GUIDELINES FOR METHOD VALIDATION OF TOXOCOLOGY TEST METHODS

1.0 BACKGROUND

It is necessary that the laboratory's ability to successfully carry out the toxicity test be properly validated in-house prior to the site assessment by CALA.

Method validation data required for toxicity tests in support of the CALA lab accreditation program (as required for conformance to ISO/IEC Guide 17025) is not the same as, and should be distinguished from, method validation data required prior to the publication of a new toxicity test method (e.g., an Environment Canada biological test method). This latter validation would require different types of method validation data, generated in long-term studies (e.g., an inter-laboratory/round-robin study, ruggedness testing, etc.). The type of method validation data required from a laboratory during a CALA assessment is meant to demonstrate that a laboratory can successfully conduct the method according to the most recent version of a published national or international test method; it is sometimes referred to as *verification*, rather than *validation*.

2.0 MINIMUM VALIDATION DATA REQUIRED

In order for a toxicity laboratory to be accredited for a particular test method, a minimum method validation data set is required, and should be established by laboratory personnel having the appropriate technical knowledge and experience. The following list is proposed:

- **Test Organism:** Species identification must be adequately confirmed to the species level using a taxonomic guide, a culture collection curator (in the case of green algae, bacteria, etc.), an external taxonomic expert (e.g., fish, invertebrates, etc.) or DNA barcoding.
- **Culturing and acclimation:** Demonstrated, documented evidence that the laboratory is meeting all animal and/or culture health criteria established in the published test method (e.g., reproductive criteria for Daphnia sp., such as number of young per brood, cell reproduction in green algae, etc.) when the lab cultures test organisms. If purchasing organisms for immediate use or limited holding, a lab must demonstrate these organisms meet all criteria in the specific method for purchased/imported organisms.

- **Negative Controls and Validity Criteria**: Demonstration of consistent success in meeting all test validity criteria (e.g., mortality in controls, growth, etc.) from 5 or more tests for the full test duration and in the test matrix (e.g. sediment or water).
- **Positive Control Testing:** At least 5 successful reference toxicant tests that meet all control validity criteria provided in the published test method, and the demonstration of a normal/typical concentration-response curve. When the reference toxicant test is conducted identical to the standard test (that is, the same matrix, duration and endpoints), then the controls from these reference toxicant tests may also serve as proof of meeting the evaluation of negative controls and validity criteria). The results of a laboratory's reference toxicant tests should be compared to results obtained from other laboratories, by comparison to published data for the reference toxicant used or by an inter-laboratory study (such as proficiency testing).

  For the reference toxicant test data to be representative of toxicity test precision for the laboratory, the 5 tests:
  - must not be conducted on the same day;
  - should, where practical, be conducted on different batches of test organisms; and,
  - should be conducted by at least two different analysts.

  The success of this element should also be demonstrated through the development of warning charts, and conformance to the laboratory's criteria for these warning charts.

- **Proficiency Testing**: As per CALA's policy a lab must perform satisfactorily in proficiency testing (options i, ii iv, v or vi) at least once prior to accreditation, and meet on-going proficiency testing requirements as outlined in P02-03 CALA *Program Description - Proficiency Testing Policy for Accreditation.*

3.0 RE-VALIDATION AFTER MOVING TO A NEW SITE

As stated in the CALA document entitled A115 - *Guidelines for Laboratory Relocation*, some areas of the laboratory may require special consideration (e.g., microbiology or toxicology). Considerations in toxicology include, but are not limited to:

- Maintaining environmental conditions of test organisms;
- Adequate separation of culture and testing areas;
- Ensuring the electrical supply ground fault is protected in wet lab areas;
- Health, proper acclimation, and response to reference toxicants of organism cultures in the new facility prior to resumption of testing; and,
- Quantity and quality of test organism culture water supply.

Labs should not have to go through a full validation providing they still have the same trained proficient personnel and quality management system that they had before they moved.

Two reference toxicant tests (conducted on separate days, preferably conducted by separate proficient analysts) would be sufficient to demonstrate continued proficiency after the move, provided that:

- Control response is acceptable;
- Reference toxicant result was within historical limits (± 2 standard deviations) for that lab (otherwise a new control chart needs to be started); and,
- Holding conditions in the new facility are suitable as evidenced by acceptable health of test organisms during holding.

Chemical analysis of the quality of the lab dilution/control waters might be useful to demonstrate that the new facility, water systems, and water supply are suitable.

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