Fact Sheet: Detection and Quantitation — What Project Managers and Data Users Need to Know

As a Project Manager or decision-maker, you may use environmental data to accomplish one or more of the following tasks:

- Determine whether a chemical substance is present in an environmental sample at or above some threshold value or action level;
- Verify that a pollutant concentration remains below a permit limit;
- Evaluate potential risks to human health or the environment;
- Monitor changes in concentrations of contaminants; or
- Determine the effectiveness of remediation activities.

Making correct decisions in these cases often depends on the ability of an analytical method to detect and measure extremely low concentrations of a substance.

This fact sheet has been prepared to: 1) provide Project Managers and data users with basic information about detection and quantitation concepts; and 2) acquaint the reader with detection and quantitation terminology and requirements contained in the *DoD Quality Systems Manual for Environmental Laboratories (DoD QSM)*, Version 4.1. This information should help clarify the uncertainty associated with reporting low-concentration data. It should also help project teams understand the importance of selecting analytical methods that are sensitive enough for their intended uses, i.e., capable of generating reliable data (data of known precision and bias) at the project-specific decision levels.

Measures of Sensitivity — Basic Concepts

The following terms are used to describe the routine sensitivity of analytical procedures:

- DL – Detection Limit
- LOD – Limit of Detection
- LOQ – Limit of Quantitation

All measures of sensitivity are specific to the analyte, sample matrix, test method, instrumentation, and analyst/laboratory performance. Therefore, analytical performance must be demonstrated for each variable (e.g., it is possible that two "identical" instruments from the same manufacturer may exhibit different sensitivities).

The Detection Limit (DL) is the smallest analyte concentration that can be demonstrated to be different from zero or a blank concentration at the 99% level of confidence. In other words, if a substance is detected at or above the DL, it can be reliably stated (with 99% confidence) that the analyte is present (there is a 1% chance that the analyte is not present (a false positive)). Note that for reporting purposes, any result at or above the DL must also meet qualitative identification criteria required by the test method. Although a result at or above the DL indicates that the analyte is present, the absence of a result at or above the DL is inconclusive (i.e., one cannot confidently state whether the analyte is present or absent), because the false negative rate at the DL is 50%.

The Limit of Detection (LOD) is the smallest amount or concentration of a substance that must be present in a sample in order to be detected at a 99% confidence level. In other words, if a sample has a true concentration at the LOD, there is a minimum probability of 99% of reporting a "detection" (a measured value $\geq$ DL) and a 1% chance of reporting a non-detect (a false negative).

The failure to obtain a "detection" should be reported as "<LOD," because the false negative rate at the LOD is 1%. Reporting the sample result as "<DL" is inappropriate because, as stated above, the false negative rate at the DL is 50%.

The Limit of Quantitation (LOQ) is the lowest concentration of a substance that produces a quantitative result within specified limits of precision and bias. The LOQ is typically larger than the LOD (but may be equal to the LOD, depending upon the acceptance limits for precision and bias); therefore, the following is true:

$$DL < LOD \leq LOQ$$

Quantitative results can only be achieved at or above the LOQ. Measurements between the DL and the LOQ assure the *presence* of the analyte with confidence, but their numeric values are estimates.

Types of Procedures for Estimating Sensitivity

Numerical estimates of the DL, LOD, or LOQ for a specific analyte, matrix, and method can be calculated using various statistical procedures, which involve spiking reagent water or other specific matrix with low concentrations of the analyte of interest. At this time, unfortunately, universally accepted statistical procedures do not exist.

The estimator that has been most commonly used by environmental laboratories is the EPA Method Detection Limit (MDL), which is an approximation of the DL. EPA has defined the MDL 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."[1] Calculating the MDL at 99% confidence means there is a 1% probability that a sample having a result at or above the MDL is a false positive. The EPA MDL was designed to protect against false positives.

Uses and Limitations of the MDL

When performed correctly and consistently, MDLs determined using the EPA procedure can be useful for comparing different laboratories' performance using the same methods, or the performance of different methods within the same laboratory. Laboratories typically determine the MDL in reagent water, resulting in a "best-case" MDL, which provides limited information about method performance on real-world samples.

The EPA MDL procedure has been criticized as a poor estimator of the DL for the following reasons:

1. It is a single laboratory, short-term estimator that fails to account for analytical bias, changing instrument conditions, or analyst skill.
2. It assumes uniform variance across all possible spike concentrations, failing to account for the fact that variance increases at higher concentrations.
3. It assumes that measured values at the spike concentration are normally distributed. By using this procedure and spiking at very low concentrations, laboratories have been able to calculate MDLs that cannot be achieved in practice.

DoD QSM Requirements

For the reasons discussed in the previous paragraph, the DoD QSM requires that laboratories verify measures of method sensitivity, in terms of the LOD and LOQ, at least quarterly. Requirements for the LOD and the LOQ are contained in DoD QSM Boxes D-13 and D-14, respectively, which follow:

[1] 40 Code of Federal Regulations (CFR) Part 136, Appendix B, rev. 1.11.

Box D-13

Limit of Detection (LOD): Determination and Verification (Requirement)

A laboratory shall establish a detection limit (DL) using a scientifically valid and documented procedure for each suite of analyte-matrix-method, including surrogates. The detection limit shall be used to determine the LOD for each analyte and matrix as well as for all preparatory and cleanup methods routinely used on samples, as follows:

After each detection limit determination, the laboratory must immediately establish the LOD by spiking a quality system matrix at approximately two to three times the detection limit (for a single-analyte standard) or one to four times the detection limit (for a multi-analyte standard). This spike concentration establishes the LOD. It is specific to each combination of analyte, matrix, method (including sample preparation), and instrument configuration. The LOD must be verified quarterly. The following requirements apply to the initial detection limit/LOD determinations and to the quarterly LOD verifications.

- The apparent signal to noise ratio at the LOD must be at least three and the results must meet all method requirements for analyte identification (e.g., ion abundance, second-column confirmation, or pattern recognition.) For data systems that do not provide a measure of noise, the signal produced by the verification sample must produce a result that is at least three standard deviations greater than the mean method blank concentrations.
- If a laboratory uses multiple instruments for a given method the LOD must be verified on each.
- If the LOD verification fails, then the laboratory must repeat the detection limit determination and LOD verification at a higher concentration or perform and pass two consecutive LOD verifications at a higher concentration and set the LOD at the higher concentration.
- The laboratory shall maintain documentation for all detection limit determinations and LOD verifications.

Box D-14

Limit of Quantitation (LOQ): Establishment and Verification of LOQ (Requirement)

For DoD projects, the LOQ must be set within the calibration range prior to sample analysis. At a minimum, the LOQ must be verified quarterly.

The laboratory procedure for establishing the LOQ must empirically demonstrate precision and bias at the LOQ. The LOQ and associated precision and bias must meet client requirements and must be reported. If the method is modified, precision and bias at the new LOQ must be demonstrated and reported.

Establishing Project-Specific Requirements for Method Sensitivity

Project teams should establish their project-specific requirements for method sensitivity in terms of a Reporting Limit (RL) for each analyte and matrix. As defined in the DoD QSM, the RL is the lowest concentration value specified by the client that meets project requirements for reporting quantitative data with known precision and bias for a specific analyte in a specific matrix. The LOQ cannot be greater than the RL, if precision and bias of the RL and LOQ are the same. If the LOQ for a particular analytical method or laboratory cannot meet the RL, then a project team has three options:

1. Improve analyst performance or modify the method to achieve a lower LOQ.
2. Select a different method with an LOQ less than or equal to the RL.
3. Raise the RL.

Please note that precision and bias must be taken into consideration when assessing the LOQ versus the RL. Also note that data below the RL can be reported; however they are estimated values if less than the LOQ.

Reporting and Flagging Analytical Data

Although data reporting and flagging requirements are project-specific, all reported LOD and LOQ shall be adjusted for the size of sample aliquots, concentration/dilution factors, and percent solids. In addition, the following example (based on Box 47 of DoD QSM Version 4.1) illustrates the proper use of the "U" and "J" data qualifier flags for non-detect and estimated analytical results, respectively.

U – Analyte was not detected and is reported as less than the LOD or as defined by the client. The LOD has been adjusted for any dilution or concentration of the sample (* see Example, below).

J – The reported result is an estimated value (e.g., matrix interference was observed or the analyte was detected at a concentration outside the quantitation range, see Box 33).

Example: DL = 2, LOD = 4, LOQ = 20, and RL = 30 with the precision and bias of the LOQ meeting those of the RL and all samples are undiluted.

Sample #1: Analytical result: Non-detect	Reported result: <4 U
Sample #2: Analytical result: 3	Reported result: 3 J
Sample #3: Analytical result: 10	Reported result: 10 J
Sample #4: Analytical result: 20	Reported result: 20
Sample #5: Analytical result: 30	Reported result: 30

Understanding and Documenting Uncertainty for Low-Concentration Data

As mentioned above, detection and quantitation limits are laboratory specific. Following are some steps Project Managers can take to document measurement uncertainty for low concentration data.

- As part of the laboratory selection process, provide the laboratory with project-specific RLs, including precision and bias, for each analyte and matrix. Ask the laboratory to provide its DL, LOD, and LOQ with associated precision and bias for each target analyte, in each matrix of concern (e.g., reagent water, clean sand, etc.), and verify that these values meet project-specific RLs. Request laboratory SOPs for establishing the DL and for establishing and verifying the LOD and LOQ.
- Ask the laboratory to verify the LOD by processing an LOD verification check sample with each batch of samples. This is a quality control sample that is spiked at a concentration at or slightly above the LOD to evaluate whether the analyte of interest is in fact "detectable" in the matrix of interest. To confidently report non-detects, set the reporting for non-detects to less than the LOD.
- If the project involves the collection of unusual or difficult matrices, or if the project-specific RL is near the LOQ, ask the laboratory to verify the LOQ in the project-specific matrix by analyzing a minimum of four replicate samples with known concentrations at the LOQ.
- Review the raw data (e.g., chromatograms) for low-concentration data. If a result is reported above the DL, make sure that the signal-to-noise ratio is at least 3.
- Compare sample results with blank results. If sample results (including chromatograms) cannot be distinguished from blank results, then they are not meaningful.