hexane	benzene for all the aromatics.
Desideri *et al.* (1984)	silica gel and alumina	sea water	n-pentane	20% (v/v) CCl_4 in n-pentane for 1-ring; 10%, 30% and 80% (v/v) $MeCl_2$ in n-pentane for 2-rings, 3-4 rings and 5-6 rings aromatics, respectively.

Note: $MeCl_2$ = methylene chloride; CCl_4 = carbon tetrachloride.

Most of the current fractionation procedures found in the literature were not toxicity-based. Toxicity tests were not performed on the obtained fractions due to the sensitivity of the organism(s) used in the toxicity tests to the artifactual toxicity caused by the organic solvents. In addition, most of these organic solvents are also immiscible with

water and cause analytical problems in the toxicity tests. Therefore, it is desirable to develop a toxicity-based fractionation procedure in which non-toxic and miscible solvent(s) are used to separate the hydrocarbons. The method developed by Burkhard *et al.* (1991) which uses a mixture of water and methanol is one example. However, it has been found that those highly non-polar organics (where $pK_{ow} > 5$) such as the polyaromatic hydrocarbons could not be separated or fractionated by these mixtures of water and methanol. Therefore, alternative solvents that may be immiscible with water and toxic to the test organism(s) may have to be used. The obtained fractions will have to be solvent-exchanged into a suitable solvent that acceptable in the subsequent toxicity tests. Lukasewycz and Durhan (1992) provide an excellent literature review on the strategies for the development of a toxicity-based fractionation procedure.

The objective of this study is to develop a toxicity-based fractionation procedure for the oil and grease extract obtained from the C18 SPE method (described in Chapter 4). By conducting toxicity tests on the obtained fractions, the toxic components of the oil and grease can be determined and further identification of the toxic compound(s) can be performed through gas chromatography/mass spectrometry. After the compounds are known, it may be possible to determine their source and develop suitable control measure(s) to prevent future discharge of these contaminant(s) into the receiving waters.

5.2 Experimental Procedures

Chemicals

Aliphatic [C16, C17, C20, C23, C29, C30 and C33] and aromatic [sec-butylbenzene, napthalene, 2,6-dimethylnapthalene, acenapthene, phenanthrene, anthracene,

fluoranthene, pyrene, chrysene and benzo(a)pyrene] hydrocarbon standards (purity > 98%) used for recovery studies of fractionation procedure were obtained from Aldrich Chemical Co. (Milwaukee, WI). Reagent grade methylene chloride, n-hexane and isopropanol were obtained from Fisher Scientific (Tustin, CA).

SPE column

500 mg size Bond Elut™ columns silica gel columns used in the fractionation procedure were obtained from Analytichem (Harbor City, CA).

Sample preparation

Stock solutions of 0.1 mg/ml of hydrocarbons were prepared in n-hexane and used to prepare samples for the recovery studies by injecting 34 µl into 0.3 ml n-hexane. The sample was cooled at 4°C prior to fractionation.

A similar sample preparation procedure was followed in the sample matrix studies. Stormwater runoff samples were first collected from a storm drain and then oil and grease was extracted using the proposed C18 SPE procedure described in Chapter 4. The dried oil and grease extract was redissolved into 0.3 ml hexane in which a known amount of hydrocarbon standards (aliphatics and aromatics) was added. These spiked extracts were also cooled at 4°C prior fractionation.

Fractionation of oil and grease

The 500 mg Si column was first conditioned with 3 ml n-hexane. The cooled sample in hexane (0.3 ml) was introduced into the column at a flow rate of 3 ml/min. The sorbed hydrocarbons on the Si column were fractionated into four fractions using the

following elution scheme: (1) 2 x 0.27 ml n-hexane; (2) 2 x 0.40 ml 5% (v/v) methylene chloride in n-hexane; (3) 2 x 0.25 ml 10% (v/v) methylene chloride in n-hexane, and (4) 2 x 0.35 ml 20% (v/v) methylene chloride in n-hexane. Each eluate was collected into a separate clean vial. The Si column was allowed to dry (by continuing the pumping) prior addition of next elution solvent. Air was allowed to pass through the column after each elution; however the column never became "bone dry". No channeling or short-circuiting was observed.

Solvent Exchange Procedure

The objective of the fractionation procedure was to separate the oil and grease components into various fractions on which toxicity testings can be performed. However, both hexane and methylene chloride elution solvents are known to be toxic to the organisms used in the toxicity tests. Therefore, all four fractions need to be solvent-exchanged to isopropanol. Previous toxicity screening tests showed high tolerance [0.5 - 1% (v/v) fraction] of testing organisms to this particular solvent.

The fractions collected from the silica gel column (in hexane or mixture of hexane/ methylene chloride) were slowly dried under a slow stream of nitrogen gas (~ 40 - 50 µl/min). The flow rate of the nitrogen gas was controlled by a needle valve. As the volume of the fraction was reduced to half, approximately 0.3 ml of isopropanol was added into the collection vial and evaporation process continued. The procedure (i.e., addition of isopropanol added into the collection vial as the volume of the solvent was reduced to half) was repeated twice and it is then assumed all the hexane and methylene chloride originally present in the collection vial had been evaporated and solvent-exchanged to isopropanol.

Gas Chromatography Analysis

The concentration of each standard in the four fractions was then analyzed using GC/FID. A Varian Vista 6000 (Varian, Sunnyvalle, CA) equipped with a splitless injector (at 275°C) and flame ionization detector (at 320°C) was used. A capillary column DB5.625 column (30 m x 0.25 mm id) obtained from J&W Scientific was used to separate the hydrocarbons where the column temperature was programmed from 50°C - 300°C at 8°C/min; 2 min initial and 10 min final hold.

Recovery Calculation

During the recovery studies using standard hydrocarbons, the percentage recovery of each hydrocarbon was calculated by comparing its concentration in each Si fraction with its initial concentration in the 0.3 ml sample. All the concentrations were measured using the gas chromatography. Before the sample was fractionated, 1 µl of the sample was injected into the gas chromatograph to determine the initial concentration of the each standard hydrocarbon. After fractionation, 1 µl of each Si fraction was injected into the gas chromatogram to determine the obtained concentration. The following percentage recovery formula was used:

$$\% \text{ Recovery} = \frac{[\text{Concentration x Volume}]_{Si}}{[\text{Concentration x Volume}]_{initial}} \text{ x } 100\% \qquad (5.1)$$

where Si = silica gel fraction, and initial = initial sample, in both standard recovery and matrix interference studies.

Toxicity Recovery Study

Phase I - Toxicity screening of standard hydrocarbons. Two standard hydrocarbons from each Si fraction were selected for this study:

F1: hexadecane (C16) and eicosane (C20)

F2: napthalene and 2,6-dimethylnapthalene

F3: phenanthrene and pyrene

F4: chrysene and benzo(a)pyrene.

Individual stock solutions of these eight hydrocarbons were prepared in isopropanol (350 μg/3.5 ml except napthalene which had 3500 μg/3.5 ml). These stock solutions were used to prepared the aqueous samples for the subsequent sea urchin fertilization tests. The percentage fertilization was determined at two concentrations, i.e., 0.01% (v/v) and 0.1% (v/v) except phenanthrene which was tested at 0.1% and 1% (v/v) concentrations. The nominal concentration of hydrocarbons in the aqueous samples were calculated based on the dilution of stock solutions of known concentration. For example, 0.1% (v/v) concentration of napthalene (in aqueous sample) is equivalent to a nominal concentration of 1000 μg/L.

Phase II - Toxicity of oil and grease fractions. Based on the toxicity results of Phase I, four hydrocarbons (C20, 2.6-dimethylnapthalene, phenanthrene and benzo(a)pyrene) were selected for the second phase study. Figure 5.1 shows the schematic diagram of the overall process where the above described fractionation procedures were followed. A 0.6 ml standard mixture of the selected hydrocarbons (60 μg for C20, 2,6-dimethylnapthalene and benzo(a)pyrene and 120 μg for phenanthrene) was prepared in

hexane. The first 0.3 ml sample mixture was used for fractionation. The second 0.3 ml sample mixture and four Si fractions were solvent-exchanged to 0.3 ml isopropanol and tested for toxicity.

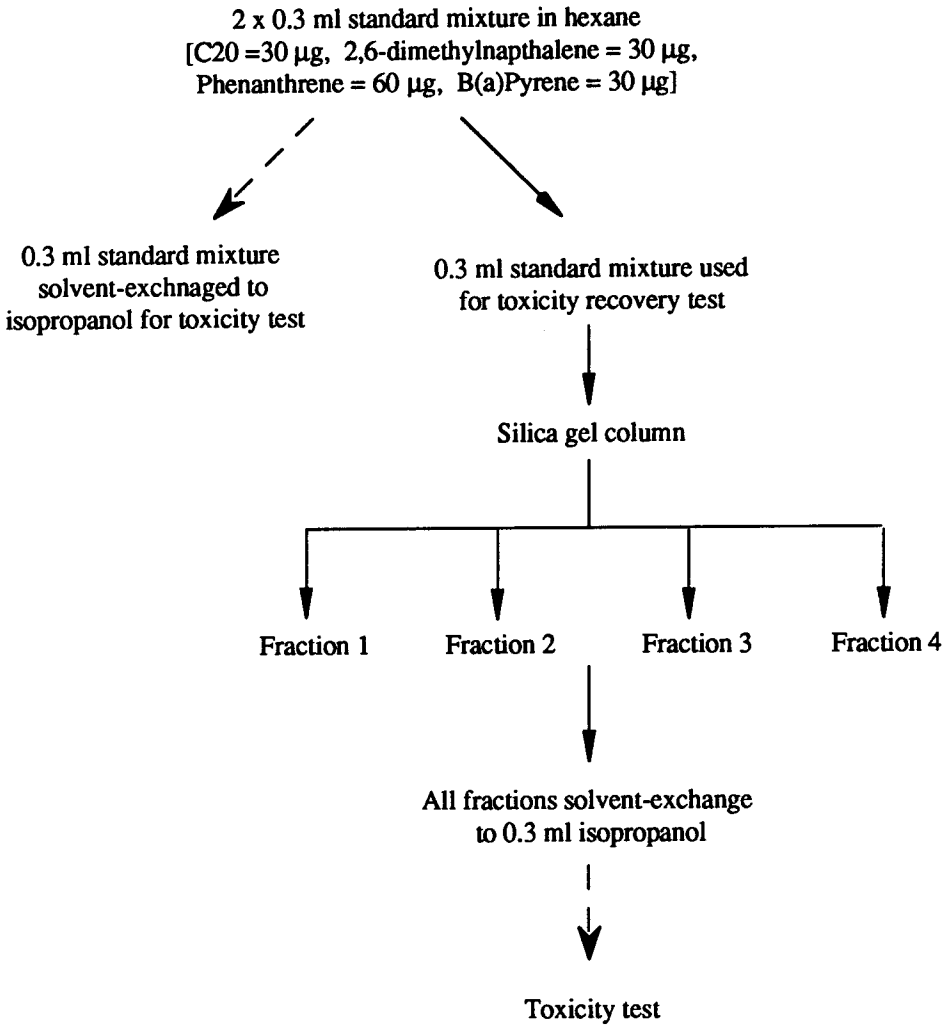


Figure 5.1 Schematic diagram of the Phase II of toxicity recovery study.

Toxicity Tests

The purple sea urchin (*Strongylocentrotus purpuratus*) fertilization test, as described in the EPA West Coast testing manual (Chapman *et al.*, 1995) was used for toxicity testing. It is one of the recommended marine test methods in the California Ocean Plan (SWRCB, 1990). The toxicity tests were performed at the Southern California Coastal Water Research Project's (SCCWRP) laboratory in Westminister, CA. Seawater dilutions of each Si fraction were prepared by adding appropriate amounts of seawater to achieve the desired dilutions and maintain a salinity of 33 mg/g. The concentrations of sample used in the toxicity tests were expressed in percentage of sample used in the dilutions. For example, a concentration of 0.5% corresponds to a diluted sample consisting of 0.5% (v/v) of sample and 99.5% of dilution water. Therefore, 0.2 ml of Si fraction would be needed in order to prepare a 40 ml sample with a concentration of 0.5%. The concentrations of each Si fraction tested for toxicity were 0.06%, 0.12%, 0.25% and 0.5% (v/v), and each concentration was tested at least twice (duplicate).

The purple sea urchin test consisted of a 20 minute sperm exposure followed by a 20 minute fertilization period. Percentage fertilization was measured on preserved samples using a compound microscope. Toxic effects were indicated by a reduction in the percentage of fertilized eggs from that observed in a control sample (seawater). All tests were conducted at 15°C.

The fertilization of sea urchin eggs was first measured in the highest concentration samples. If no toxic effect was found in the 0.5% samples, examination of samples with lower concentrations (i.e., 0.06% and 0.12%) was omitted to reduce the cost of analysis.

5.3 Results and Discussion

Recovery Studies of Fractionation Procedures

Silica gel is the most commonly used sorbent in fractionation of organic compounds. Some examples of silica gel fractionation of organics include Fam *et al.* (1988), Theobald (1988), Bundt *et al.* (1991), and Wang *et al.* (1994). A literature review of the currently available fractionation procedures also revealed that both hexane and methylene chloride had been successfully used in fractionating aliphatic and aromatic hydrocarbons. Therefore, an elution scheme using hexane and mixtures of methylene chloride-hexane was followed in the recovery studies for separation of standard aliphatics and aromatics with commercially available (i.e., the pre-packed by the manufacturer) silica gel columns. Most of the research cited in Table 5.1, with the exception of Theobald (1988), used self-made or self-packed chromatographic columns. Packing and cleaning columns is tedious and time consuming, and may introduce more variability into the testing procedure, as compare to commercially prepared column.

Elution solvent. As mentioned in previous section, the elution of aliphatics from the silica gel column is very straight forward. In general, greater than 90% of the aliphatics are successfully separated into the 100% hexane fraction. Separation or fractionation of aromatics according to their structure or their number of rings is more complicated. The amount of methylene chloride used to mix with hexane is critical. Our preliminary studies had shown that 5% (v/v) of methylene chloride in hexane can separate the 1- and 2-ring aromatics from other higher aromatics. An additional 5% (v/v) of methylene chloride, i.e., 10% (v/v) of methylene chloride in hexane can further separate those of 3- and 4-ring compounds. The remaining aromatics (i.e., those having more than 4 rings) can be eluted as the final fraction by using a 20% (v/v) methylene chloride in hexane.

Elution volume. Once the elution solvent and solvent mixtures are chosen, the optimum elution volume for each fraction is determined. To show the utility of the proposed fractionation, a simulated stormwater sample, containing 3.4 μg of each hydrocarbon standard, was fractionated. To determine the optimum elution volume for each fraction, a trial-and-error approach was used until the best separation of aliphatics from the aromatics and of aromatics according to the number of rings was obtained. Table 5.2 shows the results of the recovery studies. As expected, the aliphatic hydrocarbons concentrated into the first fraction (100% hexane), where greater than 90% were recovered. The elution volume used for the elution of aliphatics were ranged from 2 x 0.27 ml. However, as observed in Table 5.2, 33% of the 1-ring aromatic (i.e., sec- butylbenzene) was also eluted into the first fraction. Decreasing the elution volume of hexane decreased concentration of 1-ring aromatic into the first fraction, but also decreased the concentration of aliphatic compounds. Therefore, an elution volume of 2 x 0.27 ml 100% hexane was chosen for elution of the first fraction.

The aromatic hydrocarbons were separated according to the number of rings by varying the amount of methylene chloride in the hexane-methylene chloride mixture. Preliminary results had shown that a 5% (v/v) methylene chloride in hexane is efficient in separating the 1- and 2-ring aromatics into a single fraction. However, one-half of the 1-ring aromatic separated into the first fraction, and the remaining 1-ring aromatic was then eluted into the second fraction. Greater than 65% of the 2-ring aromatics such as 2,6-dimethylnapthalene and acenapthene were recovered in the second fraction. The optimum elution volume was 2 x 0.40 ml of 5% (v/v) methylene chloride in hexane. Increasing the elution volume increased the recovery of 2,6-dimethylnapthalene and acenapthene in the second fraction, but more of the 3-ring aromatics, such as anthracene and phenanthrene were also eluted.

Table 5.2 Recovery of 3.4 µg of each hydrocarbon from a standard mixture following fractionation on a silica gel column.

Compounds	Average percentage recovery ± standard deviation*				
	F.1	F.2	F.3	F.4	Total
C16	90 ± 4	0	0	0	90 ± 4
C17	91 ± 4	0	0	0	91 ± 4
C20	91 ± 4	0	0	0	91 ± 4
C23	91 ± 5	0	0	0	91 ± 5
C29	94 ± 4	0	0	0	94 ± 4
C30	92 ± 3	0	0	0	92 ± 3
C33	91 ± 4	0	0	0	91 ± 4
Sec-butylbenzene (1-ring)	33 ± 4	66 ± 8	0	0	99 ± 9
Napthalene (2-ring)	0	83 ± 3	15 ± 5	0	98 ± 6
2,6-dimethylnapthalene (2-ring)	0	67 ± 4	27 ± 7	0	94 ± 5
Acenapthene (2-ring)	0	68 ± 3	26 ± 3	0	94 ± 4
Anthracene (3-ring)	0	10 ± 5	80 ± 8	0	90 ± 8
Phenanthrene (3-ring)	0	7 ± 4	78 ± 6	7 ± 5	92 ± 8
Pyrene (4-ring)	0	5 ± 3	77 ± 6	9 ± 5	91 ± 7
Fluoranthene (4-ring)	0	0	53 ± 11	41 ± 13	94 ± 4
Chrysene (4-ring)	0	0	9 ± 11	86 ± 6	95 ± 3
Benzo(a)pyrene (5-ring)	0	0	7 ± 4	89 ± 3	96 ± 4

Note : * Based on 6 replicate extractions. F.1 = 2 x 0.27 ml 100% hexane; F.2 = 2 x 0.40 ml 5% (v/v) methylene chloride in hexane; F.3 = 2 x 0.25 ml 10% (v/v) methylene chloride in hexane; F.4 = 2 x 0.35 ml 20% (v/v) methylene chloride in hexane.

The aromatics that contain 3- and 4-rings (except chrysene) were eluted in the third fraction, i.e., 2 x 0.25 ml of 10% (v/v) methylene chloride in hexane. The recovery of was aromatic was greater than 75%, except for fluoranthene where 10% (v/v) methylene chloride only eluted as much as 53%. Further manipulation of elution volume and amount of methylene chloride did not improve the separation of fluoranthene without perturbing the

separation of the other 3- and 4-ring aromatics. The remaining 2-ring aromatics that were not elute into the second fraction eluted in this third fraction. A small amount (< 10%) of other 4- and 5-ring aromatics (e.g., chrysene and benzo(a)pyrene) also eluted into the third fraction. Finally, the highly hydrophobic aromatics (4- and 5-ring aromatics) were eluted from the silica gel column by 2 x 0.35 ml of 20% (v/v) methylene chloride in hexane, where greater than 85% were recovered. The remaining fluoranthene was also eluted into this fourth fraction.

Final elution scheme for fractionation of oil and grease using a pre-packed silica gel column is as follows:

(F.1) 2 x 0.27 ml 100% hexane;

(F.2) 2 x 0.40 ml of 10% (v/v) methylene chloride in hexane;

(F.3) 2 x 0.25 ml of 10% (v/v) methylene chloride in hexane;

(F.4) 2 x 0.35 ml of 20% (v/v) methylene chloride in hexane.

Column drying during the elution step is accomplished by continuing the pumping until the entire volume of that particular elution solvent is collected into the vial. It does not refer to a drying process that removes 100% of the liquid. It is very important to prevent the silica gel from completely drying ("bone dry") during loading of the initial sample, as channeling effects will cause poor sorption of compounds onto the sorbent, producing low recoveries. However, it is necessary to pump the column dry between solvent addition. This is to ensure that all the appropriate compounds are collected into the corresponding fraction and the elution problem such as sec-butylbenzene and fluoranthene to two fractions can be minimized.

Hydrocarbon mass. The amount of aliphatic hydrocarbons present in the oil and grease are generally greater than that of aromatics. For example, Eganhouse and Kaplan (1981) found 88% of the measured hydrocarbons were in the form of aliphatics. Hunter *et al.* (1979), however, only measured about 73% aliphatics in their urban runoff samples. Hoffman *et al.* (1984) found lesser amounts of aromatics in their studies. They only found 5.7% of the total hydrocarbons were aromatics. Therefore, a simulated stormwater sample, containing 75% of aliphatics (10.2 - 20.4 μg) and 25% of aromatics (3.4 - 6.5 μg), was used to determine the effect of total hydrocarbon mass on the elution volume of each fraction. Tables 5.3 and 5.4 show the results of the recovery of each hydrocarbon in this study.

It was observed that as the mass of each aliphatic hydrocarbon increased from 3.4 μg to 10.2 μg, the elution volume of the first fraction, i.e., 2 x 0.27 ml, was insufficient to elute all the aliphatic hydrocarbons. An increase of elution volume to 2 x 0.29 ml was sufficient to elute greater than 85% of the aliphatics at this higher concentration (see Table 5.3). A small amount of aliphatics (≤ 5%) was also eluted into the second fraction. Increasing the elution volume of first fraction (beyond 2 x 0.29 ml) caused more of the 1-ring aromatic to be eluted together with the aliphatics. As the mass of aliphatics increase again to 20.4 μg each, only a slight increase of volume to 2 x 0.30 ml was needed to obtain greater than 85% recovery (Table 5.4). It was also observed that the elution volume of fractions 2 - 4 did not change as the mass of aromatics increase from 3.4 μg to 6.5 μg.

If the distribution of aliphatics and aromatics of a real sample is not known and only the total mass of oil and grease (as determined gravimetrically from the C18 SPE) is known, the elution volume of 2 x 0.29 ml for the first fraction can be used as the initial trial

for the elution of aliphatics from the silica gel column (as shown in later section of the matrix interference study). It may be necessary to fine tune the volume of each fraction, based upon the mass of extracted oil and grease and the relative amount of each fraction. The efficiency of separation can be confirmed by gas chromatography.

Table 5.3 Recovery of 10.2 µg of each aliphatic and 3.4 µg of each aromatic from a standard mixture following fractionation on a silica gel column.

Compounds	Average percentage recovery ± standard deviation*				
	F.1	F.2	F.3	F.4	Total
C16	92 ± 4	5 ± 3	0	0	97 ± 4
C17	92 ± 4	5 ± 3	0	0	97 ± 4
C20	92 ± 4	5 ± 3	0	0	97 ± 4
C23	92 ± 4	4 ± 2	0	0	96 ± 5
C29	91 ± 6	3 ± 2	0	0	94 ± 7
C30	91 ± 7	3 ± 2	0	0	94 ± 8
C33	88 ± 9	3 ± 2	0	0	91 ± 10
Sec-butylbenzene (1-ring)	30 ± 16	66 ± 15	0	0	96 ± 5
Napthalene (2-ring)	0	78 ± 8	12 ± 7	0	90 ± 7
2,6-dimethylnapthalene (2-ring)	0	66 ± 12	25 ± 9	0	91 ± 5
Acenapthene (2-ring)	0	65 ± 8	23 ± 5	0	88 ± 3
Anthracene (3-ring)	0	11 ± 11	77 ± 9	4 ± 5	92 ± 5
Phenanthrene (3-ring)	0	8 ± 8	79 ± 7	5 ± 6	92 ± 6
Pyrene (4-ring)	0	2 ± 4	80 ± 7	7 ± 6	89 ± 8
Fluoranthene (4-ring)	0	0	60 ± 14	37 ± 13	97 ± 6
Chrysene (4-ring)	0	0	12 ± 8	83 ± 13	95 ± 7
Benzo(a)pyrene (5-ring)	0	0	8 ± 7	88 ± 12	96 ± 9

Note : * Based on 7 replicate extractions. F.1 = 2 x 0.29 ml 100% hexane; F.2 = 2 x 0.40 ml 5% (v/v) methylene chloride in hexane; F.3 = 2 x 0.25 ml 10% (v/v) methylene chloride in hexane; F.4 = 2 x 0.35 ml 20% (v/v) methylene chloride in hexane.

Table 5.4 Recovery of 20.4 µg of each aliphatic and 6.5 µg of each aromatic from a standard mixture following fractionation on a silica gel column.

Compounds	Average percentage recovery ± standard deviation*				
	F.1	F.2	F.3	F.4	Total
C16	86 ± 7	0	0	0	86 ± 9
C17	86 ± 7	0	0	0	86 ± 9
C20	85 ± 7	0	0	0	85 ± 9
C23	87 ± 7	0	0	0	87 ± 9
C29	87 ± 7	0	0	0	87 ± 9
C30	90 ± 8	0	0	0	90 ± 10
C33	90 ± 9	0	0	0	90 ± 11
Sec-butylbenzene (1-ring)	43 ± 6	54 ± 3	0	0	97 ± 7
Napthalene (2-ring)	0	88 ± 3	7 ± 2	0	95 ± 1
2,6-dimethylnapthalene (2-ring)	0	78 ± 6	15 ± 3	0	93 ± 6
Acenapthene (2-ring)	0	75 ± 3	15 ± 4	0	90 ± 1
Anthracene (3-ring)	0	26 ± 7	70 ± 6	0	96 ± 5
Phenanthrene (3-ring)	0	19 ± 6	74 ± 3	1 ± 3	94 ± 3
Pyrene (4-ring)	0	15 ± 6	80 ± 6	2 ± 4	97 ± 10
Fluoranthene (4-ring)	0	0	72 ± 10	22 ± 9	94 ± 2
Chrysene (4-ring)	0	0	22 ± 11	74 ± 9	96 ± 6
Benzo(a)pyrene (5-ring)	0	0	17 ± 9	74 ± 7	91 ± 3

Note : * Based on 5 replicate extractions. F.1 = 2 x 0.30 ml 100% hexane; F.2 = 2 x 0.40 ml 5% (v/v) methylene chloride in hexane; F.3 = 2 x 0.25 ml 10% (v/v) methylene chloride in hexane; F.4 = 2 x 0.35 ml 20% (v/v) methylene chloride in hexane.

Solvent-Exchange of Oil and Grease Fractions

Both hexane and methylene chloride used in the fractionation of oil and grease are known to be toxic to the testing organism used in the toxicity tests, and also immiscible with water. Therefore, it is necessary to solvent-exchange the obtained fractions to a solvent that is miscible with water and not toxic to the testing organisms. The solvent of

choice is isopropanol. In addition to the miscibility of isopropanol with water, preliminary toxicity tests showed that a dilution concentration of 1% isopropanol to be acceptable for purple sea urchin when reconstituting the fractions for the subsequent toxicity test. Table 5.5 shows the average total percentage recovery of each hydrocarbon standard with and without the solvent-exchange procedure. A slight decrease in more volatile hydrocarbons

Table 5.5 Average percentage recovery of hydrocarbons with and without solvent-exchange.

	Average percentage recovery ± standard deviation	
	Without solvent-exchange	With solvent-exchange
C16	90 ± 4	82 ± 4
C17	91 ± 4	83 ± 5
C20	91 ± 4	87 ± 5
C23	91 ± 5	89 ± 5
C29	94 ± 4	94 ± 4
C30	92 ± 3	96 ± 4
C33	91 ± 4	96 ± 5
Sec-butylbenzene (1-ring)	99 ± 9	85 ± 5
Napthalene (2-ring)	97 ± 6	81 ± 5
2,6-dimethylnapthalene (2-ring)	94 ± 5	85 ± 3
Acenapthene (2-ring)	93 ± 4	91 ± 3
Anthracene (3-ring)	97 ± 8	88 ± 7
Phenanthrene (3-ring)	93 ± 8	87 ± 7
Pyrene (4-ring)	94 ± 7	94 ± 6
Fluoranthene (4-ring)	94 ± 4	95 ± 9
Chrysene (4-ring)	95 ± 3	94 ± 6
Benzo(a)pyrene (5-ring)	96 ± 4	98 ± 7

Note : * Based on 6 replicate extractions and mixtures contained 3.4 µg of each standard hydrocarbon. F.1 = 2 x 0.27 ml 100% hexane; F.2 = 2 x 0.40 ml 5% (v/v) methylene chloride in hexane; F.3 = 2 x 0.25 ml 10% (v/v) methylene chloride in hexane; F.4 = 2 x 0.30 ml 20% (v/v) methylene chloride in hexane.

such as hexadecane (C16), heptadecane (C17), sec-butylbenzene, napthalene and 2,6-dimethylnapthalene was observed after the solvent-exchange procedure. Decreasing the flow rate of the nitrogen stream did not decrease the evaporation of these semi-volatile compounds. Since the overall percentage recoveries of these compounds were still greater than 80%, it is believed that the solvent-exchange procedure did not impact the efficiency of the fractionation of oil and grease.

Matrix Interference Study

A matrix interference study is very important in order to determine the feasibility of the proposed fractionation procedure for oil and grease in actual aqueous environmental samples. Stormwater runoff samples collected from a storm drain were used for the matrix interference studies of the proposed fractionation procedure. The collected samples were first extracted using C18 SPE columns using the procedures described in the Chapter 4. The weighed, dried oil and grease C18 extracts were then redissolved back into hexane and standard hydrocarbons were spiked into the hexane. Fractionation using a silica gel SPE column was then performed, and the results are shown in Table 5.6.

The results show that the recovery and separation of each of the hydrocarbon standard were not affected by the sample matrix. The mass of oil and grease extract used in this study was less than and equal to 250 μg and the elution volume of the aliphatics fraction (1st fraction) was 2 x 0.29 ml. When the mass of oil and grease extract used for the matrix study increased to greater than 250 μg, the elution volumes for the first and second fractions were changed accordingly in order to elute the aliphatics and 1 - 2 ring aromatics efficiently. Breakthrough may have occurred due to the overloading of hydrocarbons onto the 500 mg silica gel column. Satisfactory fractionation of

hydrocarbons can be obtained by increasing the mass of silica gel sorbent, or reducing the total mass of oil and grease extract applied to the column.

Figures 5.2 and 5.3 shows example of the gas chromatogram of the C18 extract of the stormwater runoff sample with and without the spiked standard hydrocarbons. The

Table 5.6 Recovery of hydrocarbons from a spiked environmental sample following fractionation on a silica gel column.

Compounds	Average percentage recovery ± standard deviation*				
	F.1	F.2	F.3	F.4	Total
C16	97 ± 8	0	0	0	97 ± 8
C17	97 ± 8	0	0	0	97 ± 8
C20	97 ± 8	0	0	0	97 ± 9
C23	98 ± 8	0	0	0	98 ± 8
C29	95 ± 7	0	0	0	95 ± 7
C30	83 ± 5	0	0	0	83 ± 6
C33	90 ± 5	0	0	0	90 ± 6
Sec-butylbenzene (1-ring)	46 ± 6	50 ± 7	0	0	96 ± 9
Napthalene (2-ring)	0	87 ± 11	0	0	87 ± 11
2,6-dimethylnapthalene (2-ring)	0	74 ± 11	11 ± 4	0	85 ± 13
Acenapthene (2-ring)	0	67 ± 12	16 ± 4	0	83 ± 14
Anthracene (3-ring)	0	13 ± 7	70 ± 7	0	83 ± 10
Phenanthrene (3-ring)	0	8 ± 5	74 ± 5	0	82 ± 8
Pyrene (4-ring)	0	7 ± 3	78 ± 3	1 ± 2	86 ± 9
Fluoranthene (4-ring)	0	0	72 ± 7	18 ± 5	90 ± 6
Chrysene (4-ring)	0	0	17 ± 7	73 ± 11	90 ± 9
Benzo(a)pyrene (5-ring)	0	0	14 ± 6	79 ± 12	93 ± 11

Note : * Based on 9 replicate extractions and samples contained ≤ 250 µg oil and grease and 2.2 µg of each standard hydrocarbon. F.1 = 2 x 0.29 ml 100% hexane; F.2 = 2 x 0.40 ml 5% (v/v) methylene chloride in hexane; F.3 = 2 x 0.25 ml 10% (v/v) methylene chloride in hexane; F.4 = 2 x 0.30 ml 20% (v/v) methylene chloride in hexane.

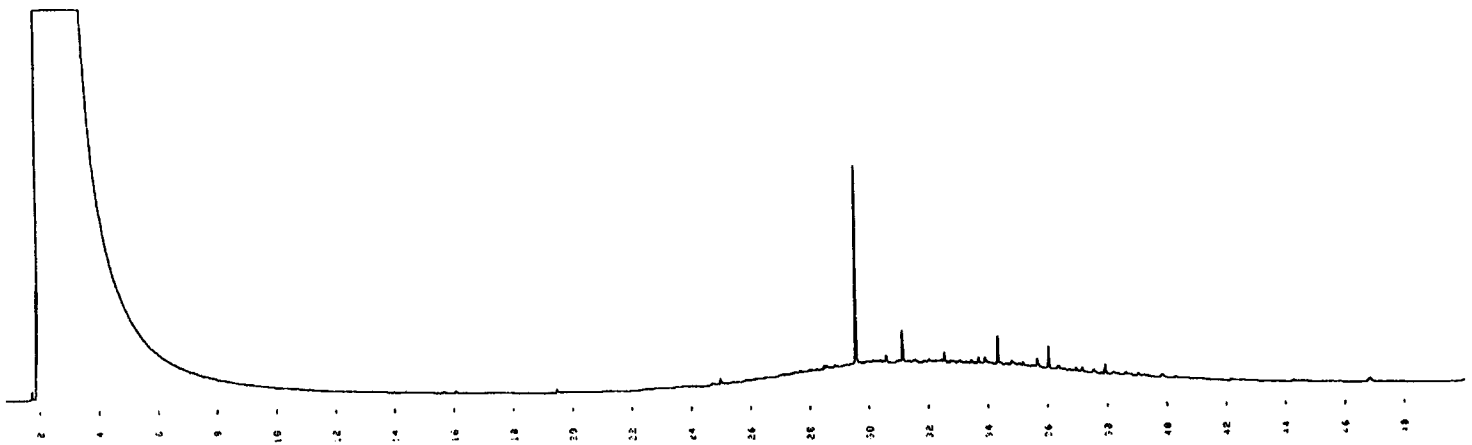


Figure 5.2 Gas chromatogram of C18 extract of a stormwater runoff sample used in the matrix interference study.

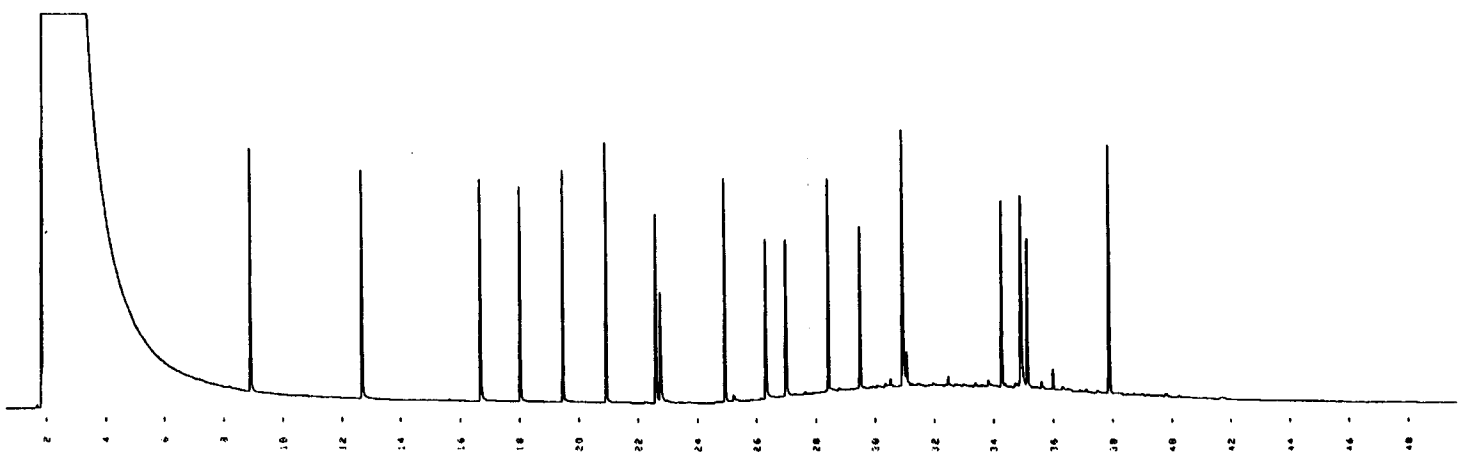


Figure 5.3 Gas chromatogram of C18 extract of a stormwater runoff sample spiked with standard hydroacrbons used in the matrix interference study.

concentration (as indicated by the peak height) standard hydrocarbons spiked into the runoff sample was at least 10 times greater than the concentration of hydrocarbons originally present in the sample. Therefore, the recovery calculated in the matrix interference study were of those standard hydrocarbons spiked into the C18 extract.

Toxicity Recovery Study

The results of the recovery studies which used standard hydrocarbons showed the feasibility of the proposed fractionation in separating oil and grease into four different groups. The toxicity recovery study was performed to determine the feasibility of this fractionation for the subsequent toxicity tests (not part of dissertation). Appendix C includes all the raw toxicity data generated from this study.

Phase I - Toxicity screening of standard hydrocarbons. Two standard hydrocarbons from each Si fractions were selected based on the availability of their water solubility data. Tables 5.7 shows the average percentage fertilization results of each standard from the toxicity screening test. Toxic effects were not observed for any hydrocarbon at the lowest nominal concentration tested, 10 μg/L. Toxic effects were observed for 2,6-dimethylnapthalene and pyrene at the nominal concentration of 100 μg/L, at which less than 60% of sea urchin eggs were fertilized (see Table 5.7).

Napthalene exhibited toxicity when its nominal concentration was increased to from 100 μg/L to 1000 μg/L; the percentage fertilization decreased from 87% to 61%. The percentage fertilization for phenanthrene decreased drastically to 0% as its nominal concentration was increased from 100 μg/L to 1000 μg/L. It is believed, however, that the apparent toxic effect of phenanthrene at this concentration was mainly caused by

isopropanol. In the control for this particular compound which contained 1% isopropanol, 31% fertilization was observed. Unknown artifactual toxicity was introduced by the use of 1% isopropanol in the aqueous sample containing 1000 µg/L (nominal concentration) of phenanthrene. In subsequent tests, only 0.5% or less isopropanol was introduced into toxicity analysis.

Table 5.7 Average percentage fertilization of each standard obtained from the Phase I of toxicity recovery study.

Si Fraction	Compound	Fraction concentration (v/v, %)	Nominal concentration (µg/L)	% fertilization Avg. ± SD
1	C16	0.1	100	91 ± 7
1	C20	0.1	100	92 ± 6
2	Napthalene	0.01	100	87 ± 6
		0.1	1000	61 ± 6
2	2,6-dimethylnapthalene	0.01	10	80 ± 11
		0.1	100	59 ± 6
3	Phenanthrene	0.1	100	89 ± 8
		1.0	1000*	0 ± 0
3	Pyrene	0.01	10	88 ± 3
		0.1	100	46 ± 10
4	Chrysene	0.1	100	92 ± 4
4	Benzo(a)pyrene	0.1	100	91 ± 6

*Treatment contained 1% isopropanol, all other treatments in experiment contained ≤ 0.1%. All samples except control were analyzed in duplicate.

The lack of toxic effects observed in both the chrysene and benzo(a)pyrene exposures may have been related to these compounds' low solubility in water. The water solubility of chrysene and benzo(a)pyrene are 1.8 µg/L and 0.172 µg/L, respectively

(Readman *et al.*, 1982). By introducing the compounds using a water miscible solvent, such as isopropanol, solubility limits will be temporary exceeded. The supersaturated compound will begin to precipitate (float or sink) or absorbed to the container wall. This process will occur over an unknown length of time. Therefore, it is suspected that actual concentration of these two compounds in the toxicity tests were much less than the nominal concentration of 100 μg/L and thus the fertilization of the sea urchin was not adversely affected.

Phase II - Toxicity of oil and grease fractions. Based on the results obtained from this study, C20, 2,6-dimethylnapthalene, phenanthrene and benzo(a)pyrene were selected for the subsequent toxicity tests. In order to increase the chance of observing toxic effects, the mass of phenanthrene used in this phase was twice as large as that used in the previous phase. Therefore, 30 μg of C20, 2,6-dimethylnapthalene and benzo(a)pyrene and 60 μg of phenanthrene in 0.3 ml hexane were used as standard samples in the fractionation procedure. The initial samples and all four Si fractions were solvent-exchanged to isopropanol prior to toxicity testing, and an aliquot of these samples was injected into the gas chromatograph to determine the actual concentrations of each standard in the samples. The concentrations of each standard in the aqueous samples for toxicity testing were not determined. Therefore, the discussion of the results are based on the nominal concentration of each standard and not their actual concentrations in the aqueous samples. Two fractionation tests were conducted in this second phase of the toxicity tests.

Table 5.8 shows the results of the sea urchin fertilization tests of the first fractionation test. In the standard mixture, the percentage fertilization of sea urchin decrease from 65% to 2% as the nominal concentration increased from 0.06% to 0.5%. This shows that the standard mixture was toxic before the fractionation. The next step was

to identify which hydrocarbon(s) caused the toxicity by examining the percentage fertilization with the Si fractions. The results in Table 5.8 show that moderate toxicity was present in the fraction (1) sample, where the percentage fertilization of sea urchin decreased from 88% at 0.06% concentration to 44% at 0.5% concentration. This result was unexpected as the toxicity results in the first phase study showed no toxic effect of the C20 compound. The gas chromatography results of fraction (1) [Table 5.9] showed that the actual concentration of C20 in the fraction (1) [in isopropanol] was 60,107 μg/L. In order to obtain a concentration of 0.5% in the aqueous sample used for toxicity tests, a 200x dilution of fraction (1) was made and the resulting nominal concentration was expected to be 301 μg/L. The nominal concentration of C20 tested in the first phase of the toxicity

Table 5.8 Percentage fertilization of sea urchin from the first fractionation test in Phase II of toxicity recovery study.

Sample	Concentration (%)	No. replicate	Avg % fertilization ± SD
Control		4	92 ± 10
Initial sample	0.06	3	65 ± 13
	0.12	3	14 ± 4
	0.25	3	1 ± 1
	0.50	3	2 ± 1
Fraction 1	0.06	3	88 ± 11
	0.12	3	81 ± 5
	0.25	2	65 ± 14
	0.50	2	44 ± 11
Fraction 2	0.50	3	93 ± 4
Fraction 3	0.25	3	91 ± 2
	0.50	3	81 ± 5
Fraction 4	0.25	3	90 ± 2
	0.50	2	89 ± 7

Table 5.9 Nominal concentration of samples from first fractionation test based on gas chromatography results in Phase II of toxicity recovery study.

Sample	Compound	Actual conc. in Si fraction (µg/L)*	Nominal conc. in aqueous sample (µg/L) **
Initial sample	C20	94,892	363
	2,6-dimethylnapthalene	148,549	474
	Phenanthrene	72,543	743
	Benzo(a)pyrene	78,167	391
Fraction 1	C20	60,107	301
Fraction 2	2,6-dimethylnapthalene	71,418	357
	Phenanthrene	22,244	111
Fraction 3	2,6-dimethylnapthalene	13,492	67
	Phenanthrene	105,489	527
	Benzo(a)pyrene	12,580	63
Fraction 4	Phenanthrene	7,298	36
	Benzo(a)pyrene	61,548	308

Note: * = actual concentration in the Si fraction as measured by GC; ** = expected nominal concentration of compounds after 200x dilution (i.e., at 0.5% concentration of toxicity tests).

recovery tests (see Table 5.7) was only 100 µg/L, one third of the nominal concentration found in the fraction (1). Therefore, it is possible that the moderate toxicity found in the first fraction of the silica gel column may be due to an increased C20 concentration, as compared to the Phase I study.

Table 5.8 also shows that no toxicity was detected in the other three Si fractions. This observation was unexpected as previous toxicity tests showed that 2,6-dimethylnapthalene exhibited toxic effects to the sea urchin (see Table 5.7) at a nominal concentration of 100 µg/L. The nominal concentration of 2,6-dimethylnapthalene in the aqueous sample of the fraction (2) was three times higher, i.e., 357 µg/L (Table 5.9).

Unlike benzo(a)pyrene, the nominal concentration 2,6-dimethylnapthalene did not exceed its solubility in water (~ 27,000 μg/L).

At a nominal concentration of 527 μg/L, phenanthrene in fraction (3) did not cause any toxicity to the sea urchin test. This shows that toxicity of the standard mixture was not caused by this compound. As expected, toxicity was also not observed in the fraction (4) samples. The gas chromatography results in Table 5.9 show that the nominal concentration of benzo(a)pyrene was 308 μg/L in the fraction (4) sample. It is suspected that a majority of benzo(a)pyrene was not dissolved to the water as the water solubility of this compound was only 0.172 μg/L. Therefore, the performance of sea urchin test for fraction (4) may be limited by the low water solubility of benzo(a)pyrene.

Based on the toxicity results of the first fractionation, it is found that only one Si fraction, i.e., fraction (1), of the standard mixture caused toxicity to the sea urchin. When comparing the percentage fertilization of the standard mixture with those measured in the fraction (1) samples, a majority of the toxicity of the standard mixture was not accounted for the percentage fertilization of fraction (1) at its highest nominal concentration (0.5%) was 44%. The percentage fertilization of standard mixture was only 2% at the same concentration. Therefore, a second fractionation of standard mixture was conducted in order to gain a better understanding of the toxicity results. Table 5.10 shows the toxicity results of the second fractionation tests.

The toxicity results of the initial standard mixture, fractions (1) and (2) in the second fractionation test, were consistent with the first test. Table 5.10 shows that toxicity was detected in both standard mixture and fraction (1) samples, where the fertilization of

Table 5.10 Percentage fertilization of sea urchin from the second fractionation test in Phase II of toxicity recovery study.

Sample	Concentration (%)	No. replicate	Avg % fertilization ± SD
Control		4	93 ± 6
Initial sample	0.06	2	77 ± 1
	0.12	3	49 ± 3
	0.25	3	7 ± 2
	0.50	3	1 ± 1
Fraction 1	0.06	n/a	n/a
	0.12	3	93 ± 4
	0.25	3	78 ± 17
	0.50	3	25 ± 5
Fraction 2	0.06	n/a	n/a
	0.12	n/a	n/a
	0.25	3	93 ± 1
	0.50	3	82 ± 17
Fraction 3	0.06	n/a	n/a
	0.12	3	92 ± 4
	0.25	3	86 ± 5
	0.50	3	43 ± 22
Fraction 4	0.06	n/a	n/a
	0.12	3	94 ± 5
	0.25	3	87 ± 3
	0.50	3	47 ± 20

Note: n/a = not measured

sea urchin was reduced as the concentration of samples increased from 0.06% to 0.5%. Similarly, fraction (2) samples did not cause any toxicity in the sea urchin test. The nominal concentrations of C20 and 2,6-dimethylnapthalene in fractions (1) and (2), as measured by gas chromatography, were similar to those samples of the first test (see Tables 5.9 and 5.11).

Unlike the first fractionation test, toxicity was detected in both fractions (3) and (4)

Table 5.11 Nominal concentration of samples from second fractionation test based on gas chromatography results in Phase II of toxicity recovery study.

Sample	Compound	Actual concentration in Si fraction (µg/L)*	Nominal concentration in aqueous sample (µg/L)**	% Different$^{\xi}$
Initial sample	C20	76,029	380	+ 5
	2,6-dimethylnapthalene	75,037	375	- 21
	Phenanthrene	161,529	808	+ 9
	Benzo(a)pyrene	64,548	323	- 17
Fraction 1	C20	70,863	354	+ 18
Fraction 2	C20	4,292	21	+100
	2,6-dimethylnapthalene	69,942	350	- 2
	Phenanthrene	29,032	145	+ 31
Fraction 3	2,6-dimethylnapthalene	13,061	65	- 3
	Phenanthrene	130,656	653	+ 24
	Benzo(a)pyrene	8,837	44	- 30
Fraction 4	Benzo(a)pyrene	53,148	266	- 14

Note: * = actual concentration in the Si fraction as measured by GC; ** = expected nominal concentration of compounds after 200x dilution (i.e., at 0.5% concentration of toxicity tests); $^{\xi}$ = % different of nominal concentrations as compared to those in Table 5.9 (+ = increase, - = decrease).

samples. Table 5.10 shows that both fractions (3) and (4) reduced the percentage fertilization of sea urchin to less than 40% as the concentration increased to 0.5%. The gas chromatographic analysis of these two fractions (Table 5.11) shows the expected nominal concentrations of phenanthrene and benzo(a)pyrene of 653 μg/L and 266 μg/L, respectively (at 0.5% concentration). The increased nominal concentration of phenanthrene in fraction (3) from 527 μg/L in the first test to 653 μg/L (~ 120 μg/L increase) may have contributed to the reduction of sea urchin fertilization in the second test. In the fraction (4) sample, the reduced percentage fertilization of sea urchin may be due to variability in the amount of benzo(a)pyrene dissolved in the seawater during the sample preparation procedure and due, for example, to variations in temperature. Thus the actual concentration of this compound in the aqueous sample might have been greater in the second test.

The toxicity results of the second fractionation test show only a partially consistent pattern of the recovery of toxicity, as compared to the standard mixture. A consistent toxicity pattern was observed with fractions (1) and (2) from both fractionation tests. Inconsistent toxicity pattern was observed with both fractions (3) and (4).

EC50 and Toxic Units

The percentage fertilization results obtained from both fractionation tests can be used to determine the EC50 and toxic units (TU) of the oil and grease. EC50 is the effective concentration that causes 50% toxic effect on the test organisms and this value can be generally obtained by interpolation from the dose-response plots (% fertilization of sea urchin vs. concentration of samples). The EC50 value can also be calculated by probit analysis. The lower EC50 value, the greater toxicity present in the sample. Figures 5.4 and 5.5 are the dose-response plots of the initial standard mixture, fractions (1), (3) and (4)

samples from both fractionation tests. Percentage fertilization at 0.01% concentration in Figures 5.4 and 5.5 is the percentage fertilization of the control samples (i.e., seawater).

Table 5.12 shows the EC50 results from the Phase II of toxicity recovery study. These values were calculated using the probit analysis provided by the EPA (Chapman *et al.*, 1995). In the second fractionation test, the EC50 values of fractions (1), (3) and (4) were 0.38%, 0.48% and 0.50%, respectively. The confidence interval at $\alpha = 0.05$ for the EC50 value of fraction (1) shows that fraction (1) is significantly more toxic than fractions (3) and (4) [Table 5.12]. However, the EC50 values of both fractions (3) and (4) are not significantly different (at $\alpha = 0.05$).

The toxic unit (TU) is another useful parameter for toxicity testing (EPA, 1989b and 1993). The toxic unit is calculated from the obtained EC50 values, i.e.,

$$\text{Toxic units (TU)} = \frac{100\%}{\text{EC50}} \qquad (5.3)$$

The calculated TU values for the initial standard mixture, fractions (1) - (4) are tabulated in Table 5.12. In general, TU values are used to track toxicity as the sample mixture is separated into different fractions. In an ideal case, the sum of TU values of fractions (1) to (4) should be equivalent to the TU value of the initial sample. The fraction that cause the toxicity can then be identified. However, this is not usually the case. If more than one toxicant is present, they may not be strictly additive in their toxicities, and when separated into different fractions the sum of the fraction toxicities will be low even if extraction of elution were 100%. A single toxicant may occur in more than one contiguous fraction, in which case a small amount of the toxicant in one fraction may not be detectable

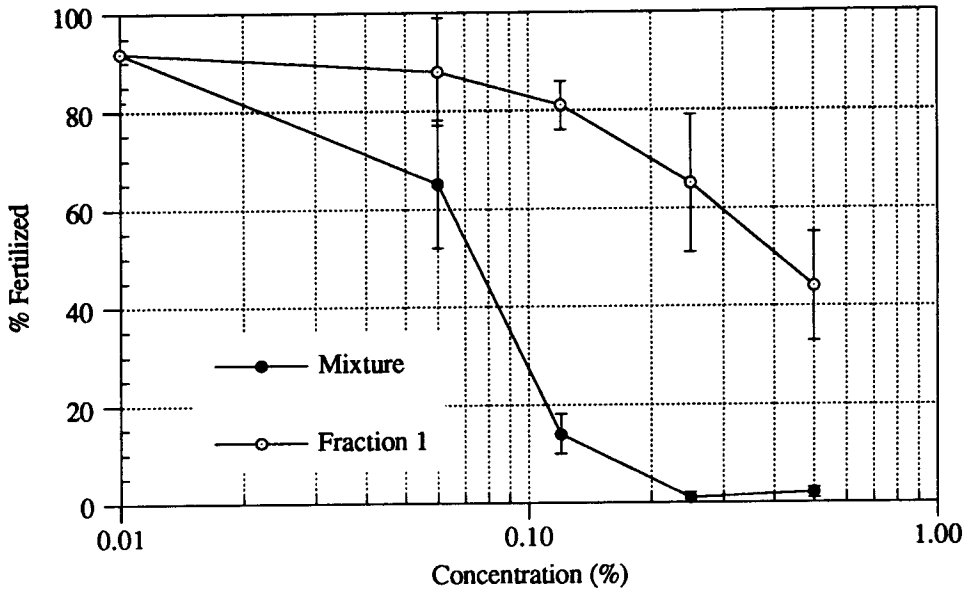


Figure 5.4 Dose-response plot of the initial standard mixture and fraction (1) from the first fractionation test in Phase II of toxicity recovery study. Control value is plotted at a concentration of 0.01%.

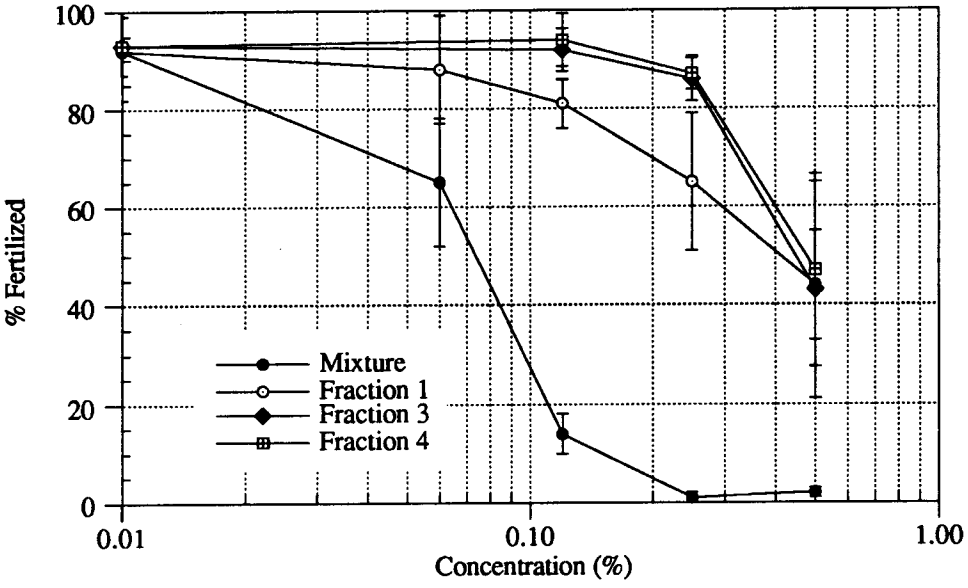


Figure 5.5 Dose-response plot of the initial standard mixture, fractions (1), (3) and (4) from the second fractionation test in Phase II of toxicity recovery study. Control value is plotted at a concentration of 0.01%.

Table 5.12 EC50 and TU values calculated from the Phase II of toxicity recovery studies.

Test	Sample	EC50 (%)*		Toxic units
		Estimated	CI (α = 0.05)	
1	Initial	0.08	0.07 - 0.09	1250
	Fraction 1	0.47	0.37 - 0.67	213
	Fraction 2	n/t	-	-
	Fraction 3	n/t	-	-
	Fraction 4	n/t	-	-
	Total$^{\xi}$			213
2	Initial	0.12	0.10 - 0.13	833
	Fraction 1	0.38	0.34 - 0.42	263
	Fraction 2	n/t	-	-
	Fraction 3	0.48	0.43 - 0.55	208
	Fraction 4	0.50	0.45 - 0.59	200
	Total$^{\xi}$			671

Note: n/t = no toxic effect was observed; - = not determined; * = estimated from the probit analysis; CI = confidence interval; $^{\xi}$ = total TU of fractions 1 - 4.

because it is present below the EC50 concentration (EPA, 1989a and 1993). Similar observations were made in the second fractionation test. The total TU of fractions (1), (3), (4) of the second fractionation test was 671, 162 less than the TU of the initial standard mixture. As observed from the GC analyses of Si fractions (Table 5.11), the nominal concentrations of C20 (~ 21 µg/L) and phenanthrene (~ 145 µg/L) in fraction (2) were present below the detectable EC50 concentration. Similar observation was made on benzo(a)pyrene in the fraction (3). These unmeasurable toxicities may have contributed to the combined TU values of fractions (1) to (4).

Since both fractionation tests showed that fraction (2) was not toxic to sea urchin, it is suspected that a synergistic effect occurs when the compounds from fractions (1) - (4) are mixed together, thus causing greater toxicity in the initial standard mixture. According to EPA (1989a and 1993), this problem can be solved by recombining the fractions (1) -

(4) and measuring toxicity to determine if the toxicity of the original mixture is recovered. Due to the small volume of each Si fraction collected from both fractionation tests, the toxicity of the combined fractions were not measured.

In the first fractionation test, only one fraction showed the presence of toxicity, and the remaining toxicity of the initial mixture was not recovered. Only one-sixth, as measured by toxic unit, was recovered. There are many possible effects and interferences that may have caused the anomalous results. Synergistic effects are possible but unknown. The availability of the sparingly soluble aromatics may have impacted the analysis. The combination of all compounds in the mixture may have increased the availability of the toxic compounds to the test organisms.

5.4 Conclusions

Oil and grease consist of different types of organic compounds which range from harmful hydrocarbons to non-toxic compounds such as animal and vegetable oils. Fractionation of oil and grease can help to better define the nature and potential environmental significance of oil and grease pollution by separating the unharmful and toxic compounds. A toxicity-based fractionation method for the oil and grease was developed in this study. The proposed fractionation utilizes the commercial pre-packed silica gel SPE column and an elution scheme using hexane and methylene chloride-hexane as elution solvents. Good separation of aliphatics (first fraction), 1- & 2-ring aromatics (second fraction), 3- & 4- ring aromatics (third fraction) and > 4-ring aromatics (fourth fraction) was obtained using the proposed fractionation procedure. More than 80% of hydrocarbons were recovered. The elution volume of each fraction is a critical parameter for the separation of hydrocarbons. A slight loss (< 10%) of semi-volatile compounds

such as sec-butylbenzene, napthalene and C16 (< 10%) was observed when oil and grease fractions were solvent-exchanged into isopropanol for the toxicity tests. The proposed toxicity-based fractionation of oil and grease was also not affected by the sample matrix.

The mass of organics to be separated, as well as the relative amount of each fraction, may impact the elution volume. A 600% increase of aliphatic mass require 10% additional hexane volume (first fraction). Doubling the aromatic mass did not affect the separation in the second, third and fourth fractions. The elution volumes may need to be tuned for specific situations.

The toxicity recovery study results showed consistent patterns in only two of the oil and grease fractions. Fraction (1), which consists of aliphatic hydrocarbons (C20 in the study), was toxic to the sea urchins in both tests. Similarly, no toxicity was detected in the fraction (2) [1- and 2-ring aromatics: 2,6-dimethylnapthalene] from both fractionation tests. Fractions (3) and (4) showed inconsistent toxicity patterns as opposite toxicity results were obtained in both tests. The source of variability for these two fractions requires further investigation. It is believed that the solubility of the aromatics eluted into the third and fourth fractions of oil and grease is critical to the toxicity tests. Extra care should be taken during the sample dilution of these fractions to dissolve the mass of hydrocarbons consistently in the seawater.

The EC50 values of the samples were also calculated, and ranged from 0.08% for the initial standard mixture to 0.5% for the fraction (4) sample. Based on the calculated TU values, most of the toxicity in the second fractionation test was accountable and found to be caused by fractions (1), (3) and (4). No such conclusion was possible for the first fractionation test. It is suspected that a synergistic effect may have caused the increased toxicity in the initial sample when the compounds were tested as a mixture. Additional research is needed to assess the availability of the toxic components to the test organisms as well as synergistic effects.

6.0 APPLICATION OF OIL SORBENTS IN OIL AND GREASE REMOVAL FROM STORMWATER RUNOFF

ABSTRACT

A bench-scale study of an oil sorbent system to remove oil and grease from urban runoff has been conducted. Three different types of studies were conducted using a mixture of used crankcase oil and water: batch adsorption isotherms, continuous flow micro-column and continuous flow adsorption/filtration tests using a field scale device. Adsorption capacity of each sorbent varied among tests. This may be due to the variation of each adsorption test and different filtration efficiencies. Greater than 50% removal of motor oil was obtained for all the sorbents in the micro-column adsorption and continuous flow experiments. The installation of an oil sorbent system in catch basins of storm drains is a feasible treatment method for removing oil and grease from stormwater runoff prior discharge into the storm drain system.

6.1 Introduction

Oil and grease are considered one of the major contaminants in the stormwater runoff. The sources of oil and grease in the runoff include vehicle exhaust, vehicle drippings, crankcase oil spillage at gas stations and illegal discharges.

Land-use had been found to be the most significant factor that affects oil and grease pollution in stormwater runoff (Stenstrom *et al.*, 1984). Runoff from commercial properties and parking lots contained oil and grease concentrations nearly 3 times higher than runoff from residential areas. In addition, the mass of oil and grease pollution per unit area from these types of land uses (i.e., commercial and parking lots) is typically more than 10 times greater than pollution from open land or residential areas. This results in part because commercial property and parking lots usually have higher runoff coefficients (e.g., runoff coefficients for commercial property and single-family residential areas are 0.70 - 0.95 and 0.3 - 0.50, respectively) (ASCE, 1960). Oil and grease from commercial property contains more anthropogenic compounds than oil and grease from residential areas (Fam *et al.*, 1987). Therefore, it is important to control the runoff of oil and grease from these properties prior discharge into the storm drain system.

A detailed review of various available treatment methods in removing oil and grease from urban runoff is provided by Stenstrom *et al.* (1982) and Silverman *et al.* (1986). The device proposed by Hannon (1980) to stop rainflow to a sanitary sewer can be modified with oil sorbent materials where it can be used to remove the oil and grease from the stormwater runoff. Oil sorbents are used extensively for oil spill clean-up, and typically can sorb several times their weight in oil. In these applications the sorbent is exposed to oil/water mixtures that are often comprised more of oil than water. Oil is trapped in the interstitial volumes and the material function as an absorbent. In the present application, the oil sorbent is exposed to stormwaters and wastewaters that contain only low oil and

grease concentrations, typically in the range of 10 - 50 mg/L. In this application, the removal mechanism is adsorption and filtration, where soluble oil and oil particles adhere to the surfaces of the fine fibers that make-up the sorbent. Therefore we use the terms adsorption and filtration throughout this paper to describe the removal process, and sorbent to describe the material since it can perform both adsorption and absorption.

Currently, limited information is available in the literature of the adsorption capacity of these oil sorbent materials in removing oil and grease from the runoff. Currently, Silverman *et al.* (1993) and Pitt *et al.* (1994) are conducting field studies of this treatment method. However, Pitt *et al.* (1994) analyzed the efficiency of several filter fabrics based on the efficiencies removal of particles as well as oil and grease. Silverman *et al.* (1993) conducted a field study where an oil sorbent material was placed in a catch basin of a parking lot. Detailed study of the adsorption behavior of these materials was not performed by these researchers.

It is the goal of this study to gain a better understanding of the behavior of several selected sorbents so that the feasibility of using these oil sorbents to remove oil and grease from the runoff can be determined. Three different types of studies were conducted in the laboratory: batch adsorption isotherms, continuous flow micro-column adsorption/filtration tests and continuous flow tests using a field scale device. Four different synthetic commercial products were evaluated.

6.2 Experimental Procedures

The following sections describe the analytical procedures and experimental setup of three different tests.

Oil Sorbents

Four sorbents were used, i.e., *Spill Tech* sorbent (*Spill Tech* Industries, Inc., Ontario, Canada), *Type 210 Oil Sorbent* (3M, Los Angeles, CA), *Alsorb® II* (Applied Fabric Tech Inc., Orchard Park, NY) and *Nanofiber* (Nanofiber Technology Inc., Southern Pines, NC). All materials are made from polypropylene. *Type 210 Oil Sorbent*, *Spill Tech* and *Nanofiber* are in particulate form, shredded pieces and loose layers, respectively. The *Alsorb® II* was supplied by the manufacturer in a roll of pads, and 2.5 cm width strip were cut to provide contact area. The synthetic sorbents were virtually 100% organics, as revealed by volatile suspended solids analysis.

SPE Columns

The 1000 mg octadecyl C18 columns (Mega Bond Elut™) were obtained from Varian (Harbor City, CA). The used motor oil was obtained from a filling station and was removed from a gasoline engine crankcase. A stock solution of motor oil-water solution was prepared by mixing a known amount of used motor oil with 100 ml deionized water in a wrist action shaker (Burrell Scientific, Pittsburgh, PA). Reagent grade methylene chloride, hexane and isopropanol from Fisher Scientific (Tustin, CA) were used for the SPE procedures.

Balance

A Sartorius Model 1712MP8 (Brinkman Instrument Co., Westbury, NY) analytical balance was used for the gravimetric analysis of the recoverable oil and grease.

Solid Phase Extraction Procedures

See the above Section 4.2 for the detailed C18 SPE procedures for the oil and grease analysis.

Batch Adsorption Isotherm Study

Batch adsorption studies were used to evaluate the adsorption capacity of motor oil on the four sorbents. In each adsorption test, 500 ml of motor oil-water sample and 7 different sorbent masses were placed into a series of bottles (Wheaton) and shaken for 24 hours. Figure 6.1 shows the schematic diagram of setup of the batch adsorption isotherm tests. After 24 hours of equilibration, the concentration of motor oil left in the water sample, i.e., the equilibrium concentration (C_e), was then determined using the C18 SPE procedure.

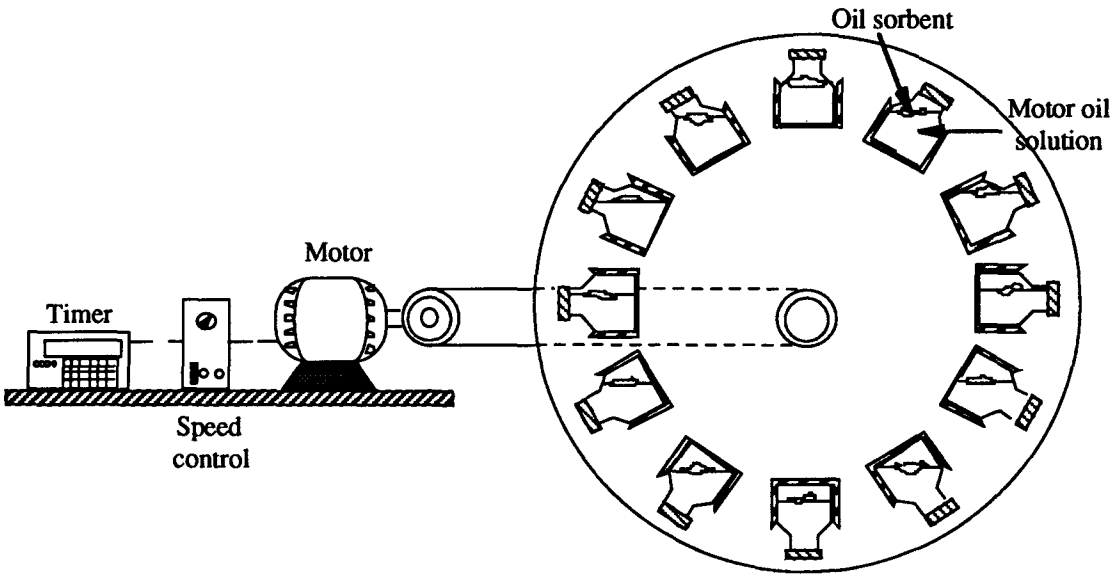


Figure 6.1 Schematic diagram of batch adsorption isotherm study.

Micro-Column Continuous Study

Micro-columns were used to study the maximum adsorption capacities of the sorbents (per unit of sorbent mass). Figure 6.2 shows the schematic diagram of the setup for the micro-column adsorption study. The sorbent (weighed 0.125g) was packed in an empty reservoir (Varian) and a series (8 - 12) of 500 ml of motor oil-water sample (with initial motor oil concentration ranging from 35 mg/L to 37 mg/L) were continuously pumped through the column under vacuum. The effluent water samples from the column were simultaneously collected and the concentration of motor oil was determined using the C18 SPE procedure. The superficial velocity through the column ranged from 16.47 cm/min to 31.43 cm/min. The velocity changed, reflecting the increased pressure drop across the column as it became saturated with oil and grease.

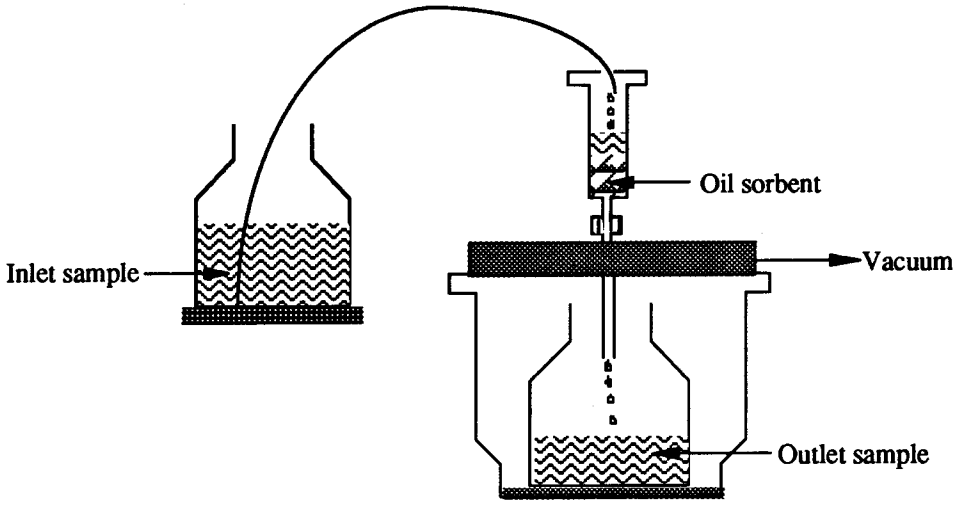


Figure 6.2 Schematic diagram of micro-column continuous flow study.

Continuous Flow Study

Figure 6.3 shows the schematic diagram of the setup for the continuous flow experiment. The weight of each sorbent placed in the basket was approximately 2.27 kg. The sorbents used in this continuous flow study were *Type 210 Oil Sorbent, Spill Tech* and *Alsorb® II* sorbents. *Alsorb® II* sorbent pads were cut into 2.5 cm width strips so that surface area if sorbent in contact with the flow through water-motor oil mixtures was maximized.

Tap water was pumped using a centrifugal pump (Dayton Electric Mfg. Co., Chicago, IL) at a flow rate of 11.4 L/min. Motor oil was introduced into the pump suction using a chemical metering pump (Cole-Parmer Instrument Co., Chicago, IL). The basket was 55 cm x 55 cm x 28.75 cm deep. A cover was constructed to simulate the traffic grate that normally covers the basket and distributed the stormwater. The cover was 56.5 cm x 62.5 cm and was perforated with 121 0.625 cm diameter holes on 5 cm x 5 cm centers. The motor oil-water mixture was initially pumped through the grate/sorbent for 8 hours, which was selected as an approximate storm duration period. Duplicate 500 ml size grab samples were collected at the inlet and outlet of the basket every hour. The samples were preserved with 1 ml of concentrated HCl, and stored at 4°C until analyzed using the developed C18 SPE procedures. After 8 hours of operation, the results were analyzed. The sorbents were only partially saturated, and the experiments were restarted for another 72 hours. In a similar fashion to the initial test, 500 ml grab samples were collected at the inlet and outlet of the basket at specific time intervals.

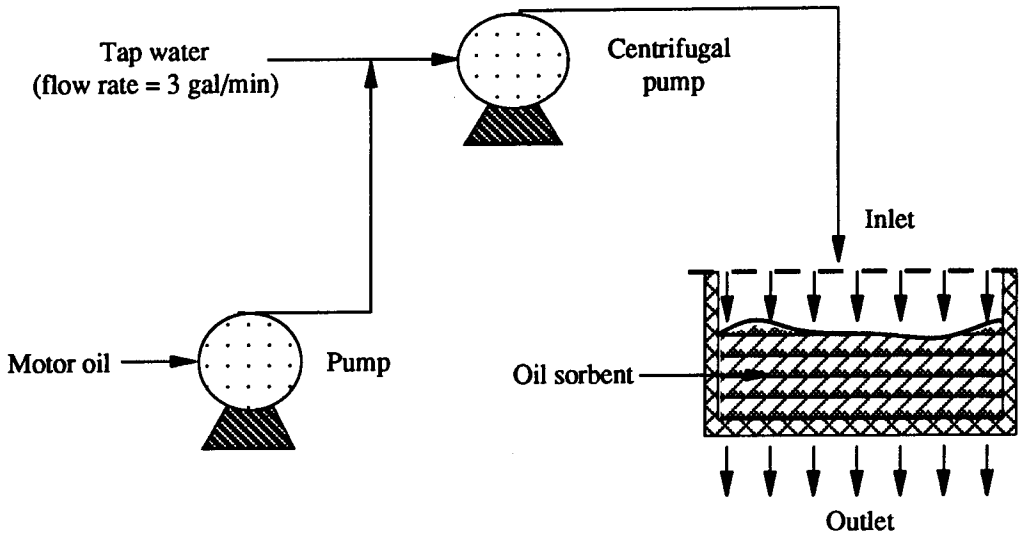


Figure 6.3 Schematic diagram of continuous flow study.

6.3 Results and Discussion

The following sections discuss the results obtained from each adsorption test where the percentage removal of motor oil and the adsorption capacity (as measured by the mass of motor oil sorbed per unit mass of sorbent) of each oil sorbent were determined. Finally a general comparison of the adsorption capacities of the selected sorbents were compared.

Batch Adsorption Isotherm Study

Preliminary adsorption isotherm tests showed that the contact efficiency between the sorbent and motor oil-water sample played an important role in determining the adsorption isotherm. Initial batch adsorption isotherm tests used a shaker that only provided horizontal shaking motion. We believe that only a limited section of the sorbent (usually the bottom layer of the sorbent) came in contact with the motor oil with this type of

shaking. Therefore, a Ferris wheel-type shaker (as shown in Figure 6.1) was constructed where the bottles were turned in a circular fashion instead of horizontally. With this circular motion, the contact between the sorbent and motor oil was increased and improved the adsorption efficiency.

Linear adsorption isotherm can be expressed as follows:

$$\frac{q}{m} = KC_e \tag{6.1}$$

where $\frac{q}{m}$ is the mass of motor oil adsorbed per unit sorbent mass and C_e is the equilibrium concentration. Linear adsorption isotherms were obtained by plotting $\frac{q}{m}$ versus the corresponding C_e. The mass of adsorbed motor oil was calculated from the known initial concentration of motor oil (C_i) in the water sample as follows:

$$\frac{q}{m} = \frac{\text{mass of motor oil adsorbed (mg)}}{\text{mass of sorbent (g)}} = \frac{(C_i - C_e) * V}{\text{mass of sorbent}} \tag{6.2}$$

where C_i and C_e are the initial and equilibrium concentrations of motor oil (mg/L), respectively, and V is the sample volume (L). Figure 6.4 shows the obtained adsorption isotherms for *Type 210 Oil Sorbent*, *Nanofiber*, *Alsorb® II* and *Spill Tech* sorbents, respectively. The adsorption isotherms were obtained based on a set of 2 to 4 replicate adsorption tests for each sorbent. The adsorption capacity (as indicated by the slope of the above linear adsorption isotherm equation, K) of *Type 210 Oil Sorbent* is greatest among the four tested sorbents, followed by *Nanofiber*, *Alsorb® II*, and *Spill Tech.*

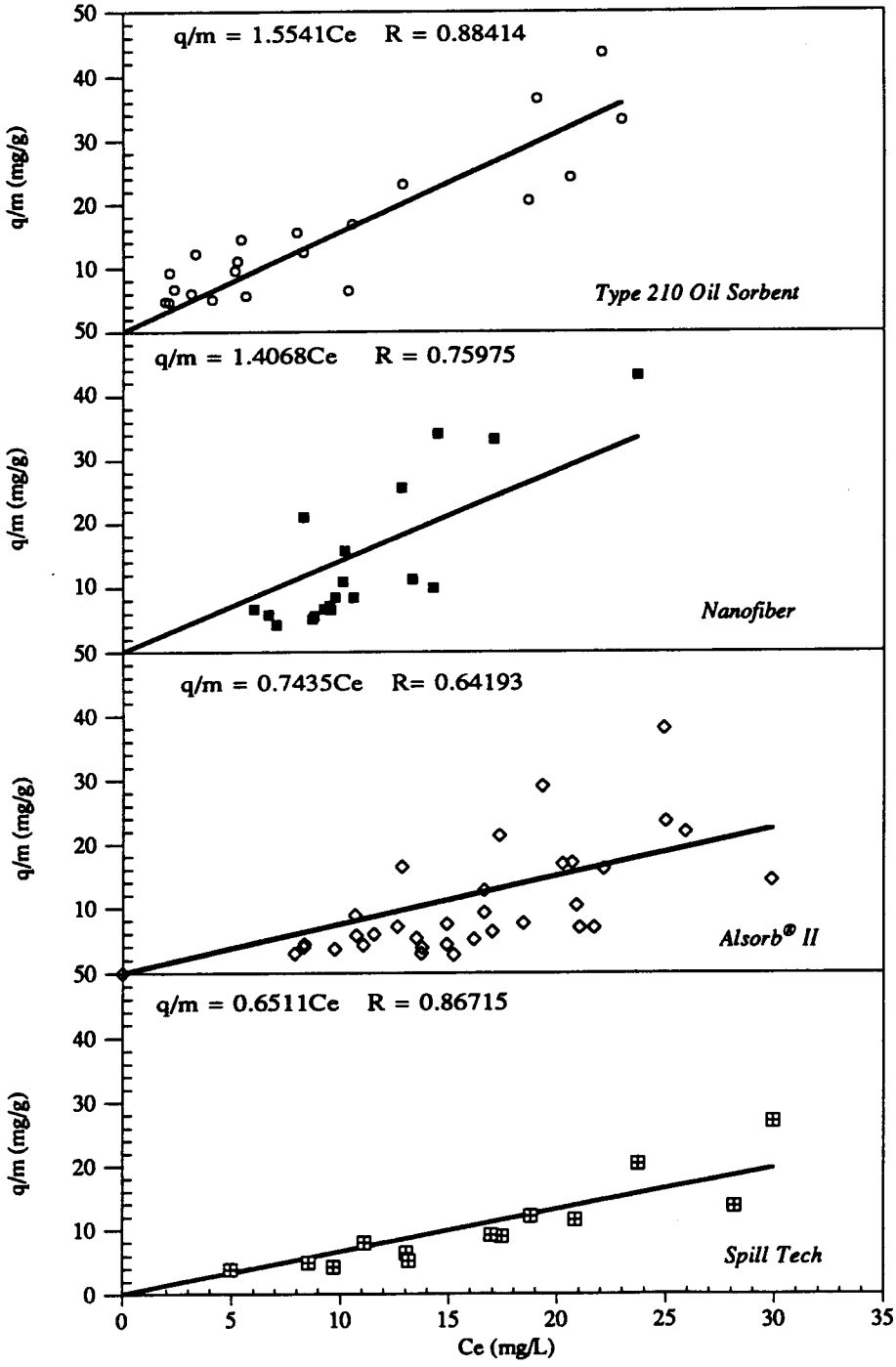


Figure 6.4 Linear adorption isotherm of *Type 210 Oil Sorbent*, *Nanofiber*, *Alsorb® II* and *Spill Tech.*

Micro-Column Continuous Flow Study

In this study, the percentage removal of motor oil was calculated from the known inlet concentration of motor oil and the analyzed motor oil concentration of the collected outlet effluent, as follows:

$$\% \text{ Removal} = \frac{(C_{in} - C_{out})}{C_{in}} \times 100 \qquad (6.3)$$

where C_{in} and C_{out} are the motor oil concentration at the inlet and outlet, respectively. With the measured C_{in} and C_{out} of each sample that passed through the sorbent, the mass of motor oil adsorbed (q) can be calculated using Equation (6.4), as follows:

$$q = (C_{in} - C_{out}) * V \qquad (6.4)$$

The V in Equation (6.4) represents the sample volume, i.e., 0.5 L in our case. Thus, the mass of motor oil removed per unit mass of sorbent ($\frac{q}{m}$) for the column study can be determined as follows:

$$\frac{q}{m} = \frac{\sum_{i=1}^{n} q_i}{\text{mass of sorbent}} \qquad (6.5)$$

where q_i is the mass of motor oil removed from the i-th bottle.

Figure 6.5 shows the plot of $\frac{q}{m}$ versus the total mass of motor oil passed through the sorbent for *Type 210 Oil Sorbent*, *Nanofiber*, *Spill Tech* and *Alsorb® II* sorbents.

From Figure 6.5, two distinctive trends are observed among the four sorbents. Both *Type 210 Oil Sorbent* and *Spill Tech* sorbents have similar adsorption trends where a saturation adsorption condition was observed after approximately 130 mg and 100 mg, respectively, of motor oil passed through the columns. However, no distinct saturation condition was observed for both *Alsorb® II* and *Nanofiber* sorbents. Under the column test conditions (e.g., 35 - 37 mg/L of initial motor oil concentration, and 0.125 g of sorbent mass), the maximum $\frac{q}{m}$ was found to be 660 mg/g, 629 mg/g, 502 mg/g and 457 mg/g for *Nanofiber, Type 210 Oil Sorbent, Alsorb® II* and *Spill Tech* sorbents, respectively.

Figure 6.6 shows the percentage removal of motor oil versus the total mass of motor oil passed through the sorbents. The obtained results show that *Nanofiber* sorbent

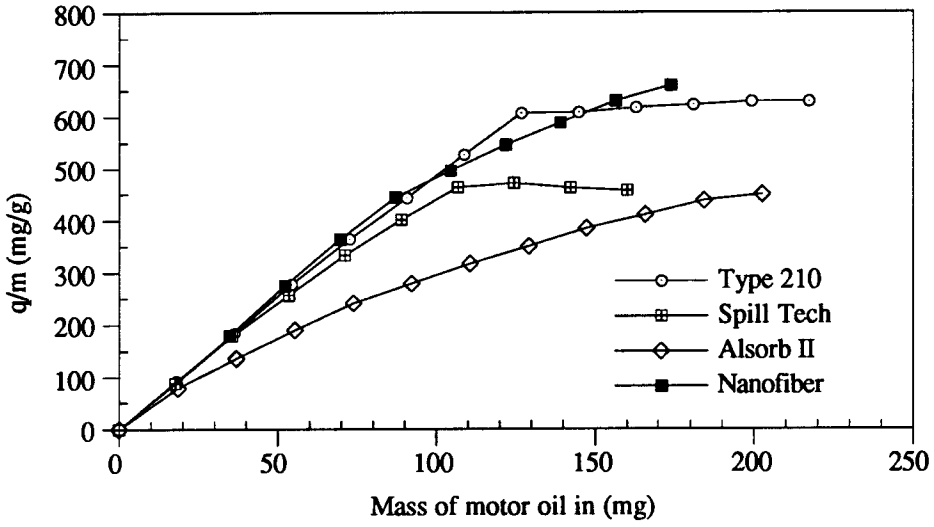


Figure 6.5 $\frac{q}{m}$ as a function of mass of motor oil from the micro-column continuous flow study.

has the greatest initial percentage removal of motor oil among the tested sorbents. The initial percentage removal is defined as the percentage removal of motor oil by the particular sorbent before partial or full saturation condition occurred within the sorbent system. The range of initial percentage removal of motor oil for *Nanofiber*, *Type 210 Oil Sorbent*, *Spill Tech* and *Alsorb® II* are found to be 58 - 67%, 54 - 65%, 44 - 64% and 31 - 50%, respectively. Comparison of the adsorption capacity of the *Spill Tech* and *Alsorb® II* sorbents based on the percentage removal gave slightly different results than those obtained based on the isotherm values. The isotherm results show that the adsorption capacity *Alsorb® II* is greater than *Spill Tech* sorbent whereas the initial percentage removal results showed otherwise.

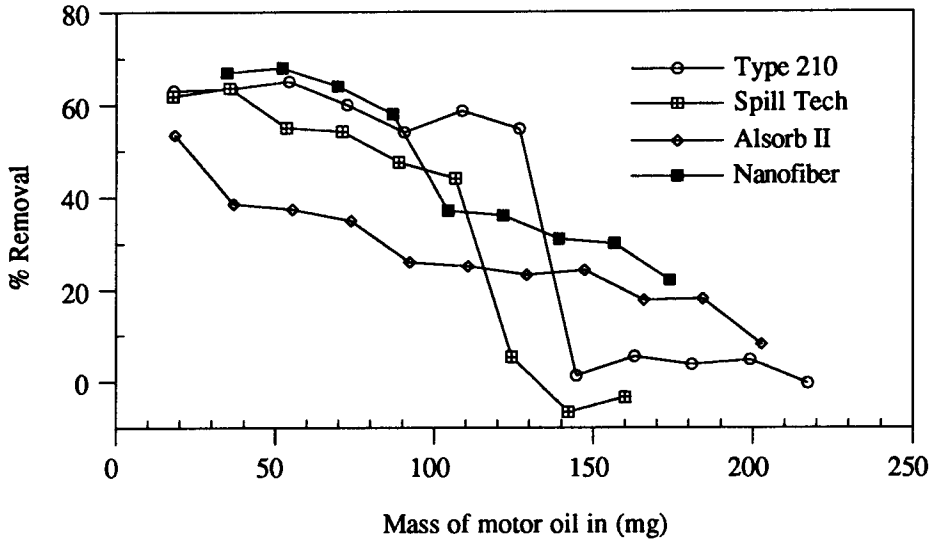


Figure 6.6 Percentage removal of motor oil as a function of mass from the micro-column study.

The adsorption capacity of the four selected sorbents measured in the micro-column is much higher than observed in the batch adsorption isotherm experiments. The batch adsorption isotherm shows that the adsorption capacity of *Type 210 Oil Sorbent* is greater than *Nanofiber*, whereas the $\frac{q}{m}$ results from the micro-column study shows otherwise. Both studies, however, shows that both *Nanofiber* and *Type 210 Oil Sorbent* have greater removal capacity than *Alsorb® II*, which is greater than *Spill Tech.*

Continuous Flow Study

Figure 6.7 shows the motor oil concentration at both inlet and outlet of the basket and the percentage removal of motor oil at various times for *Type 210 Oil Sorbent*, *Spill Tech* and *Alsorb® II* sorbents, respectively. The motor oil concentration at the inlet varied with time, because of the non-ideal mixing of motor oil with the tap water prior introduction to the sorbent system, and from deviations in tap water flow caused by power line fluctuations and tap water pressure changes. The average concentration of motor oil at the inlet and outlet and the average percentage removal of each sorbent over a duration of 82 hours are given in Table 6.1. Based on the obtained results, it was found that both *Alsorb® II* and *Spill Tech* sorbents have similar efficiencies in removing motor oil from the simulated stormwater. The average percentage removal of motor oil by Alsorb II and *Spill Tech* was 66% and 64%, respectively. The *Type 210 Oil Sorbent* only removed 55%. Table 6.1 also shows that the confidence interval at $\alpha = 0.10$ for the inlet, outlet and percentage removal for each sorbent. The inlet average concentrations are not significantly different among the three sorbents. However, the outlet average concentration and

percentage removal for the *Type 210 Oil Sorbent* were significantly worse than *Alsorb® II* and *Spill Tech* at $\alpha = 0.10$. Thus, *Alsorb® II* and *Spill Tech* are more efficient in removing the motor oil from the stormwater as compared to *Type 210 Oil Sorbent.*

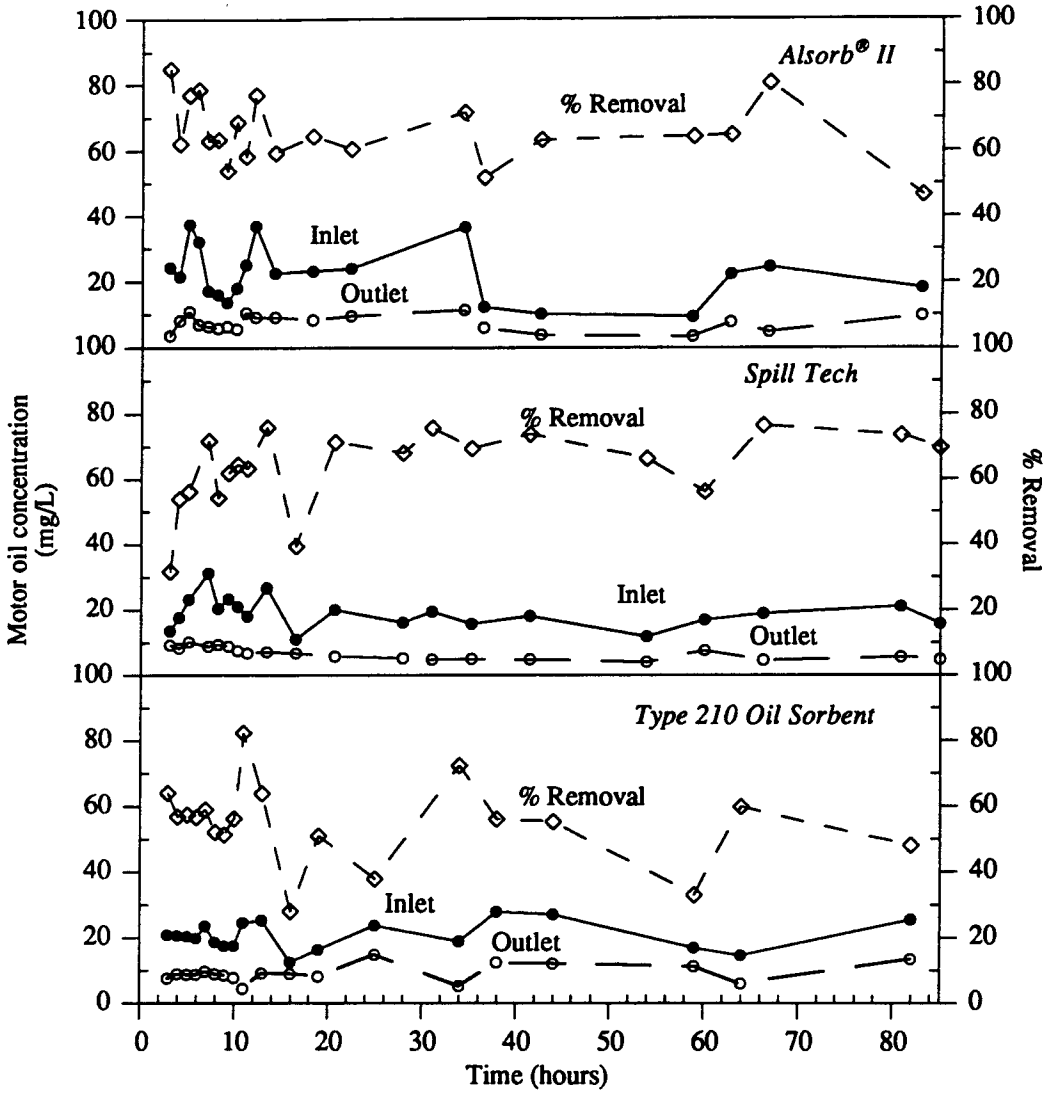


Figure 6.7 Percentage removal, inlet and outlet concentrations of motor oil from the continuous flow study.

Table 6.1 Results obtained from an 82-hours continuous flow experiment for *Alsorb® II*, *Spill Tech* and *Type 210 Oil Sorbent.*

Sorbent	Inlet (mg/L)		Outlet (mg/L)		% Removal	
	Avg ± SD	CI (α = 0.10)	Avg ± SD	CI (α = 0.10)	Avg ± SD	CI (α = 0.10)
Alsorb® II	22.17 ± 8.41	19.08 - 25.26	7.32 ± 2.45	6.42 - 8.22	66 ± 10	62 - 69
Spill Tech	18.92 ± 4.81	17.15 - 20.69	6.67 ± 1.92	5.96 - 7.38	64 ± 12	59 - 68
Type 210	20.63 ± 4.35	18.99 - 22.27	9.13 ± 2.68	8.15 - 10.12	55 ± 13	50 - 59

Note: Avg = average; SD = standard deviation; CI = confidence interval

Based on the obtained continuous flow results, the $\frac{q}{m}$ of sorbent at various times was calculated slightly differently than those in the micro-column study. The total mass of motor oil removed by the sorbent is described by the following equation:

$$\frac{q}{m} = \frac{\int_0^{82}(C_{in} - C_{out}) * Q\, dt}{\text{mass of sorbent}} \tag{6.6}$$

where Q is the flow rate of the tap water, C_{in} and C_{out} are the concentrations of motor oil at the inlet and outlet of the basket, respectively.

Since only discrete samples were collected, Equation (6.6) was modified as follows:

$$\frac{q}{m} \cong \frac{\sum_{i=1}^{n}(C_{in,i} - C_{out,i}) * V}{\text{mass of sorbent}} \tag{6.7}$$

The volume of water, V, was estimated from the measured flow rate, as follows:

$$V = \sum_{i=1}^{n} Q\,(\frac{L}{min}) * 60\,(\frac{min}{hour}) * [t_i - t_{i-1}](hour) \tag{6.8}$$

where t_i is the time of sample collection with t_0 being the start time. The obtained $\frac{q}{m}$ values for each sorbent [using Equation (6.8)] were then plotted against the time of sample collection as shown in Figure 6.8. A linear regression analysis was performed on the obtained plots and the results are shown in Table 6.2.

The slope of the linear equation represent the adsorption rate of each sorbent. The

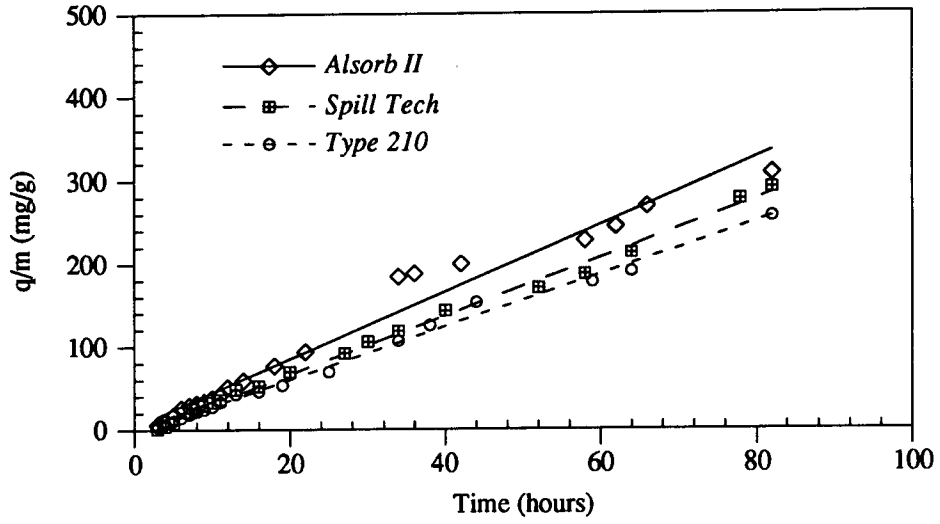


Figure 6.8 $\frac{q}{m}$ as a function of time from the continuous flow study.

Table 6.2 Linear regression analysis $\frac{q}{m}$ as a function of time from the continuous flow study.

Sorbent	Linear equation	Correlation coefficient
Alsorb® II	$\frac{q}{m} = 8.5427 + 3.2171\ t$	R = 0.9859
Spill Tech	$\frac{q}{m} = -4.2692 + 3.5252\ t$	R = 0.99802
Type 210 Oil Sorbent	$\frac{q}{m} = -3.4803 + 3.1737\ t$	R = 0.99718

results show that *Alsorb® II* has greater adsorption rate than both *Spill Tech* and *Type 210 Oil Sorbent*, which seems to agree with the above percentage removal results (Table 6.1). Similar to Figure 6.7, Figure 6.8 also shows that saturation of adsorption was not achieved

by each sorbent at the end of 82-hours of continuous input of motor oil through the sorbent system. The difference of the obtained results may be explained by comparing the maximum $\frac{q}{m}$ value of the sorbents. For example, the maximum value of the $\frac{q}{m}$ for *Type 210 Oil Sorbent* is approximately 629 mg/g and 252 mg/g in micro-column study and continuous flow study, respectively. Therefore, saturation was not observed in the continuous flow study of *Type 210 Oil Sorbent.* Table 6.3 shows the comparison of the maximum $\frac{q}{m}$ values obtained from the micro-column and continuous flow studies for *Type 210 Oil Sorbent, Spill Tech* and *Alsorb® II.*

Table 6.3 Comparison of the $\frac{q}{m}$ values of micro-column and continuous flow studies.

	$\frac{q}{m}$ ($\frac{mg}{g}$)	
Sorbent	Micro-column study	Continuous flow study
Type 210 Oil Sorbent	629	257
Spill Tech	457	293
Alsorb® II	502	309

The results obtained from the continuous flow study are different than those obtained in the batch adsorption isotherm and micro-column studies. Both batch adsorption and micro-column results show that *Type 210 Oil Sorbent* has greater adsorption capacity than both *Spill Tech* and *Alsorb® II* sorbents. The difference may be caused by uneven packing of sorbents in the basket thus causing an inefficiency of motor

oil removal by the sorbents. *Type 210 Oil Sorbent* used in this study is in the particulate form where numerous void space occurred within the sorbent itself. Therefore, when motor oil-water mixture passed through the sorbent, lack of contact between the motor oil and sorbent material caused low adsorption as observed in the continuous flow study of the *Type 210 Oil Sorbent.* Alternatively, *Spill Tech* and *Alsorb® II* sorbents used in the study were in shredded pieces and strip form, respectively. These two sorbents had less void space when packed into the basket. Therefore, greater adsorption capacity (as shown by the $\frac{q}{m}$ values) and percentage removal of motor oil were observed in both *Spill Tech* and *Alsorb® II* sorbents as compared with *Type 210 Oil Sorbent* in the continuous flow study.

In the micro-column study, however, the sorbent was packed tightly in the empty reservoir in order to minimize the void space within the sorbent. The contact opportunity between the sorbent and motor oil as the water sample passed through the sorbent are thus assumed to be equal among all the tested sorbents.

Comparison of Sorbents

The adsorption capacity of the four selected sorbents varies with the type of adsorption test, and the method used to interpret the results (e.g., percentage removal vs. $\frac{q}{m}$). Table 6.4 shows the ranking of adsorption capacity of each sorbent based on the results obtained from batch adsorption, micro-column and continuous flow studies.

Both batch adsorption isotherm and $\frac{q}{m}$ data of the micro-column study showed that *Alsorb® II* adsorbed greater amount of motor oil than *Spill Tech.* However, this observation seems to be otherwise when we compared the percentage removal of these two

sorbents where *Spill Tech* showed greater removal of motor oil than *Alsorb® II* sorbent. This contradiction of results also observed in the cases of *Type 210 Oil Sorbent* and *Nanofiber.*

Table 6.4 Comparison of adsorption capacity of sorbents based on different study (Rank: 1= most adsorbed and 4 = least adsorbed).

Sorbent	Batch	Micro-column		Continuous	
	slope[1]	$\frac{q}{m}$	Percentage removal[2]	$\frac{q}{m}$	Percentage removal[3]
Nanofiber	2	1	1	N/A[4]	N/A
Type 210	1	2	2	3	3
Alsorb® II	3	3	4	1	1
Spill Tech	4	4	3	2	2

Note: [1] slope of the linear adsorption isotherm equation.
[2] initial percentage removal, i.e., before saturation occurs.
[3] average percentage removal
[4] not available.

In the continuous flow study, *Alsorb® II* sorbent has greater average percentage removal and $\frac{q}{m}$ than *Spill Tech* and *Type 210 Oil Sorbent.* However, t-test analysis shows that there is no significant difference for the average percentage removal between *Alsorb® II* and *Spill Tech* based on the obtained confidence interval at $\alpha = 0.10$. *Type 210 Oil Sorbent* showed a significantly poorer average percentage removal than both *Alsorb® II* and *Spill Tech* at $\alpha = 0.10$.

6.4 Conclusions

Comparison of the removal capacity of each sorbents varies from one test to another. This may be due to the variation of each test, and the hydrophobicity of the motor oil in the water sample. However, the main objective of these tests was not to make comparison of the selected sorbents but is to determine the feasibility of using polypropylene oil sorbents to remove the oil and grease from the runoff water. From the results of the micro-column adsorption and continuous flow experiments where greater than 50% removal of motor oil was obtained for all the sorbents, we can conclude the installation of an oil sorbent system in the catch basin of the storm drain is a feasible treatment method in removing oil and grease from the storm water runoff prior discharge into the storm drain system. However, a pilot-scale demonstration of this oil sorbent system in a parking facility, for example, need to be performed so that a final design of the system can be made. The design parameters that need to be considered include the flow rate of the runoff, the total runoff area, the amount the rainfall, etc. The authors urge the readers not to make comparison using these results for gross oil sorption, as performed in oil clean-up. Other test procedures are available for the original use of these sorbents.

7.1 Conclusions

The main objective of this study is to develop a suitable toxicity identification evaluation (TIE) procedure that can collect and fractionate the total extractable organics in the stormwater runoff samples into different homogeneous groups to facilitate toxicity testing. Another main objective, only partially complete, was to develop a best management practices to reduce the toxicity of urban runoff.

A modified fractionation method using octadecyl (C18) solid phase extraction (SPE) columns was developed and used to fractionate the non-polar organic compounds in dry weather urban runoff samples. The C18 SPE column was found to be efficient in extracting the "dissolved" oil and grease in the urban runoff samples. However, the C18 column did not efficiently fractionate the hydrocarbons, especially those compounds which are highly hydrophobic. Similar results were observed by Durhan *et al.* (1993). An improved alternative elution scheme, proposed as part of dissertation, for fractionation non-polar organic compounds from C18 SPE columns consisted of methanol-water and methanol-methylene chloride mixtures.

The short-term chronic toxicity results from the dry weather urban runoff showed potential organic pollutants present in some of the SPE eluates. Other chemical manipulation of the samples (i.e., EDTA and sodium thiosulfate addition tests) also indicated the presence of pollutants such as oxidative compounds and cationic metals. Due to the limited number of samples collected during the dry weather season and problems with extraction procedures (C18 SPE/methanol), identification of the toxic components

present in the samples was not possible. Therefore, further work to identify the toxic components through gas chromatography/mass spectrometry is needed. More sampling is also needed to determine the variability of the toxicity. Toxicity was generally measurable in samples that contained more than 10% and less than 50% storm drain effluent. This suggests that a 10 fold dilution would reduce the toxicity below the detection limits used in this analysis.

An alternative analytical method using the commercially available C18 SPE columns was developed in this study for soluble oil and grease analysis. The proposed method has some advantages over the conventional liquid-liquid extraction (LLE) method. The method uses much less solvent and more reproducible results are obtained. Higher recovery of semi-volatile compounds was also obtained. The proposed C18 SPE method was also found to be comparable to other commercial SPE columns and disks.

An additional toxicity-based silica gel fractionation was also developed in which the C18 SPE extract can be further fractionated into a single aliphatic and three aromatic fractions. Good separation of hydrocarbons was observed from the recovery studies. In addition, it was not affected by the sample matrix, and the solvent-exchange procedure only slightly reduced the mass of semi-volatile compounds (< 10%).

Sea urchin fertilization tests were conducted on the oil and grease fractions of synthetic samples and a consistent toxicity pattern was observed in the first two fractions. Fraction (1) exhibited toxic effects to the sea urchin whereas no toxicity was detected in fraction (2). Fractions (3) and (4) did not show consistent toxicity results. The first test showed no toxicity in either fraction whereas as in the second test, toxicity was detected in both fractions. However, the proposed toxicity-based fractionation of oil and grease is still considered to be viable for identifying the toxic fraction(s) of oil and grease qualitatively. Additional research is needed to assess the availability of the toxic components to the test organisms as well as synergistic effects.

A bench-scale feasibility study of an oil sorbent system to remove oil and grease from the runoff samples was also performed. This bench-scale study involved three adsorption tests which used four commercial oil sorbents. No conclusive results can be made based on the obtained adsorption data. However, both micro-column adsorption and continuous flow experiments showed potential for removing oil and grease from runoff samples. The sorbents removed greater than 50% of the motor oil from spiked samples. A pilot-scale study of this oil sorbent system is needed to develop a prototype design which includes considerations such as flow rate and maintenance requirements.

7.2 Future Work and Recommendations

Based on the obtained results from the studies discussed in this dissertation (Chapters 3 - 6), further work is still needed in order to obtain a better understanding of the toxicity of urban runoff. The recommended works include the following:

I. Toxicity of dry weather flow

Due to the high variability in the toxicity results obtained from the dry weather study, additional samples and analyses are needed in order to determine and identify the toxic component(s). The developed toxicity-based fractionation procedure for oil and grease can be used to fractionate the extractable organics into aliphatic and aromatic fractions for subsequent toxicity testing.

II. Oil and grease analysis

Further studies are needed to determine whether the proposed C18 SPE method can also be used to analyze the oil and grease content in different types of samples such as industrial wastewaters from petroleum refineries and chemical plants.

III. Toxicity tests of oil and grease fractions

Additional toxicity tests are needed to determine the toxic fraction(s) of oil and grease. The proposed scheme can separate aliphatics and aromatics into several fractions. The methodology to determine the toxicity of these fractions, by introducing mixtures into seawater containing test organisms, needs further development. Of particular concern is the availability of the sparingly soluble organic compounds to the test organisms.

IV. Oil sorbent study

A pilot-scale study of the proposed oil sorbent system for oil and grease removal is needed in order to develop a prototype design which includes considerations such as flow rate and maintenance requirements. In addition, a toxicity study is also recommended in order to determine whether this oil sorbent system can also reduce the toxicity of urban runoff by reducing the oil and grease discharge to the storm drain.

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APPENDIX A
DEVELOPMENT OF A MODIFIED ELUTON SCHEME FOR C18 SPE TOXICITY-BASED FRACTIONATION

A.1 Introduction

The toxicity-directed method for fractionating non-polar organic toxicants using solid phase extraction (SPE) described in Phase II of EPA's *Methods for Aquatic Toxicity Identification Evaluations (TIE)* were used the dry weather flow study (Chapter 3). The proposed SPE method used octadecylsiloxane (C18) columns and an elution scheme with decreasing polarity. Prior to the extraction of actual storm drain samples, recovery studies using standard solutions of a combination of eight common polyaromatic hydrocarbons (PAHs) were conducted. Low recoveries of PAHs from the C18 SPE column were initially observed. The proposed methanol-water elution solvent system by the EPA did not elute highly non-polar PAHs (as indicated by their high log K_{ow} values), such as chrysene and benzo(a)pyrene. Therefore, a modified solvent scheme was developed so that compounds such as chrysene and benzo(a)pyrene could be fractionated for toxicity tests.

After completion of the development of the improved procedures, US EPA (Durhan *et al.*, 1993) published an improved procedure which was nearly identical to the procedure we developed. A short discussion of the alternative elution scheme developed in this study was presented by Lau and Stenstrom (1993).

A.2 Experimental Procedures

Materials

SPE column. The 500 mg and 1000 mg octadecyl C18 columns used were obtained from Burdick and Jackson (Muskegon, MI).

Polyaromatic hydrocarbons. The polyaromatic hydrocarbons (PAHs), i.e., naphthalene, 2-methylnapthalene, acenapthene, fluorene, anthracene, pyrene, chrysene, and benzo(a)pyrene, used in the recovery study were obtained from Aldrich (Milwaukee, WI). The PAHs mixture in methanol was spiked into one liter deionized water and used as working solution for the SPE procedure.

Solvents. HPLC grade methanol, methylene chloride, hexane, carbon tetrachloride and isopropanol from Fisher Scientific (Tustin, CA) were used for SPE.

Pump. A Masterflex® microprocessor pump equipped with cartridge pump drive (Cole-Parmer, IL) were used for the solid phase extraction.

SPE Procedures

The 1000 mg C18 SPE column was conditioned with 25 ml of methanol and 25 ml of deionized water. Before the sorbent dried, 1000 ml standard water solution containing PAHs was then pumped through the column at a rate of 5 ml/min. The sorbent was dried by continuing pumping for approximately 15 minutes after the whole 1000 ml sample passed through the column. Then 2 successive 1.0 ml volume of elution solvent(s) were added into the column. The concentration of PAHs were then analyzed using GC/FID. With the known concentration of each PAHs in the standard solution, the percentage recovery of each PAH was determined as follows:

$$\% \text{ Recovery} = \frac{[\text{Concentration x Volume}]_{C18}}{[\text{Concentration x Volume}]_{\text{initial}}} \times 100\% \qquad \text{(A.1)}$$

where C18 = C18 eluate, initial = initial sample.

Gas Chromatography Analysis

The PAHs were analyzed using a Varian Vista 6000 gas chromatography equipped with a splitless injector and flame ionization detector (FID). A 30 m x 0.25 mm i.d. DB5.625 capillary column (J&W Scientific) was used to analyze the PAHs in the C18 SPE eluates. The GC temperature program was 40°C for 2 min, 40°C - 140°C at 25°C/min, 140° - 290°C at 10°C/min, and 290°C for 20 min. The splitless injector and FID temperature were set at 275°C and 300°C, respectively.

The above procedure is a general description of C18 SPE procedures used in the recovery study. Changes on the elution volume and elution solvents were made when the effect of these parameter on the percentage recovery of PAHs were studied.

A.3 Results and Discussion

Methanol as the Elution Solvent

The first recovery study used only one fraction of 2 x 1.5 ml of methanol to elute the sorbed PAHs from a 500 mg C18 column. Low recoveries were obtained for most of the PAHs (Table A.1). Anthracene, acenapthene, fluorene, and 2-methylnapthalene were recovered most efficiently, greater than 40%. Both napthalene and benzo(a)pyrene have low recovery, less than 10%. It is suspected that low recoveries might be caused by

insufficient elution volume. Therefore, additional three successive 1.5 ml (3 x 1.5 ml) of methanol was used to elute the sorbed PAHs from the 500 mg and 1000 mg C18 (2nd recovery). The obtained results only showed slight improvement of the recoveries of PAHs (Table A.1). Therefore, it was suspected that maybe the methanol might be too polar to be able to elute the strongly hydrophobic PAHs. Low recovery of napthalene may be also due to it's loss through volatilization as napthalene is considered semi-volatile (H_c = 0.018).

Table A.1 Total percentage recovery of PAHs from the initial C18 SPE recovery studies.

	1st Recovery	2nd Recovery		3rd Recovery*
	500 mg C18	500 mg C18	1000 mg C18	1000 mg C18
Anthracene	58	67	70	103
Acenapthene	40	66	57	90
Fluorene	49	89	65	99
2-methylnapthalene	44	62	53	70
Pyrene	18	38	34	112
Chrysene	13	19	27	12
Benzo(a)pyrene	9	6	0	0
Napthalene	8	13	16	27

*Combination of methanol and methylene chloride eluates.

To determine the suitability of methanol as the elution solvent for PAHs, 200 µL of methylene chloride was added after the methanol. The results show that PAHs were only partially desorbed from the C18 sorbent by 100% methanol. Subsequent addition of methylene chloride helped to further elute some of the PAHs. The overall recovery (combination of methanol and methylene chloride eluates) of PAHs improved for most of

the PAHs (Table A.1). However, the percentage recovery of chrysene and benzo(a)pyrene was still not good.

The improvement of the percentage recovery of most PAHs helps to reconfirm the suspicions that methanol is an inappropriate elution solvent for PAHs. Therefore, the effect of other solvents, i.e., hexane, carbon tetrachloride, methylene chloride isopropanol, as the elution solvent for PAHs was studied. The results of this recovery study is shown in Table A.2 where the percentage recovery of most of the PAHs approached 100% in the hexane, carbon tetrachloride and methylene chloride eluates. The percentage recovery of benzo(a)pyrene has improved from about 20% to almost 50%. No improvement of the recovery was observed when isopropanol was used as the elution solvent.

Table A.2 Percentage recovery of PAHs using different solvents.

PAH compound	Total % recovery			
	n-Hexane	CCl_4*	$MeCl_2$**	Isopropanol
Napthalene	134	165	142	46
2-methylnapthalene	128	156	134	47
Acenapthene	146	161	139	56
Fluorene	149	165	142	68
Anthracene	123	150	134	53
Pyrene	138	160	129	98
Chrysene	116	85	55	47
Benzo(a)pyrene	49	58	42	19

Note: The excessive recovery (> 100%) of several PAHs were due to negligence in final volume measurement; * carbon tetrachloride; ** methylene chloride.

Even though strong non-polar solvents such as hexane, carbon tetrachloride and methylene chloride improved the recovery of the PAHs, these solvents are not desirable as the elution solvent as (1) they are not miscible in water, and (2) they are toxic to the marine

organisms used in the toxicity tests. A solvent exchange (into non-toxic solvent) procedure is usually required before they can be used in toxicity assays. Loss of volatile and semi-volatile compounds may occur during this process. Therefore, an alternative elution solvent system which meet the above two criteria is needed .

The tolerance of the marine organisms in the toxicity tests limited the choice of organic solvents. Preliminary tolerance tests showed that methanol-water, methanol, and methanol-methylene chloride were acceptable elution mixtures, although it was very desirable to limit the quantity of methylene chloride to less than 0.1% in the toxicity assay. Therefore, the effort was concentrate on the development of a modified elution system which used both methanol-water and methanol-methylene chloride mixtures.

Composition Methanol-Water And Methanol-Methylene Chloride

A total of six fractions were used to fractionate the PAHs from the C18 columns. Two different compositions of methanol-water and methanol-methylene chloride were studied, i.e., a 10% (except 5th and 6th fractions) and 25% gap between each fraction. The 10% gap in the first proposed elution solvent system consisted of 80% and 90% of methanol (v/v) in water, 100% methanol, 10%, 20% and 50% methylene chloride (v/v) in methanol. The second proposed solvent system, which has a 25% gap between each fraction, consisted of 50%, 75% of methanol (v/v) in water, 100% methanol, 25%, 50%, 75% of methylene chloride (v/v) in methanol. The results of the first and second proposed elution systems are shown in Tables A.3 and A.4.

Tables A.3 and A.4 show that more fractionation occurs in the 10% gap solvent system than the 25% gap system. It is observed that at least 3 distinct fractions could be collected in the 10% gap system (Table A.3). For example, both napthalene and 2-

Table A.3 Percentage recovery of PAHs using the 10% gap elution solvent system.

PAH compound	Percentage recovery						Total % recovery
	80% MeOH	90% MeOH	100% MeOH	10% $MeCl_2$	20% $MeCl_2$	50% $MeCl_2$	
Napthalene	5	93	0	0	0	0	98
2-methylnapthalene	0	47	0	0	0	0	47
Acenapthene	0	10	79	0	0	0	89
Fluorene	0	9	81	0	0	0	90
Anthracene	0	0	97	0	0	0	97
Pyrene	0	0	74	14	0	0	88
Chrysene	0	0	24	53	5	13	95
Benzo(a)pyrene	0	0	0	63	23	0	86

Note: MeOH = methanol; $MeCl_2$ = methylene chloride; elution volume = 2 x 1.0 ml

Table A.4 Percentage recovery of PAHs using the 25% gap elution solvent system.

PAH compound	Percentage recovery						Total % recovery
	50% MeOH	75% MeOH	100% MeOH	25% $MeCl_2$	50% $MeCl_2$	75% $MeCl_2$	
Napthalene	0	0	84	33	0	0	117
2-methylnapthalene	0	0	84	5	0	0	89
Acenapthene	0	0	73	19	0	0	92
Fluorene	0	9	76	16	0	0	101
Anthracene	0	0	53	35	0	0	88
Pyrene	0	0	13	74	0	0	87
Chrysene	0	0	0	124	0	0	124
Benzo(a)pyrene	0	0	0	98	18	0	116

Note: Elution volume = 2 x 1.0 ml

methylnapthalene were fractionated into the 2nd fraction (i.e., 90% methanol), while most of the acenapthene, fluorene, anthracene and pyrene were found in the 3rd fraction (i.e., 100% methanol). Both chrysene and benzo(a)pyrene were fractionated into the 4th fraction (i.e., 10% methylene chloride).

For the 25% gap elution solvent system, less fractionation of PAHs was observed (Table A.4). There were only two distinct fractions collected in this system. Most of the napthalene, 2-methylnapthalene, acenapthene, fluorene, and anthracene were fractionated in the 3rd fraction (i.e., 100% methanol). Pyrene, chrysene and benzo(a)pyrene were found in the 4th fraction (i.e., 25% methylene chloride). There was no or insignificant PAHs found in the first and last two fractions.

Tables A.3 and A.4 also show that the overall percentage recovery of PAHs were greater in the 25% gap solvent system. However, as better fractionation of PAHs was obtained in the 10% gap system, it was decided that to use this proposed composition of methanol-water and methanol-methylene chloride mixtures for the PAHs elution.

Elution Volume

The fractionation of PAHs caused by the volume of the elution solvent used was also studied. Two different elution volumes were compared, i.e., 2 x 1.0 ml and 2 x 1.5 ml of 80% and 90% of methanol (v/v) in water, 100% methanol, 10%, 20%, and 50% of methylene chloride (v/v) in methanol. The results of the percentage recovery of PAHs using 2 x 1.0 ml and 2 x 1.5 ml of elution volume are shown in Tables A.3 and A.5, respectively. The total percentage recovery of both volumes each PAHs are quite similar, except for 2-methylnapthalene in which a total of 76% was recovered when 2 x 1.5 ml elution volume was used and only 46% was recovered when 2 x 1.0 ml of elution volume was used.

Table A.5 Percentage recovery of PAHs using the elution volume of 2 x 1.5 ml.

PAH compound	Percentage recovery						Total % recovery
	80% MeOH	90% MeOH	100% MeOH	10% $MeCl_2$	20% $MeCl_2$	50% $MeCl_2$	
Napthalene	5	83	0	0	0	0	88
2-methylnapthalene	0	76	0	0	0	0	76
Acenapthene	0	61	19	0	0	0	80
Fluorene	0	63	17	0	0	0	80
Anthracene	0	33	55	0	0	0	88
Pyrene	0	0	104	0	0	0	104
Chrysene	0	0	85	11	0	3	99
Benzo(a)pyrene	0	0	16	78	0	0	94

Table A.3 shows that for the 2 x 1.0 ml elution volume, greater recovery was observed in 100% methanol fraction than 90% methanol. For the 2 x 1.5 ml elution volume (Table A.5), the opposite was observed. Few PAHs were recovered in the 80% methanol and 50% methylene fractions (for both 2 x 1.0 ml and 2 x 1.5 ml cases). No conclusion can be made here regarding which volume is better, except the volume of solvent and solvent make up can interact to affect recovery.

Based on the results of the above mentioned recoveries, it was decided to use the following elution scheme for the fractionation of PAHs from the C18 columns: 2 x 1.0 ml of 80% and 90% of methanol (v/v) in water, 100% methanol, 10%, 20% and 50% of methylene chloride (v/v) in methanol. The following section discussed the repeatability of this modified system based on a total eight similar extractions.

Repeatability of the Modified Elution Solvent System

A total of eight extractions were conducted using the modified methanol-water and methanol-methylene chloride system so that the variability of this SPE procedures can be determined. The extraction procedures of these eight extractions were identical except for the concentration of PAHs. For each extraction, the concentration of all PAHs, except benzo(a)pyrene were equal; the range of concentrations of each PAH (in water solution) was varied from 10 µg/L to 40 µg/L. The concentration of benzo(a)pyrene ranged from 20 µg/L to 80 µg/L. The average percentage and standard deviation of each PAH recovery in each SPE fraction which was obtained from these eight extractions are shown in Table A.6. Repeatability of the extraction procedures, as measured by the standard deviation of the recovery, was generally within 5% for fluorene as the most repeatable, and 21% for 2-methylnapthalene as the least repeatable.

Table A.6 Average percentage recovery and standard deviation of each PAH from eight extractions using the modified elution scheme.

PAH compound	Log K_{ow}	Percentage recovery ± standard deviation					
		80% MeOH	90% MeOH	100% MeOH	10% $MeCl_2$	20% $MeCl_2$	50% $MeCl_2$
Napthalene	3.54	0	86 ± 11	3 ± 4	0	0	0
2-methylnapthalene	-	0	37 ± 10	43 ± 21	0	0	0
Acenapthene	-	0	7 ± 6	77 ± 14	0	0	0
Fluorene	4.12	0	9 ± 9	82 ± 6	0	0	0
Anthracene	4.45	0	0.5 ± 1	84 ± 12	0.5 ± 1	0	0
Pyrene	4.88	0	0	62 ± 9	22 ± 9	0	0
Chrysene	5.61	0	0	15 ± 17	53 ± 11	3 ± 7	2 ± 5
Benzo(a)pyrene	6.04	0	0	0	57 ± 14	23 ± 13	0

From Table A.6 it is observed that the 80% methanol-water fraction (1st fraction) eluted no PAHs. Most of the napthalene was recovered in the 90% methanol fraction (2nd fraction). Anthracene, fluorene and acenapthene were eluted almost entirely in the 100% methanol fraction (3rd fraction). 2-methylnapthalene, pyrene, chrysene and benzo(a)pyrene were not well separated. Table A.6 also shows that elution with methylene chloride (4th and 5th fractions) is required to recover those PAHs with high log K_{ow} (such as chrysene and benzo(a)pyrene). Most were recovered with a maximum of 20% methylene chloride.

APPENDIX B
RAW DATA OF TOXICITY TESTS FOR DRY WEATHER FLOW STUDY

Table B-1 Summary of purple sea urchin fertilization for samples 8/24/92; conducted 8/26/92. Abbreviations: % Ref = mean response expressed as a percentage of the appropriate reference group(s); NS = not statistically significant difference relative to reference; S = statistically significant difference; NT = not tested (no need or data not sufficient).

Group		Reference group	% Fertilized				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		89	8			98, 83, 87
2	Brine control 18%	1	86	4	NS	97	85, 80, 89, 90
3	Brine control 32%	1	81	5	NS	90	75, 78, 86, 84
4	Brine control 56%	1	42	5	S	47	42, 45, 35, 47
5	Pico-Kenter filtrate 5.6%	1-3	88	3	NS	104	91, 88, 86
6	Pico-Kenter filtrate 10%	1-3	88	6	NS	104	82, 90, 93
7	Pico-Kenter filtrate 18%	1-3	88	5	NS	104	88, 92, 93
8	Pico-Kenter filtrate 32%	1-3	84	2	NS	99	84, 82, 86
9	Pico-Kenter filtrate 56%	4	71	1	NT	170	71, 71, 72
10	Pico-Kenter 10% (unfilt.)	1-3	92		NT	108	92
11	Pico-Kenter 18% (unfilt.)	1-3	79		NT	93	79
12	Pico-Kenter 32% (unfilt.)	1-3	82		NT	97	82
13	Ashland filtrate 5.6%	1-3	88	6	NS	104	95, 83, 87
14	Ashland filtrate 10%	1-3	82	4	NS	97	80, 80, 87
15	Ashland filtrate 18%	1-3	38	11	S	45	28, 37, 50
16	Ashland filtrate 32%	1-3	0	0	S	0	0, 0, 0
17	Ashland filtrate 56%	4	0	0	NT	0	0, 0, 1
18	Ashland 10% (unfilt.)	1-3	1		NT	1	1
19	Ashland 18% (unfilt.)	1-3	0		NT	0	0
20	Egg control (Seawater)	1-3	0		NT	0	0
21	Egg control (brine 32%)	1-3	0		NT	0	0
22	Egg control (Pico 5.6%)	1-3	0		NT	0	0
23	Egg control (Pico 18%)	1-3	0		NT	0	0
24	Egg control (Pico 56%)	4	0		NT	0	0
25	Egg control (Ashland 5.6%)	1-3	0		NT	0	0
26	Egg control (Ashland 18%)	1-3	0		NT	0	0
27	Egg control (Ahland 56%)	4	0		NT	0	0

Table B-2 Summary of purple sea urchin fertilization test for samples 9/8/92; conducted 9/9/92.

Group	Sample	Reference group	% Fertilized				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		98	1			99, 98, 97
2	Brine control 18%	1	96	2	NS	98	84, 94, 96, 99
3	Brine control 32%	1	93	6	NS	95	96, 94, 86, 98
4	Brine control 56%	1	73	6	S	75	70, 72, 70. 82
5	Ballona filtrate 5.6%	1-3	82	6	S	86	83, 88, 76
6	Ballona filtrate 10%	1-3	77	0	S	80	76, 77, 76
7	Ballona filtrate 18%	1-3	28	5	S	29	25, 33, 25
8	Ballona filtrate 32%	1-3	13	10	S	14	6, 9, 24
9	Ballona filtrate 56%	4	2	2	NT	2	1, 0, 4
10	Ballona 10% (unfilt.)	1-3	96		NT	101	96
11	Ballona 18% (unfilt.)	1-3	38		NT	40	38
12	Ballona 32% (unfilt.)	1-3	58		NT	61	58
13	Sepulveda filtrate 5.6^	1-3	90	4	NS	94	94, 86, 87
14	Sepulveda filtrate 10%	1-3	95	3	NS	100	92, 97, 96
15	Sepulveda filtrate 18%	1-3	42	15	S	43	59, 32. 34
16	Sepulveda filtrate 32%	1-3	63	7	S	66	59, 72, 58
17	Sepulveda filtrate 56%	4	19	8	NT	26	10, 26, 21
18	Sepulveda 10% (unfilt.)	1-3	64		NT	65	64
19	Sepulveda 18% (unfilt.)	1-3	40		NT	41	40
20	Sepulveda 32% (unfilt.)	1-3	85		NT	87	85
21	Egg control (seawater)	1-3	0		NT	0	0
22	Egg control (brine 18%)	1-3	0		NT	0	0
23	Egg control (Ballona 18%)	1-3	0		NT	0	0
24	Egg control (Ballona 56%)	4	0		NT	0	0
25	Egg control (Sepul. 18%)	1-3	0		NT	0	0
26	Egg control (Sepul. 56%)	4	0		NT	0	0

Table B-3 Summary of 48 hour red abalone larval development test for samples 8/24/92; conducted 8/26/92.

Group	Sample	Reference group	% Normal development				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		93	6			97, 89, 10*
2	Brine control 18%	1	83	6	NS	98	91, 82, 79
3	Brine control 32%	1	84	6	NS	99	86, 89, 76
4	Brine control 56%	1	85	8	NS	99	75, 89, 90
5	Pico-Kenter filtrate 5.6%	1-4	85	9	NS	99	93, 75, 86
6	Pico-Kenter filtrate 10%	1-4	84	5	NS	98	79, 83, 86
7	Pico-Kenter filtrate 18%	1-4	91	5	NS	106	92, 94, 85
8	Pico-Kenter filtrate 32%	1-4	73	9	S	86	72, 65, 82
9	Pico-Kenter filtrate 56%	1-4	13	8	S	15	13, 5, 21
10	Pico-Kenter 10% (unfilt.)	1-4	79		NT	93	79
11	Pico-Kenter 18% (unfilt.)	1-4	77		NT	90	77
12	Pico-Kenter 32% (unfilt.)	1-4	29		NT	34	29
13	Ashland filtrate 5.6%	1-4	69	2	S	81	71, 68, 68
14	Ashland filtrate 10%	1-4	3	2	S	4	2, 6, 2
15	Ashland filtrate 18%	1-4	0	0	NT	0	0, 0, 0
16	Ashland filtrate 32%	1-4	0	0	NT	0	0, 0, 0
17	Ashland filtrate 56%	1-4	0	0	NT	0	0, 1, 0
18	Ashland 10% (unfilt.)	1-4	0		NT	0	0
19	Ashland 18% (unfilt.)	1-4	0		NT	0	0
20	Ashland 32% (unfilt.)	1-4	0		NT	0	0

* Outlier value was not included in statistical calculations.

Table B-4 Summary of 48 hour red abalone larval development test for 9/8/92 samples; conducted 9/9/92.

Group	Sample	Reference group	% Normal development				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		97	1			97, 96, 98
2	Brine control 18%	1	98	1	NS	101	98, 99, 97, 98
3	Brine control 32%	1	96	0	NS	99	96, 96, 95, 96
4	Brine control 56%	1	97	2	NS	100	97, 96, 95, 100
5	Ballona filtrate 5.6%	1-4	96	4	NT	99	99, 96, 92
6	Ballona filtrate 10%	1-4	98	1	NT	101	98, 97, 98
7	Ballona filtrate 18%	1-4	97	1	NT	100	98, 97, 97
8	Ballona filtrate 32%	1-4	97	1	NT	100	96, 98, 97
9	Ballona filtrate 56%	1-4	96	2	NT	99	97, 94, 97
10	Ballona 10% (unfilt.)	1-4	96		NT	99	96
11	Ballona 18% (unfilt.)	1-4	99		NT	102	99
12	Ballona 32% (unfilt.)	1-4	98		NT	101	98
13	Sepulveda filtrate 5.6^	1-4	97	1	NT	100	96, 97, 97
14	Sepulveda filtrate 10%	1-4	96	1	NT	99	95, 96, 98
15	Sepulveda filtrate 18%	1-4	97	2	NT	100	95, 98, 97
16	Sepulveda filtrate 32%	1-4	97	2	NT	100	97, 95, 98
17	Sepulveda filtrate 56%	1-4	98	1	NT	101	98, 99, 98
18	Sepulveda 10% (unfilt.)	1-4	99		NT	102	99
19	Sepulveda 18% (unfilt.)	1-4	94		NT	97	94
20	Sepulveda 32% (unfilt.)	1-4	96		NT	99	96

Table B-5 Summary of kelp spore germination endpoint for samples 8/24/92; conducted 8/26/92.

Group	Sample	Reference group	% Germinated				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		86	1			88, 85, 86
2	Brine control 18%	1	86	4	NT	100	86, 90, 88, 81
3	Brine control 32%	1	92	4	NT	106	94, 94, 93, 86
4	Brine control 56%	1	93	2	NT	108	92, 95, 93
5	Pico-Kenter filtrate 5.6%	1-4	90	5	NT	101	85, 95, 90
6	Pico-Kenter filtrate 10%	1-4	89	4	NT	100	87, 87, 93
7	Pico-Kenter filtrate 18%	1-4	91	2	NT	101	92, 91, 89
8	Pico-Kenter filtrate 32%	1-4	94	4	NT	105	94, 89, 97
9	Pico-Kenter filtrate 56%	1-4	90	6	NT	101	94, 84, 93
10	Pico-Kenter 10% (unfilt.)	1-4	91		NT	102	91
11	Pico-Kenter 18% (unfilt.)	1-4	85		NT	95	85
12	Pico-Kenter 32% (unfilt.)	1-4	76		NT	85	76
13	Ashland filtrate 5.6%	1-4	93	2	NS	104	91, 94, 93
14	Ashland filtrate 10%	1-4	91	4	NS	102	95, 89, 89
15	Ashland filtrate 18%	1-4	85	1	NS	95	85, 84, 86
16	Ashland filtrate 32%	1-4	48	6	S	53	49, 41, 52
17	Ashland filtrate 56%	1-4	3	2	S	3	4, 4, 0
18	Ashland 10% (unfilt.)	1-4	ND*				
19	Ashland 18% (unfilt.)	1-4	ND*				

* Slide was unreadable due to particulates in sample.

Table B-6 Summary of kelp spore germ tube length endpoint for samples 8/24/92; conducted 8/26/92.

Group	Sample	Reference group	Germ tube length (µm) Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		15	2			13, 17, 16
2	Brine control 18%	1	12	0	S	80	12, 12, 13, 12
3	Brine control 32%	1	13	1	NS	87	12, 14, 14, 12
4	Brine control 56%	1	14	2	NS	93	13, 12, 16
5	Pico-Kenter filtrate 5.6%	1,3,4	15	1	NT	107	14, 16, 14
6	Pico-Kenter filtrate 10%	1,3,4	14	1	NT	100	15, 13, 14
7	Pico-Kenter filtrate 18%	2	15	3	NT	125	17, 12, 15
8	Pico-Kenter filtrate 32%	1,3,4	13	2	NT	93	15, 13, 11
9	Pico-Kenter filtrate 56%	1,3,4	14	2	NT	100	13, 16, 14
10	Pico-Kenter 10% (unfilt.)	1,3,4	12		NT	86	12
11	Pico-Kenter 18% (unfilt.)	2	16		NT	133	16
12	Pico-Kenter 32% (unfilt.)	1,3,4	16		NT	114	16
13	Ashland filtrate 5.6%	1,3,4	18	1	NS	129	18, 17, 19
14	Ashland filtrate 10%	1,3,4	17	1	NS	121	17, 18, 16
15	Ashland filtrate 18%	2	14	1	NT	117	13, 16, 13
16	Ashland filtrate 32%	1,3,4	10	2	S	71	8, 11, 12
17	Ashland filtrate 56%	1,3,4	10	1	S	71	11, 9, 10
18	Ashland 10% (unfilt.)	1,3,4	ND*				
19	Ashland 18% (unfilt.)	2	ND*				

* Slide was unreadable due to particulates in sample.

Table B-7 Summary of kelp spore germination endpoint for samples 9/8/92; conducted 9/9/92.

Group	Sample	Reference group	% Germinated				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		78	5			79, 82, 73
2	Brine control 18%	1	67	8	S	86	73, 58, 63, 75
3	Brine control 32%	1	75	4	NS	96	81, 74, 71, 76
4	Brine control 56%	1	78	4	NS	100	82, 77, 79, 73
5	Ballona filtrate 5.6%	1,3,4	75	12	NT	98	61, 85, 80
6	Ballona filtrate 10%	1,3,4	79	7	NT	103	85, 71, 81
7	Ballona filtrate 18%	2	75	7	NT	112	82, 76, 68
8	Ballona filtrate 32%	1,3,4	78	1	NT	101	78, 77, 80
9	Ballona filtrate 56%	1,3,4	78	1	NT	101	79, 77, 77
10	Ballona 10% (unfilt.)	1,3,4	78		NT	101	78
11	Ballona 18% (unfilt.)	2	79		NT	118	79
12	Ballona 32% (unfilt.)	1,3,4	74		NT	96	74
13	Sepulveda filtrate 5.6^	1,3,4	67	7	NT	86	66, 73, 60
14	Sepulveda filtrate 10%	1,3,4	70	3	NT	91	68, 73, 69
15	Sepulveda filtrate 18%	2	78	8	NT	116	78, 71, 86
16	Sepulveda filtrate 32%	1,3,4	82	11	NT	106	86, 91, 69
17	Sepulveda filtrate 56%	1,3,4	76	5	NT	99	72, 75, 83
18	Sepulveda 10% (unfilt.)	1,3,4	69		NT	90	69
19	Sepulveda 18% (unfilt.)	2	70		NT	91	70
20	Sepulveda 32% (unfilt.)	1,3,4	69		NT	90	69

Table B-8 Summary of kelp spore germ tube length endpoint for samples 9/8/92; conducted 9/9/92.

Group	Sample	Reference group	Germ tube lenth (µm) Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		15	2			15, 17, 14
2	Brine control 18%	1	15	0	NT	100	15, 15, 14, 15
3	Brine control 32%	1	15	0	NT	100	15, 15, 14, 15
4	Brine control 56%	1	16	3	NT	107	19, 12, 15, 16
5	Ballona filtrate 5.6%	1-4	17	2	NT	113	16, 16, 19
6	Ballona filtrate 10%	1-4	18	1	NT	120	19, 17, 19
7	Ballona filtrate 18%	1-4	18	1	NT	120	18, 18, 19
8	Ballona filtrate 32%	1-4	17	1	NT	113	17, 17, 18
9	Ballona filtrate 56%	1-4	15	1	NT	100	14, 14, 16
10	Ballona 10% (unfilt.)	1-4	18		NT	120	18
11	Ballona 18% (unfilt.)	1-4	16		NT	107	16
12	Ballona 32% (unfilt.)	1-4	18		NT	120	18
13	Sepulveda filtrate 5.6^	1-4	16	1	NT	107	16, 17, 15
14	Sepulveda filtrate 10%	1-4	17	1	NT	113	16, 18, 16
15	Sepulveda filtrate 18%	1-4	16	2	NT	107	15, 15, 19
16	Sepulveda filtrate 32%	1-4	16	2	NT	107	19, 15, 15
17	Sepulveda filtrate 56%	1-4	17	2	NT	113	18, 18, 15
18	Sepulveda 10% (unfilt.)	1-4	17		NT	113	17
19	Sepulveda 18% (unfilt.)	1-4	15		NT	100	15
20	Sepulveda 32% (unfilt.)	1-4	17		NT	113	17

Table B-9 Summary of purple sea urchin fertilization test for samples 9/28/92; conducted 10/1/92.

Group	Sample	Reference group	% Fertilized				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		81	2			79, 81, 82
2	Brine control 25%	1	87	3	NS	107	87, 90, 84
3	Brine control 56%	1	75	1	S	93	75, 76, 74
4	Ballona filtrate 5.6%	1-2	86	2	NS	103	84, 88, 85
5	Ballona filtrate 12%	1-2	83	6	NS	99	76, 88, 85
6	Ballona filtrate 25%	1-2	64	3	S	76	61, 63, 68
7	Ballona filtrate 56%	3	69	8	NS	92	67, 77, 61
8	Ashland filtrate 5.6%	1-2	87	5	NS	104	87, 92, 82
9	Ashland filtrate 12%	1-2	62	12	S	74	49, 63, 73
10	Ashland filtrate 25%	1-2	0	0	S	0	1, 0, 0
11	Ashland filtrate 56%	3	0	0	NT	0	0, 0, 0
12	Pico-Kenter filtrate 5.6%	1-2	83	6	NS	99	90, 80, 78
13	Pico-Kenter filtrate 12%	1-2	77	3	NS	92	74, 79, 77
14	Pico-Kenter filtrate 25%	1-2	82	3	NS	98	82, 85, 78
15	Pico-Kenter filtrate 56%	3	77	4	NS	103	75, 82, 75
16	Egg control (seawater)	1-2	0		NT	0	0
17	Egg control (Ballona 25%)	1-2	0		NT	0	0
18	Egg control (Ashland 25%)	1-2	0		NT	0	0
19	Egg control (Pico 25%)	1-2	0		NT	0	0

Table B-10 Summary of purple sea urchin fertilization for samples 10/12/92; conducted 10/13/92.

Group	Sample	Reference group	% Fertilized				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		84	10			87, 91, 72
2	Brine control 25%	1	92	2	NS	110	90. 91, 94
3	Brine control 56%	1	78	10	NS	94	70, 90, 75
4	Ballona filtrate 5.6%	1-3	96	2	NT	114	93, 96, 98
5	Ballona filtrate 12%	1-3	97	2	NT	115	99, 96, 96
6	Ballona filtrate 25%	1-3	86	7	NT	102	80, 94, 83
7	Ballona filtrate 56%	1-3	88	5	NT	104	92, 91, 82
8	Ashland filtrate 5.6%	1-3	0	1	NT	0	0, 1, 0
9	Ashland filtrate 12%	1-3	0	0	NT	0	0, 0, 0
10	Ashland filtrate 25%	1-3	0	1	NT	0	0, 0, 1
11	Ashland filtrate 56%	1-3	0	0	NT	0	0, 0, 0
12	Pico-Kenter filtrate 5.6%	1-3	97	2	NS	115	95, 99, 97
13	Pico-Kenter filtrate 12%	1-3	94	6	NS	111	86, 98, 96
14	Pico-Kenter filtrate 25%	1-3	90	2	NS	107	88, 90, 92
15	Pico-Kenter filtrate 56%	1-3	9	3	S	11	10, 12, 6
16	Egg control (seawater)	1-3	0		NT	0	0
17	Egg control (Ballona 25%)	1-3	0		NT	0	0
18	Egg control (Ashland 25%)	1-3	0		NT	0	0
19	Egg control (Pico 25%)	1-3	0		NT	0	0

Table B-11 Summary of 48 hour red abalone larval deveelopment test for samples 9/28/92; conducted 9/30/92.

Group	Sample	Reference group	% Normal development				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control	1	7				7
2	Brine control 25%	1	7	1	NT	100	8, 6, 6
3	Brine control 56%	1	6	1	NT	86	7, 6, 6
4	Ballona filtrate 56%	1	9	1	NT	128	9, 10, 9
5	Pico-Kenter filtrate 56%	1-3	2	2	NT	29	1, 4. 1
6	Ashland filtrate 5.6%	1-3	5	2	NT	71	4, 4, 7
7	Ashland filtrate 12%	1-3	1	1	NT	14	0, 1
8	Ashland filtrate 25%	1-3	0	0	NT	0	0, 0
9	Ashland filtrate 56%	1-3	0		NT	0	0

Table B-12 Summary of 48 hour red abalone larval development test for samples 10/12/92; conducted 10/13/92.

Group	Sample	Reference group	% Normal development				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control	1	68	12			59, 82, 63
2	Brine control 25%	1	67	1	NS	99	66, 67
3	Brine control 56%	1	17	2	NT	25	18, 17, 15
4	Ballona filtrate 5.6%	1-2	70	3	NS	103	73, 68, 68
5	Ballona filtrate 12%	1-2	67	8	NS	100	66, 60, 76
6	Ballona filtrate 25%	1-2	66	8	NS	98	69, 57, 71
7	Ballona filtrate 56%	3	60	4	NT	89	58, 57, 65
8	Ashland filtrate 5.6%	1-2	61	4	NS	91	57, 63, 64
9	Ashland filtrate 12%	1-2	62	8	NS	92	71, 57, 59
10	Ashland filtrate 25%	1-2	24	4	S	35	28, 24, 20
11	Ashland filtrate 56%	3	0		NT	0	0
12	Pico-Kenter filtrate 5.6%	1-2	61	2	NS	90	62, 62, 58
13	Pico-Kenter filtrate 12%	1-2	25	8	S	37	23, 35, 18
14	Pico-Kenter filtrate 25%	1-2	0	0	NT	0	0, 0, 0
15	Pico-Kenter filtrate 56%	3	0		NT	0	0

Table B-13 Summary of kelp spore germ tube length endpoint test for samples 9/28/92; conducted 9/3092.

Group	Sample	Reference group	Germ tube length (µm) Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		15	2			17, 15, 14
2	Brine control 25%	1	13	1	NT	87	14, 12, 14
3	Brine control 56%	1	15	2	NT	100	15, 13, 16
4	Ballona filtrate 56%	1-3	16	1	NT	107	17, 17, 15
5	Ashland filtrate 5.6%	1-3	14	1	NT	93	14, 15, 14
6	Ashland filtrate 12%	1-3	14	2	NT	93	14, 16, 13
7	Ashland filtrate 25%	1-3	13	1	NT	87	14, 13, 12
8	Ashland filtrate 56%	1-3	13	1	NT	87	14, 14, 12
9	Pico-Kenter filtrate 25%	1-3	13	1	NT	87	13, 14, 13
10	Pico-Kenter filtrate 56%	1-3	16	2	NT	107	15, 18, 16

Table B-14 Summary of kelp spore germination endpoint for samples 10/12/92; conducted 10/13/92.

Group	Sample	Reference group	% Germinated				
			Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		87	5			91, 88, 81
2	Brine control 25%	1	95	1	NS	109	95, 95, 94
3	Brine control 56%	1	92	2	NS	106	90, 92, 94
4	Ballona filtrate 5.6%	1-3	88	3	NT	96	87, 91, 85
5	Ballona filtrate 12%	1-3	87	4	NT	95	90, 88, 83
6	Ballona filtrate 25%	1-3	93	2	NT	102	95, 91, 92
7	Ballona filtrate 56%	1-3	94	1	NT	103	93, 93, 95
8	Ashland filtrate 5.6%	1-3	94	4	NS	103	96, 95, 89
9	Ashland filtrate 12%	1-3	79	8	S	87	87, 71, 79
10	Ashland filtrate 25%	1-3	38	27	S	42	66, 13, 37
11	Ashland filtrate 56%	1-3	2	2	S	2	1, 0, 4
12	Pico-Kenter filtrate 5.6%	1-3	93	2	NT	102	92, 93, 95
13	Pico-Kenter filtrate 12%	1-3	91	4	NT	100	96, 89, 88
14	Pico-Kenter filtrate 25%	1-3	90	3	NT	99	87, 94, 91
15	Pico-Kenter filtrate 56%	1-3	89	4	NT	98	92, 92, 84

Table B-15 Summary of kelp spore germ tube length endpoint for samples 10/12/92; conducted 10/13/92.

Group	Sample	Reference group	Germ tube length (µm) Mean	SD	Sig.	% Ref.	Raw data
1	Seawater control		17	1			17, 18, 17
2	Brine control 25%	1	14	1	S	82	15, 13, 14
3	Brine control 56%	1	14	2	S	82	15, 14, 12
4	Ballona filtrate 5.6%	2,3	19	1	NS	136	18, 19, 19
5	Ballona filtrate 12%	2,3	19	1	NS	136	19, 19, 18
6	Ballona filtrate 25%	2,3	18	1	NS	129	19, 17, 17
7	Ballona filtrate 56%	2,3	17	1	NS	121	18, 16, 18
8	Ashland filtrate 5.6%	2,3	14	2	NS	100	12, 17, 14
9	Ashland filtrate 12%	2,3	12	1	S	86	13, 11, 11
10	Ashland filtrate 25%	2,3	9	1	S	64	10, 9, 8
11	Ashland filtrate 56%	2,3	6	1	S	43	7, 6
12	Pico-Kenter filtrate 5.6%	2,3	18	1	NS	129	17, 18, 18
13	Pico-Kenter filtrate 12%	2,3	17	1	NS	121	17, 16, 17
14	Pico-Kenter filtrate 25%	2,3	15	1	NS	107	14, 15, 16
15	Pico-Kenter filtrate 56%	2,3	12	1	S	86	12, 12, 11

Table B-16 Summary of purple sea urchin fertilization test for sample 11/23/92.

Sample	% Fertilized	Mean
Seawater control	94	
Brine control 25% DIW	92	
Brine control 56% DIW	38	
SCCWRP DIW control 56%	76	
EDTA blank 56%	78	
EDTA 250 mg/L 56%	97	
Thiosulfate blank 56%	0	
Thiosulfate 1 g/L 56%	64	
pHo filter blank 12%	0	
pHo filter blank 56%	0	
pHo filtrate 56%	83	
pHo column blank 56%	0	
pHo post column 25 ml 56%	94	
pHo post column 950 ml 56%	87	
pH3 filter blank 12%	94	
pH3 filter blank 56%	15	
pH3 column blank 12%	58	
pH11 filter blank 56%	61	
pH11 filtrate 56%	73	
pH9 column blank 56%	34	
Ballona PM filtrate 12%	91, 95	
Ballona PM filtrate 25%	84, 99	
Ballona PM filtrate 56%	79, 3	
Methanol 0.5% 1x sperm	85	
Methanol 0.5% 2x sperm	97	
Methanol 0.5% 5x sperm	0	

Table B-17 Summary of purple sea urchin fertilization test for samples 12/14/92.

Sample	% Fertilized	Mean
Seawater control	96, 98	97
Brine control 25% DIW	100, 98	99
Brine control 56% DIW	69, 78	74
Ballona PM filtrate 12%	82, 91	86
Ballona PM filtrate 25%	67, 65	66
Ballona PM filtrate 56%	18, 12	15
Filter blank 12%	94, 97	96
Filter blank 25%	82, 75	78
Filtare blank 56%	5, 5	5
Column blank 12%		
Column blank 25%	52, 51	52
Column blank 56%	21, 23	22
Post column 12%		
Post column 25%	89, 94	92
Post column 56%	69, 82	76
EDTA 3 mg/L 12%		
EDTA 3 mg/L 25%	90, 90	90
EDTA 3 mg/L 56%	39, 50	44
EDTA 8 mg/L 12%		
EDTA 8 mg/L 25%	91, 98	94
EDTA 8 mg/L 56%	11, 13	12
Thiosulfate blank 12%		
Thiosulfate blank 25%	13, 25	24
Thiouslfate blank 56%	2, 0	1
Thiousulfate 10 mg/L 12%		
Thiousulfate 10 mg/L 25%	86, 91	88
Thiousulfate 10 mg/L 56%	99, 99	99
Thiousulfate 25 mg/L 12%		
Thiousulfate 25 mg/L 25%	98, 100	99
Thiousulfate 25 mg/L 56%	96, 99	98
50% methanol blank 0.1%		
50% methanol blank 0.2%	99, 100	100
100% methanol blank 0.1%		
100% methanol blank 0.2%	97, 96	96
50% MeCl2 blank 0.1%	98, 98	98
50% MeCl2 blank 0.2%	73, 78	76
50% methanol eluate 0.1%	100, 99	100
50% methanol eluate 0.2%	100, 99	100
100% methanol eluate 0.1%	92, 88	90
100% methanol eluate 0.2%	8, 6	7
50% MeCl2 eluate 0.1%	97, 99	98
50% MeCl2 eluate 0.2%	50, 47	48

Table B-18 Summary of purple sea urchin fertilization test for sample 1/19/93.

Sample	% Fertilized	Mean
Seawater control	87, 94	90
Brine control 25% DIW	80, 71	76
Brine control 56% DIW	89, 81	85
Ballona PM filtrate 12%	57, 43	50
Ballona PM filtrate 25%	31, 28	30
Ballona PM filtrate 56%	14, 17	16
Filter blank 12%	68, 69	68
Filter blank 25%	63, 70	66
Filtare blank 56%	58, 66	62
Column blank 12%	76, 71	74
Column blank 25%	23, 19	21
Column blank 56%	7, 15	11
Post column 12%	75, 69	72
Post column 25%	44, 42	43
Post column 56%	20, 19	20
EDTA 3 mg/L 12%	90, 87	88
EDTA 3 mg/L 25%	92, 94	93
EDTA 3 mg/L 56%	95, 90	92
EDTA 8 mg/L 12%	91, 95	93
EDTA 8 mg/L 25%	93, 95	94
EDTA 8 mg/L 56%	96, 95	96
EDTA 30 mg/L 12%	95, 97	96
EDTA 30 mg/L 25%	95, 97	96
EDTA 30 mg/L 56%	95, 90	92
Thiosulfate blank 12%	89, 88	88
Thiosulfate blank 25%	100, 89	94
Thiouslfate blank 56%	97, 96	96
Thiousulfate 10 mg/L 12%	25, 30	28
Thiousulfate 10 mg/L 25%	4, 5	4
Thiousulfate 10 mg/L 56%	9, 12	10
Thiousulfate 25 mg/L 12%	27, 28	28
Thiousulfate 25 mg/L 25%	23, 14	18
Thiousulfate 25 mg/L 56%	10, 14	12
50% methanol blank 0.1%	77, 85	81
50% methanol blank 0.2%	82, 81	82
100% methanol blank 0.1%	79, 86	82
100% methanol blank 0.2%	69, 78	74
50% MeCl2 blank 0.1%	83, 82	82
50% MeCl2 blank 0.2%	61, 55	58
50% methanol eluate 0.1%	80, 65	72
50% methanol eluate 0.2%	89, 85	87
100% methanol eluate 0.1%	46, 40	43
100% methanol eluate 0.2%	46, 39	42
50% MeCl2 eluate 0.1%	86, 67	76
50% MeCl2 eluate 0.2%	77, 77	77

APPENDIX C
RAW DATA OF TOXICITY RECOVERY STUDY FOR OIL AND GREASE FRACTIONS

Table C-1. Toxicity results of eight hydrocarbon standards (Phase I).

Sample	Conc. (µg/L)	R1	R2	R3	Mean	SD	% Control
Control		79	64	91	78	14	
Solvent blank 1%		32	30		31	1	40
C16	100	96	86		91	7	117
C20	100	88	96		92	6	118
Napthalene	1000	65	56		61	6	78
Napthalene	100	83	91		87	6	112
2,6-dimethylnapthalene	100	55	63		59	6	76
2,6-dimethylnapthalene	10	88	72		80	11	103
Phenanthrene*	1000	0	0		0	0	0
Phenanthrene	100	94	83		89	8	113
Pyrene	100	53	39		46	10	59
Pyrene	10	86	90		88	3	113
Chrysene	100	94	89		92	4	117
Benzo(a)pyrene	100	95	87		91	6	117

Table C-2. Toxicity results of first fractiontion test of 4 standard hydrocarbons.

Sample	Conc. (%)	% fertilization R1	R2	R3	R4	Mean	SD
Control		94	96	77	99	92	10
S1 (solvent blank)	0.50	96	97			97	1
S1 (solvent blank)	0.25	96				96	
S2 (elution blank 1)	0.50	92	98			95	4
S2 (elution blank 1)	0.25						
S3 (elution blank 2)	0.50	95	92			94	2
S3 (elution blank 2)	0.25						
S4 (elution blank 3)	0.50	98	94			96	3
S4 (elution blank 3)	0.25						
S5 (elution blank 4)	0.50	47	16	54		39	20
S5 (elution blank 4)	0.25	74	77	94		82	11
S6 (standard mix)	0.50	1	2	2		2	1
S6 (standard mix)	0.25	0	1	2		1	1
S6 (standard mix)	0.12	16	9	17		14	4
S6 (standard mix)	0.06	56	59	80		65	13
S7 (fraction 1)	0.50	47	31	53		44	11
S7 (fraction 1)	0.25	49	75	72		65	14
S7 (fraction 1)	0.12	78	85			81	5
S7 (fraction 1)	0.06	80	96			88	11
S8 (fraction 2)	0.50	97	89	93		93	4
S8 (fraction 2)	0.25						
S8 (fraction 2)	0.12						
S8 (fraction 2)	0.06						
S9 (fraction 3)	0.50	83	75	84		81	5
S9 (fraction 3)	0.25	89	93	91		91	2
S9 (fraction 3)	0.12						
S9 (fraction 3)	0.06						
S10 (fraction 4)	0.50	84	87	97		89	7
S10 (fraction 4)	0.25	88	91			90	2
S10 (fraction 4)	0.12						
S10 (fraction 4)	0.06						

Table C-3. Toxicity results of the second fractionation test of 4 standard hydrocarbons.

Sample	Conc. (%)	% fertilization R1	R2	R3	R4	Mean	SD
Control		94	84	94	98	93	6.0
S1 (solvent blank)	0.25						
S1 (solvent blank)	0.50	95	93	98		95	2.5
S2 (elution blank 1)	0.25	95				95	
S2 (elution blank 1)	0.50	84	*	94		89	7.1
S3 (elution blank 2)	0.25	95	95	94		95	0.6
S3 (elution blank 2)	0.50	80	20	65		55	31.2
S4 (elution blank 3)	0.25	82	98	97		92	9.0
S4 (elution blank 3)	0.50	73	55	79		69	12.5
S5 (elution blank 4)	0.25						
S5 (elution blank 4)	0.50	87	84	92		88	4.0
S6 (standard mix)	0.06	*	76	78		77	1.4
S6 (standard mix)	0.12	47	53	48		49	3.2
S6 (standard mix)	0.25	6	7	9		7	1.5
S6 (standard mix)	0.50	1	1	0		1	0.6
S7 (fraction 1)	0.06						
S7 (fraction 1)	0.12	97	90	93		93	3.5
S7 (fraction 1)	0.25	83	60	92		78	16.5
S7 (fraction 1)	0.50	30	20	25		25	5.0
S8 (fraction 2)	0.06						
S8 (fraction 2)	0.12						
S8 (fraction 2)	0.25	92	93	94		93	1.0
S8 (fraction 2)	0.50	90	93	63		82	16.5
S9 (fraction 3)	0.06						
S9 (fraction 3)	0.12	87	95	94		92	4.4
S9 (fraction 3)	0.25	90	81	87		86	4.6
S9 (fraction 3)	0.50	28	68	32		43	22.0
S10 (fraction 4)	0.06						
S10 (fraction 4)	0.12	88	98	96		94	5.3
S10 (fraction 4)	0.25	85	91	86		87	3.2
S10 (fraction 4)	0.50	27	66	47		47	19.5

* Problem with test.

APPENDIX D
EPA PROBIT ANALYSIS OUTPUT
FOR CALCULATING EC50 VALUES OF CHAPTER 5

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING EC VALUES
Version 1.4

First Fractionation Test Standard Mix

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	8	0.0800	0.0000	0.0749
0.0600	100	35	0.3500	0.2974	0.3648
0.1200	100	86	0.8600	0.8487	0.7746
0.2500	100	99	0.9900	0.9892	0.9725

Chi - Square Heterogenity = 0.6000

Mu = -1.117425
Sigma = 0.204718

Parameter	Estimate	Std. Err	95% Confidence Limits	
Intercept	10.458358	0.639695	(9.204556	11.712160)
Slope	4.884763	0.596716	(3.715200	6.054326)
Spontaneous Response Rate	0.078897	0.026921	(0.026131	0.131663)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upeper 95% Confidence Limits
EC1.00	0.0255	0.0168	0.0331
EC5.00	0.0351	0.0255	0.0431
EC10.00	0.0417	0.0318	0.0497
EC15.00	0.0468	0.0369	0.0548
EC50.00	0.0763	0.0674	0.0847
EC85.00	0.1244	0.1109	0.1450
EC90.00	0.1396	0.1230	0.1671
EC95.00	0.1657	0.1426	0.2073
EC99.00	0.2285	0.1867	0.3130

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First Fractionation Test Fraction (1)

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	8	0.0800	0.0000	0.0811
0.0600	100	12	0.1200	0.0423	0.0390
0.1200	100	19	0.1900	0.1185	0.1210
0.2500	100	35	0.3500	0.2926	0.2937
0.5000	100	56	0.5600	0.5212	0.5198

Chi - Square Heterogenity = 0.0140

Mu = -0.326315
Sigma = 0.508185

Parameter	Estimate	Std. Err	95% Confidence Limits	
Intercept	5.642119	0.243936	(5.164003	6.120234)
Slope	1.967787	0.387972	(1.207362	2.728212)
Spontaneous Response Rate	0.081095	0.025627	(0.030866	0.131323)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upeper 95% Confidence Limits
EC1.00	0.031	0.0066	0.0627
EC5.00	0.0688	0.0238	0.1131
EC10.00	0.1053	0.0468	0.156
EC15.00	0.1403	0.0735	0.1952
EC50.00	0.4717	0.3683	0.6741
EC85.00	1.5862	0.9868	4.3549
EC90.00	2.1134	1.2228	6.8998
EC95.00	3.2329	1.6745	13.6893
EC99.00	7.1759	3.0025	49.7627

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Second Fractionation Test Standard Mix

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	7	0.0700	0.0000	0.0752
0.0600	100	23	0.2300	0.1674	0.1384
0.1200	100	51	0.5100	0.4702	0.5230
0.2500	100	93	0.9300	0.9243	0.8981
0.5000	100	99	0.9900	0.9892	0.9922

Chi - Square Heterogenity = 2.2100

Mu = -0.936009
Sigma = 0.262813

Parameter	Estimate	Std. Err	95% Confidence Limits	
Intercept	8.561502	0.353053	(7.869518	9.253486)
Slope	4.58		(3.045285	4.564687)
Spontaneous Response Rate	0.075165	0.025887	(0.024427	0.125902)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upeper 95% Confidence Limits
EC1.00	0.0284	0.0184	0.038
EC5.00	0.0428	0.0307	0.054
EC10.00	0.0534	0.0402	0.0652
EC15.00	0.0619	0.0481	0.0741
EC50.00	0.1159	0.1006	0.1309
EC85.00	0.2170	0.1903	0.2556
EC90.00	0.2517	0.2179	0.3042
EC95.00	0.3135	0.2648	0.3956
EC99.00	0.4736	0.3782	0.6538

EPA PROBIT ANALYSIS PROGRAM
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Second Fractionation Test Fraction (1)

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	7	0.7000	0.0000	0.0684
0.1200	100	7	0.0700	0.0017	0.0036
0.2500	100	22	0.2200	0.1627	0.1623
0.5000	100	75	0.7500	0.7316	0.7319

Chi - Square Heterogenity = 0.0050

Mu = -0.417117
Sigma = 0.187731

Parameter	Estimate	Std. Err	95% Confidence Limits	
Intercept	7.221883	0.350544	(6.534817	7.908948)
Slope	5.326760	0.793954	(3.770610	6.882910)
Spontaneous Response Rate	0.068401	0.018787	(0.031578	0.105224)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upeper 95% Confidence Limits
EC1.00	0.1400	0.0896	0.1802
EC5.00	0.1880	0.1350	0.2276
EC10.00	0.2199	0.1676	0.2583
EC15.00	0.2445	0.1938	0.2817
EC50.00	0.3827	0.3434	0.4234
EC85.00	0.5990	0.5267	0.7353
EC90.00	0.6660	0.5753	0.8488
EC95.00	0.7793	0.6537	1.0530
EC99.00	1.0462	0.8267	1.8855

EPA PROBIT ANALYSIS PROGRAM
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Second Fractionation Test Fraction (3)

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	7	0.0700	0.0000	0.0745
0.1200	100	8	0.0800	0.0060	0.0009
0.2500	100	14	0.1400	0.0708	0.0712
0.5000	100	27	0.5700	0.5354	0.5353

Chi - Square Heterogenity = 0.0320

Mu = -0.318161
Sigma = 0.193572

Parameter	Estimate	Std. Err	95% Confidence Limits	
Intercept	6.643637	0.417342	(5.825647	7.461628)
Slope	5.166050	1.104551	(3.001130	7.330969)
Spontaneous Response Rate	0.074452	0.019013	(0.037186	0.111718)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upeper 95% Confidence Limits
EC1.00	0.1704	0.0827	0.2312
EC5.00	0.2309	0.1386	0.2882
EC10.00	0.2715	0.1820	0.3250
EC15.00	0.3028	0.2183	0.3534
EC50.00	0.4807	0.4284	0.5522
EC85.00	0.7629	0.6369	1.1392
EC90.00	0.8510	0.6910	1.3689
EC95.00	1.0006	0.7780	1.8007
EC99.00	1.3556	0.9685	3.0221

EPA PROBIT ANALYSIS PROGRAM
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Second Frcationation Test Fraction (4)

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	7	0.0700	0.0000	0.0646
0.1200	100	6	0.0600	-0.0050	0.0012
0.2500	100	13	0.1300	0.6990	0.0692
0.5000	100	53	0.5300	0.4975	0.4978

Chi - Square Heterogenity = 0.0540

Mu = -0.299891
Sigma = 0.203922

Parameter	Estimate	Std. Err	95% Confidence Limits	
Intercept	6.470615	0.410896	(5.665259	7.275971)
Slope	4.903832	1.081898	(2.783313	7.024352)
Spontaneous Response Rate	0.064646	0.17938	(0.029487	0.099804)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upeper 95% Confidence Limits
EC1.00	0.1682	0.0778	0.2305
EC5.00	0.2316	0.1357	0.2902
EC10.00	0.2746	0.1819	0.3292
EC15.00	0.3082	0.2211	0.3596
EC50.00	0.5013	0.4457	0.5906
EC85.00	0.8156	0.6655	1.3094
EC90.00	0.9151	0.7241	1.5975
EC95.00	1.0853	0.8191	2.1486
EC99.00	1.4945	1.0289	3.7579