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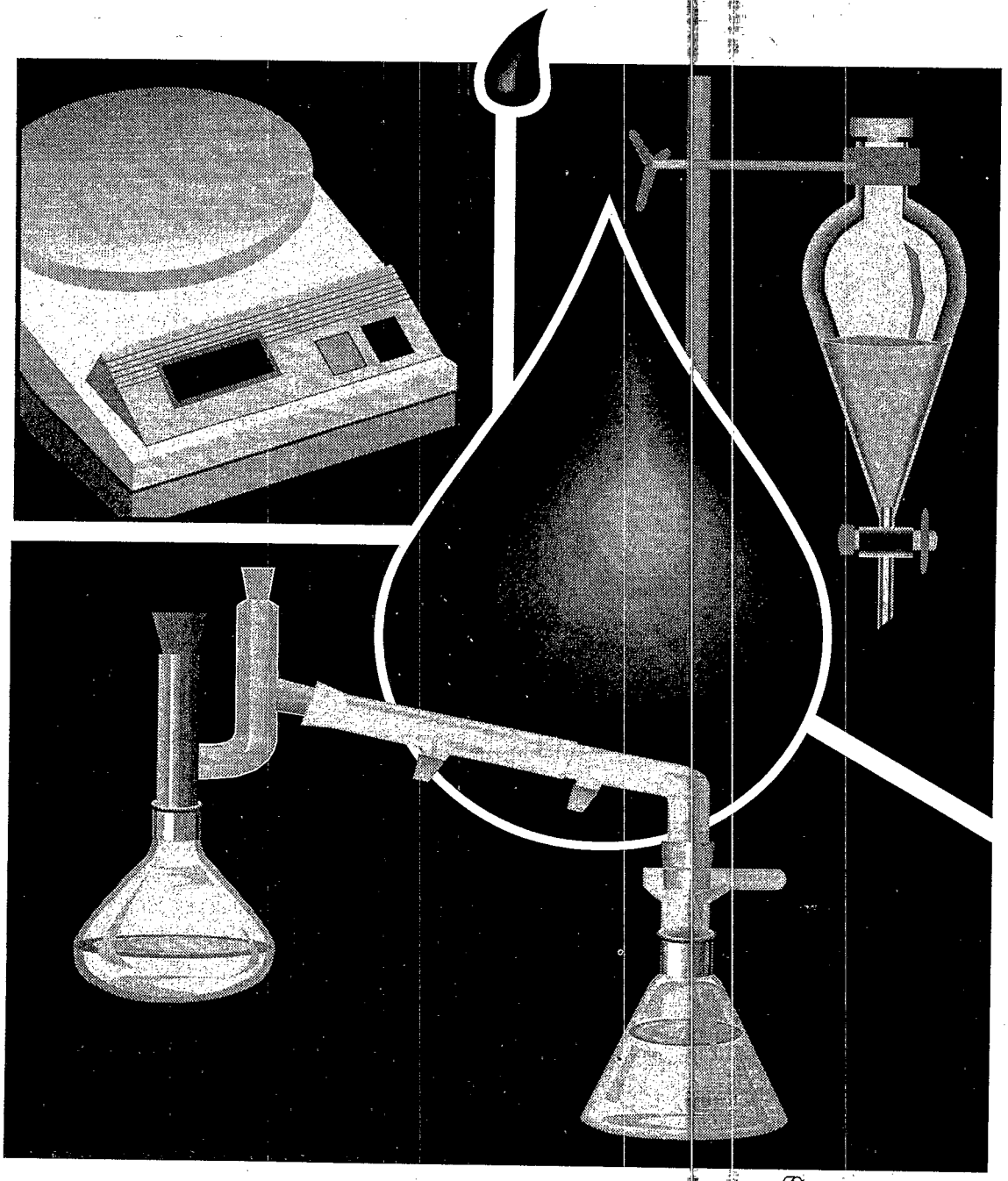
Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease and Total Petroleum Hydrocarbons: Phase II



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Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease and Total Petroleum Hydrocarbons: Phase II

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EXECUTIVE SUMMARY

In support of the Montreal Protocol on Substances that Deplete the Ozone Layer and in order to meet the chlorofluorocarbon (CFC) phaseout requirements of the Clean Air Act Amendments of 1990, the Environmental Protection Agency (EPA) initiated a multiphase study to determine a suitable replacement solvent for Freon-113, a class I CFC used in several EPA wastewater and solid waste methods for the determination of oil and grease and petroleum hydrocarbons. In Phase I of the Freon Replacement Study, five solvents were evaluated for separatory funnel extraction and gravimetric determination of oil and grease in aqueous samples. In addition, alternative techniques that included sonication extraction, solid phase extraction (SPE), and a non-dispersive infra-red (NDIR) technique were evaluated.

Conclusions from the Phase I study were used to narrow the list of alternative solvents to be considered in Phase II to n-hexane and cyclohexane. These solvents were evaluated for separatory funnel extraction and gravimetric determination of both oil and grease and total petroleum hydrocarbons (TPH) in aqueous samples. Triplicate analyses were performed for each of the solvents tested (i.e. Freon-113, n-hexane, and cyclohexane) on each of 34 samples from a combination of in-process and effluent waste streams collected from 25 facilities encompassing 16 different industrial categories. The objectives of Phase II were to find the alternative solvent that produced results closest to the results produced by Freon-113 and to develop an analytical method that incorporated this extraction solvent.

In addition to studies of alternative solvents, solid phase disk extraction, solid phase cartridge extraction (also known as solid phase column extraction), non-dispersive infra-red spectroscopy, and immunoassay were voluntarily evaluated by vendors of the products using splits of each sample collected as part of the Phase II study.

Equivalency to Freon-113 separatory funnel extraction was established by generating Acceptance Limits for the root mean square deviations (RMSDs) of the results on the basis of three sample matrix classifications (all samples, non-petroleum samples, and petroleum samples). Examination of the separatory funnel extraction results demonstrated that in all three matrix categories, oil and grease and TPH results from both n-hexane and cyclohexane extraction were not equivalent to results from Freon-113 extraction. These findings were consistent with the Phase I study conclusion that, when all sample matrices were collectively considered, none of the alternative solvents produced results statistically equivalent to results produced by Freon-113. Further evaluation of the Phase II data led to the conclusion that the results produced when using n-hexane and cyclohexane were statistically equivalent to one another.

Therefore, the decision of which alternative solvent was best suited to replace Freon-113 was influenced by practical analytical considerations, of which the primary factor was the difference between the boiling points of n-hexane (69°C) and cyclohexane (81°C). Based on laboratory comments regarding the extensive amount of time required to evaporate cyclohexane, n-hexane was chosen to replace Freon-113.

Evaluation of vendor data was limited to the SPE disk and cartridge extraction techniques with gravimetric determination, and concluded that these techniques did not produce results equivalent to results produced by separatory funnel extraction using either Freon-113 or n-hexane. NDIR and immunoassay results were not considered in this report, since they represent completely different determinative techniques. These results will be considered in other studies.

The final product of Phase II was Method 1664, a performance-based method that uses n-hexane as the extraction solvent. The most significant changes in Method 1664 compared to other oil and grease and petroleum hydrocarbons methods that use separatory funnel extraction and gravimetric determination are 1) the use of n-hexane as the extraction solvent, 2) the use of standards of known composition and purity, specifically hexadecane and stearic acid, as the spiking materials for QC

analyses, and 3) the introduction of extensive quality control (QC), including initial precision and recovery analysis (IPR), ongoing precision and recovery analysis (OPR), reagent water method blanks, and matrix spike/matrix spike duplicates (MS/MSDs). Though not specifically incorporated into Method 1664, the use of alternative extraction and concentration techniques are allowed under the performance-based option of this method, provided that performance specifications are met.

SECTION 1
BACKGROUND

The discharge of chlorofluorocarbons (CFCs) has been shown to be a primary contributor to the depletion of the earth's stratospheric ozone layer. The United States, as a party to the Montreal Protocol on Substances that Deplete the Ozone Layer and as required by law under the Clean Air Act Amendments of 1990 (CAAA), is committed to controlling and eventually phasing out CFCs. Under both the Montreal Protocol and the CAAA, Class I CFCs will be phased out by January 1, 1996.

Freon-113 is a Class I CFC that is required for use in several U.S. Environmental Protection Agency (EPA) wastewater and solid waste methods for the determination of oil and grease and petroleum hydrocarbons. As part of the effort to eliminate the use of CFCs, EPA is studying the use of alternate solvents to replace Freon-113. This effort is complicated by the fact that oil and grease is a method defined parameter and, consequently, changing the extraction solvent could produce different results. Because oil and grease is included in Clean Water Act effluent guidelines for 25 major industries and is a regulated pollutant in over 10,000 National Pollutant Discharge Elimination System (NPDES) permits as well as RCRA operating permits, any change to the analytical protocol has the potential to affect permit compliance. For this reason, the objective of this study was to find a solvent that would produce results comparable to results produced with Freon-113 for these analytes.

Initial efforts to find an alternative solvent to Freon-113 were conducted by the Office of Research and Development's Environmental Monitoring Systems Laboratory in Cincinnati, Ohio (EMSL-Ci). EMSL-Ci focused its study on MCAWW Method 413.1 (promulgated at 40 *CFR* Part 136), which is used in Clean Water Act (CWA) programs to gravimetrically determine the oil and grease content of surface and saline waters and domestic and industrial wastes. Aqueous samples, some of which were synthetically prepared by spiking reagent water with various oils and greases and others that were collected from industrial facilities, were analyzed using several different extraction solvents in place of Freon-113. Results of the study, presented in the document titled *A Study to Select a Suitable Replacement Solvent for Freon-113 for the Gravimetric Determination of Oil and Grease*, by F.K. Kawahara, October 2, 1991, suggested the use of an 80/20 mixture of n-hexane and methyl tertiary butyl ether (MTBE) in place of Freon-113 for oil and grease determination. Following this study, an Office of Air and Radiation (OAR) proposal (56 FR 30519) suggested replacement of Freon-113 by the n-hexane:MTBE mix in CWA and RCRA analytical methods for determination of oil and grease.

Based on comments submitted concerning the EMSL-Ci study results, and the need to further investigate alternative solvents, the Office of Water and the Office of Solid Waste organized a multi-phase Freon Replacement Study. Phase I of this study was initiated to evaluate alternative solvents and extraction systems for equivalency across a range of real world effluent and solid waste samples from a variety of industrial categories. This phase of the study focused on 1) the use of five alternative solvents for gravimetric determination of oil and grease in aqueous samples by MCAWW Method 413.1 (with modifications) and in solid samples by SW-846 Method 9071A (with modifications) and 2) the use of alternative techniques for oil and grease determinations including sonication extraction, solid phase extraction (SPE) using cartridges and disks, and a solvent/non-dispersive infrared (NDIR) technique.

The results of Phase I yielded the following conclusions: n-hexane should be retained as a possible extraction solvent for further study using gravimetric techniques; perchloroethylene should be retained for consideration in the use of infra-red techniques; and cyclohexane should be introduced for consideration with gravimetric techniques based on its similarity to n-hexane and because of its lower neurotoxicity when compared to n-hexane. Results of the alternative techniques indicated that only sonication extraction of high solids samples produced results equivalent to existing techniques that use

Freon-113. Specifics of the study design, results, and conclusions can be found in the *Preliminary Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease*, September 1993.

Phase II of the Freon Replacement Study is a continuation of these efforts to determine an appropriate replacement solvent for Freon-113 and to develop a revised method for determination of oil and grease. Based on the conclusions from Phase I of the Freon Replacement Study, Phase II was designed to focus on the evaluation of n-hexane and cyclohexane as extraction solvents in the gravimetric determination of oil and grease in aqueous samples by Method 413.1 (with modifications). In addition, gravimetric determination of petroleum hydrocarbons was included. The remainder of this document is a report on this study.

SECTION 2
PHASE II STUDY DESIGN

The second phase of the Freon Replacement Study evaluated n-hexane, cyclohexane, and alternative extraction and concentration techniques as possible substitutes to extraction using Freon-113 in the gravimetric determination of oil and grease and TPH in aqueous samples. The original Phase II study design is detailed in the *Draft Study Plan for Phase II of the Freon Replacement Study*, September 29, 1993. The final study design is summarized below.

2.1 Study Objectives

The purposes of the second phase of the study were to:

- Continue EPA's investigation of n-hexane as a potential replacement solvent for Freon-113 in the gravimetric determination of oil and grease in aqueous samples
- Evaluate cyclohexane as an alternative solvent
- Assess n-hexane and cyclohexane for determination of TPH in aqueous samples
- Evaluate alternative techniques, including solid phase extraction by both disk and cartridge, non-dispersive infrared spectrophotometry, and immunoassay

The final objectives of the Phase II study were to use the results to choose a replacement solvent and develop a method using this solvent. Though not evaluated in this report, as part of the development of revised analytical procedures, validation studies to support the recommended method revisions and quality control specifications were planned. Details of these studies are presented in the document titled *Report of the Method 1664 Validation Studies*, April 1995.

2.2 Sample Source Selection

Sample matrices included in Phase II of the study represented wastewaters from a variety of industrial categories and facilities. The Office of Water's Engineering and Analysis Division coordinated all facility contacts and planning for the sample collection efforts. Wastewater samples containing between 40-300 mg/L oil and grease, some from petroleum and some from non-petroleum sources, were targeted for collection. The study focused on this concentration range to avoid the problems associated with the comparison and evaluation of non-detect results. In order to increase the types of matrices considered by the Agency and to better assess the effect of different matrices on solvent extraction performance, a concerted effort was made to collect samples from a variety of facilities that were different from those collected during Phase I of the study. Further details are provided in Section 3 of this report, and a site summary is presented in Appendix A.

2.3 Analytical Study Design

The study focused on evaluation of three solvents, including Freon-113, for the analysis of aqueous samples using conventional separatory funnel extraction followed by gravimetric determination. As with Phase I, several manufacturers of alternative extraction devices and

measurement techniques volunteered to analyze splits of the EPA samples by their techniques, and to provide the results of their analyses to EPA at no cost to the Agency.

Of the data submitted by vendors, only those results from the SPE disk and cartridge analyses were considered. The results for analyses by infra-red spectroscopy and immunoassay were not presented in this report because they represent completely different determinative techniques. Infra-red determinative procedures for oil and grease and TPH will be the focus of future efforts under Phase III of the Freon Replacement Study. The immunoassay procedure may be considered for use as a screening tool but, with the measurement limitations inherent to the procedure at present, cannot be used as a quantitative measurement technique, since the analysis was only capable of determining "less than" or "greater than" values. Though not included in this report, summary results of the infra-red and immunoassay analyses can be obtained from the Sample Control Center (operated by DynCorp Environmental Programs Division), 300 N. Lee Street, Alexandria, VA 22314, (703) 519-1140.

The analytical protocol for each concentration and extraction technique considered in Phase II is presented below.

Separatory Funnel Extraction

A total of 34 aqueous sample sets were collected and sent to a single contract laboratory for analysis in triplicate. An individual split sample in its own container was collected for each analysis. To compare the performances of n-hexane and cyclohexane to Freon-113 in real world sample matrices, wastewater samples were extracted using the separatory funnel techniques described in Method 413.1. Since n-hexane and cyclohexane are less dense than water, the specifics of the extraction procedure in Method 413.1 were adjusted to explicitly deal with the removal of each solvent from the separatory funnel and the removal of residual water from the extract. For determination of TPH, the oil and grease extract residue was redissolved in n-hexane and subjected to the silica gel cleanup procedure in Method 5520F from *Standard Methods for the Examination of Water and Wastewater*, 18th ed., to adsorb polar materials.

In addition, the laboratory was required to implement more stringent quality assurance/quality control (QA/QC) tests than those required in either Method 413.1 or Method 5520F. This QC included performance of initial precision and recovery (IPR) analyses prior to the analysis of field samples. IPRs consisted of the extraction, concentration, and analysis of a set of four 1-L aliquots of reagent water that had been spiked with hexadecane and stearic acid. In order to evaluate any effects that might result from the revised analytical procedures, all IPR analyses incorporated the modifications that were necessitated by differences in solvent densities, as well as any other changes that were implemented during the study.

An ongoing precision and recovery (OPR) analysis, the equivalent of a single IPR sample, was required with each analytical batch for each alternative solvent. An analytical batch consisted of a set of samples extracted at the same time, to a maximum of ten samples.

In addition, a reagent water method blank was analyzed with each IPR set and with each analytical batch for each alternative solvent. These reagent water blanks were run through the entire extraction and analysis procedure by which the samples were run. The analytical protocol required that the concentration of oil and grease in method blanks not exceed 5 mg/L and, if contamination above this level was detected in any method blank, the laboratory was required to isolate and remove the source of contamination.

Multiple aliquots of each sample were collected to accommodate the numerous analyses required. For each sample, the aliquots were split from a homogenized sample and, to the extent practicable, contained identical concentrations of oil and grease. Within each of the three different solvent procedures and two modified methods (413.1 and 5520F), it was expected that the relative standard

deviation of the triplicate measurements would be less than 10 percent for those results at or above 25 mg/L and less than 20 percent for those results less than 25 mg/L.

Several procedures that were incorporated into the analytical requirements evolved as the study progressed. These included the routine use of sodium sulfate to ensure that all residual water was removed from the extraction solvent, the use of hexadecane and stearic acid as spiking standards for the QC analyses, and the protocol for the use of silica gel in samples containing greater than 100 mg of extractable material.

In current oil and grease methods, such as Methods 413.1 and 5520B, sodium sulfate use had been limited to breaking up emulsions. With the use of extraction solvents that are lighter than water, however, residual water from the bottom layer had a tendency to mix with the extraction solvent when the upper n-hexane or cyclohexane phase was being drained from the separatory funnel. To remove these trace amounts of water, it was recommended that the extraction solvent be drained through granular, anhydrous sodium sulfate on filter paper. Phase separator paper was also shown to be effective in the removal of water. As these or other analytical techniques evolved, they were incorporated into the QC analyses to ensure that subsequent measurements were not adversely affected by these changes.

Hexadecane and stearic acid were chosen as spiking standards because they are compounds of known composition and purity and therefore allow for accurate measurement of recovery and precision. The decision to use these compounds as spiking standards required determination of an appropriate solvent and stock solution concentration. Various solvents and concentrations were tested before it was determined that a hexadecane/stearic acid stock solution of 8 mg/mL total (i.e. 4 mg hexadecane and 4 mg stearic acid) in acetone was satisfactory.

Another issue was the applicability of the silica gel extraction procedure to oil and grease (and therefore potential TPH) concentrations in excess of 100 mg/L. The adsorptive capacity of silica gel needed to be studied in order to determine the amount of silica gel required to adsorb increasing concentrations of oil and grease. In addition, it was necessary to determine an appropriate cutoff for the maximum amount of silica gel that realistically could be used.

For the purposes of this study, it was assumed (based on MCAWW Method 418.1 and Standard Method 5520F) that 3 g of silica gel was capable of adsorbing 100 mg of adsorbable materials. Therefore, any sample containing more than 100 mg of oil and grease that was going to be subjected to the silica gel TPH procedure would require proportionately more silica gel to ensure that all potentially adsorbable materials were adsorbed. It was speculated, however, that at some level contamination from substances in the silica gel would begin to affect the TPH measurement.

In order to determine the limit at which additional amounts of silica gel might affect results, a silica gel capacity study was conducted in which 100 mL aliquots of n-hexane spiked with different amounts of stearic acid were subjected to the silica gel procedure. With increasing spike amounts (20 mg, 200 mg, and 2,000 mg), proportionately more silica gel (3 g, 6 g, and 60 g, respectively) was added. Blanks comprised of n-hexane with these corresponding amounts of silica gel were also tested. Each of the three stearic acid/silica gel combinations were prepared in triplicate. As part of this evaluation of the capacity of silica gel, the conditions for activating silica gel were revised from the existing Method 5520F protocol, which specified drying at 110°C for 24 hours, to drying at 200-250°C for 24 hours, to ensure that activation was complete.

Analysis of the 20 mg stearic acid/3 g silica gel and 200 mg stearic acid/6 g silica gel combinations produced non-detect results, thereby demonstrating that at these levels silica gel adsorbed the stearic acid. Analysis of the 2,000 mg stearic acid/60 g silica gel combination resulted in an average value of 9 mg/L, indicating that either the silica gel did not adsorb all of the stearic acid or substances in the silica gel itself were contributing to contamination.

From these results, it was estimated that the maximum amount of silica gel that could be used before potential contamination or adsorptive capacity would be of concern was 30 g. To confirm that

this was an appropriate choice, the procedure described above was performed on an n-hexane aliquot spiked with 1,000 mg of stearic acid and 30 g of silica gel. Results were below the 5 mg/L detection limit. Consequently, the maximum amount of material in a sample that could be subjected to the silica gel procedure was limited to 1,000 mg. For those instances in which the oil and grease concentrations exceeded 1,000 mg/L, it was necessary to split off a portion of the extract containing no more than 1,000 mg of oil and grease for TPH determination.

Though hexadecane was not spiked into the solvent for these tests to determine if the increase in silica gel would affect hexadecane recoveries, OPRs, which by method protocol were spiked with hexadecane and stearic acid, were prepared using the maximum amount of silica gel that had been required in each associated sample batch. All OPRs generated acceptable recoveries, thereby demonstrating that increasing amounts of silica gel did not impair hexadecane recoveries.

Solid Phase Extraction (SPE)

The performance of solid phase extraction (SPE) techniques was evaluated by two manufacturers of SPE devices, 3M Corporation and Varian Sample Preparation Products. The SPE techniques tested have the advantage of using significantly less solvent than conventional separatory funnel extraction techniques and, by nature of the mechanics of the technique, minimize emulsions.

The 3M Corporation extracted 29 aqueous samples for oil and grease using Empore SPE disks, with n-hexane and cyclohexane as elution solvents, and determined results gravimetrically. The 3M Corporation did not test samples for TPH as part of the Phase II study. Each sample was analyzed using either 47 mm SPE disks (20 samples) or 90 mm SPE disks (9 samples), the larger of the two being used for samples with high concentrations of particulates or matrices that might slow the extraction process. Each sample was analyzed in triplicate. As with the separatory funnel analysis, an individual split sample in its own container was supplied for each analysis.

Varian Sample Preparation Products extracted 33 aqueous samples with EnvirElute SPE cartridges using n-hexane, cyclohexane, methylene chloride, and pentane as elution solvents for gravimetric determination of both oil and grease and TPH. As with the separatory funnel analysis and SPE disk extractions, each of the samples was analyzed in triplicate.

3.1 Sample Source Selection

The Phase II study design required collection of a wide variety of sample matrices in order to better assess the effect of sample type on solvent extraction performance. Both effluent and in-process wastewater samples were collected from a variety of facilities. When selecting these sites, various factors were considered and included the following:

- The expected range of oil and grease concentrations, with a target range of 40 - 300 mg/L
- The goal of collecting both petroleum and non-petroleum based matrices
- Industrial categories with existing oil and grease effluent limitations
- Waste streams with known analytical interference problems, such as industrial laundries
- Accessibility of waste streams

Representatives of candidate facilities were initially contacted to request cooperation, verify plant and waste stream characteristics, and to coordinate sampling schedules. Facilities volunteering to participate were selected from EPA Regions I, II and III to minimize the costs of travel and equipment transportation. A summary of the wastewater sources is presented in Appendix A, which shows the facility types, industrial categories, and waste streams included in the study.

3.2 Sample Collection and Handling Activities

Field crews comprised of personnel from the Office of Water's Engineering and Analysis Division (EAD) and/or from EAD's Sample Control Center (SCC, operated by DynCorp Environmental) traveled to the selected sites and collected samples during one-day episodes. As with Phase I, the purpose of this sampling effort was to evaluate alternative extraction solvents and techniques, and not to characterize selected wastewaters for regulation development. Thus, short-term grab sampling of the selected sources was deemed sufficient to meet the requirements of the study.

Most of the industrial facilities selected for sampling were indirect dischargers to Publicly Owned Treatment Works, since these industrial facilities generated primary effluents that were more likely to contain oil and grease within the desired concentration range of this study. In some instances, untreated process waste streams were collected for analysis. In other instances, samples were collected as mixtures of treated and untreated streams to ensure that the desired oil and grease levels were obtained.

At each site, sample material was collected in a clean polyethylene barrel either by peristaltic pump or by hand with a clean polyethylene beaker. Transfer of material was minimized in order to prevent loss of extractable material during the collection process. To assure homogeneity, wastewater in the barrel was mixed by stirring with a polyethylene paddle while sample was siphoned from the center portion of the barrel directly into individual sample containers. Sample containers were unused, pre-cleaned, 1-liter, wide-mouth, clear glass bottles with PTFE-lined caps.

Each sample set collected at a facility included approximately sixty 1-liter aliquots. Aliquots were preserved on-site with HCl (1:1) to a pH of 2. Sample bottles were cooled with wet ice prior to

shipment to assigned laboratories. Each sample bottle was labeled prior to collection with a unique SCC sample number and identifying information, including source location, collection date, and preservatives used. Pre-assigned sample numbers were the primary method of identifying and tracking samples to ensure proper control. SCC Traffic Reports were completed at each site, and accompanied each shipment to the receiving laboratory. Copies of these reports were used by SCC field and office personnel for tracking purposes.

Information regarding site-specific activities were recorded on field log sheets for each sampling episode, and included SCC sample numbers, collection date and time, description of sample location, sample data, and preservatives used. Field personnel double-checked all labels and Traffic Reports to ensure accuracy and consistency. Communications were maintained among SCC field and office personnel and the receiving laboratories for sample tracking.

SECTION 4
DATA VALIDATION AND STATISTICAL ANALYSIS

This section describes the approaches to evaluation of the results from those analytical techniques specified in Section 2. Data validation, as detailed in Section 4.1, was straightforward. Statistical analysis, as detailed in Section 4.2, included preliminary screening of data to remove outliers. Several statistical evaluations were considered and are summarized below.

One measure of comparison, the "solvent-to-Freon ratio", indicates the amount of oil and grease or TPH extracted by a given solvent or technique relative to the amount extracted by separatory funnel Freon-113 extraction. A limitation of this measurement is evident when this ratio is averaged across samples, as is required to collectively assess the data. In the process of averaging, ratios above and below 1.00 offset one another to yield an average ratio biased toward 1.00, even when all of the individual sample ratios are significantly different than one, thereby generating a misleading indication of similarity to Freon-113 results.

A better indicator of similarity, though still not the most comprehensive, is the measurement of the median absolute deviation of the result obtained using the alternate solvent or technique from the result obtained using separatory funnel extraction with Freon-113. The median absolute deviation in a data set is that absolute deviation value below and above which 50% of the deviations fall.

The primary measure of agreement between alternative solvents or techniques and Freon-113 used in this study is the "normalized root mean square deviation" (RMSD). Analysis for this parameter was complicated by the non-Gaussian nature of the error distribution of the data, which necessitated logarithmic transformation of the data prior to performing this calculation. A more inclusive measurement than those previously noted, the RMSD accounts for the variability within the triplicate results of individual samples as well as the variability across samples (i.e., intra- and inter- sample variability, respectively).

The statistics cited above were also used to compare SPE results to the results produced by separatory funnel n-hexane extraction.

4.1 Data Validation

All data from Phase II of the study were submitted to the U.S. Environmental Protection Agency (EPA) by May 1994. The contract laboratory and other study participants submitted data in computer-readable or hardcopy formats. The computer-readable result files were verified against the hardcopy data, then loaded into the study database. Results were entered into the study database manually if no computer-readable data were provided. Hardcopy data, including all calculations submitted by the contract laboratory, were verified from the bench sheets provided.

A few of the samples containing low concentrations of oil and grease and/or TPH yielded negative results or results below the nominal detection limit of Method 413.1 for aqueous samples (5 mg/L). The negative numbers may have resulted from either the subtraction of blanks containing concentrations larger than those in the associated samples or from measurement error in the tare weights of the sample bottles (which weighed significantly more than the oil and grease residue, i.e., grams vs. milligrams, respectively). As a general rule, data results at or below the detection limit of 5 mg/L were set to 0.5 times the detection limit (i.e., 2.5 mg/L) for statistical analysis.

4.2 Statistical Analysis

Preliminary statistical analyses of gravimetric results from Phase II separatory funnel extraction and gravimetric determination were presented by the Office of Water (OW) at *EPA's 17th Annual Conference on Pollutants in the Environment* in Norfolk, VA on May 3, 1994. This report includes revised statistical analysis of those results as well as statistical analysis of the results generated by participating vendors using solid phase extraction (SPE) techniques. A table containing all results for the study in unreduced form is provided in Appendix B.

Outlier Screening

Prior to calculating the descriptive statistics (solvent-to-Freon ratios, solvent-to-hexane ratios and median absolute deviations), individual ratios were screened for outliers using a robust quantile method based on quartiles and the interquartile distance, as described in Appendix C. Ratios identified as outliers by this method were omitted from the remainder of the statistical analyses.

This screen estimates the limits above and below which lie one percent of the data, assuming the data are normally distributed. For the data sets evaluated in this study, the percentage of data points falling outside of the limits ranged from 3.4% to 15.3%. Most often, those data sets with higher percentages of outliers were associated with either of two conditions: values near or below the detection limit, where a small difference in concentration can result in a large difference in relative recovery; or highly variable data results from a particular solvent extraction, as was the case for methylene chloride/SPE cartridge analysis. Only those data points for a particular data set that were outside of the limits were removed. Data used in generating RMSDs was not subjected to this screen since the natural logarithm transformation of the data adequately reduced the effects of potential outliers on statistical analyses.

Data Stratification

Initial statistical analyses of all oil and grease and TPH results within each extraction technique yielded no solvents that were within the respective RMSD Acceptance Limits for Freon-113 equivalency. Further stratification of the study database was undertaken to determine whether results equivalent to Freon-113 could be achieved by testing subclasses of the data. Because it was expected that oil and grease of mineral origin (petroleum) might behave differently than oil and grease of biological origin (animal or vegetable), the data were stratified into "petroleum" and "non-petroleum" samples, respectively. For example, the effluent from a refinery was categorized as petroleum, whereas the effluent from a meat packing plant was categorized as non-petroleum. For the purposes of this report, data have been assessed in terms of three sample strata, which consist of 1) all samples, 2) petroleum samples, and 3) non-petroleum samples.

Solvent-to-Freon Ratio

A solvent-to-Freon ratio was computed to allow comparison of the average amount of oil and grease or TPH extracted by various solvents and measurement techniques with the amount extracted by the separatory funnel extraction technique using Freon-113. The solvent-to-Freon ratio for each sample was formed by dividing the mean of the triplicate results produced with the alternative solvent or technique by the mean of the triplicate results produced with the separatory funnel Freon-113 technique. The mean, standard deviation (SD), and relative standard deviation (RSD) of the solvent-to-Freon ratios were calculated across all samples in each of the three data sets representing an

alternative solvent or technique, i.e., separatory funnel, SPE disk, and SPE cartridge (Exhibits 1 through 8, presented in Section 5).

Because averaging gives no indication of the variability of the data, the solvent-to-Freon ratio is a less powerful measure of the agreement between an alternative solvent (or technique) and Freon-113 than the median absolute deviation and the normalized RMSD described below. Therefore, mean and standard deviation values of the solvent-to-Freon ratio were limited to use as an aid in describing the distribution of the data. The median absolute deviation and the normalized RMSD were used as the main criteria of similarity, with more emphasis being placed on the RMSD.

Median Absolute Deviation

The median absolute deviation provides a means of gauging the difference of the alternative solvent or technique results from separatory funnel Freon-113 results. The absolute deviation for each sample is determined according the formula: 100 x |solvent concentration/Freon concentration - 1|. The median absolute deviation in a data set is that value below and above which 50% of the deviations fall. Unlike the solvent-to-Freon ratio, this value is an absolute difference. Therefore, when averaged across all samples, alternative solvent or technique results that are greater than or less than Freon-113 results do not cancel each other. Though the median absolute deviation does provide an indication of central tendency for how different the alternative solvent results are in relation to the results from Freon-113, it does not consider the range of differences across all samples, and does not show how variable this measurement of difference is across all samples. Median absolute deviations were calculated across all samples for each of the three data sets and are presented in Exhibits 1 through 8.

Logarithmic Data Transformation

Analytical data often exhibit variability proportional to the magnitude of the signal over the calibration range of the measuring technique (i.e., as the concentration increases, the standard deviation will increase proportionally). Concentration dependent standard deviation was demonstrated in this study for Freon-113, n-hexane, and cyclohexane separatory funnel extraction (results are shown in Attachment 1-A of Appendix C). In order to evaluate data using Analysis of Variance (ANOVA) techniques, the results of which were required for the RMSD calculations described below, it was necessary to transform the data to eliminate the associated heteroscedasticity (non-Gaussian distribution of standard deviations) reflected by the concentration dependent standard deviations. This was accomplished by applying a natural log transformation using the following equation: $z = \ln(x)$, where x is the concentration (in mg/L). Results are shown in Attachment 1-B of Appendix C.

As stated in Section 4.1, data results at or below the detection limit of 5 mg/L were set to 0.5 times the detection limit (i.e., 2.5 mg/L). The transformed data (z) were then subjected to ANOVA.

Root Mean Square Deviation (RMSD)

The primary measure of similarity used to compare each of the other solvents and techniques to separatory funnel Freon-113 extraction was the RMSD. For each technique, the RMSD represents the standard deviation of the differences between the alternative solvent-determined concentrations for each sample and the Freon-113 separatory funnel extraction determinations. A smaller RMSD indicates better agreement with the Freon-113 separatory funnel procedure.

In performing this statistical analysis, the data were transformed using the logarithmic equation listed above and were subjected to ANOVA. The triplicate concentration results for each sample and solvent or technique were then averaged. For each alternative procedure, the RMSD was calculated by taking the difference of the Freon-113 averages from those of the alternative procedure, squaring the

differences, averaging these squared differences across all samples, and then taking the square root of those averages. Details of this calculation are presented in Appendix C. The resulting RMSD accounts for variations in the alternative solvent/technique results regardless of whether they are above or below the results from the Freon-113 extractions.

The RMSD values were normalized by dividing each RMSD by the square root of the residual error estimate based on the replicate variability (see Appendix C for details). Normalization was implemented to correct for any background variability produced by the procedure itself. This ensures that the variability measured is isolated to the use of a particular solvent and/or technique.

To determine the significance of the RMSD results, a 95 percent Acceptance Limit was calculated from each of the three data sets (separatory funnel extraction, 3M SPE disks, Varian SPE cartridges) for the three sample strata (all samples, petroleum samples, and non-petroleum samples). The Acceptance Limit is the 95th percentile of the normalized RMSD that would be observed if the two methods under comparison were exactly equivalent (i.e., if Freon-113 were compared to itself 100 times, 95 times the RMSD would be at or below the Acceptance Limit). Solvents or techniques with RMSDs less than or equal to the associated Acceptance Limit are not statistically different from Freon-113 extraction with the approved technique. Normalized RMSD results and Acceptance Limits for each of the alternative solvent and measurement techniques evaluated are presented in Exhibits 1 through 8.

As was stated in the introduction to Section 4, the statistical analyses are described above in the context of comparing one or more techniques or solvents to the separatory funnel Freon-113 determination of oil and grease and TPH. The statistical analyses described above were also used to compare the SPE disk and cartridge techniques to separatory funnel extraction using n-hexane (i.e., all statements made above for Freon-113 apply to n-hexane).

The statistical results presented in Exhibits 1 through 8 are summarized in Sections 5.1 through 5.3 below. As explained in Section 4, the median absolute deviation and root mean square deviation (RMSD) were used as the main criteria of equivalence between the results obtained with established techniques (separatory funnel Freon-113 extraction and gravimetric determination) and all other solvents and techniques. Other descriptive statistics (mean, standard deviation, and relative standard deviation of solvent-to-Freon and solvent-to-n-hexane ratios) were provided in this report to demonstrate the general distribution of the data.

Exhibits 1 through 8 are arrayed to provide a comparison of each alternative solvent or technique for the three sample strata, i.e., 1) all samples, 2) petroleum samples, and 3) non-petroleum samples. Solvents or techniques associated with results that produced an RMSD within the Acceptance Limit for separatory funnel extraction using Freon-113 were not considered to be statistically different from Freon-113. As was stated earlier, SPE disk and cartridge techniques were also compared to separatory funnel n-hexane extraction.

5.1 Separatory Funnel Extraction

Oil and Grease

Results of the statistical analysis of data for separatory funnel extraction and gravimetric determination of oil and grease are presented in Exhibit 1. These results were based on the analysis of 18 petroleum samples and 15 non-petroleum samples, for a total of 33 samples (one of the 34 samples collected was not successfully analyzed due to the formation of severe emulsions), using Freon-113, n-hexane, and cyclohexane as extraction solvents. In some cases the number of samples listed in Exhibit 1 were less than 18 and 15, respectively, due to the exclusion of sample results on a solvent-by-stratum-specific basis by the outlier screening process.

Mean solvent-to-Freon ratios ranged from 0.74 (for petroleum samples extracted with n-hexane) to 0.85 (for non-petroleum samples extracted with cyclohexane). Relative standard deviations of the solvent-to-Freon ratios were between 25% and 31%; median absolute deviations ranged from 17.6% to 25.6%. The similar values in Exhibit 1 suggest that, on average, n-hexane and cyclohexane extracted less oil and grease than Freon-113 and produced results which vary by approximately 20% from those produced by Freon-113.

RMSD values demonstrate that when all samples were examined either as a group or on a petroleum or non-petroleum sample basis, neither of the solvents tested yielded results within the Acceptance Limit.

To determine if either alternative solvent performed more similarly to Freon, a paired t-test across all samples was used to compare the n-hexane-to-Freon ratio with the cyclohexane-to-Freon ratio for the analysis of oil and grease. The results, shown in Table 1, indicate that there was no statistical difference in the performance of the two solvents.

Total Petroleum Hydrocarbons (TPH)

Results of the statistical analysis of data for separatory funnel extraction and gravimetric determination of TPH are presented in Exhibit 2. As with oil and grease determination, the results

were based on analysis of 18 petroleum and 15 non-petroleum samples (though the number of samples listed may be less due to outlier screening).

Mean solvent-to-Freon ratios ranged from 0.80 (for all samples extracted with n-hexane) to 1.40 (for non-petroleum samples extracted with cyclohexane). Relative standard deviations of the solvent-to-Freon ratios ranged from 21% to 70%, notably wider than that for oil and grease. Median absolute deviations ranged from 14.1% to 40.4%. The data in Exhibit 2 suggest that n-hexane and cyclohexane performed similarly for petroleum samples, but that cyclohexane produced more deviate results than n-hexane in non-petroleum samples, though these differences were not statistically significant.

RMSD values demonstrate that when all samples were examined either as a group, or on a petroleum or non-petroleum sample basis, neither of the solvents tested yielded results within the Acceptance Limit.

As with oil and grease analyses, a paired t-test across all samples was used to compare the n-hexane-to-Freon ratio with the cyclohexane-to-Freon ratio for the analysis of TPH. The results, shown in Table 2, indicate that there was no statistical difference in the performance of the two solvents.

5.2 Solid Phase Extraction (SPE) Using Disks

Exhibits 3 and 4 present the results of the statistical analysis of data generated by 3M Corporation for oil and grease determination using solid phase extraction (SPE) disks and gravimetric determination. As was noted in Section 2.3, n-hexane and cyclohexane were evaluated with the SPE disk technique; Freon-113 was not evaluated with the SPE disks in this study. Analysis included determination of oil and grease only; TPH was not determined as part of the Phase II study. All results generated using SPE disk techniques were compared to the performance of separatory funnel extraction using both Freon-113 and n-hexane. SPE disk results were not compared to those from SPE cartridge techniques.

Statistical results were based on the analysis of 14 petroleum and 14 non-petroleum samples. In some cases the number of samples listed in Exhibits 3 and 4 are less than 14 due to the exclusion of sample results on a solvent-by-stratum-specific basis by the outlier screening process.

Comparison to Separatory Funnel Extraction Using Freon-113

Mean solvent-to-Freon ratios in Exhibit 3 for the SPE disk data using n-hexane and cyclohexane as elution solvents were all below 1.0, ranging from 0.82 (for petroleum samples extracted with disk + n-hexane) to 0.96 (for all samples extracted with disk + cyclohexane). This indicates that, on average, SPE disks extracted less oil and grease than separatory funnel extraction using Freon-113. RSDs of the solvent-to-Freon ratios ranged from 29% to 54%. Median absolute deviations ranged between 22.9% and 29.7%, slightly higher than those for separatory funnel extraction using the same solvents.

RMSD values in Exhibit 3 for the use of SPE disks were higher than the Acceptance Limit in all three sample strata, indicating that the results were not equivalent to separatory funnel extraction using Freon-113. Of the two solvents, cyclohexane performed more similarly to separatory funnel extraction in all three strata.

Comparison to Separatory Funnel Extraction Using n-hexane

Mean solvent-to-hexane ratios in Exhibit 4 for the SPE disk data using alternative solvents were all above 1.00, ranging from 1.05 (for non-petroleum samples extracted with disk + cyclohexane) to 1.22 (for petroleum samples extracted with disk + cyclohexane). This indicates that, on average, SPE disks extracted more oil and grease than did separatory funnel extraction using n-hexane. RSDs of the

solvent-to-hexane ratios ranged from 31% to 55%. Median absolute deviations ranged between 25.5% and 47.1%

RMSD values in Exhibit 4 for the use of SPE disks were higher than the Acceptance Limit in all three sample strata, indicating that the results were not equivalent to separatory funnel extraction using n-hexane. Of the two solvents, cyclohexane performed more similarly to separatory funnel extraction in all three strata.

5.3 Solid Phase Extraction (SPE) Using Cartridges

Exhibits 5 through 8 present the results of the statistical analyses of data generated by Varian Sample Preparation Products using SPE cartridges and gravimetric determination. As was stated in Section 2.3, four solvents (n-hexane, cyclohexane, pentane, and methylene chloride) were evaluated with this technique. Both oil and grease and TPH were determined. All results generated using SPE cartridges were compared to the performance of separatory funnel extraction using both Freon-113 and n-hexane. SPE cartridge results were not compared to those from SPE disk techniques.

Statistical results were based on the analysis of 14 petroleum and 14 non-petroleum samples. In some cases the number of samples listed in Exhibits 5 through 8 were less than 14 due to the exclusion of sample results on a solvent-by-stratum-specific basis from the outlier screening process.

Comparison to Separatory Funnel Extraction Using Freon-113

Oil and Grease

Mean solvent-to-Freon ratios in Exhibit 5 ranged from 0.80 (for petroleum samples extracted with cartridge + pentane) to 1.90 (for non-petroleum samples extracted with cartridge + methylene chloride). With the exception of methylene chloride extraction for all three sample strata, all solvent-to-Freon ratios were below 1.00, indicating that, on average, SPE cartridges extracted less oil and grease than separatory funnel extraction using Freon-113. RSDs of the solvent-to-Freon ratios ranged from 18% to 66%. Median absolute deviations ranged between 15.1% and 89.9%, appreciably higher than those for separatory funnel extraction.

RMSDs in Exhibit 5 for the SPE cartridge results were higher than the Acceptance Limits in all three strata, indicating that results were not equivalent to Freon-113. Of the four solvents, cyclohexane performed closest to separatory funnel extraction for oil and grease using Freon-113 for all three sample strata.

Total Petroleum Hydrocarbons

Mean solvent-to-Freon ratios in Exhibit 6 ranged from 0.44 (for non-petroleum samples extracted with cartridge + n-hexane) to 1.04 (for petroleum samples extracted with cartridge + methylene chloride). With the exception of petroleum samples extracted with methylene chloride, all solvent-to-Freon ratios were below 1.00, indicating that, on average, SPE cartridges extracted less TPH than separatory funnel extraction using Freon-113. RSDs of the solvent-to-Freon ratios with the SPE cartridges ranged from 41% to 65%. Median absolute deviations ranged between 18.3% and 60.0%, higher than those for separatory funnel extraction.

For all three sample strata, RMSDs in Exhibit 6 for the SPE cartridge results were higher than the Acceptance Limits, indicating that results were not equivalent to Freon-113. Of the four solvents, cyclohexane performed closest to separatory funnel extraction of TPH using Freon-113 for all samples and non-petroleum samples, while methylene chloride performed closest for petroleum samples.

Comparison to Separatory Funnel Extraction Using n-hexane

Oil and Grease

Mean solvent-to-hexane ratios in Exhibit 7 ranged from 0.89 (for non-petroleum samples extracted with cartridge + n-hexane) to 2.12 (for non-petroleum samples extracted with cartridge + methylene chloride). RSDs of the solvent-to-hexane ratios ranged from 18% to 51%. Median absolute deviations ranged between 13.5% and 133.9%.

RMSDs in Exhibit 7 for the SPE cartridge results were higher than the Acceptance Limits in all three strata, indicating that results were not equivalent to separatory funnel extraction using n-hexane. Of the four solvents, cyclohexane performed closest to separatory funnel extraction for oil and grease using n-hexane for all samples and non-petroleum samples, while n-hexane came closest for petroleum samples.

Total Petroleum Hydrocarbons

Mean solvent-to-hexane ratios in Exhibit 8 ranged from 0.70 (for non-petroleum samples extracted with cartridge + n-hexane) to 1.31 (for non-petroleum samples extracted with cartridge + pentane). RSDs of the solvent-to-hexane ratios with the SPE cartridges ranged from 38% to 89%. Median absolute deviations ranged between 21.6% and 79.9%.

RMSDs in Exhibit 8 for the SPE cartridge results were higher than the Acceptance Limits in all three sample categories, indicating that results were not equivalent to n-hexane. Of the four solvents, methylene chloride performed closest to separatory funnel extraction of TPH using *n*-hexane for all samples and petroleum samples, while cyclohexane performed closest for non-petroleum samples.

5.4 Graphical Presentation of the Solvent-to-Freon Ratios

Exhibit 9 summarizes on one graph the solvent-to-Freon ratios when considering all samples collectively for the extraction of oil and grease by all techniques. In this graph, the mean solvent-to-Freon ratio was plotted on a logarithmic scale so that reciprocal ratios were equidistant from 1.00, regardless of whether or not the solvent results were greater than or less than the Freon-113 results (e.g., a solvent result that is half as much as the Freon-113 result is the same distance from 1.00 as a solvent result that is twice as much as the Freon-113 result). These conditions apply to the graphs described below for TPH analysis and in Section 5.5 for solvent-to-hexane ratios.

Exhibit 9 shows that the solvent-to-Freon ratio ranges from approximately 0.76 to 1.69, depending on the solvent and technique. The use of n-hexane and cyclohexane in both separatory funnel extraction and SPE disk and cartridge techniques allowed the variables of solvent and technique to be considered independently of one another. This graph demonstrates that separatory funnel extraction with either n-hexane or cyclohexane extracted less oil and grease, on average, than SPE using either disks or cartridges.

Exhibit 10 summarizes the mean solvent-to-Freon ratios for the extraction of TPH. The solvent-to-Freon ratio ranged between 0.72 and 0.95, narrower than that for oil and grease. Since analysis for TPH was not performed using SPE disks as part of the Phase II study, only separatory funnel extraction and SPE cartridge extraction techniques could be compared directly for n-hexane and cyclohexane use. Contrary to the results from oil and grease analysis, separatory funnel extraction with either n-hexane or cyclohexane extracted more TPH, on average, than SPE cartridges.

5.5 Graphical Presentation of the Solvent-to-Hexane Ratios

Exhibit 11 summarizes the solvent-to-hexane ratios for the extraction of oil and grease. The conditions described above for the solvent-to-Freon ratio graphs apply to the graphs addressed in this section. The solvent-to-hexane ratios for the three techniques ranged from 1.02 to 1.93, higher than the solvent-to-Freon ratios. Because solvent-to-hexane ratios were not determined for separatory funnel extraction (i.e., hexane and cyclohexane results were not directly compared), the variables of solvent and technique could not be separated for separatory funnel comparison to SPE disk and cartridge techniques. A comparable graph for TPH analysis was not prepared, since the only alternative technique by which TPH analyses were run as part of the Phase II study was the SPE cartridge.

5.6 Graphical Presentation of RMSD Versus Acceptance Limit Results

To provide a better understanding of the relative performance of all of the alternative solvents and techniques evaluated, the RMSD and Acceptance Limit data are presented graphically in Exhibits 12 through 19. Separate graphs were prepared for each combination of extraction technique (i.e., separatory funnel, SPE disk, or SPE cartridge), oil and grease or TPH determination, and Freon-113 or n-hexane Acceptance Limits.

In these Exhibits, the RMSD for a particular solvent or technique is represented by a solid or hollow square (for petroleum and non-petroleum sample categories, respectively), and Acceptance Limits are indicated by a horizontal dotted or horizontal dashed line (for petroleum and non-petroleum sample categories, respectively). Where these RMSD squares fall within (i.e., at or below) the relevant Acceptance Limit line, that solvent or technique is equivalent to Freon-113 separatory funnel extraction. Where no solvents or techniques yielded results equivalent to Freon-113, no squares are within the Acceptance Limit line.

Exhibit 1
Summary Statistics For Alternative Solvents in the Determination of Oil and Grease by Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon	33	1.00	--	--	0.0	1.22*
Hexane	29	0.76	0.20	26	21.8	4.50
Cyclohexane	32	0.83	0.22	27	18.4	3.34
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon	15	1.00	--	--	0.0	1.33*
Hexane	13	0.80	0.21	26	25.3	4.08
Cyclohexane	14	0.85	0.26	31	25.6	2.80
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon	18	1.00	--	--	0.0	1.30*
Hexane	16	0.74	0.19	26	19.2	5.15
Cyclohexane	18	0.81	0.20	25	17.6	4.14

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Freon Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 2
Summary Statistics For Alternative Solvents in the Determination of TPH by Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon	33	1.00	--	--	0.0	1.22*
Hexane	29	0.80	0.22	28	20.2	1.68
Cyclohexane	28	0.95	0.36	38	21.9	2.66
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon	15	1.00	--	--	0.0	1.33*
Hexane	15	1.12	0.74	66	40.4	1.53
Cyclohexane	13	1.40	0.99	70	31.7	2.70
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon	18	1.00	--	--	0.0	1.30*
Hexane	18	0.85	0.22	26	16.2	2.61
Cyclohexane	16	0.85	0.18	21	14.1	2.47

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Freon Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 3
Summary Statistics For Alternative Techniques in the Determination of Oil and Grease by Solid Phase Extraction Disks - Comparison to Freon Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	26	1.00	--	--	0.0	1.25*
Hexane	23	0.88	0.42	48	29.7	6.12
Cyclohexane	25	0.96	0.44	46	27.2	5.35
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	13	1.00	--	--	0.0	1.36*
Hexane	11	0.85	0.41	48	29.7	6.29
Cyclohexane	12	0.91	0.49	54	27.2	6.02
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	13	1.00	---	---	0.0	1.36*
Hexane	11	0.82	0.30	37	28.9	5.85
Cyclohexane	11	0.87	0.25	29	22.9	4.13

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Freon Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 4
Summary Statistics For Alternative Techniques in the Determination of Oil and Grease by Solid Phase Extraction Disks - Comparison to n-hexane Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	26	1.00	--	--	0.0	1.25*
Hexane	24	1.12	0.50	45	38.0	6.22
Cyclohexane	24	1.13	0.47	42	28.7	5.29
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	13	1.00	--	--	0.0	1.36*
Hexane	12	1.08	0.56	52	47.1	5.59
Cyclohexane	12	1.05	0.55	55	40.2	5.10
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	13	1.00	--	--	0.0	1.36*
Hexane	12	1.17	0.45	38	33.1	7.25
Cyclohexane	12	1.22	0.38	31	25.5	5.63

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Hexane Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 5
Summary Statistics For Alternative Techniques in the Determination of Oil and Grease by Solid Phase Extraction Cartridges - Comparison to Freon Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	27	1.00	--	--	0.0	1.24*
Hexane	26	0.88	0.48	55	42.0	5.28
Cyclohexane	25	0.90	0.37	41	20.4	3.99
Pentane	25	0.85	0.34	40	31.0	4.05
Methylene Chloride	25	1.69	0.64	38	48.7	5.47
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	14	1.00	--	--	0.0	1.33*
Hexane	13	0.91	0.60	66	45.1	5.29
Cyclohexane	13	0.94	0.52	55	47.3	4.23
Pentane	13	0.89	0.54	61	41.9	4.28
Methylene Chloride	13	1.90	0.69	36	89.9	5.28
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	13	1.00	---	---	0.0	1.34*
Hexane	13	0.85	0.34	40	29.2	5.02
Cyclohexane	12	0.88	0.20	23	15.1	3.24
Pentane	11	0.80	0.14	18	20.0	3.32
Methylene Chloride	11	1.33	0.30	23	24.4	5.77

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Freon Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 6
Summary Statistics For Alternative Techniques in the Determination of TPH by Solid Phase Extraction Cartridges - Comparison to Freon Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	27	1.00	--	--	0.0	1.24*
Hexane	17	0.72	0.41	57	39.0	3.50
Cyclohexane	18	0.81	0.45	56	39.8	3.23
Pentane	18	0.78	0.42	54	31.5	3.43
Methylene Chloride	18	0.84	0.47	56	46.1	3.45
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	14	1.00	--	--	0.0	1.34*
Hexane	7	0.44	0.23	52	54.3	3.21
Cyclohexane	7	0.54	0.29	54	45.6	2.85
Pentane	7	0.51	0.33	65	60.0	3.15
Methylene Chloride	7	0.51	0.28	55	47.4	3.24
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Freon Sep. Funnel	14	1.00	--	--	0.0	1.35*
Hexane	10	0.92	0.39	42	27.5	4.43
Cyclohexane	11	0.98	0.45	46	26.0	4.43
Pentane	11	0.95	0.39	41	18.3	4.31
Methylene Chloride	11	1.04	0.46	44	33.1	4.06

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Freon Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 7
Summary Statistics For Alternative Techniques in the Determination of Oil and Grease by Solid Phase Extraction Cartridges - Comparison to n-hexane Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	27	1.00	--	--	0.0	1.24*
Hexane	26	1.02	0.47	46	27.0	4.68
Cyclohexane	25	1.07	0.43	40	32.2	3.66
Pentane	22	1.04	0.21	20	13.5	3.98
Methylene Chloride	24	1.93	0.72	37	79.4	7.34
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	14	1.00	--	--	0.0	1.33*
Hexane	13	0.89	0.45	51	36.5	4.81
Cyclohexane	13	0.94	0.44	47	41.0	3.15
Pentane	13	0.90	0.36	40	14.2	3.65
Methylene Chloride	13	2.12	0.90	42	133.9	6.31
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	13	1.00	--	--	0.0	1.34*
Hexane	11	1.01	0.27	27	21.2	4.01
Cyclohexane	11	1.13	0.30	27	20.4	4.80
Pentane	11	1.06	0.19	18	13.5	4.77
Methylene Chloride	11	1.71	0.34	20	75.6	9.60

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Hexane Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 8
Summary Statistics For Alternative Techniques in the Determination of TPH by Solid Phase Extraction Cartridges - Comparison to n-hexane Separatory Funnel Extraction

All Samples						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	27	1.00	--	--	0.0	1.24
Hexane	18	1.03	0.58	56	33.9	2.93
Cyclohexane	19	1.03	0.50	49	24.8	2.63
Pentane	19	1.07	0.61	57	40.4	2.84
Methylene Chloride	20	1.19	0.73	61	45.2	2.55
Non-Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	14	1.00	--	--	0.0	1.34*
Hexane	7	0.70	0.47	67	28.5	2.65
Cyclohexane	8	0.87	0.50	57	21.6	2.30
Pentane	9	1.31	1.16	89	79.9	2.63
Methylene Chloride	9	1.18	0.99	84	71.1	2.32
Petroleum						
Solvent	N	Mean	SD	RSD (%)	Median Deviation (%)	RMSD
Hexane Sep. Funnel	13	1.00	--	--	0.0	1.35*
Hexane	10	1.12	0.43	38	33.9	3.81
Cyclohexane	11	1.14	0.50	44	35.1	3.70
Pentane	10	1.11	0.44	40	34.6	3.53
Methylene Chloride	11	1.20	0.48	40	25.1	3.30

* Acceptance Limit

N = Number of Samples
Mean = Mean of Solvent to Freon Ratios
SD = Standard Deviation of Solvent to Freon Ratios
RSD = Relative Standard Deviation of Solvent to Freon Ratio = 100 x SD/Mean
Median Deviation = median, across samples, of 100 x | Solvent to Hexane Ratio - 1 |
RMSD = Normalized Root Mean Square Deviation of Sample x Solvent Means

Exhibit 9

Mean Solvent-to-Freon Separatory Funnel Extraction Ratio for Oil & Grease Determinations

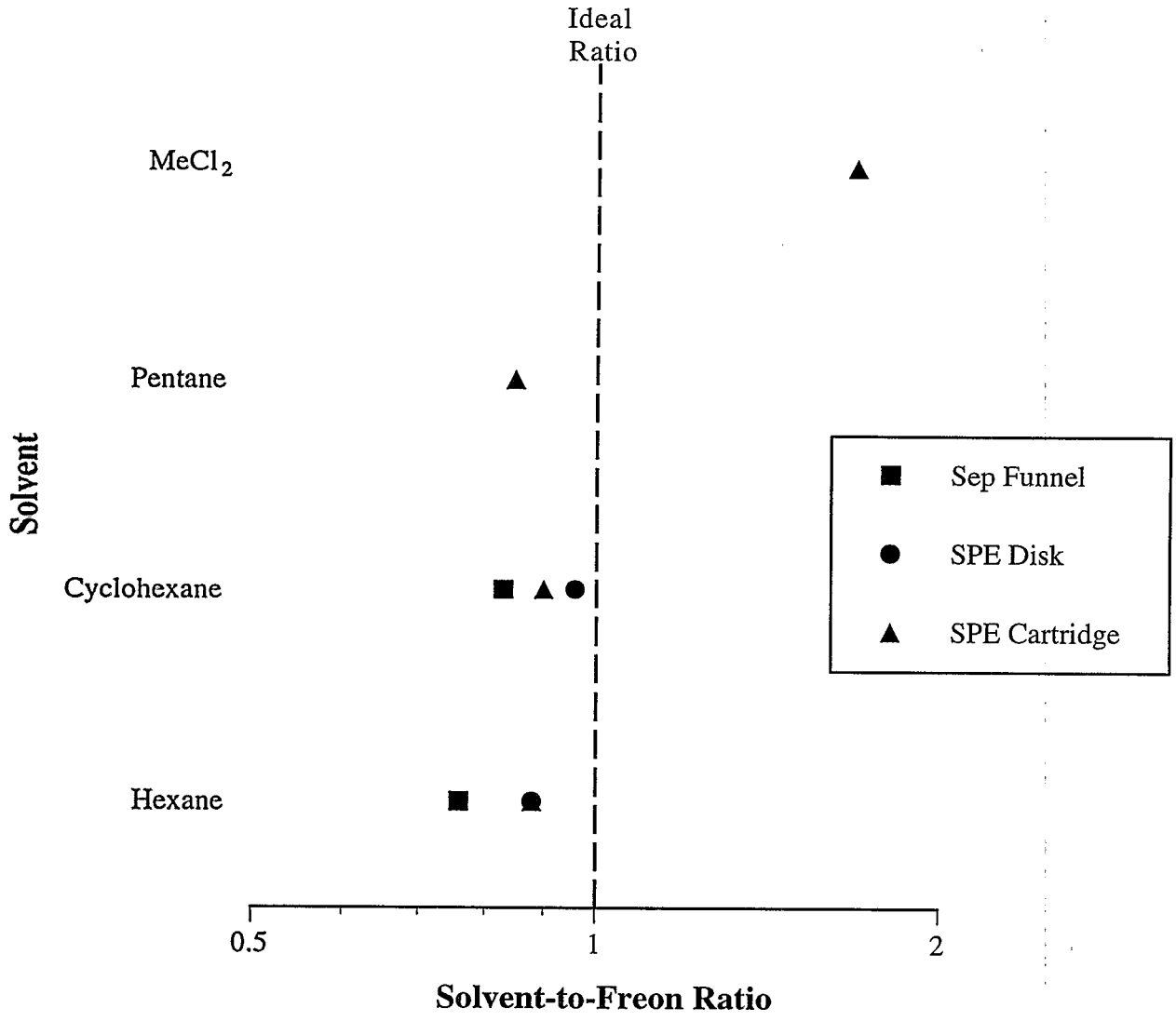


Exhibit 10

Mean Solvent-to-Freon Separatory Funnel Extraction Ratio for TPH Determinations

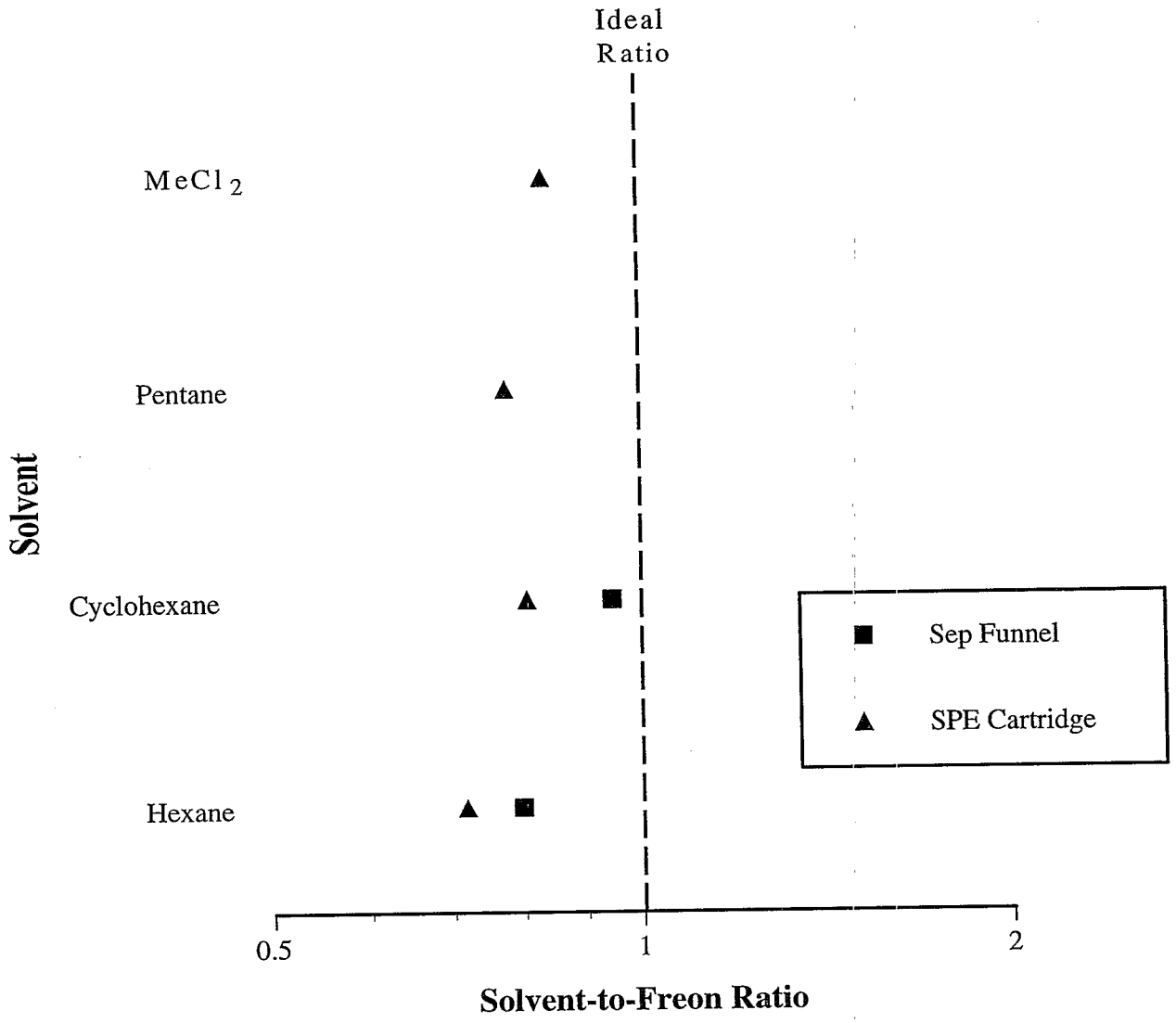


Exhibit 11

Mean Solvent-to-Hexane Separatory Funnel Extraction Ratio for Oil & Grease Determinations

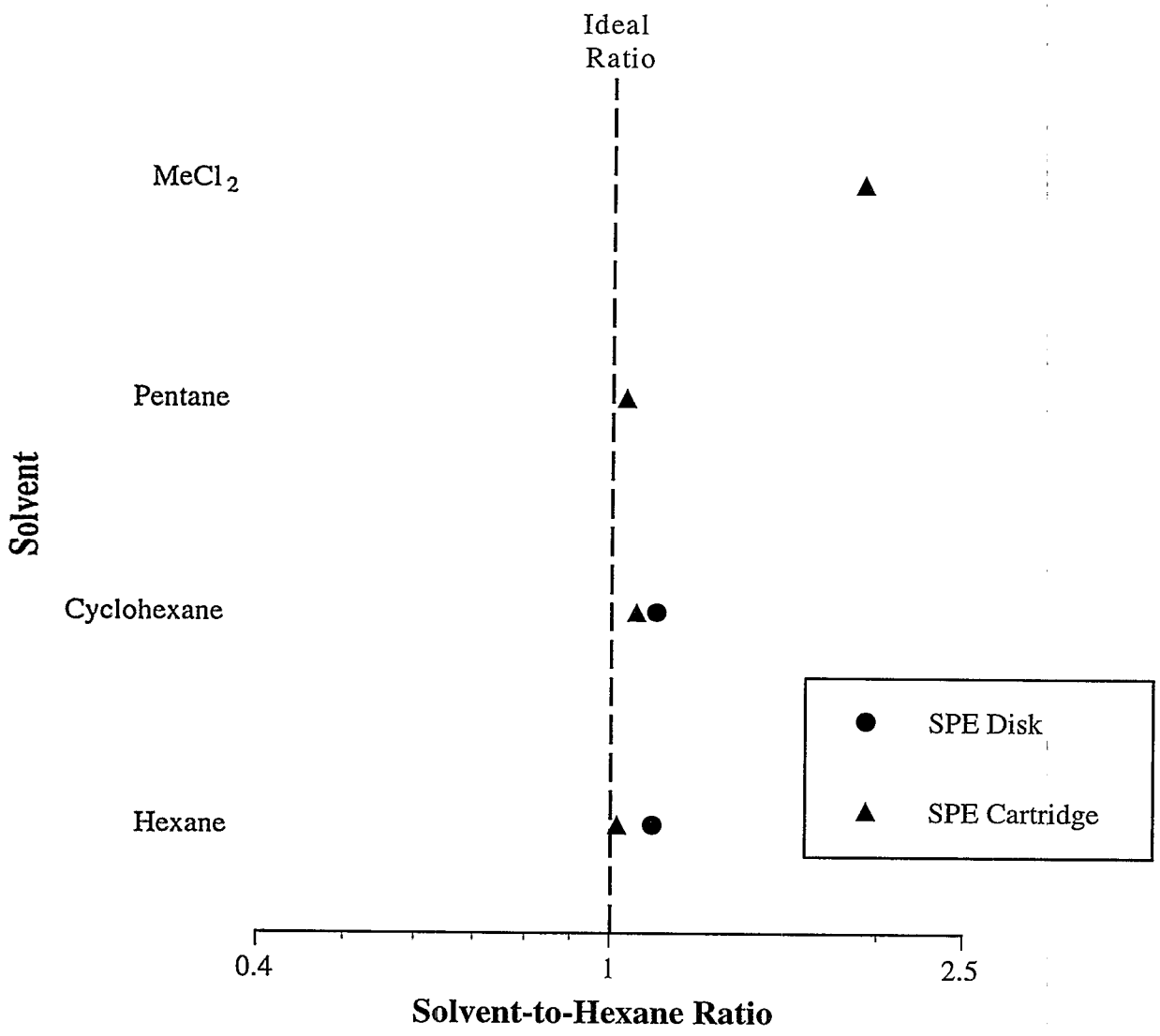
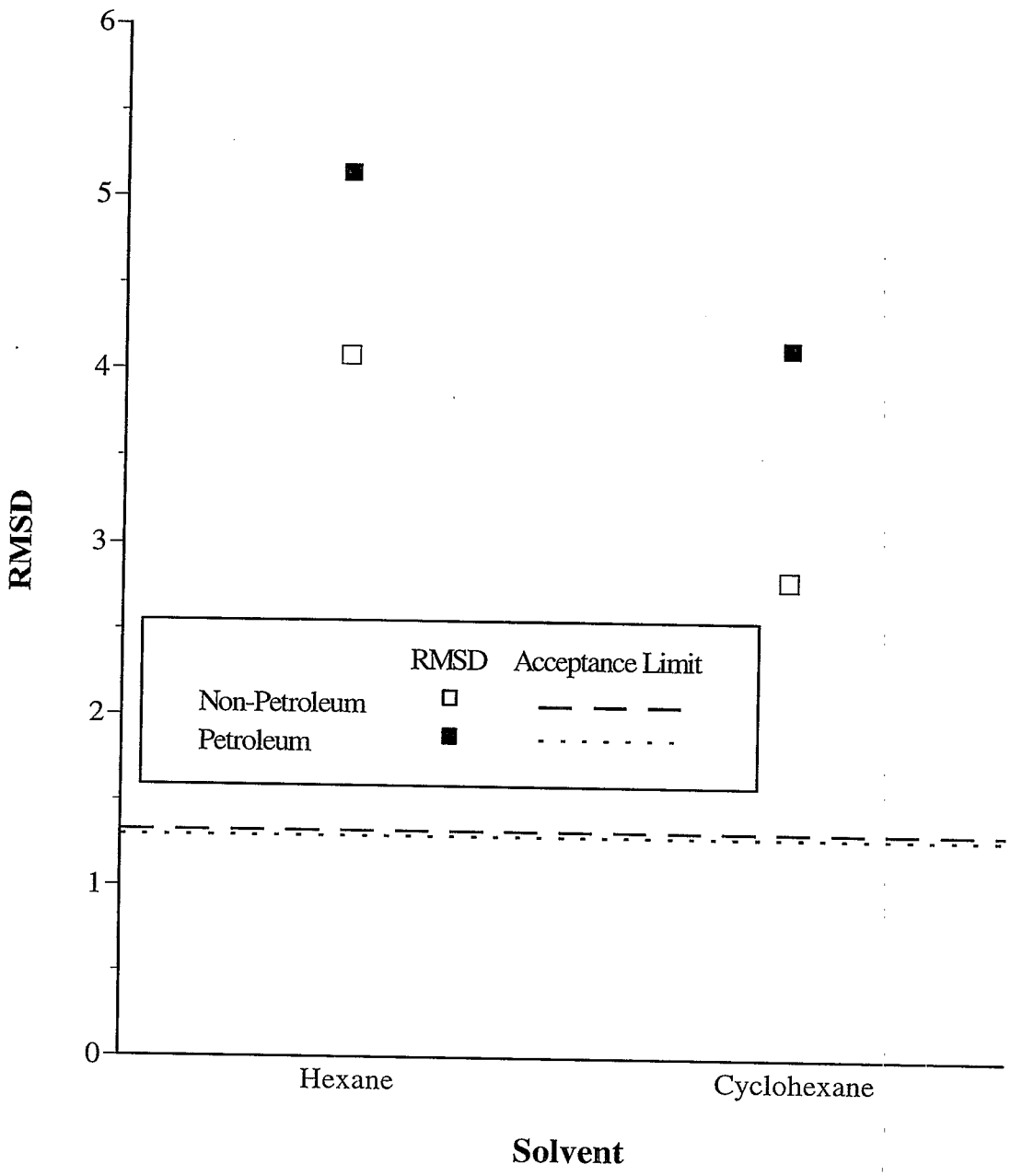


Exhibit 12

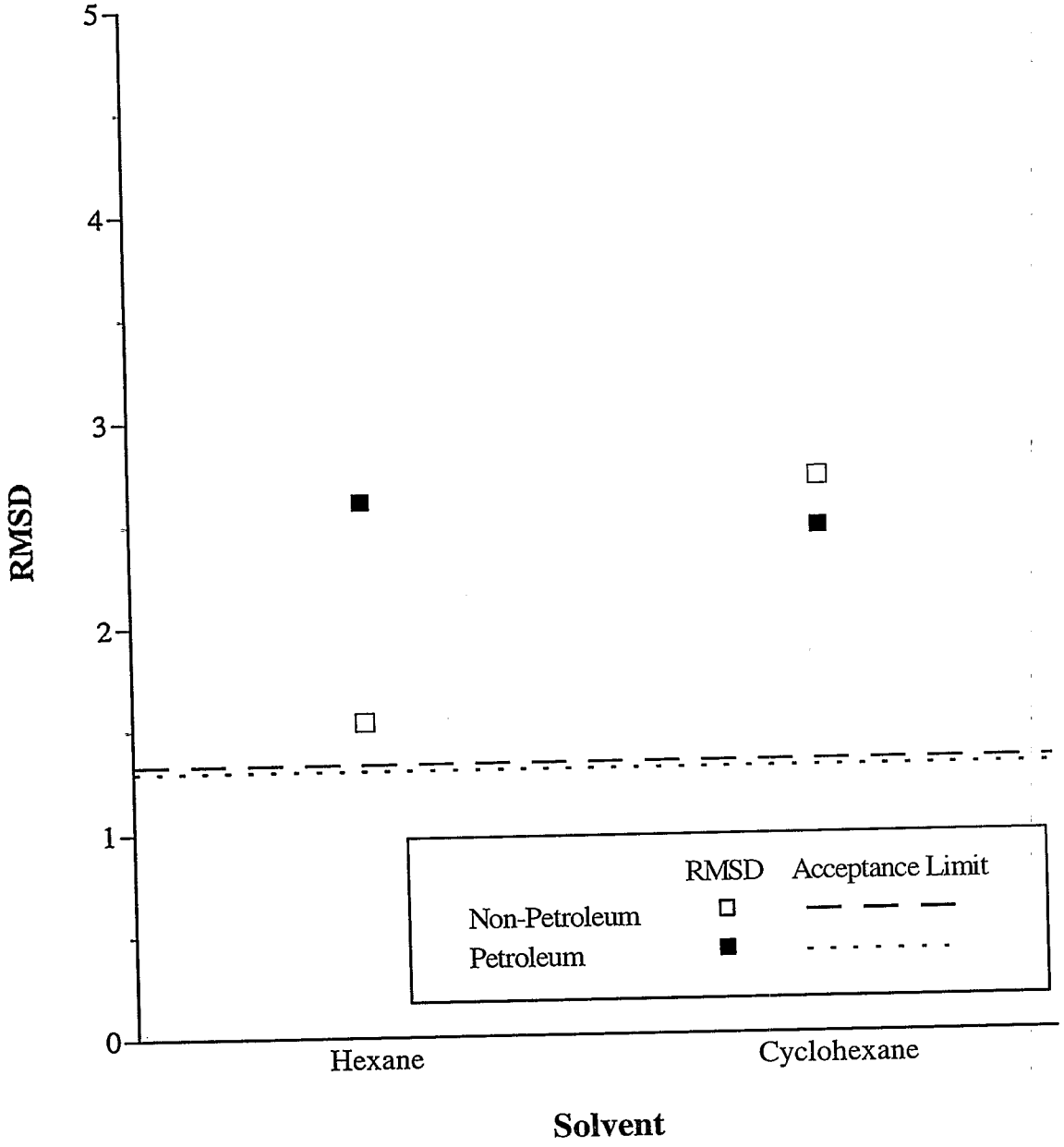
Normalized Root Mean Square Deviations
Oil & Grease Determinations By Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Freon

Exhibit 13

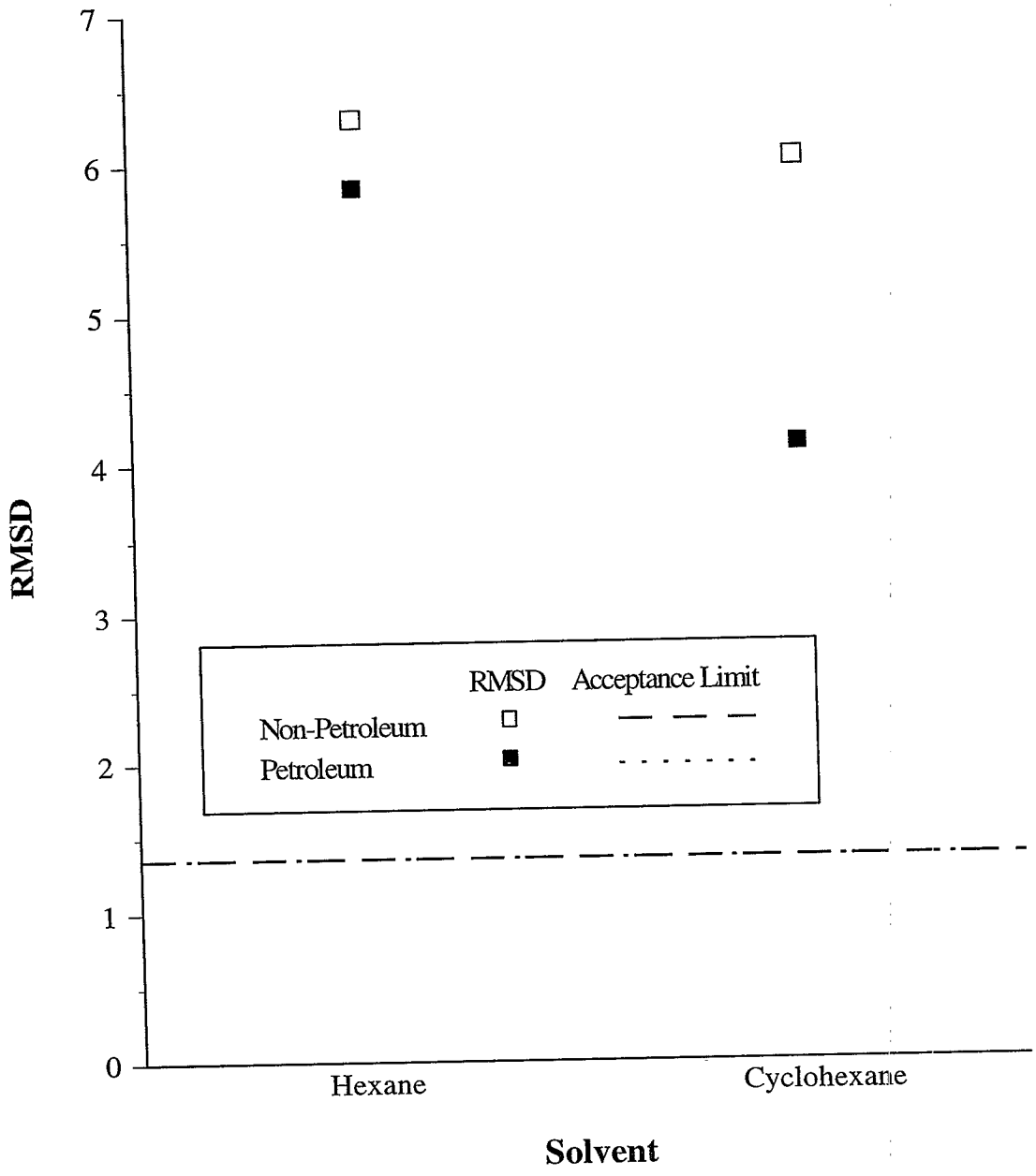
Normalized Root Mean Square Deviations
TPH Determinations By Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Freon

Exhibit 14

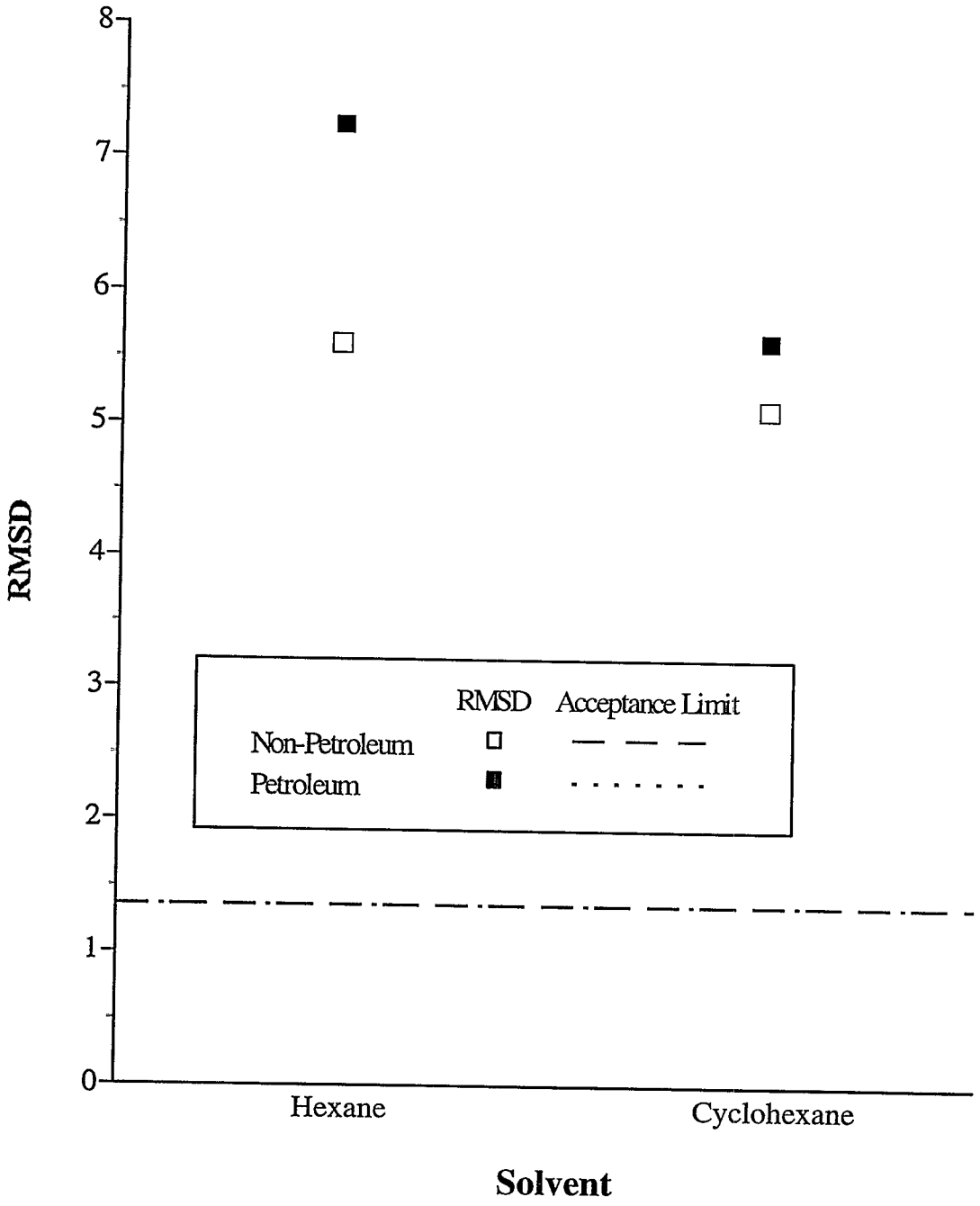
Normalized Root Mean Square Deviations
Oil & Grease Determinations By Solid Phase Extraction Disks
Relative to Freon-113 Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Freon

Exhibit 15

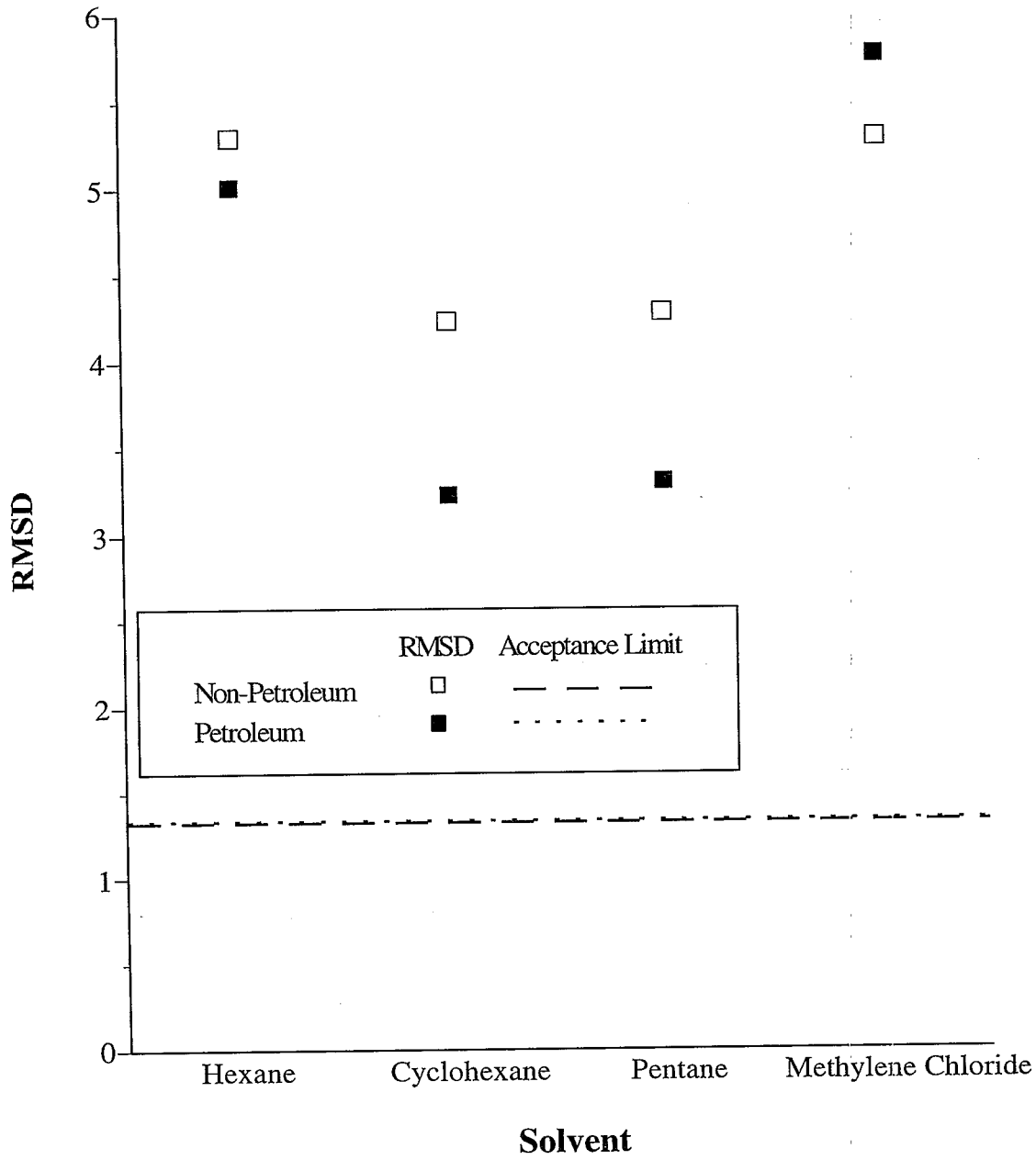
Normalized Root Mean Square Deviations
Oil & Grease Determinations By Solid Phase Extraction Disks
Relative to Hexane Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Hexane

Exhibit 16

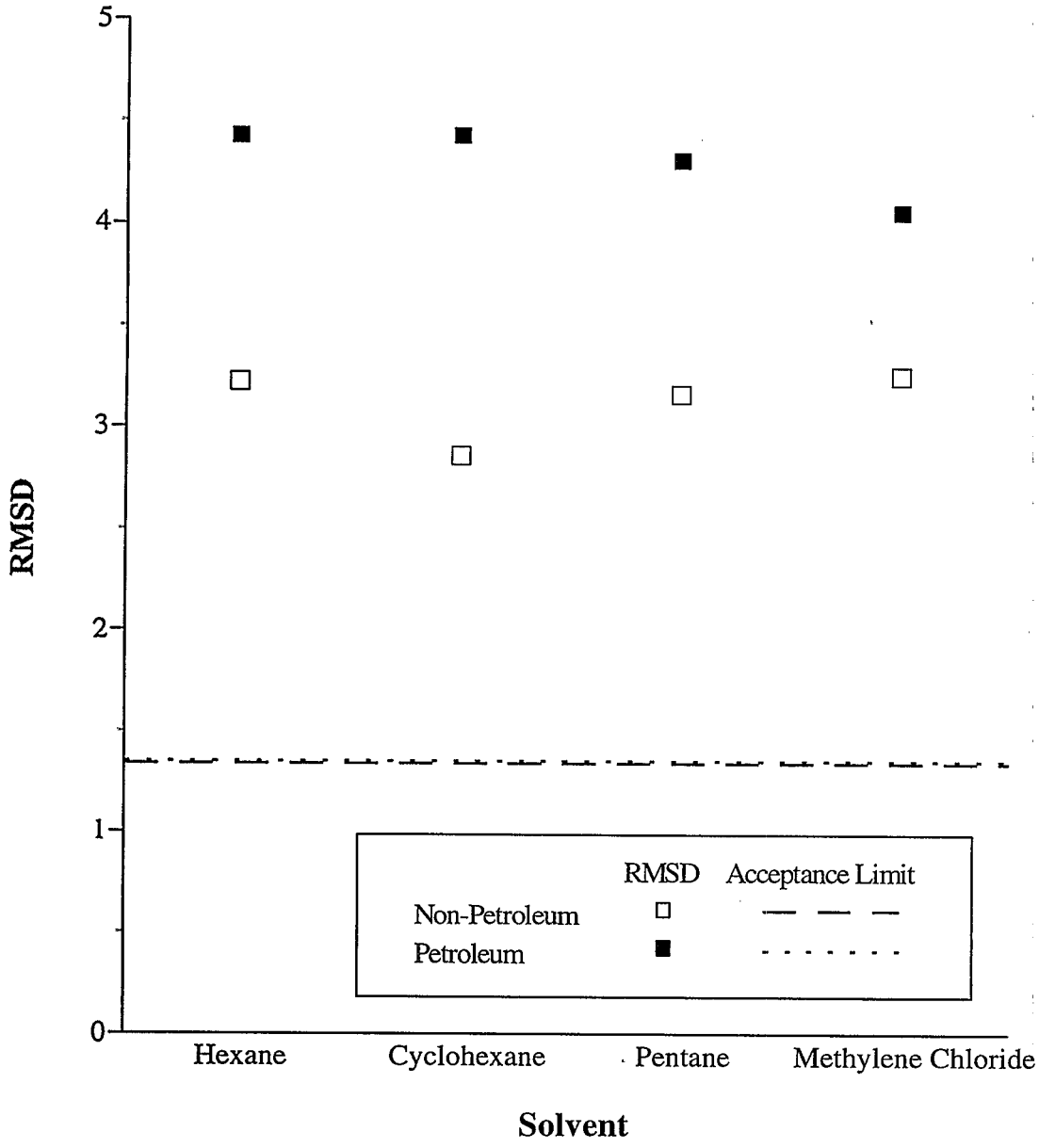
Normalized Root Mean Square Deviations
Oil & Grease Determinations By Solid Phase Extraction Cartridges
Relative to Freon-113 Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Freon

Exhibit 17

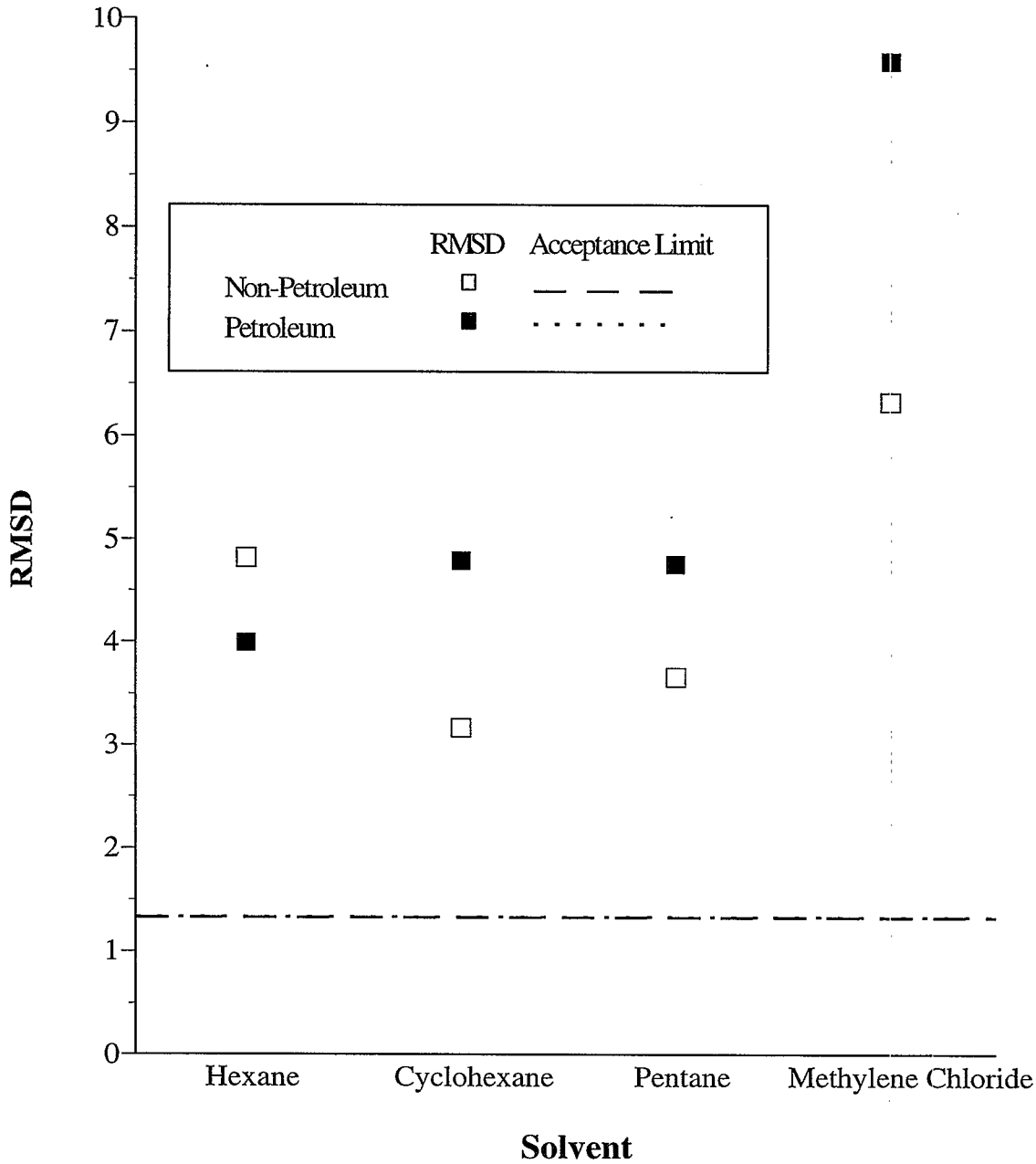
Normalized Root Mean Square Deviations
TPH Determinations By Solid Phase Extraction Cartridges
Relative to Freon-113 Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Freon

Exhibit 18

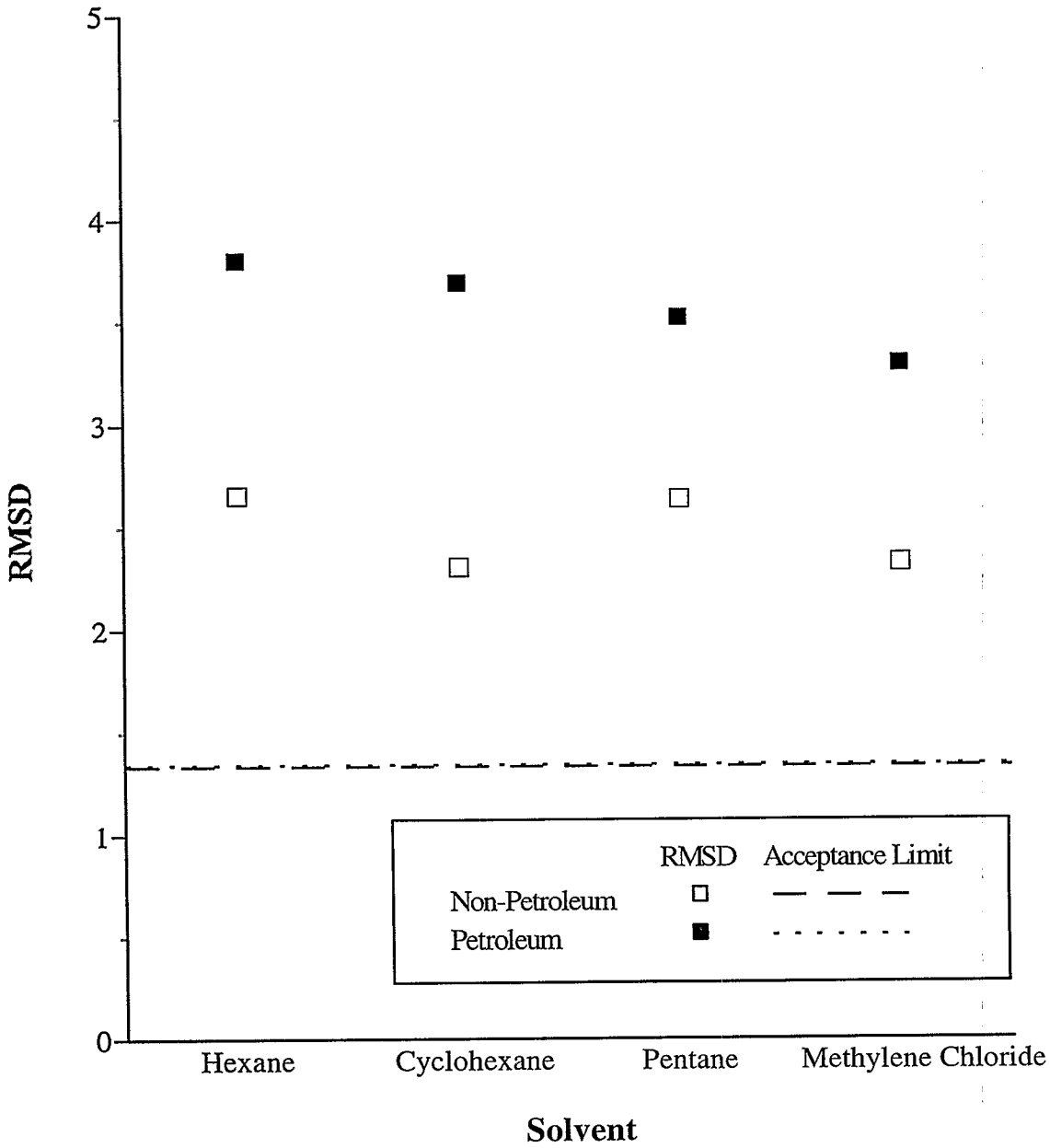
Normalized Root Mean Square Deviations
Oil & Grease Determinations By Solid Phase Extraction Cartridges
Relative to Hexane Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Hexane

Exhibit 19

Normalized Root Mean Square Deviations
TPH Determinations By Solid Phase Extraction Cartridges
Relative to Hexane Separatory Funnel Extraction



NOTE: Points below the respective Acceptance Limit are not significantly different from separatory funnel extraction with Hexane

Table 1

Student's t test on Difference Between n-Hexane and Cyclohexane in Percent Recoveries of Oil and Grease[1]

Sample Type	Average Difference	SD	t-value	Probability[2,3]
Non-Petroleum	20.6	96.6	0.82	0.424
Petroleum	3.0	30.7	0.42	0.683

[1] Percent Recoveries Relative to Recovery Using Freon-113
[2] Probability that difference = 0
[3] Probability values greater than 0.05 are not statistically significant

Table 2

Student's t test on Difference Between n-Hexane and Cyclohexane in Percent Recoveries of TPH[1]

Sample Type	Average Difference[4]	SD	t-value	Probability[2,3]
Non-Petroleum	-114.3	236.1	-1.87	0.082
Petroleum	-13.7	42.0	-1.38	0.184

[1] Percent Recoveries Relative to Recovery Using Freon-113
[2] Probability that difference = 0
[3] Probability values greater than 0.05 are not statistically significant
[4] A negative difference indicates that recoveries using cyclohexane were greater than those using n-hexane

SECTION 6
PHASE II STUDY DISCUSSION AND CONCLUSIONS

6.1 Separatory Funnel Extraction

The purpose of Phase II of the Freon Replacement Study was to evaluate n-hexane and cyclohexane as potential extraction solvent replacements for Freon-113 in the separatory funnel extraction/gravimetric determination of oil and grease and TPH in aqueous matrices. For both oil and grease and TPH determinations, neither n-hexane nor cyclohexane produced results that were equivalent to results produced by Freon-113.

On average, both n-hexane and cyclohexane extracted less oil and grease and TPH than Freon-113. RMSDs for n-hexane were higher than cyclohexane for oil and grease analysis. On the other hand, RMSDs for n-hexane were lower than cyclohexane for TPH analysis, with the exception of petroleum samples. Despite these variations, a paired t-test across all samples demonstrated that the percent recoveries between n-hexane and cyclohexane relative to Freon-113 were not statistically different from one another for both oil and grease and TPH determination (see Tables 1 and 2).

Because the performances of both solvents were similar, the decision of which alternative solvent was best suited to replace Freon-113 was based on practical analytical considerations, of which the primary factor was the difference between the boiling points of n-hexane (69°C) and cyclohexane (81°C). Based on laboratory comments regarding the extensive amount of time required to evaporate cyclohexane, and the potential for greater loss of material when extracts were heated to the higher temperatures required for cyclohexane evaporation, n-hexane was determined to be a more suitable replacement for Freon-113.

As part of this decision, health and safety concerns related to the neurotoxicity of n-hexane compared to cyclohexane were considered. The Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) for n-hexane and cyclohexane were compared and showed that n-hexane is only 1.7 times more toxic than cyclohexane. Further, the Time Weighted Average for n-hexane is 300 ppm, compared to 500 ppm for cyclohexane. Time Weighted Average is defined as the employee's average airborne exposure, which shall not be exceeded in any 8-hour work shift of a 40-hour work week. Therefore, although it is desirable to limit the amount and degree of occupational hazard presented by solvent substitution, the toxicity of n-hexane is not significantly higher than that of cyclohexane and can be minimized by implementing effective safety controls and procedures in the occupational setting.

EPA's decision to use n-hexane over all other solvents considered under the Freon Replacement Study was further supported by the following reasons: 1) n-hexane had been previously used as the extraction solvent for permit compliance analysis of oil and grease and TPH prior to the advent of Freon-113, 2) EPA Phase I and Phase II studies indicate that n-hexane produces results that are as or more comparable to Freon-113 results than other solvents (although no solvent produced results exactly equivalent to Freon-113), and 3) a t-test run on QC data from the Phase II study showed that there was no significant difference in results produced by n-hexane and Freon-113 for the analysis of reagent water samples spiked with reference standards (see Table 3).

The final product of Phase II was Method 1664 (the "Method"), a performance-based method that uses n-hexane as the extraction solvent. The quality control criteria incorporated into the Method exceeds and improves upon that of the currently approved 40 CFR part 136 oil and grease methods, and is consistent with the 40 CFR 136, Appendix A methods for determination of organic analytes. An important component of these and other QC tests required in Method 1664 is the use of hexadecane and stearic acid as the reference standards for spiking. Hexadecane was chosen to

simulate petroleum hydrocarbons; stearic acid was chosen to simulate animal fats and detergents, and serves to test the effects of the silica gel procedure.

6.2 Solid Phase Extraction

In regard to alternative techniques, neither SPE cartridge or disk extraction produced results equivalent to Freon-113. Use of these techniques are allowed, however, under the performance-based option of Method 1664, provided that the equivalency procedures in the Method are followed and all QC acceptance criteria are met.

Table 3
Student's t test on Difference Between Freon-113 and n-Hexane in Percent Recoveries of Oil and Grease in OPR Samples

Solvent	Average % Recovery	Average Difference	SD of Difference	t-value	Probability[1,2]
Freon	91.3	5.09	10.30	1.64	0.13
Hexane	86.2				

[1] Probability that difference = 0
[2] Probability values greater than 0.05 are not statistically significant

SECTION 7
FOLLOW-UP AND FUTURE FREON REPLACEMENT STUDY ACTIVITIES

7.1 Method Validation

Several method validation studies, ranging from interlaboratory studies used to derive QC specifications to single laboratory Method Detection Limit (MDL) studies, have been performed as part of the development of Method 1664. Descriptions of these studies are provided below.

Twin City Round Robin Group Interlaboratory Study

A total of eleven laboratories, working cooperatively as part of the Twin City Round Robin (TCRR) Group, performed an interlaboratory study for the determination of n-hexane extractable material (HEM) by Method 1664. Results of this study are presented in the document titled *Report of the Method 1664 Validation Studies*, April 1995. This study consisted of two parts: 1) the performance of an initial precision and recovery (IPR) test requiring the analysis of four spiked reagent water samples to demonstrate the laboratory's ability to generate acceptable precision and accuracy, and 2) the analysis of two sets of field samples, one from a petroleum source and the other from a non-petroleum source, in triplicate, for HEM.

Most laboratories did not encounter difficulties with the analysis of IPR and ongoing precision and recovery (OPR) samples and were able to achieve acceptable recoveries of hexadecane and stearic acid. Statistical evaluation of the results produced few outliers, indicating that Method 1664 is a reproducible procedure sufficiently reliable to be used by a variety of laboratories. In addition, the mean RSD of field sample results across all laboratories and all samples was 13.6, thereby demonstrating that the Method is capable of producing precise results on real world samples.

As part of the TCRR study, participants submitted comments related to the Method, most of which focused on difficulties related to extracts containing excessive amounts of water and the longer time required for the evaporation of n-hexane from the extracted material. These issues have been addressed, the former by recommending more careful separation of the aqueous and solvent phases to avoid carryover of the water into the extract and that more sodium sulfate be used in the filtering process, and the latter by allowing the use of either a water bath or steam bath set at a temperature that results in evaporation of the solvent within 30 minutes. These revisions have been incorporated into Method 1664.

MDL studies

To date, five single-laboratory MDL studies have been performed as part of the effort to determine MDLs and MLs for HEM and SGT-HEM. Results of these studies are detailed in the document titled *Report of the Method 1664 Validation Studies*, April 1995. The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero. To determine the MDL, the laboratories were required to follow the procedure in Appendix B to 40 CFR part 136. This procedure consists of the analysis of seven aliquots of reagent water that are spiked with the analyte(s) of interest. For EPA's MDL studies, the hexadecane and stearic acid specified in the quality control tests in Method 1664 were used. The MDL is calculated by multiplying the standard deviation of the seven replicate analyses by the Student's t value for (n - 1) degrees of freedom, where n equals the number of replicates. The Student's t value for seven replicates is 3.143.

The Minimum Level is defined as the level at which the entire analytical system produces a recognizable signal and an acceptable calibration point, and is determined by multiplying the MDL by 3.18 and rounding the resulting value to the nearest factor of 10 multiple of 1, 2, or 5. The value "3.18" represents the ratio between the Student t multiplier used to determine the MDL (3.143) and the 10 times multiplier used in the American Chemical Society (ACS) Limit of Quantity (LOQ) calculations (i.e. $10 \div 3.143 = 3.18$). For example, if the calculated MDL is 5.8, the ML will be equal to 5.8 times 3.18, which equals 18.4. Rounding to the nearest factor of 10 multiple of 1, 2, or 5 then establishes the ML at 20.

The first MDL study was performed in a commercial laboratory by an analyst at the Ph.D. level who has more than 20 years of experience in the determination of oil and grease and TPH. This study yielded an MDL of 0.91 mg/L and a resultant ML of 2 mg/L for HEM and an MDL of 1.6 mg/L and a resultant ML of 5 mg/L for SGT-HEM. Based on the disparity between the results obtained by this laboratory and the lower limit of the range in Method 413.1, it was decided that a second MDL study should be conducted in another commercial laboratory to verify the values obtained in the first study.

The second MDL study was also performed by a laboratory experienced in the determination of oil and grease and TPH, though the analysts performing the study were not at the Ph.D. level. In order to move expeditiously, the laboratory was required to perform the second MDL study within 24 hours. An MDL of 5.4 mg/L and an ML of 20 mg/L for HEM, and an MDL of 2.6 mg/L and an ML of 10 mg/L for SGT-HEM was determined in the second MDL study.

The second laboratory was contacted to determine if they encountered difficulties in performing the study. They stated that the results were the best that could be obtained under the imposed 24 hour turn-around time constraint, and that they believed they could achieve lower MDLs given more time. Based on these circumstances, the Agency decided that the MDLs to be included in the next revision of Method 1664 should be those representing the better performing laboratory. Therefore, the MDL and associated ML values from the original Method 1664 MDL study were incorporated into the October 1994 revision of the Method.

The high results produced in the second MDL study brought into question the reasonableness and effect of requiring a 24-hour turnaround. As a result, the second laboratory performed another MDL study (MDL study #3), this time without the turnaround constraint, and with the analytical objective of confirming the MDLs/MLs that had been obtained in the first MDL study. An MDL of 2.4 mg/L and an associated ML of 10 mg/L for HEM, and an MDL of 1.7 mg/L and an associated ML of 5 mg/L for SGT-HEM were obtained from this third MDL study. Although closer to the MDL and ML for HEM obtained in the first MDL study, the ML of 10 mg/L for HEM is still above the equivalent level in Method 413.1, and the result for SGT-HEM, the more complex procedure, was still less than the result for HEM.

From these results, the Agency concluded that the MDLs/MLs for HEM and SGT-HEM produced in the first MDL study were self-consistent, whereas the results produced in the second and third MDL studies were not. Therefore, the MDL and ML limits in the proposed January 1995 version of the Method were those from the first MDL study.

The Agency still needed to address the issue that the HEM MDL values in both the October 1994 and January 1995 versions of Method 1664 had not been verified with follow-up MDL studies. In contrast, a comparison of SGT-HEM results shows that the MDL/ML for SGT-HEM from the third MDL study supports the first MDL study results for SGT-HEM. (Both the first and third MDL studies produced an ML of 5 mg/L for SGT-HEM.)

To verify the HEM MDL and ML values specified in the October 1994 and January 1995 versions of Method 1664, which were the results obtained in MDL study #1, the laboratory that performed this MDL study conducted another study (MDL study #4). As with MDL study #1, the same Ph.D. level chemist with extensive analytical experience performed the analyses. Because the spike level in MDL study #1 was greater than five times the resulting MDL, the spike level was lowered to 5 mg/L. An

MDL of 0.88 mg/L, with a resulting ML of 2 mg/L was obtained, thereby supporting the original MDL results.

In response to comments received from laboratories and other interested parties regarding the difficulties encountered when attempting to achieve the HEM MDL of 0.91 mg/L specified in the October 1994 and January 1995 versions of Method 1664, and because most technicians performing HEM analysis for commercial laboratories will not have the experience or qualifications of the Ph.D. level chemist who performed MDL studies #1 and #4, an analyst with a bachelor's degree and one month's laboratory experience performed another HEM MDL study at this laboratory. The results of MDL study #5 were an HEM MDL of 1.4 mg/L and a resulting ML of 5 mg/L.

EPA has concluded that the MDL appropriate for Method 1664 should be representative of a better performing laboratory. However, to realistically address the qualifications of the laboratory personnel most likely to perform this procedure, the MDL should reflect the results obtained when using qualified, but not Ph.D. level, personnel. Therefore, the HEM MDL specified in the April 1995 version of Method 1664 (the version being proposed) is 1.4 mg/L and the HEM ML is 5 mg/L. Unchanged from the January 1995 version of Method 1664, the SGT-HEM MDL is 1.6 mg/L and the SGT-HEM ML is 5 mg/L.

7.2 Future Studies

Phase III - IR Study

Evaluation of alternative solvents and techniques for infra-red determination of oil and grease and TPH is being considered for future work under Phase III of the Freon Replacement Study. As was decided in Phase I of the study, the use of perchloroethylene will be evaluated as a possible replacement solvent for IR analyses. Preliminary studies of the use of perchloroethylene in place of Freon-113 indicated that the stabilizer present in the perchloroethylene solvent could interfere with analysis. As a result, J.T. Baker developed a perchloroethylene standard that contains lower levels of stabilizer to minimize these effects. Formal evaluation of perchloroethylene is planned under Phase III.

In addition to evaluating alternative solvents by separatory funnel extraction, alternative techniques are being considered for evaluation and include:

1) A procedure proposed by Chem-Check Consulting that consists of non-dispersive infra-red (NDIR) measurement of sample extracts, the residue of which are evaporated onto cavity cells. Since this technique removes the extraction solvent from the measurement step, any difficulties inherent to the solvents are eliminated.

2) 3M Disposable IR Cards, which consist of either a polyethylene substrate or a PTFE substrate. As with the NDIR/cavity cell procedure, the solvent is evaporated prior to measurement and therefore is not a concern when using IR determination.

Assessment of these techniques will most likely be accomplished through cooperative studies with the product vendors.

APPENDIX A
SITE SUMMARY

Appendix A
Site Summary

	Facility	Industrial Category	Sample	Waste Stream	Source
1	Petroleum Refinery	Petroleum Refining	24870	Separator Effluent	P
			24871	Secondary Effluent	P
2	Textile Mill	Textile Manufacturing	24873	Lagoon Effluent	NP
3	Leather Finishing Plant	Leather Tanning	24874	Primary Effluent	NP
4	POTW	POTW	24875	Primary Effluent	NP
5	Die Casting Plant	Metal Molding & Casting	24876	Primary Effluent	NP
6	Metal Finishing Plant	Metal Finishing	24877	Process Wastewater (1:24 Eff)	P
			24878	Process Wastewater (1:10 Eff)	P
7	Pump Mfg. Plant	Metal Finishing	24879	Process Wastewater	P
8	Bacon Processing Plant	Meat Products	24880	Process Wastewater (2:3 Eff)	NP
			24902	Process Wastewater (1:1 Eff)	NP
9	Shore Reception Fac.	Shore Reception Facility	24881	Oily Wastewater (1:3 Sep Eff)	P
			24882	Oily Wastewater (6:5 Sep Eff)	P
			25101	Oily Wastewater (1:1 Sep Eff)	P
10	Can Manufacturing Plant	Coil Coating	24883	Process Wastewater (2:7 Eff)	NP
11	Can Manufacturing Plant	Coil Coating	24884	Process Wastewater (2:3 Eff)	NP
12	Drum Handling Facility	Drum Reconditioning	24886	Filter Effluent (1:1 TW)	P
			24893	Washwater	P
13	Leather Tannery	Leather Tanning	24888	Primary Effluent	NP
14	Formulating Plant	Soap & Detergent Manuf.	24887	Primary Effluent	NP
15	Chemical Mfg. Plant	Organic Chemicals	24889	Primary Effluent	P
16	Dye Plant	Textile Manufacturing	24890	Process Wastewater	NP
17	Chemical Mfg. Plant	Organic Chemicals	24891	Primary Effluent	P
18	Meat Processing Plant	Meat Products	24894	Process Wastewater (1:1 TW)	NP
19	Olive Packaging Plant	Misc. Foods	24896	Primary Effluent	NP
			25104	Primary Effluent	NP
20	Bus Maintenance Fac.	Transportation Facility	24897	Separator Effluent	P
			25106	Separator Effluent	P
21	Meat Processing Plant	Meat Products	24898	Primary Effluent	NP
22	Rendering Plant	Meat Products	24899	Primary Effluent (1:4 TW)	NP
23	Industrial Laundry	Industrial Laundry	24900	Primary Eff (1:1 TW)	P
24	Railroad Maintenance	Transportation Facility	24901	Separator Effluent	P
			25105	Separator Effluent	P
25	Railroad Maintenance	Transportation Facility	25107	Oily Wastewater (5:6 TW)	P

P = Petroleum
NP = Non-Petroleum
TW = Tap Water
Eff = Effluent
Sep Eff = Separator Effluent

APPENDIX B
DATA SUMMARY

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
1	Petroleum Refinery	24870	Separator Effluent	LLE	Cyclohexane	5.6	5.4	5.3	5.4	0.1
					Freon	7.2	6.1	6.8	6.7	0.6
					Hexane	12.0	9.1	8.1	9.6	1.7
		24871	Secondary Effluent	LLE	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.7	7.5	5.0 <	6.1	1.3
2	Textile Mill	24873	Lagoon Effluent	LLE	Cyclohexane	16.0	21.0	17.0	18.0	2.5
					Freon	36.0	35.0	42.0	38.0	3.8
					Hexane	22.0	32.0	34.0	29.0	6.4
				SPE Col	Cyclohexane	21.0	25.0	20.0	22.0	2.5
					Hexane	21.0	20.0	22.0	21.0	0.8
					MeCl	65.0	69.0	80.0	71.0	8.0
					Pentane	21.0		19.0	20.0	1.8
				SPE Disk	Cyclohexane	23.0	26.0	33.0	27.0	5.1
					Hexane	23.0	31.0	30.0	28.0	4.4
3	Leather Finishing Plant	24874	Primary Effluent	LLE	Cyclohexane	11.0	12.0	7.6	10.0	2.3
					Freon	21.0	17.0	15.0	18.0	2.8
					Hexane	12.0	11.0	7.7	10.0	2.3
				SPE Col	Cyclohexane					
					Hexane	71.0	23.0	74.0	56.0	28.7
					MeCl	110.0	100.0		110.0	8.2
					Pentane					
				SPE Disk	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
4	POTW	24875	Primary Effluent	LLE	Cyclohexane	22.0	22.0	22.0	22.0	0.5
					Freon	23.0	25.0	25.0	24.0	1.0
					Hexane	23.0	23.0	22.0	22.0	0.8
				SPE Col	Cyclohexane	15.0	14.0	16.0	15.0	0.7
					Hexane	12.0	5.0 <	5.0 <	7.2	3.8
					MeCl	25.0	40.0	23.0	30.0	9.4
					Pentane	5.0 <	6.0	5.7	5.5	0.5
				SPE Disk	Cyclohexane	15.0	26.0	23.0	21.0	5.7
					Hexane	22.0	27.0	26.0	25.0	2.6
5	Die Casting Plant	24876	Primary Effluent	LLE	Cyclohexane	150.0	160.0	130.0	150.0	14.8
					Freon	250.0	230.0	220.0	230.0	14.2
					Hexane	150.0	190.0	180.0	170.0	17.0

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
				SPE Col	Cyclohexane	180.0	190.0	200.0	190.0	10.7
					Hexane	200.0	190.0	190.0	190.0	8.7
					MeCl	470.0	440.0	500.0	470.0	28.7
					Pentane	140.0	210.0	230.0	190.0	51.4
				SPE Disk	Cyclohexane	260.0	260.0	270.0	270.0	2.9
					Hexane	250.0	250.0	260.0	250.0	6.7
6	Metal Finishing Plant	24877	Process Wastewater (1:24 Eff)	LLE	Cyclohexane	50.0	49.0	47.0	49.0	1.6
					Freon	72.0	73.0	75.0	73.0	1.6
					Hexane	64.0	60.0	60.0	62.0	2.5
				SPE Col	Cyclohexane	68.0	74.0	68.0	70.0	3.3
					Hexane	67.0	90.0	66.0	75.0	13.3
					MeCl	83.0	99.0	84.0	89.0	9.4
					Pentane	65.0	63.0	53.0	60.0	6.8
				SPE Disk	Cyclohexane	79.0	80.0	73.0	77.0	3.8
					Hexane	73.0	73.0	70.0	72.0	1.7
		24878	Process Wastewater (1:10 Eff)	LLE	Cyclohexane	130.0	120.0	130.0	130.0	2.4
					Freon	140.0	130.0	130.0	130.0	2.6
					Hexane	120.0	120.0	120.0	120.0	3.2
				SPE Col	Cyclohexane	150.0	160.0	160.0	160.0	3.3
					Hexane	150.0	150.0	150.0	150.0	3.1
					MeCl	170.0	170.0	160.0	160.0	7.4
					Pentane	140.0	150.0	140.0	140.0	8.5
				SPE Disk	Cyclohexane	170.0	160.0	170.0	170.0	5.3
					Hexane	160.0	150.0	150.0	150.0	1.5
7	Pump Mfg. Plant	24879	Process Wastewater	LLE	Cyclohexane	310.0	280.0	290.0	290.0	15.2
					Freon	400.0	350.0	350.0	370.0	26.5
					Hexane	340.0	320.0	310.0	330.0	14.3
				SPE Col	Cyclohexane	380.0	420.0	380.0	390.0	24.3
					Hexane	390.0	410.0	420.0	410.0	13.5
					MeCl	460.0	510.0	500.0	490.0	24.2
					Pentane	340.0	340.0	310.0	330.0	16.9
				SPE Disk	Cyclohexane	400.0	390.0	410.0	400.0	8.1
					Hexane	360.0	340.0	350.0	350.0	10.0
8	Bacon Processing Plant	24880	Process Wastewater (2:3 Eff)	LLE	Cyclohexane	50.0	54.0	56.0	53.0	3.3
					Freon	52.0	57.0	56.0	55.0	2.7
					Hexane	48.0	45.0	49.0	48.0	1.9

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
				SPE Col	Cyclohexane	55.0	59.0	50.0	55.0	4.5
					Hexane	53.0	46.0	46.0	48.0	3.6
					MeCl	68.0	77.0	73.0	73.0	4.3
					Pentane	59.0	53.0	50.0	54.0	4.5
				SPE Disk	Cyclohexane	74.0	74.0	40.0	63.0	19.6
					Hexane	69.0	66.0	78.0	71.0	6.2
		24902	Process Wastewater (1:1 Eff)	LLE	Cyclohexane	140.0	160.0	150.0	150.0	10.6
					Freon	110.0	110.0	100.0	110.0	3.6
					Hexane	180.0	180.0	180.0	180.0	3.0
				SPE Col	Cyclohexane	190.0	180.0	190.0	190.0	5.7
					Hexane	200.0	190.0	190.0	190.0	1.9
					MeCl	300.0	340.0	360.0	330.0	27.0
					Pentane	260.0	230.0	200.0	230.0	29.6
9	Shore Reception Fac.	24881	Oily Wastewater (1:3 Sep Eff)	LLE	Cyclohexane	61.0	54.0	50.0	55.0	5.7
					Freon	69.0	65.0	65.0	66.0	2.5
					Hexane	63.0	53.0	47.0	54.0	8.3
				SPE Col	Cyclohexane	43.0	50.0	47.0	47.0	3.6
					Hexane	50.0	47.0	42.0	46.0	3.8
					MeCl	91.0	100.0	89.0	95.0	8.1
					Pentane	58.0	62.0	64.0	61.0	3.3
				SPE Disk	Cyclohexane	25.0	51.0	47.0	41.0	14.0
					Hexane	25.0	47.0	43.0	38.0	11.7
		24882	Oily Wastewater (6:5 Sep Eff)	LLE	Cyclohexane	69.0	68.0	70.0	69.0	0.9
					Freon	82.0	90.0	93.0	88.0	5.8
					Hexane	69.0	76.0	72.0	72.0	3.4
				SPE Col	Cyclohexane	44.0	30.0	42.0	39.0	7.4
					Hexane	42.0	37.0	27.0	35.0	7.6
					MeCl	120.0	110.0	76.0	100.0	22.5
					Pentane	50.0	36.0	56.0	47.0	9.8
				SPE Disk	Cyclohexane	63.0	66.0	62.0	64.0	2.1
					Hexane	31.0	43.0	58.0	44.0	13.5
		25101	Oily Wastewater (1:1 Sep Eff)	LLE	Cyclohexane	42.0	41.0	40.0	41.0	1.1
					Freon	62.0	75.0	65.0	67.0	6.7
					Hexane	38.0	38.0	36.0	37.0	1.0
				SPE Col	Cyclohexane	69.0	60.0	51.0	60.0	8.8
					Hexane	44.0	53.0	54.0	50.0	5.8
					MeCl	58.0	64.0	92.0	71.0	18.2
					Pentane	38.0	53.0	40.0	44.0	8.1

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
				SPE Disk	Cyclohexane	78.0	70.0	71.0	73.0	4.4
					Hexane	66.0	67.0	73.0	69.0	3.8
10	Can Manufacturing Plant	24883	Process Wastewater (2:7 Eff)	LLE	Cyclohexane	35.0	37.0	38.0	36.0	1.4
					Freon	50.0	47.0	47.0	48.0	1.9
					Hexane	31.0	30.0	33.0	31.0	1.4
				SPE Col	Cyclohexane	47.0	40.0	44.0	44.0	3.3
					Hexane	36.0	36.0	34.0	35.0	1.1
					MeCl	78.0	80.0	75.0	78.0	2.2
					Pentane	27.0	32.0	29.0	29.0	2.5
				SPE Disk	Cyclohexane	41.0	38.0	28.0	36.0	6.8
					Hexane	33.0	37.0	40.0	37.0	3.5
11	Can Manufacturing Plant	24884	Process Wastewater (2:3 Eff)	LLE	Cyclohexane	96.0	86.0	110.0	97.0	11.0
					Freon	150.0	150.0	150.0	150.0	3.0
					Hexane	84.0	97.0	110.0	96.0	11.5
				SPE Col	Cyclohexane	140.0	150.0	140.0	140.0	5.2
					Hexane	140.0	100.0	120.0	120.0	18.8
					MeCl	320.0	330.0	310.0	320.0	9.9
					Pentane	95.0	72.0	82.0	83.0	11.6
				SPE Disk	Cyclohexane	110.0	110.0	110.0	110.0	2.3
					Hexane	110.0	100.0	110.0	110.0	5.0
12	Drum Handling Facility	24886	Filter Effluent (1:1 TW)	LLE	Cyclohexane	57.0	50.0	68.0	58.0	9.3
					Freon	61.0	62.0	46.0	57.0	8.9
					Hexane	34.0	33.0	39.0	35.0	3.3
				SPE Col	Cyclohexane	50.0	39.0	59.0	49.0	9.7
					Hexane	32.0	30.0	40.0	34.0	5.6
					MeCl	160.0	160.0	150.0	160.0	4.6
					Pentane	36.0	39.0	42.0	39.0	2.6
				SPE Disk	Cyclohexane	20.0	26.0	19.0	22.0	3.8
					Hexane	18.0	17.0	17.0	17.0	0.6
		24893	Washwater	LLE	Cyclohexane	31.0	36.0	32.0	33.0	2.5
					Freon	34.0	32.0	36.0	34.0	1.6
					Hexane	27.0	31.0	24.0	27.0	3.2
				SPE Col	Cyclohexane	20.0	31.0	23.0	25.0	5.4
					Hexane	19.0	19.0	17.0	18.0	1.1
					MeCl	60.0	54.0	58.0	57.0	2.9
					Pentane	24.0	25.0	25.0	25.0	0.4

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
				SPE Disk	Cyclohexane	32.0	36.0	34.0	34.0	2.0
					Hexane	38.0	42.0	52.0	44.0	7.2
13	Leather Tannery	24888	Primary Effluent	LLE	Cyclohexane	190.0	210.0	220.0	210.0	19.3
					Freon	320.0	360.0	300.0	330.0	31.2
					Hexane	130.0	170.0	170.0	160.0	23.4
				SPE Col	Cyclohexane	89.0	78.0	83.0	83.0	5.9
					Hexane	92.0	110.0	96.0	100.0	10.7
					MeCl	200.0	220.0	240.0	220.0	18.4
					Pentane	170.0	140.0	140.0	150.0	13.3
				SPE Disk	Cyclohexane	130.0	140.0	110.0	120.0	13.0
					Hexane	140.0	160.0	170.0	160.0	12.6
14	Formulating Plant	24887	Primary Effluent	LLE	Cyclohexane	110.0	96.0	75.0	95.0	19.2
					Freon	80.0	82.0	160.0	110.0	45.8
					Hexane	63.0	93.0	110.0	88.0	23.4
				SPE Col	Cyclohexane	46.0	60.0	50.0	52.0	7.4
					Hexane	48.0	48.0	55.0	50.0	4.3
					MeCl	210.0	210.0	210.0	210.0	3.6
					Pentane	54.0	52.0	53.0	53.0	1.2
				SPE Disk	Cyclohexane	42.0	49.0	45.0	45.0	3.5
					Hexane	53.0	31.0	34.0	39.0	11.9
15	Chemical Mfg. Plant	24889	Primary Effluent	LLE	Cyclohexane	70.0	60.0	72.0	67.0	6.4
					Freon	75.0	83.0	78.0	78.0	4.0
					Hexane	72.0	58.0	69.0	66.0	7.6
				SPE Col	Cyclohexane	54.0	69.0	70.0	64.0	8.6
					Hexane	60.0	53.0	65.0	59.0	6.0
					MeCl	91.0	88.0	81.0	87.0	5.1
					Pentane	72.0	61.0	51.0	61.0	10.4
				SPE Disk	Cyclohexane	62.0	61.0	58.0	60.0	2.1
					Hexane	54.0	58.0	55.0	56.0	2.1
16	Dye Plant	24890	Process Wastewater	LLE	Cyclohexane	14.0	15.0	13.0	14.0	1.1
					Freon	12.0	6.7	14.0	11.0	3.7
					Hexane	6.9	11.0	5.0	< 7.5	2.8
				SPE Col	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
					MeCl	29.0	25.0	26.0	27.0	1.9
					Pentane	8.7	7.5	6.7	7.6	1.0
				SPE Disk	Cyclohexane	18.0	24.0	19.0	20.0	3.2

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
					Hexane	30.0	31.0	28.0	30.0	1.5
17	Chemical Mfg. Plant	24891	Primary Effluent	LLE	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Freon	6.9	6.8	7.2	7.0	0.2
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
				SPE Col	Cyclohexane	7.1	6.4	9.9	7.8	1.8
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
					MeCl	28.0	29.0	23.0	27.0	3.0
					Pentane	12.0	11.0	9.2	10.0	1.3
				SPE Disk	Cyclohexane	15.0	11.0	12.0	13.0	2.1
					Hexane	21.0	24.0	21.0	22.0	1.7
18	Meat Processing Plant	24894	Process Wastewater (1:1 TW)	LLE	Cyclohexane	230.0	260.0	230.0	220.0	27.0
					Freon	240.0	270.0	280.0	260.0	19.0
					Hexane	370.0	270.0	370.0	340.0	57.2
				SPE Col	Cyclohexane					
					Hexane		200.0	190.0	200.0	11.9
					MeCl	300.0	310.0		310.0	4.5
					Pentane					
				SPE Disk	Cyclohexane	350.0	340.0	320.0	340.0	15.6
					Hexane	340.0	270.0	310.0	300.0	34.0
19	Olive Packaging Plant	24896	Primary Effluent	LLE	Cyclohexane	40.0	38.0	34.0	37.0	2.8
					Freon	46.0	43.0	38.0	42.0	4.2
					Hexane	160.0	220.0	190.0	190.0	33.0
				SPE Col	Cyclohexane	67.0	71.0	66.0	68.0	2.5
					Hexane	74.0	47.0	64.0	62.0	13.8
					MeCl	84.0	72.0	74.0	77.0	6.6
					Pentane	59.0	59.0	57.0	58.0	1.2
				SPE Disk	Cyclohexane	52.0	52.0	58.0	54.0	3.5
					Hexane	54.0	45.0	67.0	55.0	11.1
		25104	Primary Effluent	LLE	Cyclohexane	160.0	160.0	160.0	160.0	3.4
					Freon	170.0	180.0	180.0	180.0	1.3
					Hexane	180.0	190.0	180.0	190.0	8.1
20	Bus Maintenance Fac.	24897	Separator Effluent	LLE	Cyclohexane	30.0	32.0	32.0	31.0	1.5
					Freon	71.0	64.0	60.0	65.0	5.8
					Hexane	27.0	28.0	34.0	30.0	3.3

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
				SPE Col	Cyclohexane	59.0	58.0	69.0	62.0	6.1
					Hexane	64.0	60.0	55.0	59.0	5.0
					MeCl	63.0	77.0	71.0	70.0	7.4
					Pentane	56.0	58.0	42.0	52.0	8.5
				SPE Disk	Cyclohexane	43.0	49.0	49.0	47.0	3.5
					Hexane	41.0	41.0	40.0	41.0	0.6
		25106	Separator Effluent	LLE	Cyclohexane	24.0	27.0	28.0	26.0	1.7
					Freon	38.0	37.0	40.0	38.0	1.5
					Hexane	25.0	22.0	23.0	23.0	1.6
21	Meat Processing Plant	24898	Primary Effluent	LLE	Cyclohexane	31.0	30.0	29.0	30.0	1.1
					Freon	20.0	19.0	19.0	19.0	0.1
					Hexane	25.0	28.0	21.0	25.0	3.3
				SPE Col	Cyclohexane		29.0		29.0	
					Hexane	46.0	36.0	35.0	39.0	6.2
					MeCl	55.0	58.0	60.0	58.0	2.8
					Pentane	23.0	24.0	35.0	27.0	6.8
				SPE Disk	Cyclohexane	56.0	54.0	46.0	52.0	5.3
					Hexane	54.0	54.0	54.0	54.0	
22	Rendering Plant	24899	Primary Effluent (1:4 TW)	LLE	Cyclohexane	380.0	540.0	500.0	470.0	86.0
					Freon	510.0	510.0	490.0	500.0	13.9
					Hexane	480.0	420.0	470.0	460.0	31.5
				SPE Col	Cyclohexane	780.0	770.0	770.0	780.0	5.6
					Hexane	740.0	750.0	700.0	730.0	21.6
					MeCl	740.0	770.0	740.0	750.0	17.7
					Pentane	620.0	630.0	620.0	620.0	8.0
				SPE Disk	Cyclohexane	760.0	670.0	720.0	710.0	43.7
					Hexane	690.0	740.0	690.0	710.0	29.9
23	Industrial Laundry	24900	Primary Eff (1:1 TW)	LLE	Cyclohexane	180.0	250.0	190.0	210.0	37.5
					Freon	250.0	230.0	260.0	240.0	16.6
					Hexane	170.0	190.0	160.0	170.0	14.6
				SPE Col	Cyclohexane	190.0	170.0	220.0	190.0	26.8
					Hexane	350.0	430.0	280.0	360.0	75.1
					MeCl	300.0	320.0	290.0	310.0	12.7
					Pentane	200.0	220.0	220.0	210.0	9.1
				SPE Disk	Cyclohexane	200.0	200.0	220.0	210.0	9.6
					Hexane	210.0	210.0	200.0	210.0	5.3

TW = Tap Water

Appendix B
Oil & Grease

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
24	Railroad Maintenance	24901	Separator Effluent	LLE	Cyclohexane	110.0	120.0	120.0	120.0	4.5
					Freon	120.0	87.0	110.0	110.0	17.7
					Hexane	130.0	110.0	120.0	120.0	9.9
				SPE Col	Cyclohexane	170.0	150.0	170.0	160.0	11.8
					Hexane	140.0	130.0	140.0	140.0	2.9
					MeCl	210.0	220.0	220.0	220.0	7.2
					Pentane	160.0	150.0	160.0	160.0	4.0
				SPE Disk	Cyclohexane	210.0	170.0	180.0	190.0	18.9
					Hexane	210.0	210.0	210.0	210.0	4.9
		25105	Separator Effluent	LLE	Cyclohexane	180.0	140.0	130.0	150.0	25.3
					Freon	180.0	180.0	190.0	180.0	7.6
					Hexane	97.0	98.0	130.0	110.0	16.3
25	Railroad Maintenance	25107	Oily Wastewater (5:6 TW)	LLE	Cyclohexane	30.0	28.0	30.0	29.0	1.3
					Freon	30.0	30.0	28.0	29.0	1.1
					Hexane	24.0	27.0	26.0	25.0	1.9

TW = Tap Water

Appendix B
Total Petroleum Hydrocarbons

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
1	Petroleum Refinery	24870	Separator Effluent	LLE	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
		24871	Secondary Effluent	LLE	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
2	Textile Mill	24873	Lagoon Effluent	LLE	Cyclohexane	7.5	12.0	8.1	9.1	2.2
					Freon	27.0	19.0	22.0	23.0	4.5
					Hexane	17.0	15.0	16.0	16.0	1.1
3	Leather Finishing Plant	24874	Primary Effluent	LLE	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
4	POTW	24875	Primary Effluent	LLE	Cyclohexane	6.5	5.9	5.0 <	5.8	0.7
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	6.8	5.0 <	6.0	5.9	0.9
5	Die Casting Plant	24876	Primary Effluent	LLE	Cyclohexane	100.0	100.0	83.0	96.0	11.3
					Freon	150.0	130.0	130.0	140.0	12.1
					Hexane	88.0	120.0	120.0	110.0	19.7
6	Metal Finishing Plant	24877	Process Wastewater (1:24 Eff)	LLE	Cyclohexane	41.0	33.0	38.0	37.0	3.7
					Freon	51.0	50.0	51.0	51.0	0.3
					Hexane	37.0	34.0	42.0	38.0	4.3
		24878	Process Wastewater (1:10 Eff)	LLE	Cyclohexane	91.0	82.0	89.0	87.0	4.6
					Freon	87.0	97.0	86.0	90.0	6.0
					Hexane	80.0	72.0	89.0	80.0	8.4
7	Pump Mfg. Plant	24879	Process Wastewater	LLE	Cyclohexane	190.0	180.0	190.0	180.0	4.0
					Freon	170.0	170.0	190.0	180.0	11.1
					Hexane	230.0	210.0	220.0	220.0	9.2
				SPE Col	Cyclohexane	260.0	280.0	270.0	270.0	9.4
					Hexane	290.0	300.0	300.0	290.0	6.7
					MeCl	260.0	290.0	280.0	270.0	14.7
					Pentane	250.0	240.0	230.0	240.0	8.8
8	Bacon Processing Plant	24880	Process Wastewater (2:3 Eff)	LLE	Cyclohexane	41.0	41.0	34.0	39.0	3.7
					Freon	21.0	26.0	25.0	24.0	2.5
					Hexane	17.0	13.0	18.0	16.0	2.3
				SPE Col	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
					MeCl	5.4	5.0 <	5.0 <	5.1	0.2
					Pentane	7.5	5.0 <	11.0	7.9	3.2

TW = Tap Water

Appendix B
Total Petroleum Hydrocarbons

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
		24902	Process Wastewater (1:1 Eff)	LLE	Cyclohexane	41.0	45.0	44.0	44.0	2.2
					Freon	13.0	8.8	13.0	11.0	2.3
					Hexane	34.0	34.0	29.0	32.0	3.0
9	Shore Reception Fac.	24881	Oily Wastewater (1:3 Sep Eff)	LLE	Cyclohexane	42.0	38.0	36.0	39.0	2.9
					Freon	40.0	42.0	38.0	40.0	2.1
					Hexane	36.0	41.0	34.0	37.0	3.4
				SPE Col	Cyclohexane	29.0	31.0	28.0	30.0	1.5
					Hexane	27.0	31.0	26.0	28.0	2.6
					MeCl	43.0	42.0	39.0	42.0	2.4
					Pentane	33.0	34.0	49.0	39.0	9.2
		24882	Oily Wastewater (6:5 Sep Eff)	LLE	Cyclohexane	42.0	43.0	41.0	42.0	0.8
					Freon	50.0	56.0	55.0	53.0	3.3
					Hexane	50.0	41.0	49.0	47.0	4.9
				SPE Col	Cyclohexane	28.0	25.0	36.0	30.0	5.5
					Hexane	22.0	21.0	22.0	22.0	0.8
					MeCl	40.0	34.0	33.0	36.0	3.4
					Pentane	31.0	21.0	39.0	30.0	9.4
		25101	Oily Wastewater (1:1 Sep Eff)	LLE	Cyclohexane	29.0	30.0	29.0	29.0	0.6
					Freon	45.0	53.0	45.0	48.0	4.6
					Hexane	22.0	20.0	15.0	19.0	3.3
				SPE Col	Cyclohexane	31.0	46.0	38.0	38.0	7.4
					Hexane	35.0	40.0	33.0	36.0	3.9
					MeCl	23.0	26.0	29.0	26.0	3.2
					Pentane	28.0	38.0	37.0	34.0	5.8
10	Can Manufacturing Plant	24883	Process Wastewater (2:7 Eff)	LLE	Cyclohexane	24.0	24.0	26.0	25.0	1.4
					Freon	34.0	30.0	31.0	32.0	2.1
					Hexane	17.0	24.0	16.0	19.0	4.4
				SPE Col	Cyclohexane	31.0	30.0	30.0	30.0	0.9
					Hexane	24.0	26.0	21.0	24.0	2.5
					MeCl	26.0	25.0	24.0	25.0	0.9
					Pentane	23.0	25.0	24.0	24.0	0.7
11	Can Manufacturing Plant	24884	Process Wastewater (2:3 Eff)	LLE	Cyclohexane	52.0	59.0	92.0	67.0	21.4
					Freon	95.0	84.0	79.0	86.0	8.0
					Hexane	60.0	72.0	73.0	69.0	7.1
				SPE Col	Cyclohexane	53.0	56.0		55.0	1.9
					Hexane	53.0	56.0		55.0	1.6
					MeCl	44.0	50.0	42.0	45.0	4.4
					Pentane	43.0	34.0	43.0	40.0	5.1

TW = Tap Water

Appendix B
Total Petroleum Hydrocarbons

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
12	Drum Handling Facility	24886	Filter Effluent (1:1 TW)	LLE	Cyclohexane	8.8	11.0	15.0	12.0	3.2
					Freon	21.0	18.0	11.0	17.0	5.1
					Hexane	14.0	10.0	7.6	11.0	3.1
				SPE Col	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	7.5	5.0 <	10.0	7.5	2.6
					MeCl	5.5	5.0 <	5.0 <	5.2	0.3
					Pentane	5.0 <	5.0 <	5.0 <	5.0 <	
		24893	Washwater	LLE	Cyclohexane	8.5	11.0	13.0	11.0	2.0
					Freon	5.8	7.0	8.8	7.2	1.5
					Hexane	5.2	5.8	7.2	6.1	1.0
				SPE Col	Cyclohexane	7.4	9.0	8.2	8.2	0.8
					Hexane	8.2	8.0	7.4	7.9	0.4
					MeCl	11.0	10.0	12.0	11.0	0.7
					Pentane	8.7	8.5	8.3	8.5	0.2
13	Leather Tannery	24888	Primary Effluent	LLE	Cyclohexane	77.0	130.0	82.0	97.0	30.2
					Freon	98.0	31.0	34.0	54.0	37.7
					Hexane	30.0	130.0	62.0	72.0	48.7
				SPE Col	Cyclohexane	8.4	9.1	14.0	10.0	3.0
					Hexane	7.6	7.2	6.2	7.0	0.7
					MeCl	20.0	20.0	15.0	18.0	2.7
					Pentane	14.0	12.0	12.0	13.0	1.0
14	Formulating Plant	24887	Primary Effluent	LLE	Cyclohexane	32.0	26.0	18.0	25.0	6.8
					Freon	17.0	28.0	17.0	21.0	6.6
					Hexane	18.0	14.0	17.0	16.0	1.7
				SPE Col	Cyclohexane	7.4	7.4	8.8	7.9	0.8
					Hexane	8.6	10.0	5.9	8.2	2.1
					MeCl	7.8	7.5	8.2	7.9	0.4
					Pentane	8.4	11.0	8.3	9.1	1.3
15	Chemical Mfg. Plant	24889	Primary Effluent	LLE	Cyclohexane	5.0 <	11.0	6.2	7.4	3.2
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
				SPE Col	Cyclohexane	5.0 <	5.0 <	5.8	5.3	0.4
					Hexane	11.0	10.0	11.0	11.0	0.5
					MeCl	5.0 <	5.0 <	5.0 <	5.0 <	
					Pentane	5.0 <	5.0 <	5.0 <	5.0 <	
16	Dye Plant	24890	Process Wastewater	LLE	Cyclohexane	6.0	8.2	5.5	6.6	1.4
					Freon	6.8	5.0 <	8.1	6.6	1.6
					Hexane	5.2	5.0 <	5.0 <	5.1	0.1

TW = Tap Water

Appendix B
Total Petroleum Hydrocarbons

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
				SPE Col	Cyclohexane	5.6	5.0 <	5.0 <	5.2	0.4
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
					MeCl	6.5	5.6	6.3	6.1	0.5
					Pentane	7.4	7.6	7.3	7.4	0.2
17	Chemical Mfg. Plant	24891	Primary Effluent	LLE	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	5.0 <	5.0 <	5.0 <	
				SPE Col	Cyclohexane	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane		5.0 <	5.0 <	5.0 <	
					MeCl	5.0 <	5.0 <	5.0 <	5.0 <	
					Pentane	5.0 <	5.0 <	5.0 <	5.0 <	
18	Meat Processing Plant	24894	Process Wastewater (1:1 TW)	LLE	Cyclohexane	97.0	83.0	92.0	90.0	7.2
					Freon	18.0	28.0	37.0	27.0	9.6
					Hexane	150.0	120.0	150.0	140.0	14.6
				SPE Col	Cyclohexane					
					Hexane		18.0	22.0	20.0	3.3
					MeCl	21.0	23.0		22.0	1.3
					Pentane					
19	Olive Packaging Plant	24896	Primary Effluent	LLE	Cyclohexane	19.0	12.0	20.0	17.0	4.2
					Freon	23.0	26.0	9.5	19.0	8.7
					Hexane	7.0	9.4	5.0 <	7.1	2.2
				SPE Col	Cyclohexane	9.1	8.0	6.4	7.8	1.4
					Hexane	11.0	7.4	8.1	8.8	1.9
					MeCl	5.2	5.0 <	5.8	5.3	0.4
					Pentane	8.9	7.8	6.5	7.7	1.2
		25104	Primary Effluent	LLE	Cyclohexane	120.0	120.0	110.0	120.0	4.9
					Freon	14.0	13.0	11.0	13.0	1.4
					Hexane	6.1	7.1	7.7	7.0	0.8
20	Bus Maintenance Fac.	24897	Separator Effluent	LLE	Cyclohexane	20.0	16.0	24.0	20.0	3.7
					Freon	26.0	25.0	31.0	27.0	3.7
					Hexane	20.0	16.0	17.0	18.0	2.0
				SPE Col	Cyclohexane	26.0	27.0	28.0	27.0	0.8
					Hexane	27.0	25.0	26.0	26.0	1.3
					MeCl	39.0	29.0	34.0	34.0	4.9
					Pentane	25.0	22.0	27.0	25.0	2.4
		25106	Separator Effluent	LLE	Cyclohexane	16.0	17.0	17.0	17.0	0.4
					Freon	23.0	20.0	22.0	21.0	1.3
					Hexane	15.0	16.0	17.0	16.0	0.8

TW = Tap Water

Appendix B
Total Petroleum Hydrocarbons

FACNUM	FACILITY	SCC#	WASTE STREAM	TECHNIQUE	SOLVENT	REP1	REP2	REP3	MEAN	STD. DEV.
21	Meat Processing Plant	24898	Primary Effluent	LLE	Cyclohexane	22.0	15.0	12.0	16.0	5.1
					Freon	5.0 <	5.0 <	5.0 <	5.0 <	
					Hexane	5.0 <	9.4	5.0 <	6.5	2.5
				SPE Col	Cyclohexane		6.6		6.6	
					Hexane	12.0	17.0	9.9	13.0	3.7
					MeCl	9.9	11.0	13.0	11.0	1.3
					Pentane	11.0	11.0	13.0	12.0	1.5
22	Rendering Plant	24899	Primary Effluent (1:4 TW)	LLE	Cyclohexane	270.0	260.0	360.0	300.0	54.0
					Freon	190.0	200.0	180.0	190.0	11.7
					Hexane	220.0	130.0	170.0	170.0	43.4
				SPE Col	Cyclohexane	160.0	130.0	170.0	150.0	20.7
					Hexane	93.0	110.0	99.0	100.0	8.2
					MeCl	73.0	83.0	88.0	81.0	7.6
					Pentane	29.0	35.0	31.0	32.0	3.0
23	Industrial Laundry	24900	Primary Eff (1:1 TW)	LLE	Cyclohexane	110.0	170.0	140.0	140.0	31.4
					Freon	130.0	120.0	140.0	130.0	9.3
					Hexane	100.0	120.0	110.0	110.0	7.4
				SPE Col	Cyclohexane	100.0	90.0	120.0	100.0	16.4
					Hexane	130.0	120.0	100.0	120.0	11.6
					MeCl	120.0	140.0	110.0	130.0	15.5
					Pentane	110.0	120.0	130.0	120.0	8.8
24	Railroad Maintenance	24901	Separator Effluent	LLE	Cyclohexane	76.0	65.0	79.0	73.0	7.5
					Freon	71.0	68.0	79.0	72.0	5.6
					Hexane	76.0	77.0	74.0	75.0	1.6
				SPE Col	Cyclohexane	120.0	120.0	120.0	120.0	2.1
					Hexane	100.0	100.0	100.0	100.0	0.5
					MeCl	120.0	120.0	120.0	120.0	0.3
					Pentane	130.0	120.0	110.0	120.0	7.1
		25105	Separator Effluent	LLE	Cyclohexane	92.0	79.0	85.0	85.0	6.5
					Freon	110.0	100.0	120.0	110.0	10.1
					Hexane	53.0	53.0	66.0	57.0	7.4
25	Railroad Maintenance	25107	Oily Wastewater (5:6 TW)	LLE	Cyclohexane	21.0	19.0	19.0	20.0	1.2
					Freon	21.0	20.0	19.0	20.0	1.4
					Hexane	15.0	18.0	17.0	17.0	1.4

TW = Tap Water

APPENDIX C
STATISTICAL TECHNIQUES

Outlier Screening Methods

A robust quantile screening method suggested by Emerson & Strenio (in Hoaglin, et al., 1983) was used to screen across the set of laboratory results for each compound (oil and grease, TPH) and sample stratum (all samples, petroleum samples, non-petroleum samples) for outlying values. This method estimates the levels above and below which fall 1% of the data, according to the following formulae:

$$Lower\ 1\%\ level = Q_1 - 1.5\,IQR$$

$$Upper\ 1\%\ level = Q_3 + 1.5\,IQR,$$

where Q_1 is the first quartile, i.e., the value below which fall 25% of the data; Q_3 is the third quartile, i.e., the value below which fall 75% of the data; and IQR is the interquartile range (75th percentile-25th percentile). Solvent-to-Freon ratios (or solvent-to-hexane ratios) were determined for each sample. These ratios were then subjected to the outlier screen and points outside the range were rejected and removed from descriptive analyses (i.e., solvent-to-Freon ratios, solvent-to-hexane ratios, and median absolute deviations).

Data Transformations

The standard deviations of the measured sample concentrations were roughly proportional to the mean sample concentrations, as is common with analytical data. The graph in Attachment 1-A to this Appendix, a plot of the replicate standard deviations versus the mean of the replicate analyses for Freon-113, n-hexane, and cyclohexane separatory funnel extraction, demonstrates this trend of increasing standard deviation with increasing concentration. This heteroscedasticity violates the basic assumptions of Analysis of Variance (ANOVA), and needed to be corrected prior to calculating the Root Mean Square Deviation (RMSD).

To eliminate the heteroscedasticity, the concentration results were transformed to produce data with constant standard deviations. Since the intercept in the standard deviation regression was zero, the data were transformed using the equation

$$\mathbf{z = \ln(x),}$$

where x is the sample concentration (in mg/L) for each individual result within all triplicate analyses, and ln indicates the natural logarithm function. As is demonstrated by the graph in Attachment 1-B to this Appendix, following this conversion, the data no longer showed a statistically significant correlation between standard deviation and mean concentration. Consequently, the transformed data were suitable to the ANOVA analysis described below.

Negative Concentrations

While it is counterintuitive to find negative concentrations, it can be expected to occur, at least occasionally, due to analytical variability whenever very small concentrations are measured via methods involving blank-subtraction. It is possible that some sample/solvent combinations could be

removed from this study by setting up statistical limits on whether the sample can statistically be shown to contain a non-zero amount of oil and grease, for instance simply by computing a one-sided hypothesis (at say 5%) that the mean concentration for a sample/solvent combination is greater than zero using the three replicate measurements (either with or without the data transformation). However, it is desirable that the study remain balanced across solvents, and since some solvents show concentrations clearly greater than zero while other solvents do not, such a rule may not be useful. Instead, negative concentrations were treated as non-detects, and their values were set to one-half of the detection limit (2.5 mg/L).

ANOVAs

The ANOVA model deemed best for this analysis was

$$y=a+b_i+g_j+d_{ij}+e_{ijk}$$

where i=1...I solvents (1=Freon), j = 1...J samples, and k=1...K (3) replicate analyses. Here a, b_i, g_j, and d_{ij} are all fixed effects that were estimated[1], and e_{ijk} is the random measurement error, with mean=0 and variance=σ^2. This model is a standard ANOVA model, and was fitted to obtain estimates of each of these parameters and standard errors associated with each estimate. Testing of this model in Phase I (see Appendix C, Attachment B of *Preliminary Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease*, Revision 1, September 1993) demonstrated that the interaction term is significant (i.e., the effect of solvents depends on the sample matrix). This association prompted evaluation of the results on the basis of similar matrix groupings, or strata (i.e., all samples, non-petroleum samples, petroleum samples).

Evaluation of Solvents

To adequately compare solvent performance, the statistic used needed to provide an overall index of performance that summarizes the similarity of each alternative solvent to Freon-113, while taking into account the differences in the outcomes for each solvent on different samples. One measure for this is the root mean square deviation, across samples, between the alternative solvent and the Freon-113 results. In terms of the model above, the mean result for each solvent for a sample is,[2]

$$a+b_i+g_j+d_{ij},$$

so the root mean square deviation for solvent i is

[1]With $\Sigma b_i= 0$, $\Sigma g_j= 0$, and $\Sigma d_{ij}= 0$ over i for fixed j and over j for fixed i.

[2] For typographical simplicity, the carats over each parameter are omitted from this point on even though the formulae refer to the sample estimates rather than the theoretical model values.

$$RMSD_i = \sqrt{\Sigma_j(((a+b_i+g_j+d_{ij})-(a+b_1+g_j+d_{1j}))^2)/J}$$
$$= \sqrt{\Sigma_j(((b_i+d_{ij})-(b_1+d_{1j}))^2)/J}$$
$$= \sqrt{(b_i-b_1)^2 + \Sigma_j((d_{ij}-d_{1j})^2)/J}$$

This can also be computed as the root mean square deviation between the sample*solvent cell means for the alternative solvent and Freon-113. The smaller this measure, the more closely the results using the alternative solvent approximate the results using Freon-113. RMSD computes the squared deviation of the average analytical results using alternative solvents on a sample from that of Freon-113 on the same sample, and accumulates this over all samples to provide an overall measure of agreement. The data show significant interaction between solvent and sample in the statistical model, that is, whether alternative solvents extract more or less oil and grease than Freon-113 varies according to the sample matrix. RMSD was chosen as the primary statistical measure because it assesses variations both above and below the Freon-113 results, which was necessary in order to account for the possibility that an alternative solvent may extract significantly less oil and grease than Freon-113 on some samples, and more on other samples.

Acceptance Limits were derived by computing the RMSD that would be expected by chance alone, e.g., if Freon-113 were tested by this protocol and compared with itself using separate analyses. Under the null hypothesis that there is no actual difference in the procedures, the square of the RMSD, appropriately normalized by the residual error estimate, will have an F distribution. Therefore,

$$\frac{K(RMSD)^2}{2s^2} \sim F_{J,IJ(K-1)}$$

where s is the root mean square error (RMSE) of the model, and I, J, and K are as above. A 95% acceptance region for the equality of the test is

$$RMSD < \sqrt{\frac{2s^2}{K} F^{-1}_{J,IJ(K-1)(0.95)}}$$

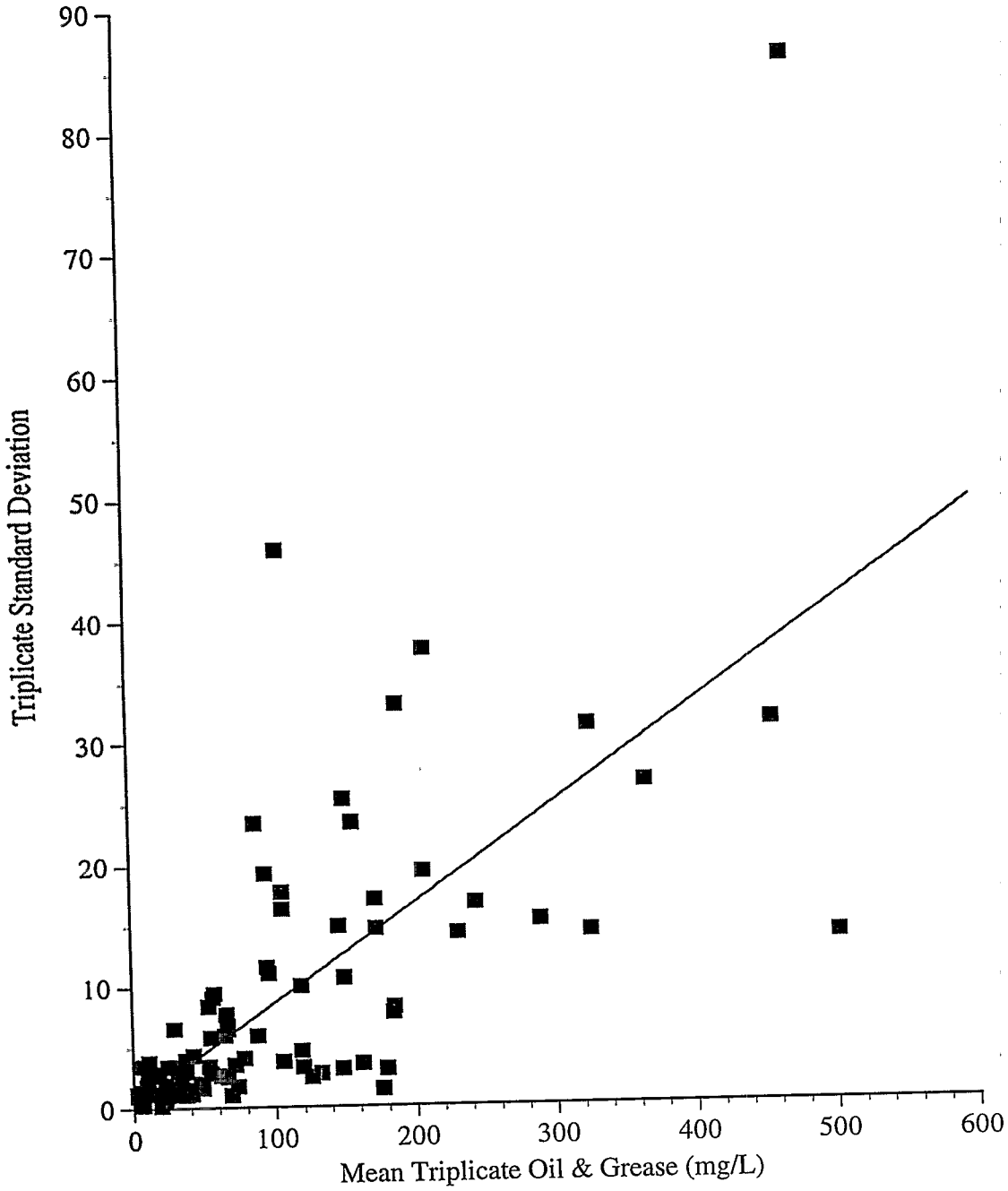
or, in terms of the normalized RMSD,

$$\frac{RMSD}{\sqrt{\frac{2s^2}{K}}} < \sqrt{F^{-1}_{J,IJ(K-1)(0.95)}}$$

Examples of these analyses are shown in Attachment 2.

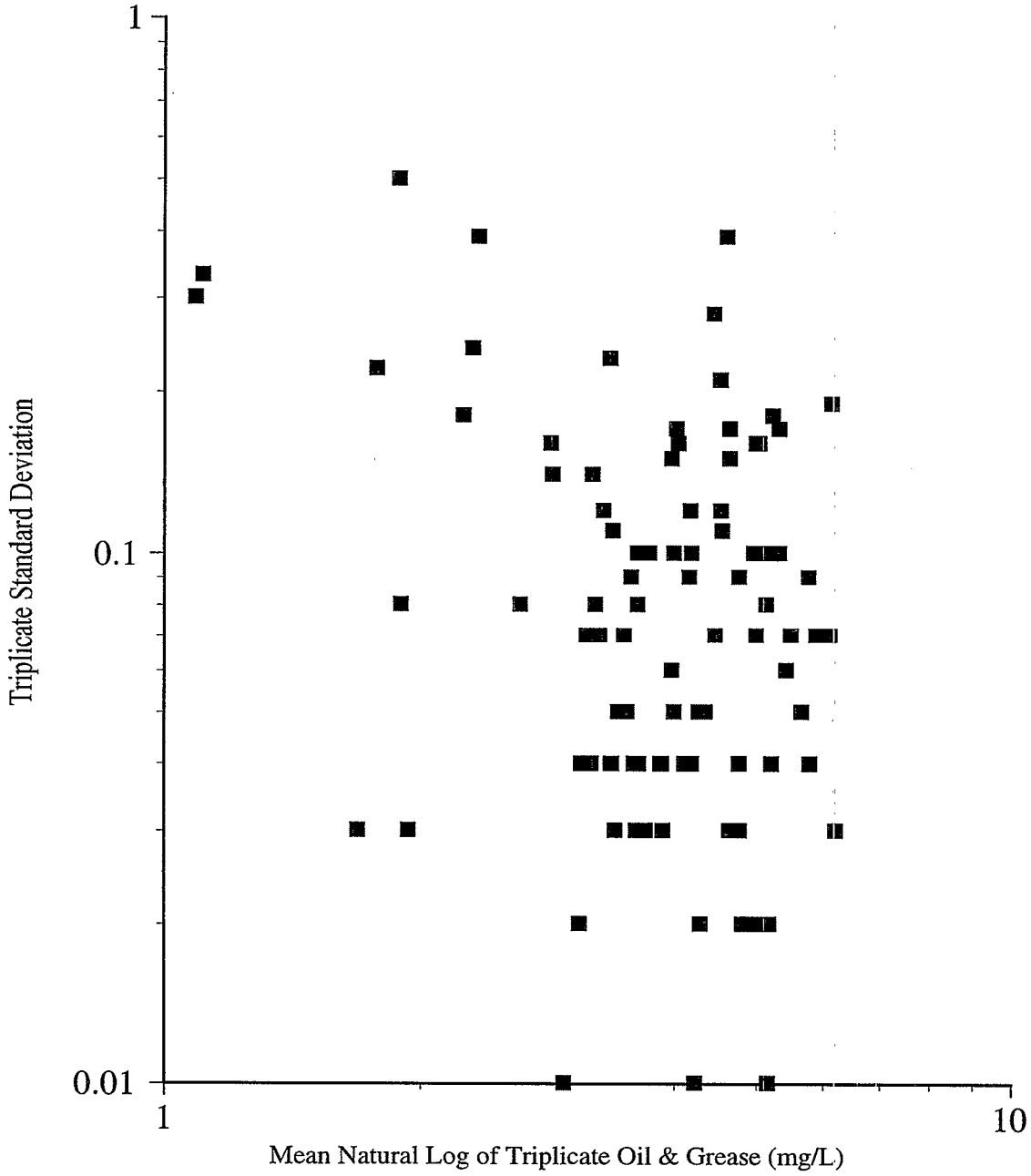
Appendix C
Attachment 1-A

Triplicate Standard Deviation as a Function of Mean Triplicate Concentration



Appendix C
Attachment 1-B

Triplicate Standard Deviation as a Function of Mean Triplicate Concentration
Following Natural Log Transformation



Appendix C
Attachment 2
Example of RMSD Acceptance Limit Analysis

Sample * Solvent Means (after Natural Log Transformation)

Sample	Freon	Hexane	Cyclohexane
24870	1.89976	2.24750	1.69108
24871	1.08922	1.78270	1.10924
24873	3.62188	3.36226	2.87459
24874	2.85957	2.31012	2.30687
24875	3.19262	3.11160	3.08939
24876	5.44335	5.14898	4.98348
24877	4.29667	4.11848	3.88191
24878	4.88645	4.78586	4.83458
24879	5.90111	5.78575	5.66966
24880	4.00163	3.86160	3.97592
24881	4.19625	3.98256	4.00258
24882	4.47511	4.27638	4.23395
24883	3.87275	3.43653	3.59227
24884	4.99640	4.55492	4.56941
24886	4.02527	3.55624	4.05484
24887	4.62359	4.45701	4.53798
24888	5.78441	5.05031	5.33054
24889	4.35926	4.18910	4.20105
24890	2.34573	1.88590	2.62744
24891	1.94084	0.91629	0.91629
24893	3.52070	3.29609	3.48958
24896	3.74459	5.23774	3.61525
24897	4.16967	3.38595	3.44812
24898	2.96354	3.20073	3.41185
24899	6.22000	6.12417	6.14840
24900	5.49763	5.15191	5.33943
24901	4.65902	4.77981	4.77752
24902	4.66147	5.18971	5.00874
25101	4.20046	3.60976	3.70926
25104	5.16971	5.21991	5.08786
25105	5.21582	4.66391	5.01186
25106	3.63882	3.14549	3.26684
25107	3.37937	3.23419	3.37465
RMSD		0.49173	0.36581

s (RMSE)	I	J	K
0.13397	3	33	3

$$\text{Acceptance Limit} = \sqrt{\frac{2s^2}{K} F^{-1}_{J,IJ(K-1)(.95)}} =$$

$$\sqrt{\frac{2(0.13397)^2}{3} 1.5} = 0.13376$$

$$\text{Normalized RMSD} = \frac{RMSD}{\sqrt{\frac{2s^2}{K}}}$$

$$\text{Normalized RMSD for Hexane} = \frac{0.49173}{\sqrt{\frac{2(0.13397)^2}{3}}} = 4.50$$

$$\text{Normalized Acceptance Limit} = \sqrt{F^{-1}_{33,198(.95)}} = \sqrt{1.5} = 1.22$$

Since 4.50 > 1.22, Hexane is not equivalent to Freon

APPENDIX D
REFERENCES

APPENDIX D
REFERENCES

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