

GUIDELINES FOR METHOD VALIDATION OF TOXICOLOGY 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 method 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 it can successfully conduct the method according to the 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 using a taxonomic guide, a culture collection curator (in the case of green algae, bacteria, etc.) or an external taxonomic expert (e.g., fish, invertebrates, etc.).
- **Culturing:** 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.).
- **Negative Controls and Validity Criteria:** Demonstration of consistent success in meeting validity criteria (e.g., mortality in controls) from 5 or more tests.

- **Positive Control Testing:** At least 5 successful¹ 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.

Important: If the reference test method design differs from the test design used for running test samples (e.g., in the case of freshwater sediment tests with *Hyalella azteca* where the reference toxicant test is 4-day water only acute lethality test and the definitive test is a 14-day sediment test for growth and survival) then in addition to reference toxicant tests a minimum of 5 tests should be conducted with a control using the definitive test method design to establish that the lab can consistently meet control validity criteria for all endpoints (e.g., survival, growth).

For the reference toxicant test data to be representative of toxicity test precision for the laboratory, the 5 tests should not be conducted on the same day, and should where practical be conducted on different batches of test organisms, and by 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.

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;
- 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.

¹ Prior to the generation of the first series of data, the laboratory would need to compare results to published values for known reference toxicants (e.g., phenol for fish, NaCl for daphnids).

4.0 IMPORTANT POINTS

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 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.