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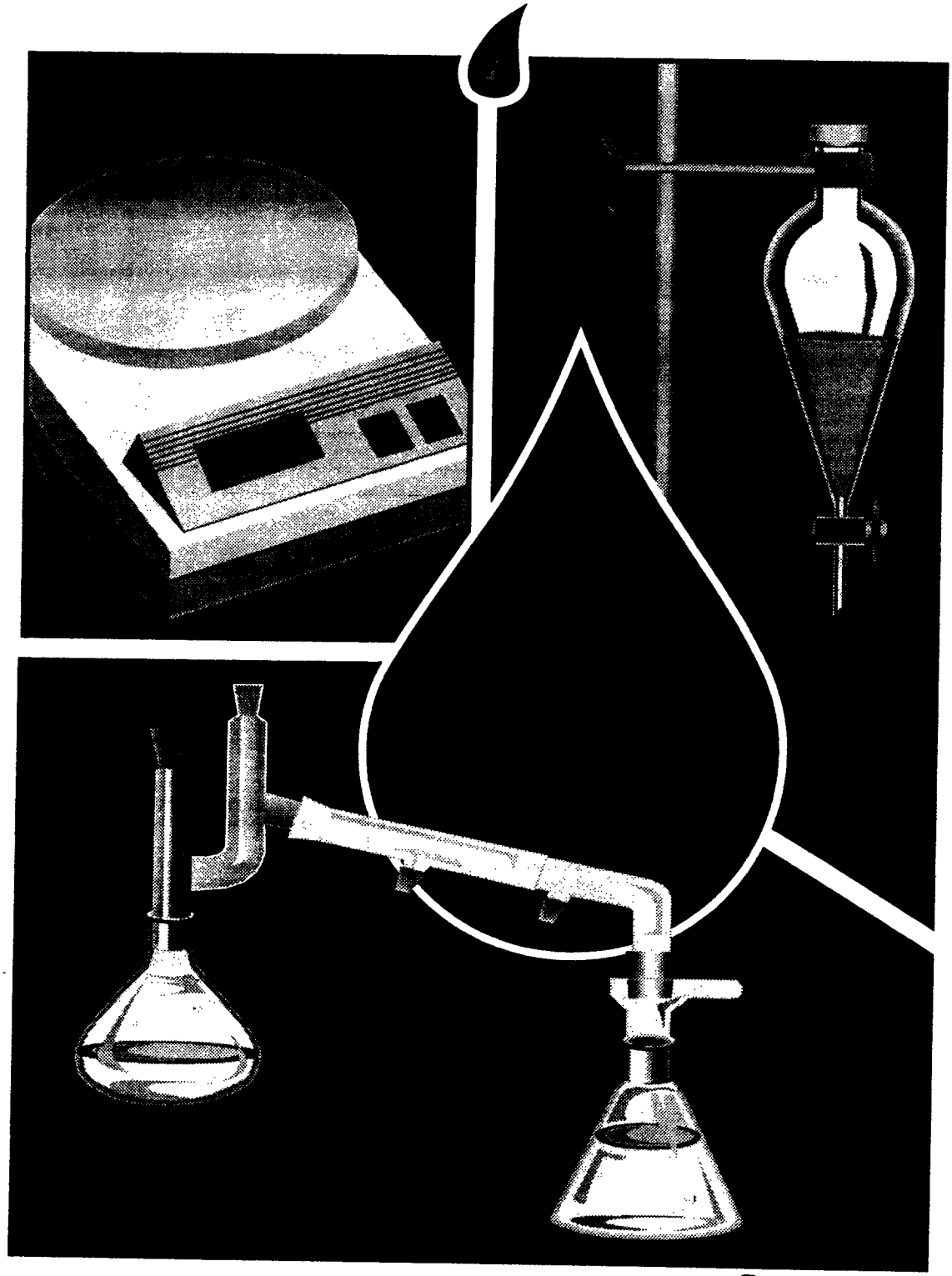

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Report of the Method 1664 Validation Studies

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NOTE: This printing of the Report of the Method 1664 Validation Studies contains an Addendum to Section 3, dated January 1996. This version of the report supersedes any previous publication of the Report of the Method 1664 Validation Studies.

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SECTION 1
BACKGROUND/INTRODUCTION

EPA Method 1664 is a gravimetric procedure applicable to aqueous matrices for the determination of n-hexane extractable material and silica gel treated n-hexane extractable material (oil and grease and total petroleum hydrocarbons, respectively). Method 1664 resulted from a multiphase study conducted by the Environmental Protection Agency (EPA) to determine a suitable replacement solvent for Freon-113, a Class I chlorofluorocarbon (CFC) used in several EPA wastewater and solid waste methods for the determination of oil and grease and petroleum hydrocarbons. Method 1664 is being proposed to replace two currently approved 40 *CFR* part 136 methods for the determination of oil and grease. These methods are EPA Method 413.1, published in *Methods for Chemical Analysis of Water and Wastes*, and Method 5520B, published in *Standard Methods for the Examination of Water and Wastewater*, 18th edition.

Development of Method 1664 was the final objective of Phase II of the Freon Replacement Study, the results and details of which are provided in the *Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease and Total Petroleum Hydrocarbons: Phase II*. Method 1664 reflects not only the information gathered under Phase II of the Freon Replacement Study, but also consideration of numerous peer review comments, survey results, data from industry studies, results from an interlaboratory method validation study, and results from several EPA single-laboratory method detection limit (MDL) studies.

The quality control (QC) requirements incorporated into Method 1664 exceed and improve upon that of the currently approved 40 *CFR* part 136 oil and grease methods, and are consistent with 40 *CFR* 136, Appendix A methods for determination of organic analytes. The QC acceptance criteria incorporated in the initial draft of Method 1664 (March 29, 1994) reflected the data quality objectives (DQOs) that were established at the beginning of Phase II of the Freon Replacement Study. These objectives are outlined in the *Draft Study Plan for Phase II of the Freon Replacement Study*, September 29, 1993 (Appendix A). A final goal of the Phase II study was the validation and/or revision of the estimated DQOs and subsequent development of final QC acceptance criteria (specifications) for the updated version of Method 1664. These final specifications were derived from the QC analyses conducted as part of Phase II of the Freon Replacement Study and from the results of an interlaboratory study conducted by 11 laboratories belonging to the Twin Cities Round Robin Group. In addition, five studies were performed to determine the method detection limit (MDL) and the minimum level of quantitation (ML).

This report gives details of the studies and associated results that were used to derive the QC acceptance criteria in the latest version of Method 1664 (April, 1995). This report is divided into three main sections: Section 2 presents the field sample results of the Twin Cities Round Robin Group interlaboratory study of Method 1664; Section 3 details the procedures used to develop the QC specifications in Method 1664; Section 4 provides the results and discussion of the five MDL studies.

SECTION 2
TWIN CITIES ROUND ROBIN STUDY

This section of this report provides the details of an interlaboratory study conducted by the Twin Cities Round Robin (TCRR) Group for the determination of HEM by Method 1664. The TCRR Group consists of a number of commercial, state, and municipal laboratories in the Minneapolis/St. Paul region that volunteer their services to evaluate new procedures. The objectives of this study were to determine if the method instructions were clear and comprehensive, and to assess whether or not the estimated QC acceptance criteria in the March 29, 1994 draft version of Method 1664 could be achieved in a variety of laboratories.

In addition, the results of the round-robin study were used to statistically derive QC specifications to replace the estimated QC specifications in the draft version of Method 1664. Field sample and QC analysis results from a total of eleven laboratories were submitted for consideration under this study. Presentation and evaluation of the QC results are included in Section 3 of this report. Results and assessment of the field sample analyses are provided below.

2.1 Study Design

2.1.1 Analytical Study Design

The TCRR study consisted of two parts: 1) 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) analysis of two sets of field samples, one from a petroleum source and the other from a non-petroleum source, in triplicate, for HEM. Laboratories were required to submit the four replicate IPR results for evaluation prior to analysis of the field samples. Laboratories were encouraged to perform analysis for silica gel treated-hexane extractable material (SGT-HEM) as well, although this analysis was optional.

In addition, analysis of a reagent water method blank with both the IPR set and the field samples was required. An ongoing precision and recovery (OPR) analysis, the equivalent of a single IPR analysis, was performed with the field sample analyses. Hexadecane and stearic acid standards from a single source were provided to the laboratories for convenience. Details of the analytical protocol and reporting requirements are provided in Appendix B.

2.1.2 Sample Source Selection

In order to avoid the problems associated with the comparison and evaluation of non-detect results, candidate field samples were screened by an independent laboratory to ensure that the HEM concentrations were within a range of 40 - 300 mg/L. The screening was performed with Method 1664. The two samples used in this study included an oily wastewater from a shore reception facility (petroleum source) and a primary effluent from an olive packaging plant (non-petroleum source).

At each site, a large-volume sample 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 of the barrel directly into individual sample containers. Sample containers were unused, pre-cleaned, 1-liter, wide-mouth, clear glass bottles with fluoropolymer-lined caps.

Each individual 1-L sample aliquot was preserved on-site with HCl (1:1) to a pH ≤ 2. Sample bottles were cooled with wet ice prior to shipment to the laboratories. Prior to collection, each sample bottle was labeled with a unique EPA sample number and identifying information, including source location, collection date, and preservatives used. Pre-assigned sample numbers were the primary means of identifying and tracking samples. Traffic Reports, which provide information on the samples collected and serve as a sample tracking sheet, were completed at each site, and accompanied each shipment to the receiving laboratory. Copies of these reports were used by field and Sample Control Center[1] personnel for tracking purposes.

2.2 Data Validation

Data generated from this study were submitted to the Sample Control Center for review and validation. All results received by August 20, 1994 were considered and are presented in this report. Final results, including all calculations, were verified from the raw data and bench sheets provided. All quality control requirements in the study plan and draft version of Method 1664 were met, and all data reviewed were determined to be of acceptable quality.

2.3 Results

Eleven laboratories submitted results for HEM determination. Only one laboratory performed the optional SGT-HEM determination; therefore, SGT-HEM results are not included in this report. For each laboratory, the HEM triplicate measurements were averaged. The standard deviation of the triplicate measurements and the relative standard deviation (RSD) were then calculated, providing an indication of the intralaboratory variability and precision.

For each sample, the average HEM concentration across all laboratories was calculated, along with the standard deviation of the HEM concentrations. In addition, the average RSD and the standard deviation of the RSDs across laboratories were calculated to estimate the interlaboratory variability and precision. These calculations, along with the analytical results, are presented by sample in Table 1.

[1] SCC, operated by DynCorp Environmental under contract to EPA's Engineering and Analysis Division.

Table 1
Twin Cities Round Robin Group Interlaboratory Study of Method 1664
HEM (mg/L) in Field Samples

Sample=25101 Source=Petroleum

Lab	Rep 1	Rep 2	Rep 3	Triplicate Mean	Triplicate Std. Deviation	RSD (%)
1	70.2	69.0	71.5	70.2	1.3	1.8
2	57.8	52.9	53.8	54.8	2.6	4.8
3	63.0	61.0	69.0	64.3	4.2	6.5
4	47.0	47.0	30.0	41.3	9.8	23.7
5	56.7	41.8	47.8	48.8	7.5	15.4
6	61.8	46.5	53.4	53.9	7.7	14.2
7	50.6	49.9	47.5	49.3	1.6	3.3
8	63.4	62.8	63.6	63.3	0.4	0.7
9	63.7	98.3	96.3	86.1	19.4	22.6
10	49.0	41.0	65.0	51.7	12.2	23.7
11	50.4	57.2	28.4	45.3	15.1	33.2

Mean HEM (mg/L)	57.2
Std. Deviation of HEM	12.9

Mean RSD Across Labs	13.6
Std. Deviation of RSDs	11.0

Table 1 (Cont.)
Twin Cities Round Robin Group Interlaboratory Study of Method 1664
HEM (mg/L) in Field Samples

Sample=25104 Source=Non-petroleum

Lab	Rep 1	Rep 2	Rep 3	Triplicate Mean	Triplicate Std. Deviation	RSD (%)
1	182.0	171.0	194.0	182.3	11.5	6.3
2	135.2	134.8	136.9	135.6	1.1	0.8
3	182.0	163.0	181.0	175.3	10.7	6.1
4	150.0	225.0	184.0	186.3	37.6	20.2
5	166.3	180.9	178.2	175.1	7.8	4.4
6	144.0	167.0	143.0	151.3	13.6	9.0
7	173.0	.*	173.0	173.0	0.0	0.0
8	194.1	187.4	186.0	189.2	4.3	2.3
9	158.5	174.1	181.9	171.5	11.9	6.9
10	157.0	167.0	167.0	163.7	5.8	3.5
11	160.0	162.0	162.0	161.3	1.2	0.7

Mean HEM (mg/L)	169.5
Std. Deviation of HEM	15.8

Mean RSD Across Labs	5.5
Std. Deviation of RSDs	5.7

Mean RSD Across Labs for Samples 25101 and 25104 Combined	9.5
Std. Deviation of RSDs for Samples 25101 and 25104 Combined	9.5

* Due to a laboratory accident, this aliquot of the sample was not analyzed.

2.4 Discussion and Conclusions

Initially, a small number of laboratories encountered some difficulties with the analysis of IPR samples but, after adjusting and improving their technique, were able to achieve acceptable recoveries of hexadecane and stearic acid in both the IPR and OPR analyses. 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 relative standard deviation across all laboratories for the petroleum sample was 13.6 percent, and for the non-petroleum sample was 5.5 percent, resulting in a combined mean RSD of 9.5 percent. These results demonstrate that Method 1664 is capable of producing precise results on real world samples.

Since the TCRR Group was the first to perform analyses according to Draft Method 1664, input from the laboratories regarding inconsistencies, unclear instructions, or suggestions for improvement in the procedure were encouraged. An initial comment related to the ambiguity surrounding the grade of n-hexane that should be used. This comment was addressed, and more specific criteria were included in the updated versions of Method 1664.

Most other comments 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. The former was addressed in revised versions of Method 1664 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. The latter was addressed in revised versions 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.

SECTION 3
DEVELOPMENT OF QUALITY CONTROL ACCEPTANCE CRITERIA

This section of this report presents details of the statistical analyses used to develop the QC acceptance criteria in Method 1664. As previously mentioned, EPA used data gathered from two studies for the development of these criteria. These two studies were the Twin Cities Round Robin (TCRR) interlaboratory method validation study and Phase II of EPA's Freon Replacement Study.

Study data that were used to develop the QC acceptance criteria that are in the April 1995 version of the method consisted of results from initial precision and recovery (IPR) and ongoing precision and recovery (OPR) tests. In the IPR test, four 1-L reagent water samples are spiked with and analyzed for the analytes of interest (in this case hexadecane and stearic acid), and the precision and recovery are measured; in the OPR test, a single reagent water aliquot is spiked, and the recovery is measured.

An advantage of using data from both studies was that the specifications ultimately developed for Method 1664 were based on results from both a breadth and depth of laboratory analyses. Use of data from the TCRR study provided EPA with a single set of IPR and OPR results from each of 11 laboratories; use of data from the Phase II study provided EPA with results from 30 OPR analyses performed by a single laboratory, along with IPR results from that laboratory. The basic design of the Phase II study and the TCRR study are described in detail in the respective study plan or analytical requirements summary (Appendices A and B). Evaluation and discussion of the Phase II field sample results is provided in the *Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease and Total Petroleum Hydrocarbons: Phase II*. Evaluation of the TCRR study field sample results are given in Section 2 of this report.

3.1 Data Validation and Statistical Analysis

3.1.1 Screening for Outlier Laboratories

Results from each of the twelve laboratories (11 from the TCRR study and one from the Phase II study) were compared to results from all laboratories to determine if any laboratories were systematically biased. First, the median percent recovery of the results from all laboratories was calculated. Then the percent recovery values of each of the four IPR samples were ranked by laboratory according to their deviation from the median percent recovery. The ranks for each laboratory were summed across the four IPR samples, and the summed ranks were analyzed by an extreme rank sum test. If all laboratories were equivalent, then theoretically the ranks of each laboratory relative to the other laboratories would be random, and the summed ranks would be equal. The extreme rank sum test determines if the summed rank of any laboratory is significantly different from those of the other laboratories. This test is based on the work of Youden in *Ranking Laboratories by Round Robin Tests* (1963).

3.1.2 QC Acceptance Criteria for Hexane Extractable Material

Criteria were developed for IPR, OPR, and matrix spike/matrix spike duplicate (MS/MSD) analyses for both HEM and SGT-HEM. For HEM, the IPR and OPR acceptance criteria were constructed using an analysis of variance (ANOVA) with laboratories as a random variance component. For each QC criterion, the mean result across laboratories was determined, and the variability was calculated from the interlaboratory and intralaboratory variance components using a formula appropriate to the particular test. The 95 percent cutoff value from the Student's *t* distribution was determined based on the appropriate degrees of freedom. The degrees of freedom are dependent on the number of laboratories, the number of sample analyses, and the variance components. The QC limit was derived by multiplying the *t* value by the variability, and subtracting this value from the mean result for the lower limit, or adding this value to the mean result for the upper limit. Details of the equations used to derive these limits are presented in the document titled *Interlaboratory Validation of U.S. Environmental Protection Agency Method 1625A*, July 1984.

3.1.3 QC Acceptance Criteria for Silica Gel Treated Hexane Extractable Material

For SGT-HEM, EPA received results from only two laboratories, one from the TCRR study and one from the Phase II study. EPA used these data to construct preliminary IPR and OPR acceptance criteria for SGT-HEM and widened these preliminary criteria to those of HEM in those instances in which the calculated SGT-HEM criteria were more stringent than those for HEM. The acceptance criteria were widened based on the knowledge that the determination of SGT-HEM follows the determination of HEM in Method 1664, and therefore the result for SGT-HEM is likely to be at least as variable as the result for HEM.

3.1.4 QC Acceptance Criteria for Matrix Spike and Matrix Spike Duplicate

For HEM and SGT-HEM, the criteria for recovery of a matrix spike (MS) or matrix spike duplicate (MSD) and for the relative percent difference between an MS and an MSD were derived from the OPR criteria, since neither the TCRR study nor the Phase II study required the spiking of field samples. EPA believes that this application of OPR criteria to MS/MSD samples is acceptable because the determinative technique in Method 1664 is gravimetry, which is not susceptible to interferences, and because nearly all of the treated effluents to which Method 1664 is to be applied in monitoring will be similar to the reagent water used in the OPR tests. This transfer of data for development of specifications for acceptance criteria is similar to that which EPA used in the organic methods that are promulgated at 40 *CFR* 136, Appendix A.

Determination of the relative percent difference (RPD) criteria consisted of setting the limit at approximately one-half of the range between the lower recovery limit and upper recovery limit. This estimation establishes the RPD limit at 18 percent for HEM and 24 percent for SGT-HEM. These limits are considered to be a reasonable first approximation of method performance in the absence of MS/MSD data. In other methods, EPA has established the default limit at 20 percent. If MS/MSD data become available, EPA may revise this limit.

3.2 Results

Few laboratories in the interlaboratory study encountered difficulties with the analysis of IPR and OPR samples, and most achieved acceptable recoveries of hexadecane and stearic acid. Statistical evaluation of the results from all twelve laboratories produced few outliers, and the extreme rank sum test showed all laboratories to be equivalent. This indicates that Method 1664 is a reproducible procedure sufficiently reliable to be used by a variety of laboratories.

Results are summarized in Table 2 below. Individual laboratory results and the statistical analyses of these data are presented in Tables 3 through 12.

Table 2
95 Percent Confidence Limits for Method 1664 QC Criteria*

Criterion	95% Lower Limit (%)	95% Upper Limit (%)
IPR		
HEM Precision		10.9
HEM Recovery	83.0	100.7
SGT-HEM Precision		13.3
SGT-HEM Recovery	83.2	116.0
MS/MSD		
HEM Recovery	79.0	113.9
HEM RPD		17.5
SGT-HEM Recovery	65.8	105.7**
SGT-HEM RPD		24.0
OPR		
HEM Recovery	79.0	113.9
SGT-HEM Recovery	65.8	105.7**

*The values for the acceptance criteria in Method 1664 were derived by rounding these values to the nearest whole number.

**These calculated values were widened to the limits generated from HEM, as explained in Section 3.1.3. This change also applies to the MS/MSD RPD calculation.

3.3 Discussion and Conclusions

The QC acceptance criteria were evaluated in several ways. First, the precision of the HEM results from the triplicate analyses of field samples in the TCRR study were compared to the QC acceptance criteria for RPD. Since there were three replicates, and RPD is determined using duplicates, RPDs were calculated for each of the three pair-wise groupings of the three replicates. These were then averaged to give the mean RPD for each laboratory. In addition, the mean RPDs of all laboratories were averaged across each sample and across both samples. The mean RPD found was 12.3 percent, which is well within the HEM RPD acceptance criterion of 18 percent. These RPD values, along with the mean and standard deviation of the three RPDs for each laboratory, are shown in Table 13.

Second, the RSD of results for the two field samples in the TCRR study was computed. As is explained in Section 2 of this report, the TCRR group analyzed two sets of field samples, one from a petroleum source and the other from a non-petroleum source, in triplicate, for HEM. The mean RSD of the results across all laboratories and all samples was 9.5 percent (see Table 1), further demonstrating that Method 1664 and the laboratories used in the study are capable of producing precise results on real world samples.

Third, IPR and OPR results from the Phase II study and the TCRR study were compared with the respective QC acceptance criteria. For the IPR test of HEM, all of the mean percent recovery values were within the IPR recovery criterion and only one of the 12 IPR sets failed the IPR precision criterion. For the OPR test, none failed the OPR recovery criterion. For the IPR test of SGT-HEM, both laboratories met the recovery criterion, but failed the precision criterion. These higher standard deviations can be attributed to one of the four IPR results for each of the labs. For the OPR test, only two of the numerous data points did not meet the recovery criterion.

From the results presented above, it can be concluded that nearly all laboratories are capable of performing Method 1664 successfully. This demonstrates that the QC acceptance criteria are realistic and reflect the performance of Method 1664 on both reagent water and on real world sample matrices.

Table 3
HEM IPR Data

Lab	% Rec. IPR 1	% Rec. IPR 2	% Rec. IPR 3	% Rec. IPR 4	Mean % Rec.	Standard Deviation
1	98.8	103.8	96.3	98.3	99.3	3.2
2	78.7	103.5	105.7	83.2	92.8	13.8*
3	87.5	92.5	85.0	87.5	88.1	3.1
4	90.3	87.5	89.0	89.3	89.0	1.2
5	93.9	97.9	82.2	90.1	91.0	6.7
6	86.8	92.0	92.5	93.8	91.3	3.1
7	82.3	83.8	81.5	86.5	83.5	2.2
8	91.0	103.5	94.5	95.3	96.1	5.3
9	85.0	93.3	89.3	102.0	92.4	7.2
10	87.5	77.5	87.5	82.5	83.8	4.8
11	89.5	89.3	92.3	89.0	90.0	1.5
12	107.5	110.0	95.0	90.0	100.6	9.7
				Mean % Recovery Across Labs	91.5	
				Std. Deviation of Mean % Recoveries Across Labs	5.3	
					Mean Std. Deviation Across Labs	5.2
					Std. Deviation of the Std. Deviations Across Labs	3.7

Table 4
HEM IPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of Mean % Rec. Values Below 95% Lower Limit	No. of Mean % Rec. Values Above 95% Upper Limit
83.0	100.7	0	0

Table 5
HEM IPR Data - Upper Limit for Precision

95% Upper Limit	No. of Standard Deviation Values Above 95% Upper Limit
10.9	1

* Result that failed the precision specification.

Table 6
HEM OPR Data

Lab	% Recovery
1	83.0
2	80.8
3	82.5
4	87.8
5	91.0
6	98.8
7	89.8
8	89.8
9	82.5
10	91.0
11	99.5
11	91.5
11	91.5
11	91.5
11	91.5
11	91.5
11	91.0
11	91.0
11	91.0
11	91.0
11	91.0
11	91.5
11	91.0
11	112.0
11	112.0
11	93.8
11	93.8
11	93.3
11	93.3
11	101.8
11	101.8
11	101.8
11	112.0
11	112.0
11	112.0
11	89.8
11	89.8
11	111.8
11	111.8
11	94.0
Mean % Recovery Across Labs	95.2
Standard Deviation of % Recoveries Across Labs	9.1

Table 7
HEM OPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of % Rec. Values Below 95% Lower Limit	No. of % Rec. Values Above 95% Upper Limit
79.0	113.9	0	0

Table 8
SGT-HEM IPR Data

Lab	% Rec. IPR 1	% Rec. IPR 2	% Rec. IPR 3	% Rec. IPR 4	Mean % Rec.	Standard Deviation
1	110.0	115.0	80.0	115.0	105.0	16.8*
2	105.0	100.0	95.0	65.0	91.3	18.0*
				Mean % Recovery Across Labs	98.2	
				Std. Deviation of Mean % Recoveries Across Labs	9.7	
					Mean Std. Deviation Across Labs	17.4
					Std. Deviation of the Std. Deviations Across Labs	0.8

Table 9
SGT-HEM IPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of Mean % Rec. Values Below 95% Lower Limit	No. of Mean % Rec. Values Above 95% Upper Limit
83.2	116.0	0	0

Table 10
SGT-HEM IPR Data - Upper Limit for Precision

95% Upper Limit	No. of Standard Deviation Values Above 95% Upper Limit
13.3	2

* Results that failed the precision specification.

Table 11
SGT-HEM OPR Data

Lab	% Recovery
1	88.0
1	82.0
1	82.0
1	82.0
1	82.0
1	82.0
1	76.0
1	76.0
1	76.0
1	76.0
1	76.0
1	82.0
1	76.0
1	75.5
1	75.5
1	100.0
1	100.0
1	107.5*
1	107.5*
1	80.0
1	80.0
1	80.0
1	75.5
1	75.5
1	75.5
1	79.0
1	79.0
1	96.0
1	96.0
1	100.0
Mean % Recovery Across Labs	84.0
Standard Deviation of % Recoveries Across Labs	10.2

Table 12
SGT-HEM OPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of % Rec. Values Below 95% Lower Limit	No. of % Rec. Values Above 95% Upper Limit
65.8	105.7	0	2

* Results that failed the percent recovery specifications.

Table 13
Twin Cities Round Robin Group Interlaboratory Study of Method 1664
Mean RPDs of the HEM Field Sample Results

Sample=25101 Source=Petroleum

Lab	Rep 1	Rep 2	Rep 3	RPD 1-2	RPD 2-3	RPD 1-3	Mean RPD	RPD Std. Deviation
1	70.2	69.0	71.5	1.7	3.6	1.8	2.4	1.0
2	57.8	52.9	53.8	8.9	1.7	7.2	5.9	3.7
3	63.0	61.0	69.0	3.2	12.3	9.1	8.2	4.6
4	47.0	47.0	30.0	0.0	44.2	44.2	29.4	25.5
5	56.7	41.8	47.8	30.3	13.4	17.0	20.2	8.9
6	61.8	46.5	53.4	28.3	13.8	14.6	18.9	8.1
7	50.6	49.9	47.5	1.4	4.9	6.3	4.2	2.5
8	63.4	62.8	63.6	1.0	1.3	0.3	0.9	0.5
9	63.7	98.3	96.3	42.7	2.1	40.7	28.5	22.9
10	49.0	41.0	65.0	17.8	45.3	28.1	30.4	13.9
11	50.4	57.2	28.4	12.6	67.3	55.8	45.2	28.8
						Mean RPD Across Labs	17.6	
						Standard Deviation of Mean RPDs Across Labs	14.5	

Table 13 (Cont.)
Twin Cities Round Robin Group Interlaboratory Study of Method 1664
Mean RPDs of the HEM Field Sample Results

Sample=25104 Source=Non-petroleum

Lab	Rep 1	Rep 2	Rep 3	RPD 1-2	RPD 2-3	RPD 1-3	Mean RPD	RPD Std. Deviation
1	182.0	171.0	194.0	6.2	12.6	6.4	8.4	3.6
2	135.2	134.8	136.9	0.3	1.5	1.2	1.0	0.7
3	182.0	163.0	181.0	11.0	10.5	0.6	7.4	5.9
4	150.0	225.0	184.0	40.0	20.0	20.4	26.8	11.4
5	166.3	180.9	178.2	8.4	1.5	6.9	5.6	3.6
6	144.0	167.0	143.0	14.8	15.5	0.7	10.3	8.3
7	173.0	.*	173.0	.*	.*	0.0	0.0	.*
8	194.1	187.4	186.0	3.5	0.7	4.3	2.8	1.8
9	158.5	174.1	181.9	9.4	4.4	13.7	9.2	4.7
10	157.0	167.0	167.0	6.2	0.0	6.2	4.1	3.6
11	160.0	162.0	162.0	1.2	0.0	1.2	0.8	0.7

Mean RPD across Labs	7.0
Standard Deviation of Mean RPDs Across Labs	7.5
Mean RPD Across Labs for Samples 25101 and 25104 Combined	12.3
Std. Deviation of RPDs for Samples 25101 and 25104 Combined	12.5

* Due to a laboratory accident, this aliquot of the sample was not analyzed, and the associated statistical analyses could not be performed.

SECTION 4
METHOD DETECTION LIMIT STUDIES

This section presents the results of five MDL studies for Method 1664. These studies were performed by two laboratories that used reagent water as the reference matrix. Global Environmental, Inc. performed MDL Studies 1, 4, and 5, and ETS Analytical Services performed MDL Studies 2 and 3. The purposes of these studies were to 1) determine and confirm the MDLs for HEM and SGT-HEM from the analysis of seven reagent water samples spiked with hexadecane and stearic acid using Method 1664 and 2) use the MDL results to establish minimum levels (MLs) for both analytes.

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 40 *CFR* 136, Appendix B. This procedure consists of analyzing seven aliquots of reagent water that are spiked with the analyte(s) of interest. 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 ML 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 number nearest to (1, 2, or 5) x 10^n, where n is an integer. The value "3.18" represents the ratio of the 10 times multiplier used in the American Chemical Society (ACS) Limit of Quantitation (LOQ) calculations to the Student's *t* multiplier of 3.143 that is used to determine the MDL (i.e., 10 ÷ 3.143 = 3.18). For example, if the calculated MDL is 5.8, the ML will be equal to 5.8 x 3.18 = 18.4. Using (1, 2, or 5) x 10^n, the number nearest to 18.4 establishes the ML at 20.

4.1 Study Design

The design of the MDL studies is described in detail in two HEM and SGT-HEM study plans (Appendix C). The study plan dated June 23, 1994 applies to MDL Studies 1 and 2; the study plan dated November 3, 1994 applies to MDL Studies 3, 4, and 5. The analytical requirements for each study were modified slightly to reflect revisions of the estimated MDL and corresponding adjustments to the suggested spike levels and blank contamination limits. Examples of these analytical requirements are provided in Appendix D. All studies were conducted in accordance with 40 *CFR* 136, Appendix B, "Definition and Procedure for the Determination of the Method Detection Limit", Revision 1.11 (Appendix E).

4.2 Implementation

4.2.1 Sample Matrix

Because EPA regulates more than 600 sub-categories of wastewater discharge, it was not possible to determine the MDL for each discharge type. EPA considers treated effluents from well-designed, well-operated best available technology (BAT) treatment systems to be

similar in nature to reagent water and, therefore, reagent water was judged to be a suitable sample matrix for the MDL studies. Reagent water is a homogeneous reference matrix available to all laboratories and is the primary matrix for determining the MDL using the procedure in 40 *CFR* 136, Appendix B. Further, because the determination of HEM and SGT-HEM are total bulk measurements, matrix interferences are unlikely.

4.2.2 Sample Preparation and Spiking

For each MDL study, the laboratories were required to prepare and analyze seven separate aliquots of spiked reagent water. The preparation of a single stock sample and subsequent distribution into seven separate containers was not allowed due to the propensity for the analyte to adhere to container walls, which would prevent equal distribution and precise allocation of the spiked analytes into each aliquot. Separate containers for each sample ensured that an exact, known amount of HEM and SGT-HEM was present in each aliquot.

MDL Studies 1, 2, and 3 were performed to determine MDL/ML values for both HEM and SGT-HEM, and therefore entailed two separate sets of analyses. In these three studies, the SGT-HEM samples had a final concentration of the combined amounts of hexadecane and stearic acid that were at least double the final concentration in the HEM samples. This was necessary in order to compensate for the adsorption of stearic acid by silica gel. MDL Studies 4 and 5 were performed to determine the MDL/ML values for HEM only. The spike concentrations in the sample aliquots for the HEM and SGT-HEM analyses in each study are provided below.

MDL Studies 1 and 2

Samples were prepared by spiking each one-liter sample with the hexadecane/stearic acid spiking solution to produce a final concentration of 10 mg/L (5 mg hexadecane and 5 mg stearic acid) for the HEM MDL study and a final concentration of 20 mg/L (10 mg hexadecane and 10 mg stearic acid) for the SGT-HEM MDL study. As recommended in the MDL procedure in 40 *CFR* 136, the spiking levels chosen represent a target analyte concentration that is one to five times the then estimated MDL of 3 mg/L for both HEM and SGT-HEM.

MDL Study 3

The final concentration of the hexadecane/stearic acid solution in the sample aliquots analyzed in Study 1 was more than five times the MDL values determined for HEM and SGT-HEM. To investigate the possible bias that may have been introduced by disproportionately high spike levels, samples in Study 3 were prepared by spiking each one-liter sample with the hexadecane/stearic acid solution to produce a final concentration of 4 mg/L (2 mg hexadecane and 2 mg stearic acid) for the HEM MDL study and a final concentration of 10 mg/L (5 mg hexadecane and 5 mg stearic acid) for the SGT-HEM MDL study.

MDL Studies 4 and 5

Because SGT-HEM results produced in Study 1 were verified by another laboratory in Study 3, Studies 4 and 5 were conducted for HEM only. Samples for Study 4 were prepared by spiking each one-liter sample with the hexadecane/stearic acid spiking solution to

produce a final concentration of 5 mg/L (2.5 mg of hexadecane and 2.5 mg of stearic acid). For Study 5, the laboratory chose to spike each one-liter sample to produce a final concentration of 2.5 mg/L (1.25 mg of hexadecane and 1.25 mg of stearic acid).

With each MDL study, the laboratories were also required to analyze a reagent water blank. The reagent water blanks were run through the entire extraction and analysis procedure by which the MDL samples were run. The HEM or SGT-HEM concentration in each blank was required to be less than the estimated MDL.

4.2.3 Method Detection Limit Calculations

Using the data obtained from the analysis of the seven replicate aliquots, the MDLs for HEM and SGT-HEM were determined in accordance with the calculations found in 40 *CFR* 136, Appendix B. The standard deviations of the replicate results were multiplied by the Student's *t* value, which is 3.143 at the 99% confidence level for six (*n* - 1) degrees of freedom, to obtain the MDLs.

4.2.4 Minimum Level Calculations

Minimum levels were calculated upon receipt of the data. Exact interim MLs were determined by multiplying the MDLs by 3.18, and final MLs were determined by rounding the interim ML to the number nearest to (1, 2 or 5) x 10^n, where n is an integer. The 3.18 factor is the quotient of the 10 times multiplier used in the American Chemical Society's Limit of Quantitation (LOQ) and the Student's *t* value used in the MDL study calculations (3.143 for seven replicates). The exact interim MLs are equivalent to the 10 standard deviations used to establish the LOQ, and the rounded final MLs are close to this value.

4.2.5 Data Verification and Validation

All data generated from this study were submitted to SCC for review and validation. Hardcopy data, including all calculations, were verified from the raw data and bench sheets provided. All QC specifications were met, and all data were determined to be of acceptable quality.

4.3 Results

4.3.1 HEM Analysis

Results of the five HEM MDL studies are provided below in Table 14. This table presents the values for the individual analyses of the seven replicate sample aliquots, the mean concentration of each set of replicates, the standard deviation of the seven results, and the calculated MDLs and MLs for each study. Results are in mg/L.

Table 14
Results and Calculated MDLs and MLs for Seven Replicate Analyses for HEM
(Units = mg/L*)

	Study 1	Study 2	Study 3	Study 4	Study 5
Spiked Concentration	10	10	4	5	2.5
Replicate Analysis 1	10.1	9.1	5.2	4.6	2.7
Replicate Analysis 2	9.8	9.3	4.0	4.4	2.8
Replicate Analysis 3	10.1	6.0	3.8	5.1	2.1
Replicate Analysis 4	10.2	9.9	4.1	4.9	1.6
Replicate Analysis 5	10.1	6.1	5.4	5.0	1.8
Replicate Analysis 6	10.2	8.0	5.7	5.1	1.9
Replicate Analysis 7	9.4	10.1	4.3	5.1	2.0
Mean (X)	9.99	8.36	4.64	4.89	2.13
Standard Deviation (s)	0.29	1.7	0.77	0.28	0.45
Student's *t* value	3.143	3.143	3.143	3.143	3.143
MDL	0.91	5.4	2.4	0.88	1.4
ML	2	20	10	2	5

*Standard Deviation (s) and Student's *t* value excluded.

4.3.2 SGT-HEM Analysis

Results of the three SGT-HEM MDL studies are provided below in Table 15. This table presents the values for the individual analyses of the seven replicate sample aliquots, the mean concentration of each set of replicates, the standard deviation of the seven results, and the calculated MDLs and MLs for each study. Results are in mg/L.

Table 15
Results and Calculated MDLs and MLs for Seven Replicate Analyses for SGT-HEM
(Units = mg/L*)

	Study 1	Study 2	Study 3
Spiked Concentration	20	20	10
Replicate Analysis 1	9.3	6.7	5.5
Replicate Analysis 2	10.1	4.7	5.7
Replicate Analysis 3	9.8	6.7	4.7
Replicate Analysis 4	9.6	5.7	4.2
Replicate Analysis 5	10.3	5.6	5.5
Replicate Analysis 6	9.6	5.4	5.1
Replicate Analysis 7	8.8	6.9	4.7
Mean (X)	9.64	5.96	5.06
Standard Deviation (s)	0.50	0.82	0.55
Student's *t* value	3.143	3.143	3.143
MDL	1.6	2.6	1.7
ML	5	10	5

*Standard Deviation (s) and Student's *t* value excluded.

4.4 Discussion

The first MDL 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. These MDL/ML results were well below those expected when using the lower limit of the ranges in EPA Method 413.1 as a guideline. The MDLs produced in this study were also less than one-fifth the spike levels used to conduct the study. Finally, though the study was conducted in a commercial laboratory, it was performed by a Ph.D. level chemist with more than 20 years' experience in the determination of oil and grease and TPH. Based on these factors, a second MDL study was conducted in another commercial laboratory to verify the values obtained in the first study.

The second MDL study was also performed by a commercial 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 expedite the proposal of Method 1664, 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 were determined in the second MDL study.

The laboratory that performed MDL Study 2 noted that the results produced were the best that could be obtained under the imposed 24 hour turnaround time constraint, and that they believed they could achieve lower MDLs given more time. As a result, this laboratory performed another MDL study (MDL Study 3) using the same turnaround constraints applied in Study 1, 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 Study 3 ML of 10 mg/L for HEM was still above the lower limit of the concentration range in EPA Method 413.1, and the result for SGT-HEM, the more complex procedure, was still less than the result for HEM.

From these results, EPA concluded that the MDLs/MLs for HEM and SGT-HEM produced in MDL Study 1 were self-consistent, whereas the results produced in MDL Studies 2 and 3 were not. Because the MDL Study 2 results were determined to have been compromised by the short timeframe required, results from MDL Study 3 were compared to those produced in MDL Study 1. The SGT-HEM results produced in MDL Study 3 supported the MDL values produced for SGT-HEM in Study 1. Results for HEM, however, were less consistent. EPA concluded that the difference in the HEM results might be explained by the elevated spike levels used in Study 1. (While the spike levels used in Study 3 were within 1 - 5 times the calculated MDLs, spike levels used in Study 1 exceeded this objective.)

Consequently, the laboratory that performed Study 1 was asked to perform a new MDL study (MDL Study 4), for HEM only, using lower spike concentrations. As with Study 1, the same Ph.D. level chemist with extensive analytical experience performed the analyses. Despite the lower spike levels used, the results obtained in Study 4 were consistent with those produced by the same chemist in Study 1 (an MDL of 0.88 mg/L, with a resulting ML of 2 mg/L).

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. This ML is consistent with the low end of the range given in EPA Method 413.1. 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.

APPENDIX A
PHASE II DRAFT STUDY PLAN

USEPA OFFICE OF WATER

STUDY PLAN FOR PHASE II OF THE FREON REPLACEMENT STUDY

1. INTRODUCTION

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. These analytes are included in various regulatory compliance monitoring programs and are therefore measured on a continuous basis. As part of the effort to eliminate the use of CFCs, EPA is studying the use of alternate solvents that would produce results nearly identical to results produced with Freon 113 for these analytes.

Initial efforts to find a solvent alternative 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 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, most of which were synthetically prepared by spiking reagent water with various oils and greases, 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 in 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 initiated a multi-phase Freon Replacement Study. The objective of Phase I was 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 analysis including sonication extraction, solid phase extraction (SPE) using cartridges and disks, and a solvent/non-dispersive infrared 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 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.

In accordance with the conclusions of Phase I of the Freon Replacement Study, Phase II will 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, determination of petroleum hydrocarbons shall be performed on the extracted oil and grease samples. Of secondary importance is the continuing evaluation of alternative techniques, which will be performed by manufacturers on splits of samples collected as part of Phase II. This study plan outlines the continuing efforts to determine an appropriate replacement solvent for Freon 113, in order to develop a revised method for oil and grease and petroleum hydrocarbons.

2. OBJECTIVES

The objectives of this study are to further assess the use of n-hexane as a replacement solvent and, as a result of concerns over the possible neurotoxic effects of hexane, to formally evaluate the use of cyclohexane as a replacement solvent for oil and grease determination in aqueous samples by Method 413.1.

In addition, the gravimetric determination of petroleum hydrocarbons by Standard Methods 5520F coupled with Method 413.1 will be evaluated using Freon 113, n-hexane, and cyclohexane as extraction solvents in order to determine an appropriate replacement solvent for this analysis. The gravimetric determination of petroleum hydrocarbons is being incorporated into the Freon Replacement Study for several reasons. These include 1) the increasing occurrence of this analyte for regulation in NPDES permits, which has resulted in the need to address the same issues that are affecting oil and grease analysis; 2) the claim that petroleum hydrocarbons measurements should replace oil and grease measurements for certain industrial categories (such as Industrial Laundries) because detergents are extracted with oil and grease, thereby misrepresenting the oil and grease measurement; and 3) the apparent need to focus on the gravimetric procedure due to concerns about the susceptibility of infra-red measurement techniques to matrix interferences. These interferences are found in matrices associated with certain industrial categories (such as Industrial Laundries) and may bias the results.

EPA will also supply additional volume of each sample collected under this study to a number of vendors who are interested in testing alternative oil and grease extraction techniques similar to those addressed in Phase I of the Freon Replacement Study. These include sonication extraction, solid phase extraction using cartridges and disks, and a non-dispersive infrared technique.

As discussed in the Phase I report, analysis of oil and grease by infra-red techniques (different than the non-dispersive technique cited in the previous paragraph) is another analytical protocol that warrants further study for replacement solvents. This investigation will not be incorporated into Phase II of the Freon Replacement Study, but will be addressed in the future as funding and time allow.

The final objective of Phase II is to utilize the results of Phase II to identify a replacement solvent for Freon 113 for the gravimetric analysis of both oil and grease and petroleum hydrocarbons. Once this is determined, a draft method will be written, and an interlaboratory study may be conducted using the replacement solvent and draft method to generate method specifications.

3. SOURCE/TYPE OF SAMPLE

Approximately 38 samples from both in-process and effluent waste streams are scheduled for collection at over 30 facilities encompassing 15-20 different industrial categories. Samples containing between 40-300 mg/L oil and grease, some from petroleum and some from non-petroleum sources, will be targeted for collection. The study will focus on this concentration range to avoid the usability problems associated with the comparison and evaluation of non-detect results. In order to increase the types of matrices considered by the Agency, many of the industrial categories sampled as part of Phase II will be different from those collected during Phase I of the study.

4. ANALYTICAL STUDY DESIGN

4.1 **Management**: The study will be managed by the Office of Water's Engineering and Analysis Division through the Analytical Methods Staff (AMS). Day-to-day management and coordination of study activities will be provided by the contractor-operated Sample Control Center (SCC) under AMS guidance. SCC will contract a laboratory experienced with the determination of oil and grease and petroleum hydrocarbons through the competitive solicitation process. SCC will then coordinate laboratory analyses, receive and validate all analytical data, and perform statistical analyses. AMS will draw conclusions from the results, and produce a report providing the results of the study. Upon request, AMS will share data and results with all interested parties.

4.2 **Analytical Plan:** The contracted laboratory will be required to analyze approximately 38 wastewater samples for oil and grease by MCAWW Method 413.1 and for petroleum hydrocarbons by Standard Method 5520F using Freon 113, n-hexane, and cyclohexane as extraction solvents. Analysis of each sample will be performed in triplicate for each of the three extraction solvents. The Method 413.1 modifications that are necessary when using n-hexane and cyclohexane as extraction solvents are included as attachments to this study plan.

Prior to the analysis of field samples, the contracted laboratory will be required to make an initial demonstration of its ability to generate acceptable accuracy and precision of each of the above required procedures. To do so, a series of Initial Precision and Recovery (IPR) analyses for oil and grease and for petroleum hydrocarbons analysis using Freon, n-hexane, and cyclohexane as extraction solvents will be performed. Performance of IPRs consists of the extraction, concentration, and analysis of a set of four 1-L aliquots of spiked reagent water using each of the analytical procedures. All IPR analyses will incorporate the sodium sulfate and filtering steps required for emulsion problems (as specified in Method 413.1) in order to test any effects these method procedures may introduce.

A reagent water method blank will be analyzed with each of the IPR sets and with each of the sample sets associated with each of the three different solvent procedures. These reagent water blanks will be run through the entire extraction and analysis procedure by which the samples are run. It will be required that method blanks contain a concentration of oil and grease less than 5 mg/L. If contamination is detected in any reagent water blank, the laboratory will need to isolate the source of contamination, and associated samples will be reanalyzed. Results for the sample analyses will be blank subtracted using the value from the reagent water blank.

Multiple aliquots will be collected for each sample in order to accommodate the numerous analyses required. For each sample, the multiple aliquots will be taken from a homogenized sample and are expected to have similar oil and grease concentrations. Within each of the three different solvent procedures and two methods (413.1 and 5520F) it is expected that the standard deviation of the triplicate measurements will be less than or equal to 10 percent. The contracted laboratory will be required to notify appropriate personnel if the triplicate results exceed a relative standard deviation of 10 percent.

4.3 **Quality Assurance/Quality Control (QA/QC) Plan:** The contracted laboratory will have a comprehensive QA program in place and operating throughout the duration of the contract to ensure that data produced are of the highest quality. The laboratory will also follow all QC procedures defined in the

methodology (except matrix spikes and duplicates) in addition to the following QC tests:

QC Sample	Frequency	Spike Level	Data Quality Objectives
Initial Precision and Recovery (IPR)	Four (4) reagent water aliquots for each of the six (6) techniques.	20 mg/L of Diesel Fuel #6	< 16 % RSD, 80 - 120% recovery
Ongoing Precision and Recovery (OPR)	One (1) with each sample batch of the six (6) analytical techniques.	20 mg/L of Diesel Fuel #6	75 - 125% recovery
Method Blanks	One (1) with each sample batch for each of the six (6) analytical techniques and for each of the six (6) IPR sets.	N/A	< 5 mg/L

4.4 **Reporting Requirements**: The laboratory will be required to submit summary data in hardcopy and electronic format. The laboratory will also be required to submit raw data, including copies of worksheets and laboratory notebooks showing tare and sample weights, sample volumes, solvent volumes, and other data that will allow the final results to be traced to the analytical steps performed. A detailed narrative describing any modifications of the analytical techniques, problems, and implemented corrective action procedures will also be provided as part of the data package.

5. SAMPLE COLLECTION ACTIVITIES

Samples will be collected between November 1993 and April 1994 from facilities in Maine, Maryland, Massachusetts, New Jersey, New York, and Virginia. It is estimated that collection activities will involve 30 facilities encompassing 15-20 industrial categories. Based on historical information from the facilities, samplers will attempt to collect samples with oil and grease concentrations between 40-300 mg/L. At each sample point, approximately 60 1-L aliquots will be collected. Samples will be distributed to the contracted laboratory as well as to vendors of alternative extraction techniques who have expressed interest in participating in the study. In addition, back-up aliquots will be stored at Gascoyne Laboratories, Inc., which provides chemical repository services for AMS activities.

6. DATA EVALUATION

Data will be evaluated for conformance with data quality objectives for the study, including completeness and validity. QC test data will be reviewed for compliance with specified

objectives, and appropriate corrective action procedures will be implemented where necessary. Results presented in the hardcopy summary forms will be verified by recalculating from the raw data. Any discrepancies found will be clarified with the analytical laboratory.

7. STATISTICAL ANALYSIS OF SAMPLE RESULTS

Standard deviations and percent recoveries will be calculated for the appropriate QC samples, and for each of the triplicate sample analyses. It is expected that within each of the six analytical techniques, the relative standard deviation of the triplicate measurements will be less than 10 percent.

It is expected that the data will be evaluated by techniques similar to those used in the Phase I study. This would include using the Root Mean Square Deviation as the main criterion of equivalence when comparing results obtained with alternative solvents to results obtained with Freon. Specifics of these statistical techniques are explained in Section 4 of the *Preliminary Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease*, September 1993.

ATTACHMENT I - Freon Replacement Study Phase II

Modifications to Method 413.1 when using n-hexane or cyclohexane as the extraction solvent:

- Replace Section 1.1 to read:

  This method includes the measurement of n-hexane (or cyclohexane) extractable matter from surface and saline water, industrial and domestic wastes. It is applicable to determination of relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related matter.

- Replace Section 1.2 to read:

  The method is not applicable to measurement of light hydrocarbons that volatilize at temperatures below 85°C. Petroleum fuels from gasoline through #2 fuel oils are completely or partially lost in the solvent removal operation.

- Replace Section 1.3 to read:

  Some crude oils and heavy fuel oils contain a significant percentage of residue-type materials that are not soluble in n-hexane (or cyclohexane). Accordingly, recoveries of these materials will be low.

- Replace Section 2.1 to read:

  The sample is acidified to low pH (<2) and serially extracted with n-hexane (or cyclohexane) in a separatory funnel. The solvent is evaporated from the extract and the residue weighed.

- Replace Section 6.2 to read:

  n-Hexane, b.p 69°C (or cyclohexane, b.p. 81°C).

- Add Section 6.4 to read:

  Silicon carbide boiling chips.

- Replace Section 7.3 to read:

  Tare a boiling flask (pre-dried in an oven at 103°C and stored in a desiccator) containing 4 small boiling chips.

- Replace Section 7.4 to read:

  Add 30 ml n-hexane (6.2) (or cyclohexane) to the sample bottle and rotate the bottle to rinse the sides. Transfer the solvent into the separatory funnel. Extract by shaking vigorously for 2 minutes. Allow the layers to separate, and drain the aqueous layer into the original sample container. Filter the solvent layer into the flask through a funnel containing solvent moistened filter paper.

  NOTE: An emulsion that fails to dissipate can be broken by pouring about 1 g sodium sulfate

(6.3) into the filter paper cone and slowly draining the emulsion through the salt. Additional 1 g portions can be added to the cone as required.

Replace Section 7.7 to read:

Connect the boiling flask to the distilling head and evaporate the solvent by immersing the low half of the flask in water at 85°C. Collect the solvent for reuse. A solvent blank should accompany each set of samples.

Replace Section 7.8 to read:

When the temperature in the distilling head reaches 70°C (this temperature is applicable when using n-hexane as the solvent. When using cyclohexane, the temperature will need to be higher) or the flask appears dry remove the distilling head. Sweep out the flask for 15 seconds with air to remove solvent vapor by inserting a glass tube connected to a vacuum source. Immediately remove the flask from the heat source and wipe the outside to remove excess moisture and fingerprints.

APPENDIX B
TCRR STUDY ANALYTICAL REQUIREMENTS

EPA EAD ANALYTICAL METHODS STAFF
ANALYTICAL REQUIREMENTS FOR
THE FREON REPLACEMENT STUDY ROUND ROBIN
3/21/94

The laboratory's strict adherence to the required analytical methods and quality control procedures is essential to assure data validity for EPA use. The laboratory shall adhere to the Quality Assurance/Quality Control (QA/QC) measures prescribed below, and shall otherwise employ accepted good laboratory practices in all aspects of analytical performance.

Summary of analytical requirements:

1) Preparation and analysis of two industrial wastewater samples, each in triplicate, for n-hexane extractable material (HEM) by Draft Method 1664.

2) The determination of silica gel treated n-hexane extractable material (SGT-HEM), as specified in Draft Method 1664, **is optional** and may be performed at the laboratory's discretion.

NOTE: The laboratory must make an initial demonstration of the ability to generate acceptable accuracy and precision of the above required procedures. To do so, a series of Initial Precision and Recovery (IPR) analyses must be performed. Performance of IPRs consists of the extraction, concentration, and analysis of a set of four 1-L aliquots of spiked reagent water, as specified in Draft Method 1664. These IPR analyses must be completed prior to the analysis of field samples. A method blank must be run with each IPR set. If SGT-HEM determination is performed, these analytical steps must be incorporated into the IPR analyses.

NOTE: A reagent water method blank must be analyzed with the IPR set and with the sample sets. These reagent water blanks shall be run through the entire extraction and analysis procedure by which the samples are run. **Method blanks must have a value less than 5 mg/L.** If contamination is detected in the reagent water blanks, the laboratory will need to isolate the source of contamination, and associated samples will need to be reanalyzed.

Special Technical Instructions:

Multiple aliquots are being collected for each of the samples. For each sample, the multiple aliquots are from a homogenized sample and are expected to have identical concentrations. It is expected that the relative standard deviation of the triplicate measurements will be 1) less than or equal to 10% for those samples with values that

are greater than or equal to the 20 mg/L minimum level and 2) less than or equal to 20% for those samples with values that are less than 20 mg/L.

All IPR analyses shall incorporate the sodium sulfate and filtering steps required for emulsion problems in order to test any effects these steps may introduce.

If a filter other than the method-specified Whatman 40 filter is necessary in order to prevent passage of fine particulates, the laboratory should incorporate such changes into the spiked reagent water and method blank analyses. These changes must be documented in the laboratory narrative.

The laboratory has the option of using whichever filter it judges to be suitable for this procedure, as long as contamination of the extract does not occur. It is advised, but not required, that 0.45 *u* membrane filters be used. If contamination occurs (i.e. method blanks produce values > 5 mg/L) samples associated with these blanks will need to be reanalyzed. The laboratory should report contamination problems to SCC as they occur.

These above mentioned considerations for particulate contamination also apply to the silica gel procedure.

Analytical protocol required:

Draft Method 1664: Determination of n-hexane extractable material and silica gel treated n-hexane extractable material (attached).

Analytical results required:

a) A narrative that details any problems with or deviations from the referenced methods and reports problems associated with the analysis of specific samples. The narrative should also provide comments on the method performance on various analytes and matrices.

b) A list of samples analyzed, and a run chronology showing (in sequence) extraction dates and times and weighing dates and times.

c) Summary reports of all sample, IPR, OPR, and blank analyses. Data reporting forms are included in this information package.

Note: The calculations performed by the laboratory in generating sample data must be able to be reproduced by a 3rd party from the data package.

QC Requirements:

The following QC parameters are required:

Initial Precision and Recovery Analyses (IPR) - IPR analysis prior to the analysis of field samples. Data quality objectives for spiked reagent water sets are as follows: An average percent recovery between 80-120% and a relative standard deviation less than or equal to 16%.

Ongoing Precision and Recovery Analysis (OPR) - One OPR analysis with the two sample sets. Data quality objectives are as follows: A percent recovery between 75-125%.

One Method Blank is required for analysis with the set of IPR samples and with the samples. Method blank values must be less than 5 mg/L.

NOTE: If contamination is detected in the method blank, the source of contamination must be identified and corrected. The blank and all samples associated with that contaminated blank must be reprepared and reanalyzed.

Instrument Calibration shall be confirmed through the use of Initial and Continuing Calibration Verification Standards per method instructions.

If any of these instructions are not clear please call Carrie Buswell at (703) 519-1140.

Reporting Format for the Twin City Interlaboratory Study

Initial Precision and Recovery (IPR) Samples and Associated Method Blank

Laboratory Name: _____

Parameter: ___ HEM ___ SGT-HEM

Extraction/Analysis Date: _____ Date Extractable Material Weighed: _____

Sample	Sample Volume (L)	Initial Weight of Flask (g)	Final Weight of Flask (g)	Sample Weight (g)	Sample Conc. (mg/L)	Blank Conc. (mg/L)	Sample - Blank (mg/L)	Spike Conc. (mg/L)	Percent Recovery
Method Blank							N/A	N/A	N/A
IPR 1									
IPR 2									
IPR 3									
IPR 4									

IPR Mean Recovery = _____%

IPR Relative Standard Deviation = _____%

Data Quality Objectives for the average IPR recovery = 80 - 120%

Data Quality Objectives for the relative standard deviation = less than or equal to 16%

Method Blank values must be less than 5 mg/L

Reporting Format for the Twin City Interlaboratory Study

Field Samples, Associated Method Blank, and Associated Ongoing Precision and Recovery Sample

Laboratory Name: _____

Parameter: ___ HEM ___ SGT-HEM

Extraction/Analysis Date: _____ Date Extractable Material Weighed: _____

Lab Sample #	EPA Sample #	Rep #	Sample Volume (L)	Initial Weight of Flask (g)	Final Weight of Flask (g)	Sample Weight (g)	Sample Conc. (mg/L)	Blank Conc. (mg/L)	Sample - Blank (mg/L)	Spike Conc. (mg/L)	Percent Recovery
	Method Blank								N/A	N/A	N/A
		1								N/A	N/A
		2								N/A	N/A
		3								N/A	N/A
		1								N/A	N/A
		2								N/A	N/A
		3								N/A	N/A
	OPR										

Data Quality Objectives for the OPR recovery = 75 - 125%

Method Blank values must be less than 5 mg/L

APPENDIX C

STUDY PLANS FOR THE METHOD 1664 MDL STUDIES

STUDY PLAN

Determination of MDL for EPA Draft Method 1664 - "Oil and Grease" and "Petroleum Hydrocarbons" [N-Hexane Extractable Material (HEM) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry]

1. Introduction

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), the United States is committed to controlling and eventually phasing out the use of chlorofluorocarbons (CFCs). In support of these efforts, the U.S. Environmental Protection Agency is searching for suitable solvents to replace Freon-113, which is required for use in several U.S. EPA wastewater and solid waste methods for the determination of oil and grease and petroleum hydrocarbons. The Freon Replacement Study, which to date has consisted of two phases, is part of these efforts.

In Phase I, five extraction solvents for liquid/liquid extraction and gravimetric determination of oil and grease in aqueous samples were evaluated, as was the use of alternative techniques for oil and grease analysis including sonication extraction, solid phase extraction, and a non-dispersive infra-red technique.

The results from Phase I were used to narrow the list of alternative solvents under consideration in Phase II to n-hexane and cyclohexane. These Phase II solvents were used for liquid/liquid extraction and gravimetric determination of both oil and grease and total petroleum hydrocarbons (TPH). Based on the results of Phase II and the analytical issues and observations consolidated from the study, Draft Method 1664 was written, with n-hexane as the extraction solvent.

The most significant changes in Draft Method 1664 compared to previous oil and grease and petroleum hydrocarbons methods were 1) the use of n-hexane as the extraction solvent, 2) the use of hexadecane and stearic acid as the spiking materials for QC analyses, and 3) the introduction of 1600 series method 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). Interlaboratory studies are currently underway to develop method specifications for IPRs and OPRs.

As part of QC development, a method detection limit (MDL) must be determined for Draft Method 1664. A previous study determined the MDL for a modified version of MCAWW Method 413.1 using Wesson oil and No. 6 Fuel oil as analytes and n-hexane as the extraction solvent. Though the modified Method 413.1 procedure was similar to Draft Method 1664, the spiking materials differed from the hexadecane and stearic acid

spiking materials specified in Draft Method 1664. This study plan describes EPA's approach to determining the MDL for n-hexane extractable material (HEM) and silica gel treated n-hexane extractable material (SGT-HEM) ("oil and grease" and "petroleum hydrocarbons", respectively), using Draft Method 1664.

2. Objective

The objective of this study is to determine the MDLs for HEM and SGT-HEM by Draft Method 1664 from the analysis of seven reagent water samples spiked with hexadecane and stearic acid. As is specified in Section 9.2.1 of Draft Method 1664, MDL analyses will be performed according to the procedure in 40 *CFR* 136, Appendix B.

Data quality objectives (DQOs) include the following:

- To achieve an MDL that is equal to or within a factor of five lower than the level spiked in order to ensure that the MDLs determined in this study are not understated or overstated.

- To achieve an MDL of 3 mg/L for both HEM and SGT-HEM.

In order to meet these DQOs, the laboratory will be required to have a comprehensive QA program in place and operating throughout the duration of this study. This will ensure that the data produced are of the highest possible quality. The laboratory will be required to follow all QC procedures defined in this study plan and in EPA Draft Method 1664 with the following exceptions:

- Demonstration of ongoing precision and recovery will not be required.

- Performance of matrix spike and matrix spike duplicate analyses will not be required.

- Instrument calibration must be performed at a range that will encompass the estimated detection limit being studied.

- Analysis of a Quality Control Sample, as defined in Section 9.7 of Draft Method 1664, will not be required.

If the procedures described in this study plan conflict with those described in Draft Method 1664 and 40 *CFR* 136, Appendix B, the study plan will take precedence.

3. Study Management/Limitations

The study will be managed by the Office of Water's Engineering and Analysis Division through the Analytical Methods Staff (AMS). Day-to-day management and coordination of study activities will be provided by the contractor-operated Sample Control Center (SCC) under AMS guidance. SCC will contract a laboratory experienced with gravimetric determination of oil and grease and petroleum hydrocarbons. SCC will then coordinate

laboratory analysis, receive and validate all analytical data, and perform statistical analyses. AMS will draw conclusions from the results, and produce a report providing the results of the study. Upon request, AMS will share data and results with all interested parties.

Analyses will be performed and results obtained by July 15, 1994, in order to incorporate these results into Draft Method 1664 prior to the proposal of this method in the *Federal Register*.

If they have not already done so, the laboratories considered for this study will be required to perform initial precision and recovery analyses (IPR) for Draft Method 1664 and to meet the IPR data quality objectives specified in this method.

4. Technical Approach/Limitations/Procedures

Two separate MDL studies will be performed, one for HEM analysis and one for SGT-HEM analysis. MDLs will be determined according to the protocol in 40 *CFR* 136, Appendix B using the apparatus, reagents, and standards that are specified in Draft Method 1664. Reagent water will be used as the sample matrix because it is homogeneous, readily available, and included as a matrix option in 40 *CFR* 136.

Targeting other matrices was not considered a viable option for a number of reasons. If samples from a particular industrial category were used, for example, results would be biased for a particular industry. It would also be nearly impossible to test every sample matrix to which this method may be applied, since EPA regulates more than 600 categories of wastewater discharge. Consequently, laboratory evaluation of Draft Method 1664 on each discharge would be both time-consuming and expensive.

Some exceptions and clarifications to the procedure in 40 *CFR* 136, Appendix B will need to be incorporated into this study. They are as follows:

- The laboratory will be required to prepare and analyze seven separate aliquots of spiked reagent water, each prepared in its own sample container, for each of the two MDL studies. Separate containers are required for each sample to ensure that an exact, known amount of HEM and SGT-HEM is present in each aliquot. If aliquots were to be taken from a single container, components of the HEM and SGT-HEM might adhere to the sample container, thereby preventing equal distribution and precise allocation of the spiked components into each aliquot.

- The MDL study protocol requires spiking seven replicates with the targeted analytes at a concentration of one to five times the estimated MDL. The estimated MDL for both HEM and SGT-HEM is 3 mg/L. For the HEM MDL study, the laboratory will be required to spike reagent water samples to produce a combined hexadecane/stearic acid concentration of 10 mg/L (5 mg of hexadecane and 5 mg of stearic acid). For the SGT-HEM MDL study, the

laboratory will need to compensate for the silica gel adsorption of the stearic acid. Consequently, the laboratory will be required to spike reagent water samples to produce a combined hexadecane/stearic acid concentration of 20 mg/L (10 mg hexadecane and 10 mg of stearic acid) for SGT-HEM analysis.

- Samples are to be prepared according to the instructions in Section 7.9 of Draft Method 1664, with the exception that the amount of the hexadecane/stearic acid spiking solution added to the one liter reagent water sample will be decreased in order to produce a final concentration of 10 mg/L (5 mg of hexadecane and 5 mg of stearic acid) in each one liter sample for the HEM MDL study and 20 mg/L (10 mg of hexadecane and 10 mg of stearic acid) in each one liter sample for the SGT-HEM MDL study.

To ensure that the laboratory is spiking at the appropriate level, the laboratory will be required to analyze two aliquots to evaluate the spike levels as described in Section 4b of the 40 *CFR* 136, Appendix B procedure. The laboratory will report the results of these two analyses to SCC before continuing with any other analyses. If these measurements indicate that the sample is in the desirable range for determination of the MDL, SCC will instruct the laboratory to proceed with the analysis of the remaining five aliquots. All seven measurements will be used for calculation of the MDL. If the first two measurements indicate that the samples are not in the desirable range, the laboratory shall repeat Section 4b (analysis of two aliquots) until the desired level is achieved.

A reagent water blank will be analyzed with each set of seven analyses. These reagent water blanks will be run through the entire extraction and analysis procedure by which the MDL samples are run. It will be required that method blanks contain a concentration of 3 mg/L or less. If contamination is detected in any reagent water blank, the laboratory will need to isolate the source of contamination, and the associated MDL studies will need to be repeated.

If the MDL resulting from the study is not equal to or within a factor of five lower than the level spiked, the spiking, measurement, and calculation process will be iterated until the measured MDL is within these specifications.

5. Reporting Requirements/Statistical Analysis of Laboratory Results

The laboratory will be required to submit summary data in hardcopy and electronic format. The summary data will consist of a table that will group data under the following categories: concentration of HEM or SGT-HEM in the seven aliquots, the mean of the seven concentrations, standard deviations, Student's value used, and calculated MDL for HEM and SGT-HEM.

The laboratory will also be required to submit raw data, including copies of worksheets and laboratory notebooks showing tare and sample weights, sample volumes, solvent volumes, and other data that will allow the final results to be traced to the analytical steps performed. A detailed narrative describing any modifications of the analytical

techniques, problems, and implemented corrective action procedures will also be provided as part of the data package.

All data from this study will be submitted to SCC for review and validation. The computer-readable results will be verified against the hardcopy data, then loaded into the study database. Hardcopy data, including all calculations, will be verified from the bench sheets provided.

The MDLs will be calculated using the procedures in 40 *CFR* 136, Appendix B.

STUDY PLAN

Determination of MDL for EPA Method 1664 - N-Hexane Extractable Material (HEM) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry (Oil and Grease and Total Petroleum Hydrocarbons)

1. Introduction

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), the United States is committed to controlling and eventually phasing out the use of chlorofluorocarbons (CFCs). In support of these efforts, the U.S. Environmental Protection Agency is searching for suitable solvents to replace Freon-113, which is required for use in several U.S. EPA wastewater and solid waste methods for the determination of oil and grease and petroleum hydrocarbons. The Freon Replacement Study, which to date has consisted of two phases, is part of these efforts.

In Phase I, five extraction solvents for liquid/liquid extraction and gravimetric determination of oil and grease in aqueous samples were evaluated, as was the use of alternative techniques for oil and grease analysis including sonication extraction, solid phase extraction, and a non-dispersive infra-red technique.

The results from Phase I were used to narrow the list of alternative solvents under consideration in Phase II to n-hexane and cyclohexane. These Phase II solvents were used for liquid/liquid extraction and gravimetric determination of both oil and grease and total petroleum hydrocarbons (TPH). Based on the results of Phase II and the analytical issues and observations consolidated from the study, Draft Method 1664 was written, with n-hexane as the extraction solvent.

The most significant changes in Draft Method 1664 compared to previous oil and grease and petroleum hydrocarbons methods were 1) the use of n-hexane as the extraction solvent, 2) the use of hexadecane and stearic acid as the spiking materials for QC analyses, and 3) the introduction of 1600 series method 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).

An interlaboratory study was conducted and the results obtained were used to develop method specifications for IPRs, OPRs, and MS/MSDs, which were incorporated into the October 1994 revision of Method 1664.

As part of this QC development, a method detection limit (MDL) also needed to be determined for Method 1664. An MDL study was conducted using reagent water as the sample matrix and the method specified hexadecane and stearic acid spiking materials. The MDL values produced were 0.91 mg/L for HEM and 1.6 mg/L for SGT-HEM.

In order to confirm the values from the original study, a second MDL study was initiated. Results from this study (5.4 mg/L for HEM and 2.6 mg/L for SGT-HEM) were higher than those produced in the original study but, after evaluating the conditions under which the laboratory performed the second study, which included considerable time constraints (i.e., a 48 hour turnaround) and what the laboratory admitted as performance not reflective of their best abilities, it was decided that the MDL values to be included in the October version of Method 1664 should be those representing the best laboratory performance. Therefore, the MDL and associated Minimum Level values from the original Method 1664 MDL study were incorporated into the October 1994 revision of Method 1664.

Though a decision was made regarding the MDL and ML specifications for the October 1994 version of Method 1664, several factors suggest the need to perform additional MDL studies. First, though a second MDL study was performed, the laboratory stated that the work performed did not reflect their ability. Therefore, the results are suspect and do not represent a reliable comparison to the original study. Second, the HEM and SGT-HEM values calculated from the first MDL study are at levels below five times the then estimated detection limit of 3 mg/L, indicating that even lower spike levels could have been used. Given these circumstances, a third MDL study will be conducted using reagent water samples spiked at lower levels than previously used to reflect the updated (lower) MDL levels in the October version of Method 1664.

This study plan describes EPA's approach to determining the MDL for n-hexane extractable material (HEM) and silica gel treated n-hexane extractable material (SGT-HEM) using Method 1664, October 1994.

2. Objective

The objective of this study is to determine the MDLs for HEM and SGT-HEM by Method 1664 from the analysis of seven reagent water samples spiked with hexadecane and stearic acid. As is specified in Section 9.2.1 of Method 1664, MDL analyses will be performed according to the procedure in 40 *CFR* 136, Appendix B.

Data quality objectives (DQOs) include the following:

- To achieve an MDL that is equal to or within a factor of five lower than the level spiked in order to ensure that the MDLs determined in this study are not understated or overstated.

- To achieve an MDL of 0.91 mg/L for HEM and 1.6 mg/L for SGT-HEM.

In order to meet these DQOs, the laboratory will be required to have a comprehensive QA program in place and operating throughout the duration of this study. This will ensure that the data produced are of the highest possible quality. The laboratory will be required to follow all QC procedures defined in this study plan and in the October 1994 version of Method 1664 with the following exceptions:

- Demonstration of ongoing precision and recovery will not be required.
- Performance of matrix spike and matrix spike duplicate analyses will not be required.
- Instrument calibration must be performed at a range that will encompass the estimated detection limit being studied.
- Analysis of a Quality Control Sample, as defined in Section 9.7 of Method 1664, will not be required.

If the procedures described in this study plan conflict with those described in Method 1664 and 40 *CFR* 136, Appendix B, the study plan will take precedence.

3. Study Management/Limitations

The study will be managed by the Office of Water's Engineering and Analysis Division through the Analytical Methods Staff (AMS). Day-to-day management and coordination of study activities will be provided by the contractor-operated Sample Control Center (SCC) under AMS guidance. SCC will contract a laboratory experienced with gravimetric determination of oil and grease and petroleum hydrocarbons. SCC will then coordinate laboratory analysis, receive and validate all analytical data, and perform statistical analyses. AMS will draw conclusions from the results, and produce a report providing the results of the study. Upon request, AMS will share data and results with all interested parties.

Analyses will be performed and results obtained by November 21, 1994, in order to incorporate these results prior to a revision of Method 1664 for proposal in the *Federal Register*.

If they have not already done so, the laboratories considered for this study will be required to perform initial precision and recovery analyses (IPR) for Method 1664 and to meet the IPR data quality objectives specified in this method.

4. Technical Approach/Limitations/Procedures

Two separate MDL studies will be performed, one for HEM analysis and one for SGT-HEM analysis. MDLs will be determined according to the protocol in 40 *CFR* 136, Appendix B using the apparatus, reagents, and standards that are specified in Method 1664. Reagent water will be used as the sample matrix because it is homogeneous, readily available, and included as a matrix option in 40 *CFR* 136.

Targeting other matrices was not considered a viable option for a number of reasons. If samples from a particular industrial category were used, for example, results would be biased for a particular industry. It would also be nearly impossible to test every sample matrix to which this method may be applied, since EPA regulates more than 600

categories of wastewater discharge. Consequently, laboratory evaluation of Method 1664 on each discharge would be both time-consuming and expensive.

Some exceptions and clarifications to the procedure in 40 *CFR* 136, Appendix B will need to be incorporated into this study. They are as follows:

- The laboratory will be required to prepare and analyze seven separate aliquots of spiked reagent water, each prepared in its own sample container, for each of the two MDL studies. Separate containers are required for each sample to ensure that an exact, known amount of HEM and SGT-HEM is present in each aliquot. If aliquots were to be taken from a single container, components of the HEM and SGT-HEM might adhere to the sample container, thereby preventing equal distribution and precise allocation of the spiked components into each aliquot.

- The MDL study protocol requires spiking seven replicates with the targeted analytes at a concentration of one to five times the estimated MDL. The estimated MDL for HEM is 0.91 mg/L and for SGT-HEM is 1.6 mg/L. For the HEM MDL study, the laboratory will be required to spike reagent water samples to produce a combined hexadecane/stearic acid concentration between 0.91 and 4.55 mg/L (note that equal amounts of hexadecane and stearic acid will comprise the total concentration). For the SGT-HEM MDL study, the laboratory will need to compensate for the silica gel adsorption of the stearic acid. Consequently, the laboratory will be required to spike reagent water samples to produce a combined hexadecane/stearic acid concentration of 3.2 to 16 mg/L (note that equal amounts of hexadecane and stearic acid will comprise the total concentration) for SGT-HEM analysis.

- Samples are to be prepared according to the instructions in Section 7.11 of Method 1664, with the exception that the amount of the hexadecane/stearic acid spiking solution added to the one liter reagent water sample will be decreased in order to produce a final concentration within the ranges specified above.

To ensure that the laboratory is spiking at the appropriate level, the laboratory will be required to analyze two aliquots to evaluate the spike levels as described in Section 4b of the 40 *CFR* 136, Appendix B procedure. The laboratory will report the results of these two analyses to SCC before continuing with any other analyses. If these measurements indicate that the sample is in the desirable range for determination of the MDL, SCC will instruct the laboratory to proceed with the analysis of the remaining five aliquots. All seven measurements will be used for calculation of the MDL. If the first two measurements indicate that the samples are not in the desirable range, the laboratory shall repeat Section 4b (analysis of two aliquots) until the desired level is achieved.

A reagent water blank will be analyzed with each set of seven analyses. These reagent water blanks will be run through the entire extraction and analysis procedure by which the MDL samples are run. It will be required that method blanks contain a concentration of 0.91 mg/L or less for HEM and 1.6 mg/L or less for SGT-HEM. If contamination is

detected in any reagent water blank, the laboratory will need to isolate the source of contamination, and the associated MDL studies will need to be repeated.

If the MDL resulting from the study is not equal to or within a factor of five lower than the level spiked, the spiking, measurement, and calculation process will be iterated until the measured MDL is within these specifications.

5. Reporting Requirements/Statistical Analysis of Laboratory Results

The laboratory will be required to submit summary data in hardcopy and electronic format. The summary data will consist of a table that will group data under the following categories: concentration of HEM or SGT-HEM in the seven aliquots, the mean of the seven concentrations, standard deviations, Student's t value used, and calculated MDL for HEM and SGT-HEM.

The laboratory will also be required to submit raw data, including copies of worksheets and laboratory notebooks showing tare and sample weights, sample volumes, solvent volumes, and other data that will allow the final results to be traced to the analytical steps performed. A detailed narrative describing any modifications of the analytical techniques, problems, and implemented corrective action procedures will also be provided as part of the data package.

All data from this study will be submitted to SCC for review and validation. The computer-readable results will be verified against the hardcopy data, then loaded into the study database. Hardcopy data, including all calculations, will be verified from the bench sheets provided.

The MDLs will be calculated using the procedures in 40 *CFR* 136, Appendix B.

APPENDIX D

ANALYTICAL REQUIREMENTS FOR THE METHOD 1664 MDL STUDIES

ANALYTICAL REQUIREMENTS FOR THE DRAFT METHOD 1664 MDL STUDY

6/24/94

The laboratories strict adherence to the required analytical methods and quality control procedures is essential to assure data validity for EPA use. The laboratory shall adhere to the Quality Assurance/Quality Control (QA/QC) measures prescribed below, and shall otherwise employ accepted good laboratory practices in all aspects of analytical performance.

Summary of analytical requirements:

· Performance of a method detection limit (MDL) study for HEM analysis by Draft Method 1664.

  Under this purchase order, a minimum of seven HEM analyses and a maximum of nine HEM analyses will be performed for the MDL determination. Note: This number does not include the four IPR analyses that may be necessary and the method blank analysis.

· Performance of a method detection limit (MDL) study for SGT-HEM analysis by Draft Method 1664.

  Under this purchase order, a minimum of seven SGT-HEM analyses and a maximum of nine SGT-HEM analyses will be performed for the MDL determination. Note: This number does not include the four IPR analyses that may be necessary and the method blank analysis.

NOTE 1: Two separate MDL studies are required, one for HEM and the other for SGT-HEM, because the concentrations at which the samples will be spiked with hexadecane and stearic acid for the HEM MDL study will be too low for satisfactory determination and quantitation of SGT-HEM. This is due to the fact that the silica gel procedure will adsorb the stearic acid, thereby halving the concentration available for SGT-HEM measurement.

NOTE 2: The maximum number of nine analyses for each MDL study accounts for the possibility that, after performing the procedure in Section 4b of 40 *CFR* 136, Appendix B, the laboratory may find that it is necessary to adjust spiking levels and repeat the analysis of those two aliquots.

NOTE 3: Laboratories who have not previously done so must satisfactorily analyze four initial precision and recovery (IPR) samples according to the instructions in Section 9.2.2 of Draft Method 1664 prior to the analysis of the MDL study samples. The data for these IPR samples must be faxed to SCC upon completion at (703) 684-0610. These data must also be submitted with the data package. For the purposes of the IPR analyses only, the same extract used for HEM analysis may be used for SGT-HEM analysis, meaning that the HEM residue may be

redissolved in n-hexane and subjected to the silica gel procedure for determination of SGT-HEM.

NOTE 4: A reagent water method blank, as is specified in Section 9.4 of Draft Method 1664, must be analyzed with the set of four IPR samples and with each of the two MDL studies (three method blanks total). Method blanks must have a value of less than 3 mg/L. If contamination is detected in the reagent water blanks, the laboratory will need to isolate the source of contamination, and the associated MDL studies will need to be repeated.

Data Turnaround Requirements: Data must be submitted to SCC by July 15, 1994.

Spiking Instructions:

- IPR samples are to be spiked as described in Section 9.2.2 of Draft Method 1664.
- For the HEM MDL study, reagent water samples are to be prepared according to the instructions in Section 7.9 of Draft Method 1664, with the exception that the amount of hexadecane/stearic acid spiking solution will be decreased in order to produce a final concentration of 10 mg/L (5 mg of hexadecane and 5 mg of stearic acid).
- For the SGT-HEM MDL study, reagent water samples are to be prepared according to the instructions in Section 7.9 of Draft Method 1664, with the exception that the amount of hexadecane/stearic acid spiking solution will be decreased in order to produce a final concentration of 20 mg/L (10 mg of hexadecane and 10 mg of stearic acid).

Analytical Protocol Required:

- EPA Draft Method 1664.
- 40 *CFR* Part 136, Appendix B - *Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11* (attached).

Special Technical Instructions:

- The laboratory must follow the technical instructions outlined in the Study Plan, along with those specified in the required analytical protocol, with the exception of the bullet listed below.
- A minimum of seven analyses and a maximum of nine analyses will be required for each of the two MDL studies (IPRs are not included). The Study Plan states that if the resulting MDL(s) does not meet the study plan criteria of being equal to or within a factor of five lower than the level spiked, the laboratory is to repeat the study. For the purposes of this project, however, the laboratory is NOT to repeat an MDL study without formal modification of this purchase order by DynCorp Viar.

Analytical Results Required:

- A narrative that details any problems with or deviations from the referenced methods and reports problems associated with the analysis of specific samples. The narrative should also provide comments on the method performance on each analyte.
- A list of samples analyzed, and a run chronology.
- Summary reports of all sample results. An explanation of the summary format table is provided in the Study Plan.
- Raw data, including bench sheets, etc.
- The laboratory must report the MDL values to three significant figures.

QC Requirements: As specified in the Study Plan - QC Minimums provided below.

- Initial Precision and Recovery Analyses - Data quality objectives are specified in Draft Method 1664.
- Analysis of a method blank is required with the IPR set and with each of the two MDL studies. Method blank values must be less than 3 mg/L.

  NOTE: If contamination is detected in the method blank, the source of contamination must be identified and corrected. The blank and any IPR sets or MDL studies associated with that contaminated blank must be reprepared and reanalyzed.

- Instrument calibration shall be confirmed per Draft Method 1664 and Study Plan specifications.

ANALYTICAL REQUIREMENTS FOR METHOD 1664 MDL STUDY

11/3/94

The laboratory's strict adherence to the required analytical methods and quality control procedures is essential to assure data validity for EPA use. The laboratory shall adhere to the Quality Assurance/Quality Control (QA/QC) measures prescribed below, and shall otherwise employ accepted good laboratory practices in all aspects of analytical performance.

Summary of analytical requirements:

· Performance of a method detection limit (MDL) study for HEM analysis by Method 1664.

  Under this purchase order, a minimum of seven HEM analyses and a maximum of nine HEM analyses will be performed for the MDL determination. Note: This number does not include the four IPR analyses that may be necessary and the method blank analysis.

· Performance of a method detection limit (MDL) study for SGT-HEM analysis by Method 1664.

  Under this purchase order, a minimum of seven SGT-HEM analyses and a maximum of nine SGT-HEM analyses will be performed for the MDL determination. Note: This number does not include the four IPR analyses that may be necessary and the method blank analysis.

NOTE 1: Two separate MDL studies are required, one for HEM and the other for SGT-HEM, because the concentrations at which the samples will be spiked with hexadecane and stearic acid for the HEM MDL study will be too low for satisfactory determination and quantitation of SGT-HEM. This is due to the fact that the silica gel procedure will adsorb the stearic acid, thereby halving the concentration available for SGT-HEM measurement.

NOTE 2: The maximum number of nine analyses for each MDL study accounts for the possibility that, after performing the procedure in Section 4b of 40 *CFR* 136, Appendix B, the laboratory may find that it is necessary to adjust spiking levels and repeat the analysis of those two aliquots.

NOTE 3: Laboratories who have not previously done so must satisfactorily analyze four initial precision and recovery (IPR) samples according to the instructions in Section 9.2.2 of Method 1664 prior to the analysis of the MDL study samples. The data for these IPR samples must be faxed to SCC upon completion at (703) 684-0610. These data must also be submitted with the data package. For the purposes of the IPR analyses only, the same extract used for HEM analysis may

be used for SGT-HEM analysis, meaning that the HEM residue may be redissolved in n-hexane and subjected to the silica gel procedure for determination of SGT-HEM.

NOTE 4: A reagent water method blank, as is specified in Section 9.4 of Method 1664, must be analyzed with the set of four IPR samples and with each of the two MDL studies (three method blanks total). Method blanks must have a value of less than 0.91 mg/L for HEM and 1.6 mg/L for SGT-HEM. If contamination is detected in the reagent water blanks, the laboratory will need to isolate the source of contamination, and the associated MDL studies will need to be repeated.

Data Turnaround Requirements: A formal data package must be submitted to SCC by November 21, 1994.

Spiking Instructions:

- IPR samples are to be spiked as described in Section 9.2.2 of Method 1664.

- For the HEM MDL study, reagent water samples are to be prepared according to the instructions in Section 7.11 of Method 1664, with the exception that the amount of hexadecane/stearic acid spiking solution will be decreased in order to produce a final concentration that is one to five times the 0.91 mg/L MDL value that is specified in the October 1994 version of Method 1664, i.e. 0.91 - 4.55 mg/L. Note that the concentration chosen will consist of equal portions of hexadecane and stearic acid. For example, if a final concentration of 3 mg/L is chosen, it will consist of 1.5 mg of hexadecane and 1.5 mg of stearic acid.

- For the SGT-HEM MDL study, reagent water samples are to be prepared according to the instructions in Section 7.11 Method 1664, with the exception that the amount of hexadecane/stearic acid spiking solution will be decreased in order to produce a final concentration that is two to ten times the 1.6 mg/L MDL value that is specified in the October 1994 version of Method 1664, i.e. 3.2 - 16 mg/L. Note that the concentration chosen will consist of equal portions of hexadecane and stearic acid. For example, if a final concentration of 10 mg/L is chosen, it will consist of 5 mg of hexadecane and 5 mg of stearic acid. Also note that the two to ten times multiplier applied to the MDL is double that in the HEM study in order to account for the adsorption of the stearic acid by the silica gel procedure.

Analytical Protocol Required:

- EPA Method 1664, October 1994.

- 40 *CFR* Part 136, Appendix B - *Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11* (attached).

Special Technical Instructions:

- The laboratory must follow the technical instructions outlined in the Study Plan, along with those specified in the required analytical protocol, with the exception of the bullet listed below.

- A minimum of seven analyses and a maximum of nine analyses will be required for each of the two MDL studies (IPRs are not included). The Study Plan states that if the resulting MDL(s) does not meet the study plan criteria of being equal to or within a factor of five lower than the level spiked, the laboratory is to repeat the study. For the purposes of this project, however, the laboratory is NOT to repeat an MDL study without formal modification of this purchase order by DynCorp Viar.

Analytical Results Required:

- A narrative that details any problems with or deviations from the referenced methods and reports problems associated with the analysis of specific samples. The narrative should also provide comments on the method performance on each analyte.

- A list of samples analyzed, and a run chronology.

- Summary reports of all sample results. An explanation of the summary format table is provided in the Study Plan.

- Raw data, including bench sheets, etc.

- The laboratory must report the MDL values to three significant figures.

QC Requirements: As specified in the Study Plan - QC Minimums provided below.

- Initial Precision and Recovery Analyses - Data quality objectives are specified in Method 1664.

- Analysis of a method blank is required with the IPR set and with each of the two MDL studies. Method blank values must be less than 0.91 mg/L for HEM and 1.6 mg/L for SGT-HEM.

  NOTE: If contamination is detected in the method blank, the source of contamination must be identified and corrected. The blank and any IPR sets or MDL studies associated with that contaminated blank must be reprepared and reanalyzed.

- Instrument calibration shall be confirmed per Method 1664 and Study Plan specifications.

APPENDIX E

40 *CFR* 136, APPENDIX B

APPENDIX B TO PART 136 - DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT - REVISION 1.11

Definition

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific, and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

(a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

(b) The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

(c) That region of the standard curve where there is a significant change in sensitivity, i.e., a break in the slope of the standard curve.

(d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the initial estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) Prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1S}^{n} X_1^2 - \frac{(\sum_{i=1}^{n} X_1)^2}{n}\right] \quad S = (S^2)^{1/2}$$

Where:

Xi; I=1 to n, are the analytical results in the final method reporting units obtained from the n sample aliquots and S refers to the sum of the X values from I=l to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1,1-\alpha = 0.99)} (S)$$

where:

MDL = the method detection limit

$t_{(n-1,1-a = .99)}$ = the students t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence interval estimates for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution ($_x^2$ / df).

LCL = 0.64 MDL

UCL = 2.20 MDL

where: LCL and UCL are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike the matrix at this calculated MDL and proceed through the procedure starting with Step 4.

(b) If this is the second or later iteration of the MDL calculation, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F-ratio. The F-ratio is calculated by substituting the larger S^2 into the numerator S^2_A and the other into the denominator S^2_B The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows: if $S^2_A / S^2_B > 3.05$, then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{\frac{1}{2}}$$

if $S^2_A / S^2_B > 3.05$, respike at the most recent calculated MDL and process the samples through the procedure starting with Step 4. If the most recent calculated MDL does not permit qualitative identification when samples are spiked at that level, report the MDL as a concentration between the current and previous MDL which permits qualitative identification.

(c) Use the S_{pooled} as calculated in 7b to compute the final MDL according to the following equation:

$$MDL = 2.681 \ (S_{pooled})$$

where 2.681 is equal to $t_{(12,\ 1-a\ =.99)}$.

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from percentiles of the chi squared over degrees of freedom distribution.

LCL=0.72 MDL

UCL=1.65 MDL

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

TABLES OF STUDENTS' T VALUES AT THE 99 PERCENT CONFIDENCE LEVEL

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1, .99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
00	00	2.326

Reporting

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL and indicate if the MDL procedure was iterated. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level of analyte in the sample was below the determined MDL or exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

[49 F.R. 43430, Oct. 26, 1984; 50 F.R. 694, 696, Jan. 4, 1985, as amended at 51 F.R. 23703, June 30, 1986]

APPENDIX F

REFERENCES

APPENDIX F

REFERENCES

1. *Interlaboratory Validation of U.S. Environmental Protection Agency Method 1625A*, July 1984. Prepared by SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025, (415) 326-6200. Available from the Sample Control Center (operated by DynCorp Environmental), 300 N. Lee Street, Alexandria, VA 22314, (703) 519-1140.

2. *Method 1664: N-Hexane Extractable Material (HEM) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry (Oil and Grease and Total Petroleum Hydrocarbons)*, April 1995, Document No. EPA-821-B-94-004b, EPA Water Resource Center, Mail Code RC-4100, 401 M Street, S.W., Washington, D.C. 20460.

3. *Methods for Chemical Analysis of Water and Wastes*, 3rd Edition; Environmental Protection Agency, Environmental Monitoring Systems Laboratory-Cincinnati (EMSL-Ci): Cincinnati, Ohio 45268, EPA-600/4-79-020, 1983; Method 413.1.

4. *Report of EPA Efforts to Replace Freon for the Determination of Oil and Grease: Phase II*, April 1995, Document No. EPA-820-R-95-003, EPA Water Resource Center, Mail Code RC-4100, 401 M Street, S.W., Washington, D.C. 20460.

5. *Standard Methods for the Examination of Water and Wastewater*, 18th Edition; American Public Health Association: 1015 Fifteenth Street, NW, Washington, D.C. 20005, 1992, Method 5520B.

6. Youden, W.J., "Ranking Laboratories by Round-Robin Tests", *Materials Research and Standards* 3, 13-17, 1963.

ADDENDUM TO SECTION 3

DEVELOPMENT OF QUALITY CONTROL ACCEPTANCE CRITERIA

JANUARY 1996

ADDENDUM TO SECTION 3

DEVELOPMENT OF QUALITY CONTROL ACCEPTANCE CRITERIA

JANUARY 1996

Section 3 of the Method 1664 validation study report details the development of the quality control (QC) acceptance criteria that were published in the April 1995 version of Method 1664, and that were included in the version of Method 1664 incorporated by reference into the proposal scheduled for publication in the *Federal Register* in January of 1996. After the preamble and regulatory language had been submitted for publication, and after it was too late to recall the proposal of Method 1664, it was noted that some of the data sets used to derive the QC acceptance criteria required modification. These discrepancies consisted of several occurrences of replicate values of the same results and two missing results. Also, data that were received too late for inclusion in the original QC derivations were included in the revised data set, and an alternate statistical approach was used for generation of the IPR and OPR acceptance criteria. This addendum gives details of changes resulting from the modifications to the data sets used for statistical analyses and provides re-derived QC acceptance criteria based on the revised data sets.

Replicate and Missing Data

A close inspection of the data sets used to establish the ongoing precision and recovery (OPR) QC acceptance criteria for both HEM and SGT-HEM showed that they contained replicate values for what should have been a single result, and were each missing one OPR result. The database was corrected by removing the replicate results and adding the missing result.

Late Data

A set of HEM data from the TCRR study were received from a laboratory that had not submitted results in time for inclusion in the database that was used for development of the original QC acceptance criteria. In addition, an SGT-HEM OPR result from the TCRR laboratory that performed this analysis was added to the SGT-HEM OPR data set. Originally, this datum had not been included. These additions served to further broaden the data set.

Alternate Statistical Analysis

The IPR and OPR QC acceptance criteria in the April 1995 version of Method 1664, and the version incorporated by reference into the proposal scheduled for publication in January of 1996, were each constructed using the respective IPR or OPR data that had been provided by the Twin Cities Round Robin (TCRR) study and Phase II of EPA's Freon Replacement Study. Because an OPR sample is the equivalent of each of the four samples that make up an IPR set, an alternate, and equally acceptable approach would be to pool the IPR and OPR data. The advantage of this approach is that it would provide a broader data set and greater

statistical power for calculation of the variance components. These variance components are factors in the equations used to determine the IPR and OPR QC acceptance criteria limits for both recovery and precision. For derivation of the revised QC acceptance criteria given in this addendum, the variance components were recalculated using the pooled data set.

Effect of Changes to the Database

With the exception of the SGT-HEM IPR precision specification, the SGT-HEM OPR upper recovery limit, and the OPR-based matrix spike (MS) and matrix spike duplicate (MSD) relative percent difference (RPD) limit, the recalculated values are not substantially different from those published in the April 1995 version of Method 1664. These recalculated values are not in the version of Method 1664 incorporated by reference into the proposal scheduled for publication in January 1996. At the close of the comment period on the proposal, EPA will consider comments as well as other data and, depending upon the conclusions reached, may find it appropriate to revise one or more of the specifications in Method 1664.

The remainder of this addendum is a revision of those subsections within Section 3 of the validation study report that required modification to reflect the recalculations. Those subsections that did not require revision have not been reprinted here.

3.1 Data Validation and Statistical Analysis

3.1.2 QC Acceptance Criteria for Hexane Extractable Material

Criteria were developed for the IPR, OPR, and matrix spike/matrix spike duplicate (MS/MSD) tests for both HEM and SGT-HEM. For HEM, the IPR and OPR acceptance criteria were constructed using an analysis of variance (ANOVA) with laboratories as a random variance component. For each QC criterion, the mean result across laboratories was determined. The IPR and OPR data were pooled, and the interlaboratory and intralaboratory variance components were calculated from the consolidated data. The individual IPR and OPR variability values were then calculated from these interlaboratory and intralaboratory variance components using a formula appropriate to the particular quality control test. The 95 percent cutoff value from the Student's *t* distribution was determined based on the appropriate degrees of freedom. The degrees of freedom are dependent on the number of laboratories, the number of sample analyses, and the variance components. The QC limit was derived by multiplying the *t* value by the variability, and subtracting this value from the mean result for the lower limit, or adding this value to the mean result for the upper limit. Details of the equations used to derive these limits are presented in the document titled *Interlaboratory Validation of U.S. Environmental Protection Agency Method 1625A*, July 1984.

3.1.3 QC Acceptance Criteria for Silica Gel Treated Hexane Extractable Material

For SGT-HEM, EPA received results from only two laboratories, one from the TCRR study and one from the Phase II study. EPA used these data to construct the revised IPR and OPR QC acceptance criteria.

3.1.4 QC Acceptance Criteria for Matrix Spike and Matrix Spike Duplicate

For HEM and SGT-HEM, the criteria for recovery of a matrix spike (MS) or matrix spike duplicate (MSD) and for the relative percent difference between an MS and an MSD were derived from the OPR criteria, since neither the TCRR study nor the Phase II study required the spiking of field samples. EPA believes that this application of OPR criteria to MS/MSD samples is acceptable because the determinative technique in Method 1664 is gravimetry, which is not susceptible to interferences, and because nearly all of the treated effluents to which Method 1664 is to be applied in monitoring will be similar to the reagent water used in the OPR tests. This transfer of data for development of specifications for acceptance criteria is similar to that which EPA used in the organic methods that are promulgated at 40 *CFR* 136, Appendix A and in other methods.

Determination of the relative percent difference (RPD) criteria consisted of setting the limit at approximately one-half of the range between the lower recovery limit and the upper recovery limit. This estimation establishes the RPD limit at 14.4 percent for HEM and 33.4 percent for SGT-HEM. These limits are considered to be a reasonable first approximation of method performance in the absence of MS/MSD data. If MS/MSD data become available, it may be appropriate to revise these limits.

3.2 Results

Few laboratories in the interlaboratory study encountered difficulties with the analysis of IPR and OPR samples, and most achieved acceptable recoveries of hexadecane and stearic acid. Statistical evaluation of the results from all thirteen laboratories produced few outliers, and the extreme rank sum test showed all laboratories to be equivalent. This indicates that Method 1664 is a reproducible procedure sufficiently reliable to be used by a variety of laboratories.

Results are summarized in Addendum Table 2 below. Individual laboratory results and the statistical analyses of these data are presented in Addendum Tables 3 through 12.

Addendum Table 2

95 Percent Confidence Limits for Method 1664 QC Criteria

Criterion	95% Lower Limit (%)	95% Upper Limit (%)
IPR		
HEM Precision		10.7
HEM Recovery	85.4	97.8
SGT-HEM Precision		27.9
SGT-HEM Recovery	86.3	101.8
MS/MSD		
HEM Recovery	78.0	106.7
HEM RPD		14.4
SGT-HEM Recovery	64.9	131.7
SGT-HEM RPD		33.4
OPR		
HEM Recovery	78.0	106.7
SGT-HEM Recovery	64.9	131.7

3.3 Discussion and Conclusions

As a check on the validity of the revised QC acceptance criteria, IPR and OPR results from the Phase II study and the TCRR study were compared with the respective criteria. For the IPR test of HEM, four of the mean percent recovery values did not meet the IPR recovery criterion, and only one of the 13 IPR sets failed the IPR precision criterion. In all cases, the IPR recoveries that did not meet the lower or upper limits were within 3 percent of that limit. For the OPR test, only three of the 22 results failed the OPR recovery criterion, one of which was within 0.09 percent of the limit. For the IPR test of SGT-HEM, one of the two laboratories did not meet the recovery criterion (3 percent outside the limit), and both laboratories met the precision criterion.

Upon comparing these recalculated QC acceptance criteria to the criteria given in the version of Method 1664 being proposed, it is evident that the majority of the QC limits are similar (Addendum Table 13B). The most obvious exceptions are the SGT-HEM IPR precision criteria, the SGT-HEM OPR upper recovery limit, and the OPR-based SGT-HEM MS/MSD RPD limit. When determining those criteria presented in Section 3 (and published in the proposed version of Method 1664), the IPR and OPR data were considered separately. This approach is acceptable because an OPR analysis, though considered to be the equivalent of one of four IPR analyses, is performed at a frequency of one per analytical batch. IPR analyses are performed at the same time and should theoretically be less susceptible to the variability that would occur were the samples prepared days apart, as they are with the OPR.

Because each individual IPR analysis is performed in exactly the same way as each OPR analysis, it is also acceptable to consider these analyses to be equivalent, and it is therefore acceptable to pool data from both analyses to derive the variance components. The advantage to this latter approach is that this will provide a broader data set, which is especially important given the limited number of laboratories that performed SGT-HEM analysis. The effect of this pooling is a widening of the specifications for SGT-HEM. Even when pooled, however, the SGT-HEM data set is limited and may not provide an adequate representation of interlaboratory effects. If more data become available, it may be necessary to re-examine these specifications.

Regardless of the approach to data pooling, nearly all results produced by the contributing laboratories are within the QC acceptance criteria contained in the April 1995 version of Method 1664 that is to be proposed, and demonstrate that the QC acceptance criteria are realistic and reflect the performance of Method 1664 on both reagent water and on real world sample matrices. For this reason, and because the April 1995 version of Method 1664 has been in circulation for more than nine months with an almost unanimously favorable response, EPA intends to retain the QC acceptance criteria currently given in the April 1995 version of Method 1664, which is to be incorporated by reference into 40 *CFR* part 136 by the proposal scheduled to be published in the *Federal Register* in January of 1996.

Addendum Table 3

HEM IPR Data

Lab	% Rec. IPR 1	% Rec. IPR 2	% Rec. IPR 3	% Rec. IPR 4	Mean % Rec.	Standard Deviation
1	98.8	103.8	96.3	98.3	99.3*	3.2
2	78.7	103.5	105.7	83.2	92.8	13.8*
3	87.5	92.5	85.0	87.5	88.1	3.1
4	90.3	87.5	89.0	89.3	89.0	1.2
5	93.9	97.9	82.2	90.1	91.0	6.7
6	86.8	92.0	92.5	93.8	91.3	3.1
7	82.3	83.8	81.5	86.5	83.5*	2.2
8	91.0	103.5	94.5	95.3	96.1	5.3
9	85.0	93.3	89.3	102.0	92.4	7.2
10	87.5	77.5	87.5	82.5	83.8*	4.8
11	89.5	89.3	92.3	89.0	90.0	1.5
12	107.5	110.0	95.0	90.0	100.6*	9.7
13	95.1	94.9	97.1	97.8	96.2	1.4
				Mean % Recovery Across Labs	91.9	
				Std. Deviation of Mean % Recoveries Across Labs	5.3	
					Mean Std. Deviation Across Labs	4.9
					Std. Deviation of the Std. Deviations Across Labs	3.7

* Results that failed the recovery or precision specification.

Addendum Table 4

HEM IPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of Mean % Rec. Values Below 95% Lower Limit	No. of Mean % Rec. Values Above 95% Upper Limit
85.4	97.8	2	2

Addendum Table 5

HEM IPR Data - Upper Limit for Precision

95% Upper Limit	No. of Standard Deviation Values Above 95% Upper Limit
10.7	1

Addendum Table 6

HEM OPR Data

Lab	% Recovery
1	83.0
2	80.8
3	82.5
4	87.8
5	91.0
6	98.8
7	89.8
8	89.8
9	82.5
10	91.0
11	99.5
11	91.5
11	91.0
11	112.0*
11	93.8
11	93.3
11	101.8
11	89.8
11	111.8*
11	94.0
11	106.8*
12	92.2
Mean % Recovery Across Labs	93.4
Standard Deviation of % Recoveries Across Labs	8.7

Addendum Table 7

HEM OPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of % Rec. Values Below 95% Lower Limit	No. of % Rec. Values Above 95% Upper Limit
78.0	106.7	0	3

* Results that failed the recovery specification.

Addendum Table 8

SGT-HEM IPR Data

Lab	% Rec. IPR 1	% Rec. IPR 2	% Rec. IPR 3	% Rec. IPR 4	Mean % Rec.	Standard Deviation
1	110.0	115.0	80.0	115.0	105.0*	16.8
2	105.0	100.0	95.0	65.0	91.3	18.0
				Mean % Recovery Across Labs	98.1	
				Std. Deviation of Mean % Recoveries Across Labs	9.7	
					Mean Std. Deviation Across Labs	17.4
					Std. Deviation of the Std. Deviations Across Labs	0.8

Addendum Table 9

SGT-HEM IPR Data - Upper and Lower Limits for Recovery

95% Lower Limit1	95% Upper Limit	No. of Mean % Rec. Values Below 95% Lower Limit	No. of Mean % Rec. Values Above 95% Upper Limit
86.3	101.8	0	1

* Result that failed the recovery specification.

Addendum Table 10

SGT-HEM IPR Data - Upper Limit for Precision

95% Upper Limit	No. of Standard Deviation Values Above 95% Upper Limit
27.9	0

* Result that failed the recovery specification.

Addendum Table 11

SGT-HEM OPR Data

Lab	% Recovery
1	88.0
1	82.0
1	76.0
1	75.5
1	100.0
1	107.5
1	80.0
1	79.0
1	96.0
1	100.0
1	111.0
2	75.0
Mean % Recovery Across Labs	89.2
Standard Deviation of % Recoveries Across Labs	13.1

Addendum Table 12

SGT-HEM OPR Data - Upper and Lower Limits for Recovery

95% Lower Limit	95% Upper Limit	No. of % Rec. Values Below 95% Lower Limit	No. of % Rec. Values Above 95% Upper Limit
64.9	131.7	0	0

Addendum Table 13B

Comparison of Method 1664 Criteria

	HEM IPR			HEM OPR		HEM MS/MSD		
	95% Lower % Rec.	95% Upper % Rec.	Precision	95% Lower % Rec.	95% Upper % Rec.	Lower % Rec.	Upper % Rec.	RPD
Published	83.0	100.7	10.9	79.0	113.9	79.0	113.9	17.5
Recalculated	85.4	97.8	10.7	78.0	106.7	78.0	106.7	14.4

	SGT-HEM IPR			SGT-HEM OPR		SGT-HEM MS/MSD		
	95% Lower % Rec.	95% Upper % Rec.	Precision	95% Lower % Rec.	95% Upper % Rec.	Lower % Rec.	Upper % Rec.	RPD
Published	83.2	116.0	13.3	65.8	105.7*	65.8	105.7*	24.0
Recalculated	86.3	101.8	27.9	64.9	131.7	64.9	131.7	33.4

*These calculated values were widened to the limits generated from HEM, as explained in Section 3.1.3 of the validation study report. This change also applies to the MS/MSD RPD calculation.