ANALYTICAL DETECTION LIMIT GUIDANCE

& Laboratory Guide for Determining Method Detection Limits

Wisconsin Department of Natural Resources
Laboratory Certification Program

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introduction and background

The Department of Natural Resources (DNR) formed a Technical Advisory Committee in November, 1990 at the request of the Secretary of the Department to examine the LOD/LOQ language in Ch. NR 106, Wis. Adm. Code, and the corresponding language in WPDES permits to determine if the permit language was consistent with the code, and if not, to formulate recommendations to achieve consistency. The committee's report (WI DNR, 1994), issued on January 2, 1994, recommended implementing new, consistent permit language based upon scientific limitations. The report also addressed related LOD/LOQ issues facing the Department raised through the investigation but not directly within the Committee's charge. These issues were deemed critically related to the LOD/LOQ issue and the Committee urged the Department "to give serious consideration to implementing (these) recommendations". Among them:

1. The Department needs to provide permittees with guidance instructing them how to report, interpret and apply sampling results that are below the LOQ.

2. The Department needs to develop uniform definitions of LOD and LOQ which are applicable to all environmental sampling programs.

The need for consistent definitions and low level data reporting across all of the agency's environmental programs compelled the Laboratory Certification Program, to play a significant role in coordinating the Department's efforts. The Laboratory Certification Code, Ch. NR 149, Wis. Adm. Code, which requires laboratories to statistically determine their detection limits, was revised to address the Committees' recommendations. An amendment requiring laboratories to report analytical data for selected substances down to their calculated detection limit becomes effective January 1, 1997. This requirement was created to supplement other Administrative Codes, many of which already require facilities and site owners to report analytical data down to a calculated detection limit. This rule change also modified the definitions of the limit of detection and limit of quantitation for consistency with Chs. NR 106, NR 140, proposed NR 507, and NR 809. Wis. Adm. Code.

At the present time, the Department requires certified and registered laboratories to calculate detection limits using the U.S. Environmental Protection Agency Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11). This method has both critics and supporters. Despite its limitations, it remains the most widely documented and one of the simplest ways to calculate a detection limit. However, the procedure is often misunderstood, and invalid MDL determinations are common. The Department conducted an interlaboratory survey of semivolatile organic compounds detection limits in April of 1993. Of the 56 labs surveyed, 23 incorrectly calculated their MDLs. The Laboratory Certification Program developed this guidance to assist laboratories follow the procedure correctly and generate meaningful detection limits. These meaningful detection limits are a critical first step toward meeting the agency's data needs of the future, where toxicology is expected to continue to push the boundaries of analytical science.

This document interprets the Laboratory Certification Program's policy on limits of detection, and contains helpful hints and suggestions to assist laboratories calculate method detection limits. It provides alternatives for analytical methods which do not lend themselves well to statistical detection limit determinations. Moreover, this document provides guidance for performing a "common sense check" on a calculated MDL. This document supplements the Code of Federal Regulations procedure for calculating the method detection limit. In all cases, the Federal Regulations protocol must be followed for calculating MDLs.

This guidance was written and edited by Jeffrey Ripp, a chemist in the Wisconsin Department of Natural Resources' Laboratory Certification Program. Additional copies may be requested by writing to the Laboratory Certification Program, Wisconsin Department of Natural Resources, 101 S. Webster St., Box 7921, Madison, WI 53707. Or, visit the Wisconsin DNR online at {http://www.dnr.state.wi.us}.

Table of Contents

Part I. Definitions 1

Part II. Method Detection Limits 3
2.1 What are Method Detection Limits (MDLs)? 3
2.2 Why do we need MDLs? 4
2.3 Discussion of the MDLs' Limitations 5

Part III. Calculating Method Detection Limits 6
3.1 Practical Considerations for Improving the MDL Procedure 6
3.1.1 Analytical Systems 6
3.1.2 Calibrating for the MDL Procedure 6
3.1.3 Choosing the Proper Spike Level 7
3.1.4 Replicate Sample Preparation 7
3.1.5 Analyzing Blanks 8
3.1.6 Accounting for Day to Day Variability 8
3.2 Calculations 8
3.3 Frequency of MDL Determinations 9
3.4 Calculating MDLs for Multiple Instruments and Analysts 9
3.4.1 The 50% Rule 9
3.4.2 The F-Ratio Test 10
3.4.3 The Upper Critical Limit Test 10
3.4.4 Screening Instruments 10
3.4.5 Safe Drinking Water Instruments 11
3.4.6 Examples of Multiple Instruments 11

Part IV. Validating MDL Determinations 11
4.1 Common Sense Check 11
4.2 The Five Point Check 12
4.2.1 Meeting Requirements 12
4.2.2 The S/N Test 12
4.2.3 Percent Recoveries 13
4.3 Serial Dilutions 14
4.4 Iterative Procedure 14

Part V. Examples and Special Cases 14
5.1 Ammonia by Ion Selective Electrode (ISE) 14
5.2 Atrazine by Gas Chromatography 15
5.3 Lead in Drinking Water by Graphite Furnace (GFAA) 16
5.4 Gasoline Range Organics (GRO) by the Wisconsin Method 17
5.5 Common Wastewater Tests and Exempt Analytes 18

5.5.1 Calculating Residual Chlorine Detection Limits 19
5.5.2 Solids (Gravimetric) 19
5.5.3 BOD and CBOD 19
5.5.4 Titrimetric Procedures 19
5.6 MDLs for Organic Analytes and Common Lab Contaminants 19
5.7 MDLs for High Precision Methods 20

Part VI. Data Reporting Requirements 20
6.1 Department Consistency Efforts 20
6.2 NR 106 - Surface Water (Wastewater) 21
6.3 NR 140 - Groundwater 21
6.4 NR 149 - Laboratory Certification 22
6.5 NR 507 - Landfills (Proposed) 22
6.6 NR 720 - Spills 22
6.7 NR 809 - Drinking Water 23

Part VII. References 24

Appendix A: 40 CFR 136, EPA MDL Procedure I

Appendix B: Procedure for Determining Outliers IV

Appendix C: Sample MDL Spreadsheet V

An enormous variety of definitions relating to detection limits and quantitation limits are used in the literature and by government agencies. Unfortunately, universally accepted procedures for calculating these limits do not exist. This can be frustrating and confusing for both regulators and the regulated community. The definitions below are not an attempt to resolve all of the confusion, but rather, an attempt to clarify the meaning of these terms as used by the Department. These definitions are consistent with the definitions found in the Wisconsin Administrative Codes.

Accuracy is a combination of the **bias** and **precision** of an analytical procedure, which reflects the closeness of a measured value to a true value. (Standard Methods, 18th edition) For the purposes of laboratory certification, accuracy means the closeness of a measured value to its generally accepted value or its value based upon an accepted reference standard. (NR 149.03(2))

Bias provides a measure of systematic, or determinative error in an analytical method. Bias is determined by assessing the percent recovery of spiked samples. Historically, the term **accuracy** has been used interchangeably with bias, although many sources make a distinction between the two. (Standard Methods, 18th edition)

Enforcement Standard (ES) means a numerical value expressing the maximum concentration of a substance in groundwater which is adopted under s. 160.07, Stats., and s. NR 140.10 or s. 160.09, Stats., and s. NR 140.12. These standards are toxicologically derived to protect human health. Analytical values above the ES trigger the procedure prescribed in s. NR 140.26. (s. NR 140.05(7))

False Positive, or Type I (alpha) error, means concluding that a substance is present when it truly is not.

False Negative, or Type II (beta) error, means concluding that a substance is not present when it truly is.

Instrument Detection Limit (IDL) is the concentration equivalent to a signal, due to the analyte of interest, which is the smallest signal that can be distinguished from background noise by a particular instrument. The IDL should always be below the **method detection limit**, and is not used for compliance data reporting, but may be used for statistical data analysis and comparing the attributes of different instruments. The IDL is similar to the "critical level" and "criterion of detection" as defined in the literature. (Standard Methods, 18th edition)

Limit of Detection (LOD) or detection limit, is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is typically determined to be in the region where the **signal to noise ratio** is greater than 5. Limits of detection are **matrix**, method, and analyte specific. (ss. NR 140.05(12) & 149.03(15))

Note: For the purposes of laboratory certification, the LOD is approximately equal to the **MDL** for those tests which the **MDL** can be calculated.

Limit of Quantitation (LOQ), or lower limit of quantitation (LOQ), is the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is

mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable **limit of detection**. Limits of quantitation are matrix, method, and analyte specific. (ss. NR 140.05(13) & 149.03(16))

Linear Calibration Range (LCR), or Range of Linearity, is the region of a calibration curve within which a plot of the concentration of an analyte versus the response of that particular analyte remains linear and the correlation coefficient of the line is approximately 1 (0.995 for most analytes). The plot may be normal-normal, log-normal, or log-log where allowed by the analytical method. At the upper and lower bounds of this region (upper and lower **limits of quantitation**), the response of the analyte's signal versus concentration deviates from the line.

Maximum Contaminant Level (MCL) is a numerical value expressing the maximum permissible level of a contaminant in water which is delivered to any user of a public water system. Maximum contaminant levels are listed in s. NR 809.09, Wis. Adm. Code. (NR 809.04(34))

Method Detection Limit (MDL) is 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. Appendix A contains the necessary equations for calculating method detection limits. (40 CFR part 136, Appendix B, rev. 1.11)

Practical Quantitation Limit (PQL) is a quantitation limit that represents a practical and routinely achievable quantitation limit with a high degree of certainty (>99.9% confidence) in the results. The PQL appears in older DNR literature and in some current EPA methods, however its use is being phased out by the Department. (WI DNR LUST Analytical Guidance, 1993 and EPA SW-846, Test Methods for Evaluating Solid Waste, 3rd edition)

Precision is a measure of the random error associated with a series of repeated measurements of the same parameter within a sample. Precision describes the closeness with which multiple analyses of a given sample agree with each other, and is sometimes referred to as reproducibility. Precision is determined by the absolute standard deviation, relative standard deviation, variance, coefficient of variation, relative percent difference, or the absolute range of a series of measurements. (s. NR 140.05(16) and Standard Methods, 18th edition)

Preventive Action Limit (PAL) is a numerical value expressing the maximum concentration of a substance in groundwater which is adopted under s. 160.15, Stats., and s. NR 140.10, 140.12 or 140.20. Reported values above the PAL trigger the procedure prescribed in s. NR 140.22. The PAL is typically set at 1/10th of the **enforcement standard** if the substance is carcinogenic, mutagenic, teratogenic or has a synergistic effect. The PAL is 20% of the enforcement standard for other substances of public health concern. (NR 140.05(17) & 140.10 note)

Reporting Limit is an arbitrary number below which data is not reported. The reporting limit may or may not be statistically determined, or may be an estimate that is based upon the experience and judgement of the analyst. Analytical results below the reporting limit are expressed as "less than" the reporting limit. **Reporting limits are not acceptable substitutes for detection limits unless specifically approved by the Department for a particular test.**

Sample Matrix, or **Matrix**, defines the general physical-chemical makeup of a particular sample. Although the actual matrix of a sample varies from discharge to discharge and from location to location, general classes of matrices include; reagent (clean) water, wastewater, public drinking water, waste, surface water, groundwater, sediments and soils. (NR 149.03(28))

Sample Standard Deviation, or **Standard Deviation (s)**, is a measure of the degree of agreement, or **precision**, among replicate analyses of a sample. (Standard Methods, 18th edition) In this document, standard deviation implies sample standard deviation (n-1 degrees of freedom). The population standard deviation (n degrees of freedom) should only be used when dealing with a true approximation of a population (e.g. greater than 25 data points). The standard deviation is defined as:

$$s = [\Sigma (x-\overline{x})^2/(n-1)]^{\frac{1}{2}}$$

Sensitivity means the ability of a method or instrument to detect an analyte at a specified concentration. (NR 149.03(28m))

Signal to Noise Ratio (S/N) is a dimensionless measure of the relative strength of an analytical signal (S) to the average strength of the background instrumental noise (N) for a particular sample and is closely related to the detection level. The ratio is useful for determining the effect of the noise on the relative error of a measurement. The S/N ratio can be measured a variety of ways, but one convenient way to approximate the S/N ratio is too divide the arithmetic mean (average) of a series of replicates by the **standard deviation** of the replicate results. (Skoog & Leary 1992)

Note: For chromatographic analyses, the S/N ratio can be calculated directly with a ruler by taking the raw chromatogram or strip chart output and measuring the distance from the signal peak to the midline between the maximum and minimum noise at baseline, off-peak. This is the signal (S). The noise (N) is the distance between the maximum and minimum baseline response, off-peak.

Statistical Outlier, or **Outlier**, is an observation or data point that appears to deviate markedly from other members of the population in which it occurs. The presence of outliers must be verified using an approved statistical method, at the 1% significance level for Wisconsin compliance. Appendix B contains one procedure for evaluating outliers. (Kelly, et. al 1992)

part ii. method detection limits

2.1 What are Method Detection Limits (MDLs)?

Method detection limits are statistically determined values that define how easily measurements of a substance by a specific analytical protocol can be distinguished from measurements of a blank (background noise). Method detection limits are matrix, instrument and analyst specific and require a well defined analytical method. Method detection limits provide a useful mechanism for comparing different laboratories' capabilities with identical methods as well as different analytical methods within the same laboratory.

The Department's definition of detection limit requires clarification of when a result is "statistically different" from a blank. The MDL procedure sets the limit of detection at the 99% confidence level, according the U.S.

Environmental Protection Agency's (EPA) MDL procedure[1] promulgated at 40 CFR (Code of Federal Regulations) Part 136, Appendix B, rev. 1.11. The EPA defines the MDL as the "minimum concentration of 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". Understanding this definition is critical to understanding exactly what the MDL represents. Statistically, the 99% confidence interval means that any substance detected at a concentration equal to the MDL is 99% likely to be present at a concentration greater than zero. It also means that there is a 1% chance that a substance detected at the MDL will be considered (falsely) "present" when in reality the true analyte concentration is zero[2]. This situation is known as a false positive, or Type I decision error, and the MDL procedure is designed to protect against making this type of error. The MDL is a statistical, rather than chemical, concept and it is quite possible that a substance can be "detected" at concentrations well below the method detection limit (hence the differentiation from the instrument detection limit). Also, the MDL tells us nothing about the numerical uncertainty of analytical results. It is assumed that because a substance was detected at a concentration equal to or greater than the MDL, that substance is 99% likely to be present and the quantitated value is the "best available estimate" of the true value. Quantitative uncertainty in a reported value exists to a greater or lesser extent in all analytical data, and must be accounted for with additional quality control information.

Method detection limits are typically calculated using reagent water spiked with the analyte of interest, although they can also be determined in specific matrices such as wastewater or soils using the same procedure. Reagent water MDLs can be described as "best case limits", and the detection limits achievable in clean samples may not be analytically achievable in other matrices. Nonetheless, calculating the MDL in reagent water is useful for comparing detection limits among many laboratories. Certain permits may require that MDLs be calculated for unique sample matrices, and the MDLs calculated using these matrices may or may not be comparable to other matrices and laboratories. Laboratories that analyze specific matrices consistently, such as a municipal wastewater treatment facility's lab testing the plant's effluent, are encouraged to calculate matrix-specific MDLs. Because the data user does not necessarily know how an MDL was calculated, it is important to specify the proper units (mg/L or ug/L) and matrix type with all reported MDL data.

2.2 WHY DO WE NEED MDLS?

Method detection limits are a relative measure of the performance of a particular lab, method or analyst. In many instances, the Department pools data from many sources prior to evaluating the data or making a compliance decision. Standardization in data reporting significantly enhances the ability of resource managers to interpret and review data because it is comparable. Reporting a method detection limit along with low-level data alerts data users of the known uncertainties and limitations associated with using the data. Data users in turn must understand these limitations in order to minimize the risk of making poor environmental decisions. Censoring data below unspecified or non-statistical reporting limits severely biases data sets and restricts its usefulness. This and can lead to erroneous decisions by data users when they calculate averages, mass balances or interpret statistics. A number reported as "<4" with no corresponding information is very difficult to interpret, and often must be discarded. Just like we were taught in grade school to turn in our homework because "zeros don't average", in analytical chemistry, "less-thans" don't average.

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[1]The EPA procedure for calculating MDLs is found in Appendix A of this document.

[2]We need to be careful with declaring a true concentration of an analyte is "zero", since Avogadro's number is extremely large, ($N = 6.0223 \times 10^{23}$ particles mol^{-1}) it could be argued that it is impossible to ever say that a substance is not present. For the purposes of this discussion, "zero" means that for all practical purposes, the analyte of interest is not present.

The ability to evaluate low-level data is critical when the MDL exceeds a health based standard for a particular contaminant, such as vinyl chloride in groundwater. Potentially harmful levels of the chemical may exist below our ability to detect them and it is unreasonable to suggest modifying these health based standards to meet analytical capabilities. History has shown that improvements in sensitivity are always possible. While this causes difficulty during the time it takes chemistry to "catch up" to toxicology, monitoring for marginal changes in the concentration of these contaminants is important for resource managers charged with protecting human health and the environment. Early detection of changes in the concentration of low-level contaminants can only be accomplished through careful statistical evaluations of low-level data.

2.3 Discussion of the MDLs' Limitations

Calculating the MDL at the 99% confidence interval allows for the probability that 1% of the samples analyzed which have a true concentration at the MDL level will be false positives (type I error). Additionally, reporting data down to the MDL does nothing to control the possibility for false negatives (type II error). Since replicate analyses of environmental samples tend to follow a Gaussian distribution around a mean, it is logical to assume that for a sample spiked at the MDL concentration, 50% of the values would fall above the MDL (detected) and 50% would fall below (not detected). False negatives are much less of an issue for the regulated community because in general "not detected" does not result in future site remediation or permit limits. The slim possibility of false positives and the high probability of false negatives are inherent drawbacks of using a method detection limit. The Department recognizes and accepts these probabilities as a reasonable balance between environmental risk and the cost of investigating false positives.

The basic assumption behind the MDL determination, that precision is indicative of detectability, does not always hold true. Moreover, the procedure does not take into account the effects of high or low bias on the MDL. It is entirely possible that a series of measurements may yield a high precision (small standard deviation) but be biased either high or low. Bias may affect the calculated detection limit. Unfortunately, it is practically impossible to pinpoint a "true" detection limit for most analytes without introducing some uncertainty about the validity of low level results.

The EPA's MDL procedure has been criticized in the literature and by regulated facilities for a variety of reasons, including what some feel to be faulty statistical assumptions (Gibbons, 1996). The Department is aware of the limitations with using this procedure and despite its limitations, feels that it is the best available method for the Laboratory Certification Program, for two primary reasons; 1) The method is codified in the federal regulations and is widely used, and 2) The procedure is relatively straightforward, and based on the Department's experience, results in reasonable estimates of the detection limit for a wide range of environmental contaminants. The limitations of the procedure are not unique, and similarly complex flaws are found with any alternative.

Nonetheless, these limitations are important to keep in mind when evaluating low level data. Data users must understand these limitations, and proceed with caution when interpreting data reported between the detection limit and the limit of quantitation. Although the MDL procedure produces a specific number above which data are considered detectable and usable, this is really an oversimplification. The LOD/LOQ region is a continuum of uncertainty, and distinct cutoff points do not exist. It is important to note that the MDL is only a mechanism for dealing with analytical uncertainty. Obviously, uncertainty is introduced in all steps of the sampling, transport, storage and analysis of a sample.

3.1 Practical Considerations for Improving the MDL Procedure

Calculating an MDL based upon low-level replicate samples can be time consuming, and most laboratories cannot afford to repeat the study numerous times before achieving a number that they are comfortable reporting. For this reason, it is important to carefully control the variables that may cause an MDL determination to be invalid; such as calibration range, spike level, and blank contamination. The following sections explain how to set up and perfom an MDL study and include several options and alternatives for calculating reasonable method detection limits. Laboratories should always document the exact procedure used to arrive at the final calculated MDL. MDLs are important pieces of data and must be traceable.

3.1.1 Analytical Systems - Method detection limit samples should be run on instruments that are functioning properly and have passed all of the necessary quality control checks specified in the method of choice. The relative cleanliness of the analytical system has an effect on the calculated MDL. An MDL calculated using gas chromatography, run on a new column will be different (maybe significantly) than an MDL calculated using an older, "dirtier" column. Analytical systems do not have to be pristine prior to analyzing MDL samples, however the laboratory should take precautions to avoid contaminant carry-over from earlier samples. For most laboratories, routine instrument maintenance ensures that the instruments are sufficiently clean and responding properly.

Many laboratories use more than one instrument to perfom a similar test; one is used for high-level "screening" type analyses and one is used for low-level compliance. Since MDLs are a measure of a laboratory's low-level capabilities, it is inappropriate to determine method detection limits on the screening instrument. Method detection limits should always be determined on the instruments that will be used to report low-level results, under realistic production conditions. Calculating MDLs for multiple instruments and analysts is discussed further in Section 3.4.

3.1.2 Calibrating for the MDL Procedure - Proper calibration is essential for determining a valid detection limits but is often overlooked. The correct calibration for an analytical method should cover the expected concentration range of the samples to be analyzed. The MDL should always be calculated using the same calibration curve that would be used for typical sample analysis. For most low-level analyses, the Department recommends that the lowest calibration standard be approximately equal to the limit of quantitation (or estimated LOQ) and the remaining standards cover the full range of sample concentrations typically encountered by the laboratory. It may not be possible to achieve a low MDL using an instrument calibrated for analysis of highly contaminated samples. If the laboratory does not regularly perfom low-level analysis at concentrations below the LOQ, many required MDLs may be impossible to achieve. Usually, the manufacturer of an analytical device will specify the instrument's limit of quantitation, which is a reasonable approximation of the lab's LOQ until sufficient data has been generated to make a statistical determination.

A new calibration curve should be generated prior to analyzing MDL samples. If this is not possible, the working calibration curve must be verified at least at the beginning of the analytical shift using the appropriate calibration check standard. Using only three standards presumes that the calibration is within the linear range of the curve for the analyte of concern. The actual number of calibration points should be based upon the width of the working range and the shape of the calibration curve, and should insure the accuracy of the determination. Non-linear, or quadratic curves require a minimum of five calibration standards to fully characterize the curve. For most inorganic analyses, the blank should be included as a point on the calibration curve. It is not acceptable to force any calibration curve through zero.

A good laboratory practice would be to first establish the linear range for the instrument with a minimum of three different concentration standards for methods which only require a one point calibration. The initial

calibration may then be verified, during the beginning and end of each analytical shift (8 - 12 hours), with a single calibration standard at a concentration in the middle of the linear range, or lower if typical of routinely encountered sample concentrations.

These are very general guidelines to establishing a defensible calibration. Always consult the method in question for additional requirements.

3.1.3 Choosing the Proper Spike Level - The MDL is based upon the variability, or precision, between seven or more replicates run at identical concentrations. Because precision is measured using the standard deviation of the sample results, and is dependent upon concentration, the initial spike level selected for the MDL samples is important. Some analytical methods will yield larger absolute standard deviations for seven replicates at concentrations above the LOQ than at concentrations near the LOD, leading to artificially high MDLs. A high approximation for the MDL censors data because any compounds below the detection limit would not be reported. Conversely, precision may be improved for some methods at concentrations above the LOQ, which could lead to unachievably low MDLs. A low approximation permits unrealistic expectations about detectability. Since the MDL is an estimate for the lower level of the calibration curve, the best spiking level is 1 - 5 times the estimated detection level, as specified in the EPA procedure. When choosing the appropriate spiking level for the MDL procedure, consider the signal response that the spiked level of the analyte will give on the system that is being used. Is the signal off scale? Is the signal distinguishable from background noise? Consideration of the signal to noise ratio (S/N), discussed in Section 4.2.2, may help to choose an appropriate spike level.

Due to the dependence of precision on concentration, the calculated MDL must be greater than one-tenth of the spike level. This is the maximum concentration for an MDL study, and concentrations below this maximum are preferable. At the other extreme, the calculated MDL must not be higher than the spike level. Logically, if the calculated MDL exceeds the spike level it is not statistically possible to differentiate the spiked samples from a blank (and the precision of the determination was very poor!). The following inequalities are useful for evaluating a calculated MDL:

Calculated MDL < Spike Level < 10 x Calculated MDL

If these conditions are met the spike level is appropriate. If these conditions are not met, it is necessary to recalculate the MDL. The Department will not accept MDL data if both of the above conditions are not met.

3.1.4 Replicate Sample Preparation - The procedure requires a minimum of seven replicates of a sample spiked at the appropriate concentration for the analyte of interest. The Department recommends using at least eight replicates, to ensure that the minimum number of replicates will be included in the event that one must be discarded as an outlier[1]. Because the limitations of the method define the MDL, the replicate samples must be prepared and processed exactly as prescribed in the analytical method. This includes extractions, surrogate additions, digestions, etc. An MDL calculated using unprocessed samples is unacceptable, and will not be representative of the true MDL.

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[1]Appendix B explains one procedure for determining whether a replicate result is an outlier. The Department does not discourage discarding statistical outliers, however laboratories should be cautious and consistent when doing so because this will have an effect on the final MDL calculation. Outliers resulting from obvious analyst error or improper sample preparation should always be discarded.

Laboratories are required at a minimum to calculate reagent water MDLs for most environmental analyses[1]. A strict reading of the MDL procedure shows that reagent water MDLs should be calculated by preparing a single stock solution large enough to divide into at least seven replicates. This is impractical for many procedures, especially methods for analyzing volatile organic constituents and methods requiring large extraction volumes. In these instances, preparing individual aliquots is preferable. The Department recommends preparing and processing each sample individually for all MDL determinations to mimic unknown sample variability. These individual samples must be analyzed exactly as ordinary samples, following all of the prescribed method steps, and the results quantitated and reported in the proper units.

Laboratories are encouraged, but not required, to determine and use matrix-specific MDLs whenever possible[2]. MDLs for solid matrices are trickier to calculate, and there is some controversy over their applicability to real-world samples (Kimbrough and Wakakuwa, 1993). Laboratories are cautioned against recalculating solid matrix MDLs based upon results from a water matrix. For example, "Back calculating" from reagent water MDLs to obtain an MDL in soil does not accurately take into account matrix effects and extraction techniques for some methods. Solid matrix MDLs should be determined by spiking the analytes of interest into clean sand or soil. The replicates must be carried through the same extraction procedure as actual samples. For metals, it may be preferable to spike a control soil matrix, a soil reference sample, or a metal free sludge. Contaminated soil may be used provided the concentration of the analyte of interest meets the spiking level guidelines in Section 3.1.3. The Department recommends validating each matrix-specific MDL by preparing and analyzing a single matrix spike at the MDL concentration to see if the analytical system can distinguish the sample from a blank.

3.1.5 Analyzing Blanks - At least one method blank should be analyzed with each set of MDL samples to measure background contamination, and the blank results reported when applying for laboratory certification. Blanks are an important quality control tool that can validate or invalidate a calculated MDL. For methods that allow blank subtraction from the sample results, a paired method blank should be analyzed for each sample and the average blank subtracted from the sample results, as specified by the procedure. Ignoring the contribution of blank variability on sample results can result in an artificially low MDL, and an increased false positive risk. For methods requiring blank subtraction, analyzing only one blank, and subtracting this result from all of the samples does not account for the true contribution of blank variability. It is not acceptable to subtract blanks for methods that do not allow blank subtraction for ordinary samples.

3.1.6 Accounting for Day to Day Variability - One of the biggest concerns with using the EPA's MDL procedure is that it doesn't take into account the real world variability that affects laboratory results. One way to introduce some real world variability into the MDL calculation is to prepare and analyze the seven or more replicate standards in different sample batches, or even on different analysis days and pool the data. Another way to account for long-range variability is to pool data from several MDL determinations over time (e.g. annual calculations) using a standard statistical protocol to determine the lab's routine MDL.

3.2 Calculations

Three important things to remember about calculating MDLs are: 1) use the sample standard deviation, 2) use the correct Student's t-value and 3) use all significant figures. The sample standard deviation, s, must be used when calculating MDLs. One of the most common mistakes is using the population standard deviation, σ.

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[1]Laboratories that analyze a limited number of sample types, such as a WWTP lab may substitute matrix-specific MDLs for reagent water MDLs (e.g. treatment plant effluent).

[2]Laboratories certified for petroleum hydrocarbons analyses (GRO, DRO and PVOCs) are required to calculate MDLs in both clean sand and reagent water. Matrix specific MDLs for other tests are required only when specified in the analytical method, Administrative Codes or in a discharge permit.

The population standard deviation can only be used when a true population of data exits. For the purposes of calculating MDLs, it is unlikely that any laboratory will analyze enough replicates to approximate a population, so always use the sample standard deviation. Most calculators and computer spreadsheets are capable of perfonming both types of computations, so it is important to be sure you use the correct one. Carry all significant figures through the calculations, and round the final MDL to the number of digits used when reporting results for that method. It is acceptable to round the calculated value up to the nearest decimal place. For example, if the calculated MDL is 0.15, it is acceptable to round the MDL to 0.2 if results are only reported to one significant figure. MDLs should never be rounded down, unless the laboratory feels it can routinely achieve the rounded value.

3.3 Frequency of MDL Determinations

Method detection limits will change over time for a variety of reasons, and it is necessary to periodically update the calculated MDL value. Many analytical methods require that the MDL be determined prior to using a new analytical system, and some even require annual updates. The frequency of the determination specified in the analytical method should be followed. If the method does not specify a frequency, the Department recommends that MDLs be recalculated whenever a new analyst begins generating data or the perfonmance of the analytical system changes (in addition to the initial MDL). Method detection limits should also be recalculated whenever the analytical procedure is modified (e.g. new extraction solvent). Laboratories may wish to give their MDLs an "expiration date" of one year, beyond which the limit is no longer valid, to help maintain current and usable results.

3.4 Calculating MDLs for Multiple Instruments and Analysts

Analytical instruments with significantly different sensitivities should be differentiated by their capabilities. Although it is more efficient to report only one MDL for each analyte of interest, this practice may not be acceptable in all instances. The Department acknowledges that the procedure is method specific, and different instruments and analysts almost always will produce different results, even with the exact same samples. Since the MDL procedure does not take this variability into account, the MDL should be run independently by each analyst and on each system which will be used to test for a particular analyte. This is necessary to insure that the method detection limit is reported consistently throughout the laboratory, and that low-level samples are not run on instruments which are not capable of detecting low levels of contaminants

The Department has included the criteria outlined in Sections 3.4.1 to 3.4.3 for determining when several calculated MDLs for the same analyte could be considered equivalent. If individual MDLs are equivalent, the laboratory will be able to report the highest of the calculated MDLs as the detection limit, provided that this value still meets the necessary regulatory criteria (such as the PAL, MCL, effluent limit, etc...). The ability to report only the highest MDL for several similar instruments will simplify data reporting requirements. Laboratories always have the option of reporting instrument specific MDLs, or following an alternate approved statistical procedure, and do not need to follow the criteria in Sections 3.4.1 to 3.4.3 to determine equivalency.

Laboratories must document whenever the calculated MDL for a particular test is modified, pooled or compared to other values to substantiate the results. The Department and other potential laboratory clients need to understand that different instruments and analysts have different capabilities, and that a laboratory may not always report the same detection limit for the same analyte.

3.4.1 The 50% Rule - The MDL procedure should be run independently on each system which will be used for the analysis of a particular analyte, and the laboratory should report the highest of the calculated MDLs with its data as long as the higher MDL meets all of the necessary regulatory requirements. If the MDLs

determined on multiple instruments or by multiple analysts differ by more than 50%, the laboratory should report the actual MDL of the instrument used. This is the simplest way to verify equivalent MDLs.

3.4.2 The F-Ratio Test - Title 40 CFR Part 136, Appendix B (the MDL procedure) includes an optional iterative procedure for verifying the reasonableness of an MDL determination that could be modified slightly to test the reasonableness of considering two MDL determinations (run on separate instruments) equivalent. Using this procedure, the F-ratio between two individual MDL determinations is calculated using the variances (S^2) from each set of replicates. The computed F-ratio is compared with the F-ratio found in a standard statistical table at a 1% significance level.

If S^2_A/S^2_B is less than the F-ratio in the table, then the laboratory may report the higher of the two MDLs as the detection limit as long as the higher MDL meets all of the necessary regulatory requirements. Alternatively, the laboratory may calculate a new MDL using the pooled standard deviation. The pooled standard deviation and new MDL can be computed using the equations in the optional iterative procedure. If S^2_A/S^2_B is greater than the F-ratio in the table, the iterative procedure requires respiking, and calculating a new MDL, but for the purpose of comparing MDLs between multiple instruments, the instrument MDLs can be reported individually instead of respiking.

The F-ratio test should be performed between each calculated MDL for multiple instruments, and must pass for each instrument if the laboratory wishes to report a single detection limit. For example, if three different instruments are used for volatiles analysis, and the variances are $S^2_1 > S^2_2 > S^2_3$, it is necessary to perform the F-ratio test three times: S^2_1/ S^2_2, S^2_2/S^2_3, and S^2_1/S^2_3. Only those MDLs for which the F-ratio test passes can be considered equivalent.

3.4.3 The Upper Critical Limit Test - The EPA's MDL procedure derives the 95% confidence interval estimates for the method detection limit using the percentiles of the chi square over the degrees of freedom distribution. For seven replicates, the 95% confidence intervals listed in 40 CFR 136 are:

Lower Critical Limit (LCL) = 0.64 x MDL
Upper Critical Limit (UCL) = 2.20 x MDL

These limits vary depending on the number of replicates. For the purposes of determining whether multiple MDL determinations are equivalent, the laboratory calculates the UCL and LCL for the lowest MDL value. If the other MDLs fall below the UCL of the lowest MDL, it is acceptable to report the highest MDL value as the detection limit, as long as the highest MDL meets all of the necessary regulatory requirements. For example, if two instruments used for the determination of lead in drinking water give MDLs of 0.5 and 1.0 ug/L, the laboratory calculates the confidence interval around the lower MDL using the formulas in the procedure. Assuming seven replicates:

LCL = (0.64)(0.5 ug/L) = 0.32 ug/L
UCL = (2.20)(0.5 ug/L) = 1.1 ug/L

Since 1.0 ug/L is less than 1.1 ug/L, and 1.0 ug/L is below the SDWA regulatory requirement of 1.5 ug/L for lead, the laboratory may report 1.0 ug/L as the detection limit. If the second MDL were 1.2 ug/L instead of 1.0 ug/L, the laboratory could not report 1.2 ug/L as the detection limit for both instruments because it falls outside of the UCL, even though 1.2 ug/L is below the regulatory requirements.

3.4.4 Screening Instruments - Many laboratories use instruments for sample screening prior to quantitative analysis. These instruments are typically older or "dirtier", and have much higher MDLs than quantitative instruments. A "screening instrument" is defined here as an analytical system used to determine future sample analysis, including on which instrument and by what method the sample must be quantitatively analyzed.

Screening instruments are also used to determine necessary dilution factors. Sample results are never reported from a screening analysis, either quantitatively or as "less than detection". If samples are excluded from future analyses based upon the results of a screen, the instrument is no longer considered a screening instrument and the MDL must be reported. Method detection limits for true screening instruments need not be reported, and should not be included in an analysis for MDL equivalency.

3.4.5 Safe Drinking Water Instruments - If multiple instruments are used for drinking water analysis, the laboratory must meet the SDWA MDL requirement on each instrument. Method detection limits for multiple instruments may be pooled, provided the pooled value meets the regulatory requirement. If the required MDL is not achieved for a particular instrument, the instrument cannot be used for SDWA analysis.

3.4.6 Examples of Multiple Instruments - The following examples illustrate when instrument specific MDLs would be required.

Example 1: Multiple Instruments for Semivolatile Organics Analysis

Instrument # & Type	Use/Method	MDL Requirement
1. GC-FID	BNA Screen	Report No MDLs (Do Not Report Sample Data!)
2. GC-ECD	Phenols/8040/604	Report MDLs for 8040/604
3. GC-FID	PAHs/8100/610	Report MDLs for 8100/610
4. GC/MS	BNAs/8250/625	Report Same MDL for #4 & #5
5. GC/MS*	BNAs/8270/625	Report Same MDL for #4 & #5

*Indicates low-level GC/MS capabilities. The highest MDL between instruments four and five should be reported for each analyte, unless the individual MDLs do not meet the criteria for equivalent MDLs.

Example 2: Multiple Instruments for Volatile Organics, including Drinking Water Analysis

Instrument # & Type	Use/Method	MDL Requirement
1. GC-FID (headspace)	Volatile Screen	Report No MDLs (Do Not Report Sample Data!)
2. GC-ELCD/PID	VOC/502.2	Report SDWA MDL for 502.2
	8020/8021/601/602	Report MDL for 8020/8021/601/602/8010
3. GC/MS	VOC/8240/624	Report Same MDL for #3 & #4
4. GC/MS**	VOC/524.2	Report SDWA MDL for 524.2
	8260/624	Report Same MDL for #3 & #4

**Indicates low-level GC/MS capabilities. The highest MDL between instruments three and four should be reported for each analyte, unless the MDLs do not meet the necessary criteria for equivalency. The "SDWA MDL" may be the same as the "MDL" for instruments #2, 3 & 4 if the MDL meets SDWA requirements.

part iv. validating mdl determinations

4.1 Common Sense Check

Prior to reporting a calculated MDL, the analyst should ask: Is this MDL reasonable and if not, what can be done to improve the determination? Analyst experience is an important factor when deciding whether or not a calculated MDL is valid and analytically achievable. It is often useful to run the MDL study at several concentration levels over a long period of time, and compare the results. This allows the analyst to become familiar with how the system operates, and what sensitivity can be expected at varying concentrations.

4.2 THE FIVE POINT CHECK

In addition to analyst experience, the calculated MDL should be evaluated using several checks to determine if it will meet all of the necessary criteria. The following five items, which will be referred to as the "Five Point Check" are simple ways to check a calculated MDL.

1. Does the spike level exceed 10 times the MDL? If so, the spike level is high.
2. Is the MDL higher than the spike level? If so, the spike level is too low.
3. Does the calculated MDL meet regulatory requirement for the necessary program(s)?
4. Is the signal/noise (S/N) in the appropriate range?
5. Are the replicate recoveries reasonable?

The maximum and minimum spike level requirements are explained in Section 3.1.3 and the maximum regulatory MDL requirements are discussed in 4.2.1. Items 1, 2, and 3 above are requirements for all MDLs. If a particular analyte does not have a maximum required MDL the third item can be disregarded. For environmental programs setting maximum MDLs, it is important to check the appropriate Administrative Code or analytical methods for the current requirements. Items 4 and 5 are not required, but are useful for evaluating the data used to generate the MDL. The S/N check and the percent recovery check are described in Sections 4.2.2 and 4.2.3, respectively.

Other ways to evaluate whether or not a calculated MDL is a good estimate of the detection limit exist. The MDL procedure in 40 CFR 136 gives an iterative procedure, utilizing pooled standard deviations, for evaluating the MDL. This procedure is found in section 7 of Appendix A. Another validation method is the analysis of serial dilutions, which is a good, physical check on the reality of an MDL. This procedure is described in Section 4.3.

4.2.1 Meeting Requirements - Maximum allowable method detection limits are specified in many analytical methods and by several Department programs, including Safe Drinking Water Act (SDWA) analyses and the Leaking Underground Storage Tank (LUST) program. Laboratories applying for certification under ch. NR 149 or submitting data to the Department under these programs are expected to obtain MDLs equal to or lower than the maximum requirement. These MDLs are generally determined by the EPA and are method specific, however the Department may specify a required MDL which differs from the federal regulations. Be sure to check each program's MDL requirements before submitting data. Data from laboratories that cannot meet the minimum detection limit will not be acceptable for Departmental decision making.

Typically, maximum MDLs for determining compliance with the safe drinking water code (NR 809) are set at one-tenth of the Maximum Contaminant Level (MCL). Compliance with the groundwater code (NR 140) is based upon the Preventive Action Limit (PAL). Some compounds regulated under NR 809 and NR 140 have MCLs or PALs which are orders of magnitude higher than what is analytically achievable, and these are not a challenge. On the other hand, some compounds have standards below what is analytically detectable and laboratories must have detection limits as low as possible. In this instance a detect may be significant, based on toxicological infomation, and may have a human health impact.

4.2.2 The S/N Test - The MDL procedure in 40 CFR Part 136 recommends that the detection limit be estimated somewhere in the range where the signal to noise ratio is 2.5 to 5. The S/N is not only useful for estimating the initial detection limit, but it is also useful for evaluating the final MDL determination. The signal to noise ratio describes the effect of random error on a particular measurement, and estimates the expected precision of a series of measurements. Samples spiked in the appropriate range for an MDL determination typically have a S/N in the range of 2.5 to 10. A signal to noise ratio less than 2.5 indicates that the random error in a series of measurements is too high, and the determined MDL is probably high. In this instance, the samples should be spiked at a higher level to increase the signal. If the signal to noise ratio

is greater than 10, the spike concentration is *usually* too high, and the calculated MDL is not necessarily representative of the LOD. In this case, the samples should be spiked at lower level. In some instances, especially with highly precise analytical techniques, the S/N may always be higher than ten. Again, the analyst's experience is crucial for determining when the S/N ratio is too high. The S/N ratio for a series of measurements can be **estimated** by:

$$(S/N)_{est} = X_{ave}/s$$

Where X_{ave} is the average of either the calculated concentrations or analytical signals for the replicates and s is the sample standard deviation for the replicates.

The S/N ratio is a useful test for MDL validity, but a high signal to noise ratio does not necessarily indicate that the MDL is invalid. For example, a laboratory calculates an MDL for lead in drinking water at 1.0 ug/L. The laboratory spiked the seven sample aliquots at 5.0 ug/L, and the required MDL is 1.5 ug/L. The laboratory meets the first three MDL requirements, but the S/N ratio was 25. Should this MDL be discarded? Not necessarily, because the laboratory may be confident that this is a reasonable detection limit for their system. The laboratory should note the high S/N, and the next time that the MDL is determined, the laboratory may wish to spike at a lower value. If the laboratory knows that 1.0 ug/L is not a reasonable detection limit, they may wish to redetermine the MDL at a lower spike concentration immediately.

There is no promulgated requirement to meet a certain S/N, and the Department will not reject an MDL determination based solely on the S/N ratio, but an extremely low or high S/N ratio usually indicates additional problems with the MDL. The analyst is expected to make the decision whether or not to use a calculated MDL based upon the S/N.

4.2.3 Percent Recoveries - One of the drawbacks of the MDL procedure is that it doesn't take into account the effects of high or low bias in a series of measurements. The effects of a bias are usually most notable in samples other than reagent water, due to matrix interferences. Bias can be measured by the average percent recovery of a series of samples. In order for an MDL to be realistic, the average percent recovery for the samples should be reasonable. Undoubtedly, this will raise the question "What is reasonable?" A reasonable recovery is subjective, and will be defined differently for different situations. Since MDL calculations involve low level analysis, the recoveries may not be comparable to samples spiked somewhere well within the "quantitation" region of the calibration curve. An analyst familiar with the analytical system should be able to judge whether or not the average percent recovery falls within the expected range for low level samples. The average percent recovery can be calculated using the following equation:

$$Ave.\%R = (X_{ave}/spikelevel) \times 100\%$$

Where X_{ave} is the average concentration of the samples and spike level is the initial spike concentration. Some analytical methods specify appropriate control limits for low-level precision and accuracy samples. If a method does not specify appropriate low level control limits, it is often useful to evaluate the average percent recovery using previously established control limits. The appropriate control limits for reagent water spikes could be the limits for fortified blank recovery. If the determination was performed in a matrix other than reagent water, use the control limits specific for the matrix spikes. The results for low-level analysis may not always fall within the acceptable range, but if a calculated MDL is questionable, evaluating the recoveries in this manner may reveal a bias which could affect the calculation.

The LOD procedure used by the U.S. Army Toxic and Hazardous Materials Agency is based upon a method developed by Andre Hubaux and Gilbert Vos (Hubaux and Vos, 1970) which takes into account the effects of

bias and concentration on the calculated LOD. Their paper gives useful insight for calculating detection limits, and demonstrates the importance of biases at different concentration levels.

4.3 Serial Dilutions

Another good way to check the determined MDL is to analyze several serial dilutions of a known stock standard. This procedure can be time consuming, and is not recommended for all analytes. To validate the MDL using serial dilutions, prepare a standard at a level that is significantly higher than the MDL (at least one order of magnitude), and analyze successive dilutions of the standard down to and below the MDL. If the analytical system cannot distinguish concentrations at or above the MDL from an uncontaminated reagent blank, calculate a new MDL using seven samples spiked near the lowest concentration that was detected. If the serial dilutions indicate that the MDL is too high, continue diluting the stock until the analyte of interest is no longer detected. The criteria that should be used to determine whether or not a sample is indistinguishable from a blank vary depending on the type of analysis performed. It is not important to accurately quantitate the dilutions which fall below the previously calculated detection limit, because the serial dilutions are only meant to verify whether or not low level samples can be detected. If dilutions below the presumed MDL are detectable, recalibrate the instrument (if necessary) and calculate a new MDL using seven samples spiked at or just above the lowest concentration that was detected.

A simplified version of this technique involves analyzing a single sample spiked at the MDL concentration. If the analytical response is indistinguishable from a reagent blank, the calculated MDL is unreasonably low. In this case, reestimate the MDL and analyze seven new replicate samples at a different spike concentration. Verify the results as before. If the analyte is detected at the presumed MDL, the MDL is defensible and should be reported.

4.4 Iterative Procedure

One other verification procedure mentioned is found in the MDL procedure itself, and may be useful in some instances. Section 7 of 40 CFR 136 describes an optional iterative procedure for verifying a calculated MDL. The procedure is detailed and requires the use of many samples, but laboratories are encouraged to use this procedure to verify questionable MDLs.

part v. examples and special cases

This section contains four examples of MDL calculations. Three examples result in acceptable, defensible method detection limits while one, Lead by Graphite Furnace, demonstrates some of the problems associated with an incorrect MDL determination.

5.1 Ammonia by Ion Selective Electrode (ISE)

This example calculates the MDL for ammonia-nitrogen using the approved ion selective electrode method. The electrode's manufacturer claims that the probe can detect ammonia as low as 0.05 mg/L. The instrument was calibrated using three standards at 1, 5, and 10 mg/L and a blank. The first step, estimating the MDL, requires familiarity with the analytical procedure. In this case, the manufacturer's claim is a convenient place to start. Multiplying the manufacturer's number, 0.05 mg/L by a factor of 5 gives 0.25 mg/L for an initial spike level (the spike level could be anywhere between 0.05 to 0.25 mg/L). The ISE method requires 100 ml of sample per analysis. A stock standard was prepared by spiking reagent water at the appropriate concentration, and seven 100 ml aliquots were prepared and analyzed as prescribed in the method. The results for the study are shown in the Table I (in mg/L).

To calculate the MDL, simply multiply the sample standard deviation by the correct Student's t-value from Appendix A. For seven replicates and six degrees of freedom, t is found to be 3.143. The MDL is calculated as follows:

MDL= (s)(t-value)= 0.013 x 3.143= 0.040859

Rounding to the correct number of significant figures, the calculated MDL becomes 0.041 mg/L, slightly lower than the manufacturer's claim. The limit of quantitation can also be calculated:

LOQ= 10 x (s)= 10 x 0.013= 0.13 mg/L

The MDL is now verified using the five point check:

TABLE I - AMMONIA

Sample	Results	% Recovery
sample 1	0.20	80%
sample 2	0.21	84%
sample 3	0.22	88%
sample 4	0.22	88%
sample 5	0.24	96%
sample 6	0.21	84%
sample 7	0.23	92%
Mean =	**0.22**	**87.4%**
Std Dev. =	**0.013**	

✓1. Spike Level (MDLx10>Spike): (0.041)(10) = 0.41 > 0.25 ug/L [OK, Meets Criteria]
✓2. Spike Level (MDL<Spike): 0.041 < 0.25 ug/L [OK, Meets Criteria]
✓3. MDL < Req'd? --- [No Requirement]
✓4. S/N Estimate (ave/sd): 0.22/0.013= 16.9 [Check for Error]
✓5. Ave. % Recovery: 87.4% [Acceptable]

Ideally, the signal to noise ratio should be between 2.5 and 10. In this instance the S/N ratio is higher than ten. However, A lower signal to noise ratio may not be achievable for this particular ion selective electrode. ISE is one example where the S/N check may not be the best way to verify the MDL. Because the response increases logarithmically with concentration, the average concentration divided by the standard deviation is not representative of a true signal to noise ratio. The S/N may still give useful hints about the data. In this case, spiking at a lower level probably wouldn't make much difference in the S/N ratio although the lab may wish to consider a lower spike level the next time the MDL is calculated. The calculated MDL meets all of the other criteria, and the analyst reports 0.041 mg/L as the MDL.

5.2 ATRAZINE BY GAS CHROMATOGRAPHY

The following is a summary of the analytical work done to statistically establish the limit of detection and the limit of quantitation of atrazine in water. The analytical method used was EPA method 507. The GC was externally calibrated using five standards at 0.5, 1, 2, 5, and 10 ug/L Ten one liter (1 L) aliquots of tap water were fortified with 0.21 ug/L of atrazine. The extracts were concentrated to 1 ml, according to the procedure

TABLE II - ATRAZINE

Sample #	Results	% Recovery
sample 1	0.23	110%
sample 2	0.21	100%
sample 3	0.24	114%
sample 4	0.19	90%
sample 5	0.18	86%
sample 6	0.23	110%
sample 7	0.22	105%
sample 8	0.17	81%
sample 9	0.16	76%
Mean =	**0.20**	**96.9%**
Std Dev. =	**0.029**	

given in the method, and were then analyzed by gas chromatography using the appropriate instrument parameters. The results (in ug/L) are found in Table II.

The number of observations is equal to the number of replicates, nine, with eight degrees of freedom. The Student's t-value for 9 replicates and 8 degrees of freedom is 2.896, much different than the value for 7 replicates. Multiplying this value by the standard deviation and rounding gives:

MDL= (s)(t-value)= 0.029 x 2.896= 0.084

The LOQ is calculated using:

LOQ= 10 x (s)= 10 x 0.029 = 0.29 ug/L

The calculated MDL can be verified using the five point check:

✓1. Spike Level (MDLx10>spike):	(0.084)(10)= 0.84 > 0.21 ug/L	[OK, Meets Criteria]
✓2. Spike Level (MDL<Spike):	0.084<0.21 ug/L	[OK, Meets Criteria]
✓3. MDL < Req'd?	PAL= 0.3 ug/L	[OK, Meets Criteria]
✓4. S/N Estimate (ave./sd):	0.20/0.029= 6.9	[OK, Meets Criteria]
✓5. Ave. % Recovery:	96.9%	[Acceptable]

Based upon the above checks, this detection limit is defensible. The S/N ratio is approximately 6.9, which is adequate for our GC MDL determination. The spike level is appropriate for the calculated MDL and the average percent recovery falls within our control limits. The MDL is lower than the PAL, and will allow the lab to report data down to appropriate levels for determining compliance.

5.3 Lead in Drinking Water by Graphite Furnace (GFAA)

The following information was used to calculate the MDL for lead in drinking water, using EPA method 200.9. Chapter NR 809 states that laboratories must achieve a minimum detection limit of 1.5 ug/L. Using this value as a starting point, the laboratory estimates its detection limit to be 1 ug/L. A stock solution containing dilute nitric acid and 5 ug/L (5 times the estimate) lead was prepared. The calibration curve was prepared using four standards at 5, 10, 20, 50 ug/L and a blank. Eight aliquots of the sample were analyzed according to the procedure specified in the method. The results (in ug/L) are summarized in Table III.

Note that sample number 5 was flagged because the percent recovery fell outside of the appropriate control limits. Before calculating an MDL with this data, the analyst should attempt to determine the cause of the aberrant result. If it was the result of a physical problem or

Table III - Lead

Sample #	Results	% Recovery
blank	-0.8	NA
sample 1	4.9	98%
sample 2	4.7	94%
sample 3	4.6	92%
sample 4	4.5	90%
sample 5	6.8	136% *error*
sample 6	4.7	94%
sample 7	4.8	96%
sample 8	4.8	96%
Mean =	**5.0**	**99.5%**
Std Dev. =	**0.75**	

analytical error, such as sample contamination or a bad calibration, the point should be rejected outright. Since the cause of this high result is unknown, the analyst decides to perform a standard statistical test to determine if the point is an outlier. Using the formula presented in Appendix B, the following calculation is performed:

$T_n=(X_n-X_{ave})/s= (6.8-5.0)/0.75= 2.4$

The value of 2.4 is compared against the critical values in the table Appendix B. With the number of observations (n) equal to 8, and at the 1% significance level, the critical value is 2.22. Since 2.4 is greater than 2.22, the result for sample 5 is indeed an outlier. This value is discarded and documented, and a new mean and standard deviation are calculated.

Average: 4.7 ug/L
Average % Recovery: 94.3%
Standard Deviation: 0.13 ug/L

Note that by rejecting the data point, the number of observations is reduced to seven, and the degrees of freedom becomes six. The MDL is calculated and rounded:

MDL= (s)(t-value)= 0.13 x 3.143 = 0.41

The LOQ is also calculated:

LOQ = 10(s) = 10 x 0.13 = 1.3 ug/L

A quick check using the five point check will verify the results.

✓1. Spike Level (10xMDL>Spike):	(10)(0.41)= 4.1 ≯5.0	[Criteria Not Met]
✓2. Spike Level (MDL<Spike):	0.41<5.0	[OK, Meets Criteria]
✓3. MDL < Req'd?	Req'd MDL = 1.5 ug/L	[OK, Meets Criteria]
✓4. S/N (ave./sd):	(4.7/0.13)= 36.2	[Too High]
✓5. Ave. % Recovery:	94.3%	[Acceptable]

The S/N ratio is found to be 36.2, which indicates that the samples were probably spiked too high. Checking the spike level against the calculated MDL confirms that the samples should have been spiked lower. Unfortunately, even though the MDL does meet the requirement for lead in drinking water it must be recalculated. If the study were repeated at a lower spike level, the result would more accurately describe the attainable detection limit. If the outlier had not been discarded, the calculated MDL would be 2.2 ug/L which exceeds the requirement for safe drinking water. This example demonstrates not only how outliers can affect MDL calculations, but also the importance of carefully choosing a spike level that will give the desired precision for calculating the detection limit.

5.4 Gasoline Range Organics (GRO) by the Wisconsin Method

The following information was used to calculate an MDL in reagent water according to the September 1995 Wisconsin GRO procedure. The GC was externally calibrated using five standards at 100, 250, 500, 750 and 1000 ug/L and a blank. Seven replicates at 20 ug/L (two times the estimated detection limit, although anywhere from 10 - 100 ug/L could have been used) were prepared following the sample preparation procedure given in the method. After purging 5 mls of each sample and analyzing, the lab obtained the results in Table IV (in ug/L).

With 7 replicates, the Student's t-value is 3.143. Multiplying the standard deviation by the t-value and rounding gives:

MDL= (t-value)(s)= 3.143 x 2.88= 9.1

The LOQ is determined to be:

LOQ= 10 x (s)= 10 x 2.88= 28.8 ug/L

The Wisconsin GRO (and DRO) method does not have an MDL requirement, but instead requires laboratories to demonstrate that their LOQ is less than or equal to the 100 ug/L reporting limit. Laboratories must also demonstrate MDLs capable of showing that blanks contain less than 50 ug/L of GRO contamination.

TABLE IV - WISCONSIN GRO

Sample #	Results	% Recovery
blank	< 50	---
sample 1	25.4	127
sample 2	22.1	110.5
sample 3	23.6	118.0
sample 4	16.9	84.5
sample 5	22.3	111.5
sample 6	19.3	96.5
sample 7	23.5	117.5
Mean =	**21.9**	**109.4**
Std Dev. =	**2.88**	

The MDL is now verified using the five point check:

✓1. Spike Level (10xMDL>Spike):	10 x 9.1= 91 > 20 ug/L	[OK, Meets Criteria]
✓2. Spike Level (MDL<Spike):	9.1 < 20 ug/L	[OK, Meets Criteria]
✓3. MDL < Req'd?	Method requires LOQ<100 ug/L	[OK, Meets Criteria]
✓4. S/N Estimate (ave./sd):	21.9/2.88 = 7.60	[OK, Meets Criteria]
✓5. Ave. % Recovery (80 - 120%):	109.4%	[Acceptable]

The analyst perfoming this MDL study followed the instructions in the method for calibrating the GC and spiking the MDL samples. The signal/noise estimate is in the appropriate range, the percent recovery meets the method specific criteria and the MDL verifies that this laboratory will be able to report GRO data down to the required reporting limit. The proper calibration is critical in achieving an appropriate MDL for the GRO and DRO methods. This is one of the few tests which the Department sets a reporting limit equal to the method required LOQ.

5.5 COMMON WASTEWATER TESTS AND EXEMPT ANALYTES

The Laboratory Certification Program does not require laboratories to calculate their MDLs for several analytes and test categories. Instead, laboratories should demonstrate that they are able to report down to the recommended reporting limits listed in Sections 5.5.1 - 5.5.4, or as specified in the methods. If an analyte does not have a recommended reporting limit, the laboratory should statistically determine the detection limit. The laboratory may justify a different reporting limit through a variance granted by the Department.

The following analytes are excluded from method detection limit calculations:

Category 1: BOD_5, and CBOD
Category 4: All Parameters
Category 5: Chlorophyll a, Color
Category 7: Ignitability, Reactivity, Corrosivity, Waste Fingerprinting Analyses
Category 20: All Parameters

Analytes which are not regulated under Ch. NR 149, including temperature, pH, nutrients in soil and sludge, physical properties of soil and sludges, residual chlorine, specific conductance, flow measurements and microbiological tests are exempt from detection limit calculation requirements. Additionally, specific tests such as titrimetric and gravimetric determinations, are exempt from a seven replicate MDL determination. For these tests, the laboratory should determine the detection limit using an alternate procedure, as described in Sections 5.5.1 to 5.5.4.

5.5.1 Calculating Residual Chlorine Detection Limits - Residual chlorine is not regulated by the Laboratory Certification Program, but monitoring requirements exist in many permits. There are several approved methods for the determination of residual chlorine including the ion selective electrode (ISE) procedure produced by Orion Research, Inc.. The Iodometric Titration (method 330.2) for residual chlorine analysis is not recommended because it is unlikely to detect residual chlorine in the range required by most wastewater permits. The detection limit for residual chlorine is difficult to calculate due to the unavailability of standards. Department data shows that the MDL can be calculated using the ISE method and household bleach. Matrix specific MDLs can also be calculated provided care is used in choosing the appropriate test sample. Laboratories should be capable of achieving an MDL of 0.05 mg/L for residual chlorine.

5.5.2 Solids (Gravimetric) - It is not practical to analyze seven spiked samples for the various residues, and the MDL for solids parameters should be determined as the smallest amount that can be distinguished from a blank. Typically, the sensitivity of the analytical balance defines the detection limit. Detection limits can always be decreased by increasing sample volume. A justifiable reporting limit for total suspended solids (and other solids measurements) is 1.0 mg/L.

5.5.3 BOD and CBOD - The MDL procedure does not correspond to the BOD test. Instead, the LOD for BOD and CBOD is identical to the minimum required blank depletion of 2 mg/L prescribed by the method.

5.5.4 Titrimetric Procedures - The detection limit for titrimetric procedures can be defined by the smallest amount of reagent that can be added during a titration to cause a chemical change. This is typically determined by the smallest size of the drop that can be produced on a particular buret or other titrating device. Drop size can be estimated by averaging the size of several (5 to 10 is a good number) repeated drops. The detection limit can then be calculated based on the titrant concentration, the sample size, and the minimum drop size. Laboratories using the titration method for ammonia determination are expected to achieve a detection limit of 1.0 mg/L.

5.6 MDLs for Organic Analytes and Common Lab Contaminants

It is important to verify that the determined MDL for an organic analyte will produce an instrument signal at that level. The method of serial dilution is usually a good way to verify the detection limit. If the calculated value is not discernable from the background, then the MDL is not reasonable. The MDLs for many common laboratory solvents should be straightforward to calculate, although the calculated value may not be attainable due to ambient contamination. Laboratories should make every attempt to minimize contamination. The MDLs for these compounds should be reported, and detects must be dealt with on a case by case basis.

Many GC and GC/MS methods have extensive analyte lists, and the use of a common MDL for all analytes is useful for reporting purposes. This is not an accurate representation of detectability, and setting such a reporting limit is discouraged for compounds which have standards near or below the LOQ. Compounds which have standards far above the detection limit are not as problematic for reporting purposes, and reasonable compromises can be allowed.

The MDL for regulated analytes should be below the standard (e.g. PAL, MCL, etc...) whenever possible. For substances with PALs below or near the current limits of detection, and if the calculated MDL is above the

PAL any detects should be appropriately confirmed. Other programs have specific requirements for confirmation that must be followed for the appropriate samples.

5.7 MDLs for High Precision Methods

Many analytical methods are very precise at low levels. The use of auto-analyzers for traditional wet chemistry analysis has greatly increased reproducibility. Ion chromatography is one method that can be exceptionally precise at low levels. In fact, it is very likely that calculated MDLs for these tests will be far below what is analytically achievable in a real sample. In this event, perform a serial dilution analysis, and recalculate the MDL until an appropriate detection limit is found. Another option is to perform the iterative procedure for calculating the MDL given in 40 CFR 136, appendix B.

part vi. data reporting requirements

6.1 Department Consistency Efforts

During 1994 and 1995, the Department of Natural Resources promulgated changes to several Administrative Codes in an attempt to unify data reporting requirements and low-level data interpretation across all of the agency's environmental programs, wherever possible. Because these programs have different data needs, the reporting requirements and use of low-level data language found in the various Administrative Codes are still slightly different. For example, the recent amendments to Ch. NR 149 place data reporting requirements on certified and registered laboratories similar to those that already exist for various facilities, but with a limited the scope of applicability. The Department feels strongly that consistent data reporting requirements will improve the quality of data coming into the agency and ultimately will improve its decisions. Department teams continue to work on the standardized reporting issue, with the ultimate goal of electronic data transmittal for all programs. Sections 6.2 through 6.7 list the reporting requirements at the time this document was printed. For the most current requirements, check the Wisconsin Administrative Code.

The strategy for implementing consistent low-level data reporting requirements involves reporting the actual laboratory MDL with all analytical results, and follows three basic ideas:

1) Any substance detected at a concentration equal to or less than the MDL is less than 99% likely to be present, with the confidence decreasing sharply the closer the value is to zero. These results are reported as "<MDL" (where "MDL" is the lab's actual MDL, or an equivalent system). **These results are dealt with on a case by case basis in the Administrative Codes when used for regulatory calculations.**

2) Any substance detected at a concentration greater than the MDL but less than the LOQ is 99% likely to be present, however the uncertainty in the quantitated value is unknown and the actual concentration is questionable. The determined concentration is reported along with a qualifier to alert data users that the result is between the MDL and the LOQ, and the MDL is included in the report. **These numbers may be used with caution for compliance or regulatory calculations, but require additional substantiation.**

3) Any substance detected at a concentration greater than the LOQ is more than 99% likely to be present, and the quantitated value can be reported with a high degree of confidence. These substances are reported without qualification. **These numbers may be used for compliance or regulatory calculations without further substantiation.**

6.2 NR 106 - Surface Water (Wastewater)

NR 106.07(5) When the water quality based effluent limitation for any substance is less than the limit of detection or the limit of quantitation normally achievable and determined to be appropriate for that substance in the effluent, an acceptable analytical methodology for that substance in the effluent shall be used to produce the lowest limit of detection and limit of quantitation.

(a) When the water quality based effluent limitation is less than the limit of detection, the permit may include conditions which provide that effluent concentrations less than the limit of detection or reported as "not detected" are in compliance with the effluent limitation.

(b) When the water quality based effluent limitation is less than the limit of detection, the permit may include conditions which provide that effluent concentrations greater than the limit of detection, but less than the limit of quantitation determined to be appropriate for that substance in the effluent, are in compliance with the effluent limitation except when confirmed by a sufficient number of analyses of multiple samples and use of appropriate statistical techniques.

(c) When the water quality based effluent limitation is greater than the limit of detection, but less than the limit of quantitation determined to be appropriate for that substance in the effluent, the permit may include conditions which provide that effluent concentrations reported as "not detected" or "not quantified" are in compliance with the effluent limitation.

History: Cr. Register, February, 1989, No. 398, eff. 3-1-89.

6.3 NR 140 - Groundwater

NR 140.14(3) In addition to sub. (2) the following applies when a preventive action limit or enforcement standard is equal to or less than the limit of quantitation:

(a) If a substance is not detected in a sample, the regulatory agency may not consider the preventive action limit or enforcement standard to have been attained or exceeded.

(b) If the preventive action limit or enforcement standard is less than the limit of detection, and the concentration of a substance is reported between the limit of detection and the limit of quantitation, the regulatory agency shall consider the preventive action limit or enforcement standard to be attained or exceeded only if:

1. The substance has been analytically confirmed to be present in the same sample using an equivalently sensitive analytical method or the same analytical method, and

2. The substance has been statistically confirmed to be present above the preventive action limit or enforcement standard, determined by an appropriate statistical test with sufficient samples at a significance level of 0.05.

(c) If the preventive action limit or enforcement standard is between the limit of detection and the limit of quantitation, the regulatory agency shall consider the preventive action limit to be attained or exceed if the concentration of a substance is reported at or above the limit of quantitation.

History: Cr. Register, September, 1985, No. 357, eff, eff. 10-1-85; am. (1)(intro) and (b), r. and recr. (2), Register, October, 1988, No. 394, eff. 11-1-88; am. (1)(b), (2) and (3)(b), Register, September, 1990, No 417, eff. 10-1-90; am.(1)(b), Register, March, 1994, No 459, eff. 4-1-94; r. and recr. (3)(intro), (a), (b), renum. (3)(c) to be 140.16 (5) and am., Register, August, 1995, No 476, eff. 9-1-95.

NR 140.16(4) The department may reject groundwater quality data that does not meet the requirements of the approved or designated analytical methods.

(5) The owner or operator of the facility, practice or activity shall report the limit of detection and the limit of quantitation with the sample results. If a substance is detected below the limit of quantitation, the owner or operator shall report the detected value with the appropriate qualifier to the regulatory agency.

History: Cr. Register, September, 1985, No. 357, eff. 10-1-85; am(1), Register, September, 1990, No. 417, eff. 10-1-90; am. (1), r. and recr. (2), Register, March, 1994, No. 459, eff. 4-1-94; (5) renum. from NR 140.14(3)(c), cr. (4), Register, August, 1995, No. 476, eff. 9-1-95.

6.4 NR 149 - Laboratory Certification

NR 149.15 Data reporting. With each set of sample results, a laboratory shall report:

(3) All analytical results greater than the limit of detection, as determined by a method specified by the department. All analytical results greater than the limit of detection and below the limit of quantitation shall be appropriately qualified.

Note: The requirement in sub. (3) becomes effective January 1, 1997 only for those substances with standards specified in chs. NR 105, 140 and 720 that are below the applicable limits of quantitation. Chapter NR 809 requires this information be reported for all regulated primary drinking water contaminants. The department shall annually publish a list of these substances. Laboratories shall use the best available analytical science to determine whether, in their best professional judgement, a substance has been detected.

History: Cr. Register, February, 1996, No. 482, eff. 3-1-96, except (3) eff. 1-1-97.

The list of compounds described in Ch. NR 149.15(3) consists of the following substances:

1,1,2-Trichloroethane
1,1,2,2-Tetrachloroethane
1,3-Dichloropropene (cis/trans)
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Alachlor
Aldicarb
Antimony
Benzidine
Benzo(a)pyrene
Beryllium
Bis(chloromethyl)ether
Bromodichloromethane
Bromoform
Bromomethane
Cadmium
Chloroform
Chloromethane
Chromium (Hexavalent)
DDT and Metabolites
Di(2-ethylhexyl)phthalate
Dibromochloropropane (DBCP)
Dimethoate
Dioxin (2,3,7,8-TCDD)
Ethylene dibromide (EDB)
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
Lead
Lindane
Mercury
Methyl tert-butyl ether (MTBE)
Methylene Chloride
Parathion
Pentachlorophenol (PCP)
Polychlorinated biphenyls (PCBs)
Thallium
Toxaphene
Trifluralin
Vinyl Chloride

6.5 NR 507 - Landfills (Proposed)

NR 507.26(3)(b) Sampling Results. The owner or operator shall submit all sampling results above the limit of detection. In addition, the owner or operator shall submit all of the following information for each sampling round:

1. The limit of detection and the limit of quantitation for each parameter. The limit of detection and the limit of quantitation shall be determined in accordance with a method specified by the department as required in s. NR 149.11(5).

2. A result qualifier for each detected parameter with a reported value between the limit of detection and the limit of quantitation.

History: Proposed Rule.

6.6 NR 720 - Spills

NR 720.07(2) COMPLIANCE WITH SOIL CLEANUP STANDARDS. (a) Contaminant concentrations in soil samples shall be determined using a department-approved and appropriate analytical method and reported on a dry weight basis. An appropriate analytical method shall have limits of detection or limits of quantitation, or both, at or below soil cleanup standards where possible. Responsible parties shall report the limit of detection and the limit of quantitation with sample results. The department may require that

supporting documentation for the reported limit of detection and limit of quantitation be submitted.

(b) If a soil contaminant concentration in a sample exceeds the soil cleanup standard at or above the limit of quantitation for that soil contaminant, the soil cleanup standard shall be considered to have been exceeded.

(c) If a soil cleanup standard for a soil contaminant is between the limit of detection and the limit of quantitation, the soil cleanup standard shall be considered to be exceed if the soil contaminant concentration is reported at or above the limit of quantitation.

(d) The following applies when a soil cleanup standard for a soil contaminant is below the limit of detection:

1. If a soil contaminant is not detected in a sample, the soil cleanup standard shall not be considered to have been exceeded.

2. If a soil contaminant is reported above the limit of detection but below the limit of quantitation, the soil cleanup standard shall be considered to have been exceeded if the presence of that soil contaminant has been confirmed by the use of an appropriate analytical method.

History: Cr. Register, March, 1995, No. 471, eff. 4-1-95.

6.7 NR 809 - Drinking Water

NR 809.12(9)(a) Compliance with s. NR 809.11 (SDWA INORGANICS) shall be determined based on the analytical results obtained at each entry point. Any contaminant listed in s. NR 809.11 which is detected shall be quantified.

NR 809.21(d) Any contaminant listed in s. NR 809.20 (SDWA SOCS) that is detected shall be quantified. Any sample below the reported method detection limit shall be calculated at zero for the purposes of determining the averages in pars. (b) and (c).

NR 809.25(d) Any contaminant listed in s. NR 809.24 (SDWA VOCS) that is detected shall be quantified. Any sample below the reported method detection limit shall be calculated at zero for the purposes of determining the averages in pars. (b) and (c).

American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992, various pages.

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Skoog, Douglas A. & Leary, James J. Principles of Instrumental Analysis, Fourth Edition, Saunders College Publishing, 1992.

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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 interferant 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 (interferant). The interferant concentration is presupposed to be normally distributed in representative samples of a give 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 detection limit. (Recommend between 1 and 5 times the estimated 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) This 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 concentration of analyte will not result in significant 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:

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

$$S=(S_2)^{1/2}$$

where:

X_i; i=1 to n, are the analytical results in the final method reporting units obtained from the sample aliquots and Σ refers to the sum of the X values from i=1 to n.

6. (a) Compute the MDL, as follows:

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

where:

MDL = the method detection limit

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

$$S_{pooled} = \left[\frac{6\,(S^2)_A + 6\,(S^2)_B}{12}\right]^{1/2}$$

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 $t_{(12,1-\alpha=.99)}$.

(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	2.002
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
∞	∞	2.326

Reporting

The analytical method used must be specifically identified by number of 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 eh 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. 265, 1984; 50 FR 694, 696, Jan. 4 1985, as amended at 51 FR 23703, June 30, 1986]

Adapted from the Code of Federal Regulations by the Wisconsin Department of Natural Resources

An outlier is defined as an observation or "data point" which does not appear to fall within the expected distribution for a particular data set. Outliers may be rejected outright if they are caused by a known or demonstrated physical reason, such as sample spillage, contamination, mechanical failure, or improper calibration. Data points which appear to deviate from the expected sample distribution for no known physical reason must be verified as outliers using statistical criteria.

Outliers can significantly alter the outcome of a method detection limit calculation. Including outliers in an MDL calculation leads to increased variability (larger standard deviation). An MDL calculated using outliers will be inaccurate and higher than the true detection limit. For this reason, it is important to recognize outliers, and to reject them from the calculation. Since the procedure requires at least seven replicates, rejecting one of only seven sample results will result in too few data points to calculate an MDL.

Table of Critical Values
(1% significance value)

# Observations	Critical Value
7	2.10
8	2.22
9	2.32
10	2.41
11	2.48
12	2.55
13	2.61
14	2.66

For the MDL procedure, all data sets will only be samples of the true population, and both the population mean (μ) and the population standard deviation (σ) will be unknown. The expected distribution for MDL observations is most closely represented by a log-normal distribution, and only one-sided outliers should be expected. Due to the nature of the MDL procedure (low-level precision), most outliers will be high-sided, and the only test necessary will be a single-sided outlier test. A low-sided outlier could occur, but the data would be unusable because it would most often appear as a "no detect".

One method for determining single sided outliers when both the population mean (μ) and the population standard deviation (σ) are unknown was described by Grubbs (F.E. Grubbs 1979) and is included in *Standard Methods*.

$T_n = X_n - X_{ave}/s$ (high sided outliers) $T_1 = X_{ave} - X_1/s$ (low sided outliers)

Where X_n (X_1) is the data point in question, X_{ave} is the sample mean, and s is the sample standard deviation. The value T_n is then compared against a table of critical values. If T_n is greater than the critical value for the appropriate number of replicates at the 1% significance level, the questionable data point is an outlier, and it may be rejected. The critical values for various numbers of replicates at the 1% significance level are given in the sidebar.

Example 1: The following results were obtained for an MDL study: [10.2, 9.5, 10.1, 10.3, 9.8, 9.9, 11.9, 10.0] with X_{ave}= 10.2 and s= 0.726. The analyst suspects 11.9 to be an outlier. Using the high-sided test:

T_n= 11.9-10.2/0.726= 2.34

The calculated T_n value is now checked against the table. Since 2.34>2.22, 11.9 is indeed an outlier.

Example 2: The following results were obtained : [0.523, 0.562, 0.601, 0.498, 0.547, 0.525, 0.578, 0.503] with X_{ave}= 0.542 and s= 0.036. Is 0.601 an outlier?

T_n= 0.601-0.542/0.036= 1.64

Checking the table shows that 1.64<2.22 and 0.601 is not an outlier and could be included in the MDL calculation.

References

Grubbs, F.E. 1979. Procedures for detecting outlying observations. In *Army Statistics Manual* DARCOM-P706-103, Chapter 3. U.S. Army Research and Development Center, Aberdeen Proving Ground, MD 21005.

American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 17th, 18th or 19th Editions, (1989, 1992 or 1996).

Below is a sample of a spreadsheet that can be used to help evaluate MDLs. This spreadsheet was adapted from a version presented by NET Laboratories at the February, 1995 WELA meeting.

Title: Sample MDL Calculation Form/Spreadsheet		
Row Number	**Column A**	**Column B**
1	Analyte	
2	Method	
3	Date	
4	Instrument	
5	Spike Conc.	
6	Units	
7	Replicate 1	
8	Replicate 2	
9	Replicate 3	
10	Replicate 4	
11	Replicate 5	
12	Replicate 6	
13	Replicate 7	
14	Replicate 8	
15	Mean	Average(B7..B14)
16	Std. Dev.	STDEV(B7..B14)
17	MDL	(t-value)*(B16)
18	LOQ	10*B16
19	High Spike Check	IF B5<10*B17,"OK","Not OK"
20	Low Spike Check	IF B5>B17, "OK","Not OK"
21	S/N	B15/B16

Note that the t-value will change. Also, laboratories may wish to include outlier checks, previous MDL data, or calibration infomation with this spreadsheet. This is only a model, and is not the required format for reporting MDLs, nor is it the only way to create a spreadsheet to calculate MDLs.