# Results of the EPA Method 1631 Validation Study

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## Acknowledgments

This report was prepared under the direction of William A. Telliard of the Engineering and Analysis Division within the EPA Office of Water. It was prepared by DynCorp Environmental under EPA Contract No. 68-C3-0337. Other contributers to this to this study included Battelle Marine Sciences (Sequim, WA); Brooks Rand, Ltd. (Seattle, WA); the University of Conneticut's Department of Marine Sciences (Groton, CT); and the University of Minnesota's Department of Soil, Water, and Climate (St. Paul, MN).

### Disclaimer

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## 1.0 Background

In 1993, EPA's Engineering and Analysis Division (EAD) began developing methods for the determination of metals at ambient water quality criteria levels. This method development initiative was driven by an increased emphasis on water quality-based permitting.

In April 1995, EPA released its first draft of *Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*. This method was designed to allow determination of mercury at EPA's lowest water quality criterion of 0.012 µg/L (12 ng/L) and was based on procedures that were already well-documented in the published literature. In January 1996, a revised version of Method 1631, (EPA 821-R-96-001), was released to correct typographical errors in Table 2 and to update several references cited in the method.

As part of its ongoing method development and validation efforts, EAD has conducted a validation study using Method 1631. The purpose of this study was to validate the estimated method detection limit of 0.05 ng/L and the quality control criteria specified in the January 1996 draft of Method 1631. An additional purpose of the study was to identify further method development needs or to further revise the method.

## 2.0 Study Design and Objectives

The study described in this report was designed as a single laboratory study aimed at providing EPA with some level of verification that the procedures and quality control (QC) acceptance criteria specified in the draft method could be met by environmental laboratories. Depending on laboratory costs and EPA budget contraints, the study was designed to be implemented by one or more laboratories. More extensive interlaboratory method validation studies may be performed at a later date.

The study design is described fully in the *Study Plan for Validation of EPA Method 1631* summarized below and provided in Appendix A of this report. The study was conducted in two phases.

The objective of Phase 1 was to use reagent water samples to determine the method detection limit (MDL) for mercury using oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry procedures described in the January 1996 version of Method 1631. The MDL stated in Method 1631 (0.05 ng/L) was used as the initial estimate of the MDL. Reagent water was chosen as the sample matrix because it is homogeneous, readily available, and included as a matrix option in the EPA MDL procedure promulgated at 40 *CFR* Part 136, Appendix B.

The objective of Phase 2 focused on validating the QC acceptance criteria specified in draft Method 1631. The objectives that were pursued included verifying that Method 1631 is capable of yielding a minimum level (ML) of 0.2 ng/L for mercury, and determining if the initial precision and recovery (IPR), calibration verification (VER), ongoing precision and recovery (OPR), and matrix spike/matrix spike duplicate (MS/MSD) QC acceptance criteria specified in Table 1 of Method 1631 can be achieved for mercury. A final overall objective was to identify any further method development needs or areas in need of revision in the method. Performance of Phase 2 was contingent on successful completion of Phase 1.

## 3.0 Study Implementation

Four laboratories performed the Phase 1 and Phase 2 studies. These were: Battelle Marine Sciences (Sequim, WA): Brooks Rand, Ltd., (Seattle, WA); the University of Conneticut's Department of Marine Sciences (Groton, CT); and the University of Minnesota's Department of Soil, Water, and Climate (St. Paul, MN). All analyses and data reporting were completed in May 1996. Day-to-day coordination of the study and review of study data was performed by the contractor-operated Sample Control Center (SCC)[1].

### *3.1 Phase 1 Methodology/Approach*

Each laboratory was instructed to perform the MDL determination in accordance with the procedures described in 40 *CFR* Part 136, Appendix B - *Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11*. The laboratory analyzed a minimum of seven replicate samples that contained mercury (Hg) at a concentration of one to five times the estimated MDL. For this study, the estimated MDL was 0.05 ng/L (Method 1631, Section 1.5). Therefore, the laboratories were instructed to choose a spike level that produced a concentration of 0.05 - 0.25 ng/L (1 - 5 times the estimated MDL of 0.05 ng/L).

Two of the laboratories prepared replicate samples by spiking reagent water with Hg at 0.25 and 0.30 ng/L. The other two laboratories used unspiked reagent water because the contributions from the reagents and the levels present in the reagent water resulted in levels within one to five times the estimated MDL. To ensure that the laboratory spike level was appropriate, laboratories were instructed to perform the two-aliquot test described in Section 4b of the 40 *CFR* Part 136 procedure. If these measurements indicated that the sample concentration was in the desired range for determination of the MDL, the laboratory was required to proceed with the five additional aliquots for a total of seven aliquots. All seven measurements were then used for calculation of the MDL. If the first two measurements indicated that the sample concentration was not in the correct range, the laboratory was required to repeat Section 4b (analysis of two spiked aliquots) until the desired spike level was achieved.

### *3.2 Phase 2 Methodology/Approach*

The technical approach for Phase 2 centered around the use of Method 1631 to analyze four spiked reagent water replicates and two spiked aqueous field sample replicates. The results of the spiked reagent water analyses were used to assess the precision and accuracy that can be achieved for Hg determinations using Method 1631. The results of the spiked field sample replicates were used to assess the precision and accuracy of the method for determining Hg in a real-world sample matrix. All QC requirements and analytical procedures stated in Method 1631 were performed with these analyses. The results of associated QC analyses (e.g., blanks, calibration verification, etc.) were used to further assess the validity of the method.

In developing Method 1631, an interim minimum level (ML) of quantitation was derived by multiplying the estimated MDL by 3.18. This 3.18 value is the ratio between the 10 sigma multiplier used

[1]The Sample Control Center is operated by DynCorp Environmental under EPA Contract No. 68-C3-0037.

to establish the American Chemical Society (ACS) and International Union of Pure and Applied Chemistry (IUPAC) limit of quantitation (LOQ) and the student t multiplier normally used to determine an MDL (i.e., 10 ÷ 3.143 = 3.18). For the purposes of this study, an interim ML was determined using the results from Phase 1 of this study. The MDL determined in Phase 1 was multiplied by 3.18 to arrive at an interim ML. This interim ML was then rounded to the nearest factor of 10 multiple of 1, 2, or 5 in order to simplify instrument calibration. Because only two of the four laboratories were able to achieve an MDL that verified the ML listed in Method 1631, a new (higher) ML using the other laboratories' MDL results was defined for Phase 2 of the study.

In accordance with Section 7.10 of Method 1631, the laboratories were instructed to initially calibrate their instruments with a minimum of five calibration points. The lowest calibration point was equal to the new ML (0.5 ng/L). IPR, OPR, and quality control sample (QCS) samples were prepared by spiking reagent water with Hg as specified in Method 1631.

Two spiked aqueous field sample replicates were used for the MS/MSD samples. Each laboratory collected a single sample from a source known to contain Hg at ambient concentrations that were at or below the levels of interest in this study. The laboratories divided this sample into replicate aliquots and spiked each aliquot at a concentration that was between one and five times the background concentration or at the concentration of the mid-level calibration standard, whichever was greater. The laboratories also collected and analyzed a field blank with the field sample to demonstrate that the replicates used for preparation of the MS/MSD were free from contamination.

## 4.0 Data Reporting and Validation

All data from this study were submitted to SCC for review and validation. The laboratory reports included hardcopy summary level data, MDL results, and raw data. Data were reviewed against the requirements of the study plan and in accordance with EPA's *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring*[2]. Completeness of the data submissions was verified by ensuring that all required data were present, including results of all required tests, sample lists, run chronologies, summaries of analytical results, raw data, and copies of laboratory notebooks. Validation of data was performed by comparing each required data element to the requirements of the study plan. This included verification that (1) reagent water and ambient water samples were utilized as appropriate, (2) proper spike levels were used in the MDL studies and QC analyses, (3) the instruments were properly calibrated and other method procedures were followed, and (4) the MDL was calculated in accordance with the MDL procedure. In those instances in which the requirements in the study plan were not met, an explanation was provided in the narrative report or was resolved through subsequent discussions with the laboratory. All data were considered to be of acceptable quality with the exceptions noted below.

[2]*Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring*, January 1996 (EPA 821-B-96-002). Available from the Sample Control Center (operated by DynCorp), 300 N. Lee Street, Alexandria, VA 22314, (703) 519-1140.

## 5.0 Results

### *5.1 Phase 1 Results*

The analytical and statistical results for Phase 1 are provided in Table 1. The MDL is calculated as the standard deviation of seven consecutive replicate analyses multiplied by the students' t value for (n-1) degrees of freedom, where n equals the number of replicates. The students' t value for six degrees of freedom (seven replicates) is 3.143.

None of the laboratories were able to achieve the estimated MDL specified in the January 1996 draft version of Method 1631 (0.05 ng/L). The MDLs determined in this study ranged from 0.067 to 0.160 ng/L. All four MDLs, however, were at least 10 times lower than EPA's lowest water quality criterion of 12 ng/L for Hg.

### *5.2 Phase 2 Results*

The analytical and statistical results for Phase 2 are provided in Table 2. Two of the laboratories (Brooks Rand, Ltd., and the University of Minnesota) reported MDLs that verified the ML listed in the method (0.2 ng/L). The other two laboratories (Battelle and the University of Connecticut) reported MDLs (0.160 and 0.138 ng/L, respectively) that yielded a slightly higher ML of 0.5 ng/L. Because the ML serves as the basis for preparing the lowest calibration standard and because the lower ML of 0.2 ng/L was not definatively confirmed by the MDL studies, all laboratories were instructed to use the higher ML of 0.5 ng/L for Phase 2 analyses. All laboratories were able to successfully calibrate to the 0.5 ng/L level.

All laboratories met the QC acceptance criteria for all QC analyses with the exception of two of the three OPR analyses performed by the University of Connecticut. All four of the laboratories used concentration levels of 5 ng/L for the OPRs. The problem with the two failed OPRs at the University of Conneticut appears to be a result of problems related to the saline sample matrix used for the MS/MSD analyses. The laboratory explained that following the analysis of the field samples collected from the Long Island Sound, a drastic loss in sensitivity was observed. A second set of the samples was analyzed with a lower level of BrCl reagent and resulted in good recovery for the matrix spikes, but the OPR recovery remained low. It should be noted that Method 1631was not intended for use with seawater (or high saline samples) and, therefore, the OPR criterion was not adjusted due to these low OPR results.

As directed, laboratories participating in this study provided EPA with comments and recommendations concerning future improvements to the method. These comments ranged from minor editorial comments to suggestions for technical clarification correction of specified procedures.

## Table 1: Method Detection Limits for Hg by Method 1631

| | | Concentration (ng/L) Replicate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Laboratory** | **Spike Levels** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **Mean** | **s** | **MDL** |
| Battelle | 0.300 | 0.371 | 0.251 | 0.232 | 0.305 | 0.353 | 0.302 | 0.328 | 0.306 | 0.051 | 0.160 |
| Brooks Rand, Ltd. | - | 0.016 | 0.035 | 0.000 | 0.050 | 0.035 | 0.050 | 0.000 | 0.027 | 0.021 | 0.067 |
| Unv. Connecticut | 0.25 | 0.31 | 0.21 | 0.21 | 0.24 | 0.27 | 0.32 | 0.27 | 0.26 | 0.044 | 0.138 |
| Unv. Minnesota | - | 0.29 | 0.27 | 0.28 | 0.26 | 0.21 | 0.26 | 0.22 | 0.26 | 0.029 | 0.092 |

## Table 2: Minimum Levels and Validation of QC Acceptance Criteria for Hg by Method 1631

| | **ML ng/L** | **IPR %** | | **OPR % Recovery** | **QCS[1] % Recovery** | **MS/MSD % Recovery** | **MS/MSD RPD** |
|---|---|---|---|---|---|---|---|
| | | **s** | **X** | | | | |
| **Laboratory** | **0.2** | **21** | **79-121** | **77-123** | **-** | **75-125** | **24** |
| Battelle | 0.5 | 3.14 | 104 | 99.3 | 98, 96 | 107/105 | 1.89 |
| Brooks Rand, Ltd. | 0.2 | 2.5 | 103 | 106 | 96, 101 | 94/100 | 6.7 |
| Unv. Connecticut | 0.5 | 4.5 | 91 | 69, 83, 75 | - | 95/103 | 4 |
| Unv. Minnesota | 0.2 | 1.0 | 98 | 99, 106 | - | 86/101 | 6 |

[1] Method 1631 does not specify acceptance criteria for QCS

## 6.0 Conclusion and Discussion

Results of Phase 1 indicated that the estimated MDL specified in the draft method could not be achieved by any of the laboratories involved in the study. In addition, all the MDLs that were determined did not verify the ML specified in the draft method. For Phase 2 of the study, the laboratories were instructed to use 0.5 ng/L as the ML (based on the results of Battelle and the University of Connecticut). All laboratories were able to calibrate to this level in Phase 2. Phase 2 also demonstrated that all other QC acceptance criteria in EPA Method 1631 are reasonable and that it should be possible for laboratories to meet these criteria on a routine basis. Results of this study, therefore, suggest that the MDL and ML specified in draft Method 1631 should be revised to 0.2 ng/L and 0.5 ng/L, respectively, but that no further modifications to the QC acceptance criteria in the method are warrented. Finally, comments submitted by each of the participating laboratories suggested that slight modifications should be made to the procedures specified in the method in order to avoid misunderstanding and inconsistency in laboratory performance. These modifications should be made when the method is revised to update the MDLs and MLs.

# APPENDIX

## Study Plan for Validation of EPA Method 1631, February 1996

# Study Plan
# for
# Validation of EPA Method 1631

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## Disclaimer

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## SECTION 1: INTRODUCTION

In 1993, EPA's Engineering and Analysis Division (EAD) began developing methods for determination of metals at ambient water quality criteria levels. This method development initiative was driven by an increased emphasis on water quality-based permitting.

In April 1995, EPA released its first draft of *Method 1631: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*. This method was designed to allow determination of mercury at EPA's lowest water quality criterion of 0.012 µg/L (12 ng/L) and was based on procedures that were already well-documented in the published literature. In January 1996, a revised version of Method 1631, (EPA 821-R-96-001), was released to correct typographical errors in Table 2 and to update several references cited in the method.

The purpose of this study plan is describe EAD's strategy for validating the estimated method detection limit of 0.05 ng/L and to validate the quality control criteria in the January 1996 draft of Method 1631.

## SECTION 2: OBJECTIVES

In order to minimize cost, the study will be pursued in two phases, each of which is designed to accomplish specific objectives. Objectives for each phase are summarized below.

### 2.1 Phase 1 Objectives

The objective of Phase 1 of this study is to use reagent water samples to determine the method detection limit (MDL) for mercury using oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry procedures described in the January 1996 version of Method 1631. The MDL stated in Method 1631, (0.05 ng/L), will be used as the initial estimate of the MDL. If this MDL cannot be met, results of the study will be used as a basis to identify further method development needs or to further revise the method.

#### 2.1.1 Data Quality Objectives for Phase 1

In addition to the overall objective described above, EAD has three principle data quality objectives (DQOs) for this phase of the study:

(1) The MDLs determined in this study must be within a factor of five of the level spiked in order to ensure that MDLs determined in the study are neither overstated nor understated.

(2) All data produced under this phase of the study must be generated in accordance with the analytical and QA/QC procedures defined in Method 1631. Alternatively, the data must be the result of pre-approved and documented changes to these procedures. This will allow EAD to use the results of this phase to identify the need for further revision of the method.

(3) All data produced under this phase of the study must be capable of being verified by an independent person reviewing the analytical data package.

### 2.1.2 Exceptions to QC Procedures for Phase 1

In order to meet these DQOs, the laboratory will be required to have a comprehensive QA program in place and operating throughout this study. This will ensure that the data produced are of the highest possible quality. During Phase 1 of the study, the laboratory will be required to follow all QC procedures defined in Method 1631 with the following exceptions:

- Demonstration of initial and ongoing precision and recovery will not be required.
- Performance of matrix spike and matrix spike duplicate analyses will not be required.
- Analysis of field blanks and equipment blanks will not be required.
- Instrument calibration must be performed at a range that will encompass the minimum level (ML) associated with the detection limit being studied.

All analyses performed in this study must be performed on a calibrated instrument, and calibration verification must be performed during each analytical batch.

## 2.2 Phase 2 Objectives

The second phase of this study will focus on validating the QC acceptance criteria that are specified in Method 1631. The primary objectives that will be pursued in Phase 2 are to:

(1) Verify that Method 1631 is capable of yielding a minimum level (ML) of 0.2 ng/L for mercury.

(2) Determine if the IPR, calibration verification, OPR, and matrix spike QC acceptance criteria specified in Table 2 of the Method 1631 can be achieved for mercury.

(3) Identify any further method development needs or areas in need of revision.

### 2.2.1 Data Quality Objectives for Phase 2

Data quality objectives for this phase of the study are as follows:

(1) All data produced under this phase of the study must be generated in accordance with the analytical and QA/QC procedures defined in Method 1631; alternatively, the data must be the result of pre-approved and documented changes to these procedures. This will allow EAD to use the results of this phase to identify the need for further revision of Method 1631.

(2) The QC data generated in this study must reflect careful laboratory attention to Method 1631. The QC data are not to be the result of repeated efforts on the part of the laboratory to achieve results that are within the QC limits currently stated in the method. Therefore, the laboratory will be instructed to utilize any stated QC specifications (i.e., limits) as data quality objectives (DQOs) rather

than as definitive requirements of the study. The results of the laboratory's QC analyses will be used to confirm that the specifications provided in Method 1631 can be met, or alternatively, to identify specifications that may not be achievable.

(3) All data produced under this phase of the study must be capable of being verified by an independent review of the analytical data package.

## SECTION 3: STUDY MANAGEMENT

The study described in this document will be managed by EAD's 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 with one or more laboratories experienced in the determination of mercury at WQC levels. The total number of laboratories participating in this study will be dependent upon laboratory capability, laboratory availability, cost, and scheduling constraints. SCC will 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. AMS will also share data and results with all interested parties upon request.

## SECTION 4: TECHNICAL APPROACH

The technical approach detailed below is designed to be consistent with the technical approach previously used by EAD for validating the procedures outlined in the *Quality Control Supplement for Determination of Metals at Ambient Water Quality Criteria Levels* (the "QC Supplement"). That approach is detailed in the *Study Plan for Validation the Quality Control Supplement for Determination of Metals at Ambient Water Quality Criteria Levels* (June 1994).

### 4.1 Phase 1 Technical Approach

Phase 1 will focus on the performance of an MDL study in accordance with the procedure described in 40 *CFR* Part 136, Appendix B - *Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11*. This procedure, which is provided as Attachment 1 to this study plan, involves the analysis of at least seven replicate samples that are known to contain the target analyte(s) at a concentration of one to five times the estimated MDL. For the purposes of this study, the estimated MDL is 0.05 ng/L (Method 1631 Section 1.5). Therefore, the laboratory will use choose a spike level that produces a concentration of 0.05 - 0.25 ng/L (1 - 5 times the estimated MDL of 0.05 ng/L).

In this phase of the study, reagent water will be spiked with mercury to produce a synthetic sample that can be divided into at least seven replicates. In order to ensure that the laboratory is spiking at the appropriate level, it is recommended that the laboratory follow the two-aliquot test described in Section 4b of the 40 *CFR* Part 136 procedure. If these measurements indicate that the sample concentration is in the desired range for determination of the MDL, the laboratory may proceed with the five additional aliquots for a total of seven aliquots. *All* seven measurements will be used for calculation of the MDL. If the first two measurements indicate that the sample concentration is not in the correct range, the laboratory will be required to repeat

Section 4b (analysis of two spiked aliquots) until the desired spike level is achieved. Application of the two aliquot test is designed to minimize the possibility of repeating the MDL study in order to obtain an MDL that is within a factor of five of the level spiked.

If the MDL resulting from the study is not within a factor of five of the level spiked, then the spiking, measurement, and calculation processes will be iterated until the measured MDL is within a factor of five, as given in the MDL procedure. If the laboratory obtains non-detect results during their MDL study, the laboratory must increase the spike level and repeat the test. The objective of these requirements is to ensure that MDLs resulting from the study are not artificially high or low due to inappropriate spiking levels. If appropriate spiking levels are chosen but yield MDLs greater than 0.05 ng/L, EAD will consider the need for revision of the method, further study, or identification of new analytical techniques.

The analytical portion of the MDL study will be performed in accordance with the procedures described in Method 1631, with the four exceptions noted in Section 2.1.2 above.

If the results of Phase 1 suggest that the MDL of 0.05 ng/L cannot be achieved using Method 1631, SCC will work with the laboratory(ies) to identify clarifications and/or revisions that must be made in order to yield the acceptable detection levels. SCC will notify EAD of these results and any laboratory recommendations. EAD will make a final determination as to whether additional method development activities are needed before Phase 2 is commenced.

## 4.2 Phase 2 Technical Approach

The technical approach for Phase 2 will be centered around the use of Method 1631 to analyze four spiked reagent water replicates and two spiked aqueous field sample replicates. The results of the spiked reagent water analyses will be used to assess the precision and accuracy that can be achieved for mercury determinations using Method 1631. The results of the spiked field sample replicates will be used to assess the precision and accuracy of the method in a real world matrix. *All* quality control requirements and analytical procedures stated in Method 1631 will be performed with each of these analyses. The results of any associated QC analyses (e.g., blanks, calibration verification, etc.) will be used to further assess the validity of the method.

### 4.2.1 Matrix Spike and Matrix Spike Duplicate

The two spiked aqueous field sample replicates analyzed in this study (known as matrix spike and matrix spike duplicate samples) will be prepared by the laboratory. To do this, the laboratory shall collect a single sample from a source known to contain mercury at ambient concentrations that are at or below the levels of interest in this study. This sample will then be divided into replicate aliquots and spiked in the laboratory to produce matrix spike (MS) and matrix spike duplicate (MSD) samples as described below and in Method 1631. The laboratory must collect and analyze a field blank with the field sample in order to demonstrate that the replicates used for preparation of the MS and MSD are free from contamination. Prior to collection of field samples, the laboratory shall verify that the sampling equipment is free from contamination by analyzing equipment blanks as described in Section 9.5.3 of Method 1631. When collecting field samples for this study, the laboratory may follow the procedures outlined in *EPA Method 1669: Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* or in other documented sampling

protocols known to be capable of yielding samples free from contamination at the levels of interest in this study. If an alternate documented sampling protocol is used, a copy of that protocol shall be provided with the results.

### 4.2.2 Minimum Level

In developing Method 1631, an interim minimum level (ML) of quantitation was derived by multiplying the estimated MDL by 3.18. This 3.18 value is the ratio between the student t multiplier normally used to determine an MDL (3.143) and the 10 sigma multiplier used to establish the American Chemical Society (ACS) and International Union of Pure and Applied Chemistry (IUPAC) limit of quantitation (LOQ). For the purposes of developing the draft method, the MDL of 0.05 ng/L was estimated from the standard deviation of replicate blank measurements. The MDL that will be determined from Phase 1 of this study will be used to verify this estimated MDL, and hence, to verify the ML of 0.2 ng/L listed in Method 1631. The MDL determined in Phase 1 will be multiplied by 3.18 to arrive at an interim ML. This interim ML may then be rounded to the nearest factor of 10 multiple of 1, 2, or 5 in order to simplify instrument calibration.

### 4.2.3 Detailed Requirements

If the results of Phase 1 verify the MDL of 0.05 ng/L, then all Phase 2 QC analyses will be based on the requirements and concentrations specified in draft Method 1631. Specifically:

(1) The instrument will be calibrated at five points, as specified in Section 7.10 of the method; the lowest calibration point is equal to the ML (0.2 ng/L).

(2) Initial precision and recovery (IPR), ongoing precision and recovery (OPR), and quality control sample (QCS) samples must be prepared by spiking reagent water with mercury at 5.0 ng/L as specified in Sections 7.9, 9.2.2, 9.5, and 9.6 of Method 1631.

(3) All blanks (bubbler, reagent, field, and equipment) must be demonstrated to be free from contamination below the MDL, as described in Section 9.4 of Method 1631.

(4) Matrix spike and matrix spike duplicate samples must be prepared by spiking field samples collected for Phase 2 at a concentration that is between one and five times the background concentration or at the concentration of the low-level working standard, whichever is greater (see Section 9.3.1.2). The field samples used for the performance of matrix spike and matrix spike duplicate analyses should be collected from sources known to contain mercury at ambient concentrations that are at or below the levels of interest in this study.

All IPR, OPR, QCS, matrix spike, and matrix spike duplicate analyses performed during this phase of the study will be performed in accordance with the procedures described in Method 1631. If, during the course of analysis, the laboratory encounters difficulties that may be resolved through modification of the written procedures, the laboratory will be required to contact SCC, explain the problem, obtain approval for the deviation, evaluate the effectiveness of the deviation, and supply SCC with results and information that will enable SCC to modify the written procedures accordingly.

*Note*: The current MDL of 0.05 ng/L is well below EPA's target MDL of 1/10 the lowest water quality criterion (12 ng/L) for mercury. Therefore, if the results of Phase 1 do not verify the MDL or ML cited in the January 1996 draft of Method 1631, EAD may choose to pursue Phase 2 without further method development activity. In this case, the MDL determined in Phase 1 of this study would be used to calculate a new interim ML for mercury. The new MDL should be low enough to demonstrate that any measurements made at the water quality criterion were free from contamination. The new MDL, therefore, should be no higher than 1/10 EPA's water quality criterion (e.g. 1.2 ng/L). A new interim ML would be calculated by multiplying the MDL determined in Phase 1 by 3.18. This new interim ML would then be used to identify appropriate calibration levels and spike concentrations for the OPR, QCS, IPR, and MS/MSD samples.

## SECTION 5: DATA REPORTING AND EVALUATION

The laboratory will be required to submit both summary level and raw data in hardcopy format. The laboratory will also be required to submit detailed explanations of any approved modifications to the analytical techniques specified in the referenced method and used in this study.

Upon receipt of the laboratory data package, SCC will review the data to ensure that the data were generated in accordance with the required procedures. The results of Phase 1 will be used to verify the MDL and ML listed in Method 1631 for mercury. The results of the analyses performed in Phase 2 will be used to verify the IPR, OPR, calibration verification, and matrix spike QC specifications that are listed in Table 2 of Method 1631. Laboratory comments and suggestions will also be used to identify further revisions that should be made to Method 1631.

The laboratory shall report the following data, at a minimum:

(1) Summary reports of all analytical results. The Phase 1 summary report shall include an MDL summary table that groups data under the following categories: spiked concentration in each of the seven replicates, the mean of the seven measured concentrations, the standard deviation of these measurements, the student's t-value used, and the calculated MDL for the technique. The MDL and the individual values that support the MDL should be reported to three significant figures. All other data that support the MDL determination including calibration, blanks, and calibration verifications must also be reported.

The Phase 2 summary report must contain a summary of analytical results for all QC and field samples. For the IPR analysis, the spiking level, individual results of the four replicates, and the mean recovery and relative standard deviation of the four replicates should be reported. For the OPR, QCS and CALVER analyses, the true (or expected) concentration of the QC sample, the measured concentration, and the percent recovery should be reported. For MS/MSD analyses, the background concentration of the field sample, the spiking level, the individual results of the MS and MSD analyses, the percent recovery for the MS and MSD, the average concentration found in the MS/MSD samples, and the RPD between the MS and MSD should be reported. The results for all

other QC, including calibration and blanks, should also be reported.

(2) A list of samples analyzed and a run chronology.

(3) Copies of all raw data, including quantitation reports, strip charts, spectra, bench sheets and laboratory notebooks showing tare and sample weights, sample volumes, and other data that will allow the final results reported to be traced back to the analytical steps performed. Each data element shall be clearly identified in the laboratory's data package.

(4) A written report that details any problems associated with the performance of the MDL study or analysis of the samples. The written report should also provide comments on the performance of the method.

(5) A detailed written description of any approved modifications to the procedures specified in the referenced method that were used during the performance of this study.

## SECTION 6: LIMITATIONS

The most significant limitation of the study is that it focuses only on the water quality criteria levels published by EPA; it does not address lower water quality criteria or criteria for other forms and species of mercury that have been promulgated by states not covered under the National Toxics Rule or the Stay of Federal Water Quality Criteria for Metals. This limitation is consistent with and does not go beyond EAD's current mission to develop methods that will allow measurement of trace metals at the lowest water quality criteria levels published by EPA.

Other limitations are that this study plan does not attempt to validate the applicability of the procedures outlined in Method 1631 to effluent samples or marine samples. This study plan is limited to a validation of mercury in ambient water samples. This approach is consistent with EAD's mission to prioritize metals in ambient waters over determination of metals in effluent and marine samples.

# *Appendix: Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11*

(Federal Register, Vol. 49, No. 209, Friday, October 26, 1984)

## 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:

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

$$S = (S^2)^{\frac{1}{2}}$$

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:

$MDL = t_{(n-1,1-a = 0.99)}$ (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 precentiles 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)*** | $t_{cn-1, .99)}$ |
|---|---|---|
| *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 ald 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 FR 43430, Oct. 26, 1984; 50 FR 694, 696, Jan. 4, 1985, as amended at 51 FR 23703, June 30, 1986]