Overview

This module of the Chapter NR 149 Implementation Guidance discusses definitions of and related to limits of detection and limits of quantitation. The module also paraphrases the requirements in NR 149.48 dealing with limits of detection and quantitation, discusses briefly the implications of those requirements for certified or registered laboratories, and at times offers suggestions for complying with some requirements.

This module does not discuss in detail the procedure for determining the method detection limit (MDL). The Department has available a separate guidance describing how to determine and optimize MDL determinations.

Definitions

NR 149.03 (41) Limit of detection or "LOD":

The lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is not a false positive value. For department purposes, the LOD approximates the MDL and is determined per the method cited in sub. (46).

Discussion

The LOD definition in Chapter NR 149 is fairly general and can accommodate various approaches to determine these limits. The definition also states that the MDL approximates the LOD, and for practical purposes, until existing regulations change or EPA promulgates a different protocol for determining detection limits, most laboratories are bound to determine LODs by performing MDL studies.

Another way of understanding detection limits is to think of LOD as the generic, most basic concept. There can be many types of LODs and they are defined by the protocols used to determine them. And so MDLs, instrument detection limits (IDLs), estimated detection limits (EDLs) and others are all types of LODs, and all attempt to reach that concentration or amount above which a laboratory can be certain that a detected analyte is not a false positive.

NR 149.03 (42) Limit of quantitation or "LOQ":

The lowest concentration or amount of an analyte for which quantitative results can be obtained.

Discussion

This definition is also sufficiently general to accommodate various approaches to determining quantitation limits. Most commonly, LOQs are established by multiplying an LOD by a confidence or coverage factor, and can be estimated from calibration functions.

LOQ is a generic term to describe any number of possible limits all attempting to reach that lowest concentration above which numerical results become reproducible. LOQs are usually associated with a confidence level, which means that depending on how accurate one needs to be, several LOQs can be established. Practical quantitation limits (PQLs) and minimum reporting limits (MRLs) are all types of LOQs.

The region between the LOD and the LOQ is a measurement zone that indicates the presence of an analyte, but cannot be relied on for quantitative soundness.

NR 149.03 (46) Method detection limit or "MDL":

The minimum concentration of an analyte that can be measured and reported with 99% confidence that the stated concentration is greater than zero, determined from a set of samples containing the analyte in a given matrix. The method detection limit is generated according to the protocol specified in 40 CFR Part 136, Appendix B.

Discussion

This is the most commonly used LOD in environmental chemistry. The procedure is based on determining the precision of a set of replicate measurements from samples processed through an entire analysis method. The MDL's reliance on precision produces unrealistic results for techniques that are inherently precise. The use of the Student-t factor in the protocol is considered by some inappropriate for establishing the stated confidence level.

NR 149.03 (71) Reporting Limit or "RL":

A concentration or amount of analyte required by the department or client above which numerical results must be reported. Reporting limits may be limits of detection, limits of quantitation, practical quantitation limits, or other concentrations, and may be specific to a project or investigation.

Discussion

Chapter NR 149 defines RL as any limit to which a laboratory is instructed to report and above which numerical results must be reported. For the Department, this can mean detection limits, for analytes that have very low enforcement standards, all the way up to a number many factors away from the LOQ, for analytes of limited toxicity.

Environmental laboratories also use the term RL to indicate a quantity above which they will report numeric results (no "less than" values). However, many laboratories equate this limit with the lowest concentration of a calibration standard in an analysis. That designation of the RL will not always correspond to Department RLs.

Limits of Detection and Quantitation [NR 149.48 (2)]

Requirement: NR 149.48 (1) (a) 2

Part of a quality control program includes detection limit studies. A laboratory must establish quality control procedures to asses the sensitivity of all tests performed.

Discussion

This is the anchoring statement in Chapter NR 149 requiring determining LODs and LOQs. As estimators of the sensitivity of a procedure, LODs and LOQs are as integral to a quality control program as method blanks, laboratory control samples, quality control standards, matrix spiked and matrix spike duplicates, replicates, surrogates, and confirmation techniques.

Requirement: NR 149.48 (2) (a)

A laboratory must determine LODs for all tests performed and all analytes reported except for biochemical oxygen demand (BOD), titrimetric tests, gravimetric tests other than hexane extractable material (HEM), and tests for which spiking is not possible.

Discussion

This part of Chapter NR 149asserts that laboratories must determine LODs for all analytes reported, and of course, all tests performed. When a laboratory sub-contracts analyses, the subcontractor's detection limits must be reported with the results. For the indicated tests, an LOD determination is not possible or required; however, NR 149.48 (2) (c) requires estimating the sensitivity of these excluded tests based on how their data will be used.

Requirement: NR 149.48 (2) (b)

A laboratory must determine the LOD of an analyte by a procedure specified in regulation or in an approved method of analysis. The laboratory must include all sample-processing steps of a method when determining LODs.

Discussion

At the moment, most regulations require determining an MDL. However, through guidance and actual practice, laboratories have been allowed to establish "common sense" detection limits when an MDL cannot be detected. Until there is change in the MDL procedure, or until it is superseded by a different protocol, the

Department will allow exceptions, as is currently done, when MDLs are demonstrated to be unrealistic for a specific analyte and method of analysis.

Requirement: NR 149.48 (2) (c)

For tests for which performing a detection limit study is not possible, a laboratory must estimate the sensitivity of the tests based on how the resulting data will be used in a given application.

Discussion

For the most commonly performed excluded tests, there are defensible ways of estimating their sensitivity. For BOD and cBOD, at the moment, notwithstanding the reporting requirements of the latest version of the method, it seems reasonable to set the test's sensitivity on the requirement for achieving a minimum dissolved oxygen depletion and the maximum sample volume processed. For example, if the maximum volume used in a BOD dilution is 200 mL, instead of using a full 300 mL of sample, and the minimum depletion allowable by the procedure is 2.0 mg/L, the detection limit of BOD in the sample would be 3 mg/L (300mL/200mL x 2 mg/L).

For gravimetric tests, the last unit of the analytical balance (or for solids test, minimum capture requirements) and the amount of sample analyzed can be used to obtain defensible estimates of sensitivity. For titrimetric tests, the concentration equivalent of the smallest burette graduation and the sample volume size can also be used to estimate sensitivity.

All of these estimates can be moderated by the necessity or requirements of a data user to be able to discern analytes or quantitate them at a certain level.

Requirement: NR 149.48 (2) (d)

A laboratory must determine LODs at least annually unless it can verify by an established and defensible protocol that an existing limit continues to apply.

Discussion

In the past, laboratories were only required to re-determine their LODs when an approved method required it. Now laboratories have to determine their LODs at least annually, unless they opt to verify the continued applicability of an existing LOD. Should an approved method require re-determination of an LOD sooner than yearly, a laboratory must comply with that directive.

Verifying LODs can be an art, but the option is meant to keep laboratories from having to repeat a set of determinations that likely will not yield different results from those already established.

Laboratories will have to determine their own protocols for verifying LODs, if they choose that option. Those protocols would of course, be subject to a review for defensibility. One protocol, included in The NELAC Institute's (TNI) Standard (Volume 1, Module 4, Chemical Testing) advises analyzing a sample fortified with the analytes of interest at no more than three times the LOD, for single analyte tests, and no more than four times the LOD for multiple analyte tests. Detecting the analytes of interest (a value above zero) would verify the LODs. This would allow the laboratory to continue to use the verified LOD and would exempt the laboratory from having to perform another full LOD determination.

Other verification schemes are possible and laboratories are encouraged to perform them, document them, and submit them to the Department for review and endorsement.

Requirement: NR 149.48 (2) (e)

A laboratory must re-determine LODs whenever there is a change in a test method or instrumentation that affects the sensitivity of an analysis.

Discussion

This is a self-evident requirement. If a laboratory feels a change does not affect the sensitivity of an analysis, the existing valid LOD can be retained and used for reporting. Laboratories, however, should be able to demonstrate that a specific change in a method or instrumentation has not increased or decreased the stated sensitivity of an analysis.

Requirement: NR 149.48 (2) (f)

A laboratory must establish procedures to relate LODs to LOQs.

Discussion

Protocols are available for determining an LOD, but protocols for determining an LOQ usually use a determined LOD to arrive at a derived LOQ. It is common to use a multiplier to arrive at an LOD from LOQ. For an MDL, dividing it by the Student-t factor used to determine it (to obtain the standard deviation) and multiplying the quotient by 10 complies with one of the definitions of LOQ. Other ways of determining the LOQ may be equally justified. Chapter NR 149 requires laboratories to establish procedures for relating the two limits. The Certification Program hopes

that this provision brings laboratories to start discussing these issues and to establish rules that may eventually culminate in more uniform guidance.

A caveat about relating LODs to LOQs: because laboratories are allowed to report results between the LOD and the LOQ with qualifiers attesting to the uncertainty of the reported result, laboratories must be careful not to set their LOQs artificially high. This is important not only because the resulting LOQ is not the "lowest" required by definition, but also, and more importantly, because numerical results that are quantitative may appear as if they were not. Laboratories that use the lowest calibration standard concentration as their reporting limit calling it also an LOQ, must be able to explain flagging results below the lowest calibration standard when that standard is significantly removed from a more sensitive estimate of the LOQ.

Requirement: NR 149.48 (2) (g)

LOQs established by a laboratory must be above determined LODs.

Discussion

This is also a fairly self-evident statement that could as validly be stated as "determined LODs must be below established LOQs". No matter how the laboratory decides to relate LOQs to LODs, the resulting LOQs must be greater than the corresponding LODs.