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 5.1. 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.[1]

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). A graphical representation of these terms is shown as Figure 1.

The Detection Limit (DL) is the smallest analyte concentration that can be demonstrated to be different from zero or a blank concentration with 99% confidence. At the DL, the false positive rate (Type I error) is 1% (red shaded region in Figure 1). A DL may be used as the lowest

[1] A discussion on the Minimum Detectable Concentration (or Minimum Detectable Activity) for radiological data is beyond the scope of this Fact Sheet. For a discussion on this, see DoD/DOE QSM 5.1 Module 6, Section 1.5.2.1.1.

concentration for reliably reporting a detection of a specific analyte in a specific matrix with a specific method with 99% confidence.

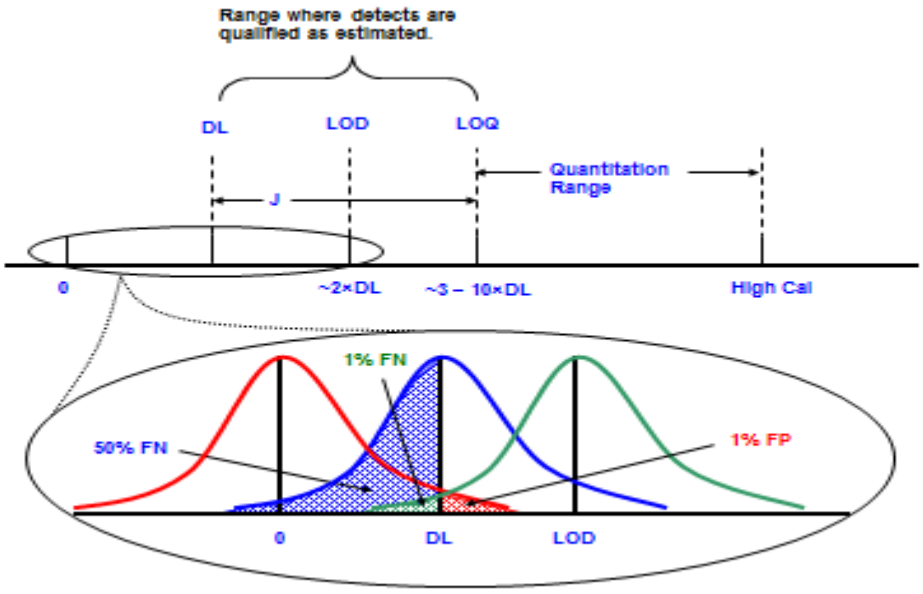

Figure 1: Summary of data quality characteristics below and above DL, LOD, and LOQ. The red trace shows the distribution of results given a sample with a true concentration of zero. The blue trace shows the distribution of results given a sample with a true concentration at the Detection Limit. The green trace shows the distribution of results given a sample with a true concentration at the Limit of Detection. The red shaded region represents those results which would yield a false positive (i.e., the true concentration is zero but the analytical result is detection). The blue and green shaded regions represent those results which would yield a false negative if the true concentration is at the reporting limit and the reporting limit is set at the DL or LOD, respectively (i.e., a sample with a true concentration at the DL has a 50% chance of yielding a false negative, and a sample with a true concentration at the LOD has a 1% chance of yielding a false negative).

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 if the analyte is present at the DL is 50% (blue shaded region in Figure 1).

The Limit of Detection (LOD) is defined as the lowest concentration for reliable reporting of a non-detect of a specific analyte in a specific matrix with a specific method at 99% confidence. At the LOD, the false negative rate (Type II error) is 1% (green shaded region in Figure 1). In other words, if a sample has a true concentration at the LOD, there is at least a 99% probability of reporting a "detection" (a measured value ≥ DL) and a 1% chance of falsely reporting a non-detect (a false negative).

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

The Limit of Quantitation (LOQ) is the smallest concentration that produces a quantitative result with known and recorded precision and bias. For DoD/DOE projects, the LOQ shall be set at or above the concentration of the lowest initial calibration standard and within the calibration range. Because of the requirements on precision and bias, the LOQ is larger than the LOD[2]; therefore, the following is true:

$$DL < LOD < LOQ$$

Quantitative results, with a known degree of precision and bias, can only be achieved at or above the LOQ. Detections between the DL and the LOQ assure the *presence* of the analyte, but their numeric values are estimates and are therefore indicated as such on test reports. Figure 2 summarizes the differences and the relationship between DL, LOD, and LOQ.

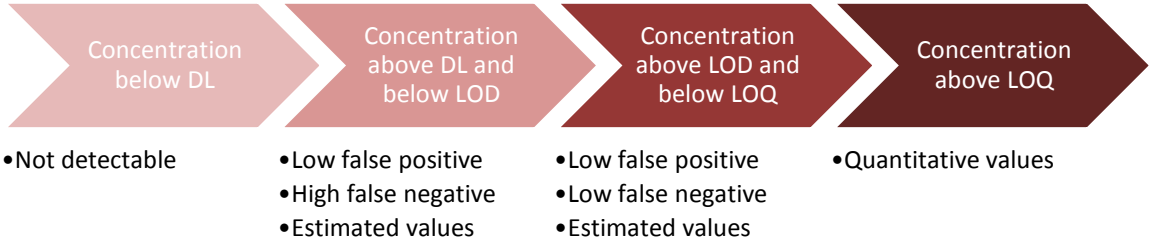

Figure 2: Summary of data quality characteristics below and above DL, LOD, and LOQ.

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."[3] Calculating the MDL at 99% confidence means there is a 1% probability of a false positive when a sample has a result at the MDL. The EPA MDL was designed to protect against false positives; however, it does not protect against false negatives.

[2] See TNI Module 4, Section 1.5.2.2d.
[3] 40 Code of Federal Regulations (CFR) Part 136, Appendix B, rev.2

Uses and Limitations of the MDL

When performed correctly and consistently, MDLs determined using the EPA procedure can be useful for comparing the performance of different laboratories 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 as originally defined in 40 CFR Part 136, (Appendix B, 1984) has been criticized as a poor estimator of the DL for numerous reasons including but not limited to:

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 changes at higher concentrations.
3. It assumes that measured values at the spike concentration are normally distributed. By using the procedure and spiking at very low concentrations, laboratories have been able to calculate MDLs that cannot be achieved in practice.
4. It does not require a demonstration of the ability to detect an analyte at the calculated MDL.
5. It is not reproducible from day-to-day, lab-to-lab, etc.

Since 2000 the EPA has increased efforts to address these issues. In 2016 the EPA updated the MDL procedure in 40 CFR 136 which did include provisions for addressing background contamination, multiple analysts and instruments, and included verification requirements; however, the MDL calculation of spikes remains unchanged.

DoD QSM Requirements

Requirements for the DL, LOD and the LOQ which are designed to address some of the concerns discussed in the previous paragraph are contained in DoD QSM Module 4 Sections 1.5.2.1 and 1.5.2.2. Requirements that may be of particular note to Project Managers and Data Users are:

- Laboratories are required to verify measures of sensitivity, in terms of the LOD and LOQ, at least quarterly.
- Laboratories shall establish a detection limit (DL) for each suite of analyte-matrix-method, including surrogates. The DL 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.
- After each DL determination, the laboratory must establish the LOD. It is specific to each suite of analyte, matrix, and method (including sample preparation).
- The laboratory must establish the LOD by spiking a quality system matrix at a concentration of at least 2 times but no greater than four times the DL.
- The signal to noise (S/N) ratio at the LOD must be at least three, and the results must meet all method requirements for analyte identification.

- The DL and LOD must be reported for all analyte-matrix-method suites unless it is not applicable to the test or specifically excluded by project requirements.
- The laboratory procedure for establishing the LOQ must empirically demonstrate precision and bias at the LOQ for each suite of analyte-matrix-method, including surrogates. 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 For DoD/DOE projects, the LOQ must be set within the calibration range, including the lowest calibration level.

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 a customer-specified lowest concentration value that meets project requirements for quantitative data with known precision and bias for a specific analyte in a specific matrix. The RL cannot be less than the LOQ, if precision and bias of the RL and the LOQ are identical. If the LOQ for a particular analytical method or laboratory cannot meet the RL, then a project team has four options:

1. Consult with the laboratory to improve method 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.
4. If no other options are available to meet project needs, allow for increased level of uncertainty such that adjusted LOQ can meet RL. This LOQ must be verified.

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 may be reported; however, they are estimated values if less than the LOQ. 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.

Reporting and Flagging Analytical Data

The following example (based on QSM 5.1 Module 2 section 5.10.3.1.1) illustrates the proper use of the “U” and “J” data qualifier flags for non-detect and estimated analytical results, respectively.

Data Qualifier flags in this example are defined as:
U- Analyte was not detected and is reported as less than the LOD or as defined by the customer. The LOD has been adjusted for any dilution or concentration of the sample.
J- The reported result is an estimated value (e.g. matrix interference was observed, or the analyte was detected at a concentration outside the calibration range).

Example: Detection Limit (DL) = 2, Limit of Detection (LOD) = 4, Limit of Quantitation (LOQ) = 20, Reporting Limit (RL) for the project = 30, with precision and bias of the LOQ meeting precision and bias of the RL. All samples are undiluted.

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

Note that the laboratory may use additional data qualifiers or different letters or symbols to denote the qualifiers as long as they are appropriately defined and their use is consistent with project-specific requirements. Additionally, the laboratory-defined data qualifiers are for laboratory use only. Data usability must be determined by the project team.

Understanding and Documenting Uncertainty for Low-Concentration Data

As mentioned above, detection and quantitation limits are laboratory specific. The 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 accurately report non-detects, set the reporting for non-detects to "less than the LOD" or report the LOD with a "U" flag.
- 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 low concentration raw data (e.g., chromatograms). If a result is reported above the DL, make sure that the signal-to-noise ratio is at least 3.
- Compare sample result with blank results. If sample results (including chromatograms) cannot be distinguished from blank results, the data may not be useable for decision making.