Introduction to Toxicity Test Methodology and Applications

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INTRODUCTION

The 1977 Clean Water Act established a regulatory framework to protect the chemical, physical and biological integrity of the nation's waterways and integrated approach to water quality protection that encompasses chemical-specific testing, biological assessments, and toxicity testing. Chemical-specific methods detect and measure individual contaminants (e.g. pesticides, metals) that pose a threat to human or aquatic life health. While useful, chemical-specific methods do not provide a complete picture, since there are many more chemicals present in water bodies than currently tested, and they do not account for effects that multiple stressors (e.g. additive or synergistic effects of chemical mixtures, or non-chemical such as flow or habitat alteration) have on aquatic life. Biological assessments (bioassessments) and toxicity tests are designed to fill this gap by evaluating the effects of multiple stressors on aquatic life. Bioassessments survey resident biota at a site of interest[1], while toxicity tests expose organisms to water or sediment collected from a sample site. The

[1] See the SWAMP Bioassessment website for more information on bioassessment.

majority of tests are conducted in a laboratory setting, but toxicity may also be assessed *in situ* depending on study objectives.

HISTORY

Toxicity tests or "bioassays" have been utilized for well over a century, with one of the first known studies dating back to 1863. In this pioneering report, researchers Penny and Adams examine the impacts of various industrial chemicals on the aquatic biota of several Scottish rivers by subjecting goldfish and minnows to spiked ambient water samples. Other toxicity studies were published sporadically until the practice became more widespread in the 1920s. State and federal agencies began adopting the practice in the 1930s, with standardized methodology first published in 1960 (Tarzwell 1978). It is interesting to note that, while test methodology has evolved since Penny and Adam conducted their experiments, the basic structure of their test design is reflected in modern-day toxicity tests.

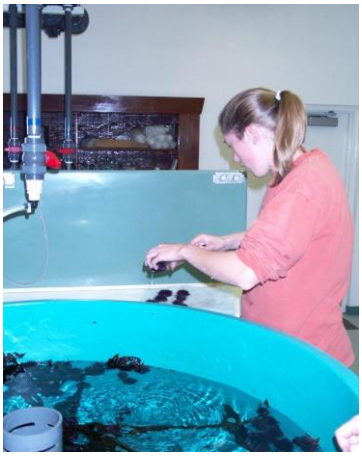

Figure 1. *S. purpuratus* fertilization test

TEST METHODOLOGY

In simplest terms, toxicity tests are used to determine the effects of pollutants, and pollutant combinations, upon aquatic biota. These tests are typically conducted by exposing laboratory-reared organisms—such as water fleas and fathead minnows—to a water sample or sediment sample for a set amount of time and comparing the resulting effects to a control consisting of uncontaminated water or sediment.

A program manager has multiple options to choose from when designing a toxicity monitoring study. Options are chosen based on how well they meet the objectives of the study (i.e. how well they answer the study questions), and whether compliance monitoring (effluent and receiving water) or ambient monitoring (inland surface waters) is required.

Sediment vs. Water Column

Water column toxicity tests detect the biological effects of contamination caused by soluble pollutants. Sediment toxicity tests are used to determine the impacts of contaminants that bind to sediments.

Acute vs. Chronic

Toxicity tests are divided into two categories that are characterized by the length of exposure to a sample: acute tests measure mortality and last 24, 48, or 96 hours, while chronic tests measure mortality as well as sub-lethal effects such as reductions in growth and reproduction over the course of seven or more days. Exceptions to this time frame exist, however, as some chronic toxicity test methods last 96 hours or less.

Test Species

Test species used for determining compliance under the National Pollutant Discharge Elimination System (NPDES) program are approved by the U.S. Environmental Protection Agency (U.S. EPA). These freshwater and marine test species are listed in U.S. EPA's seven toxicity test method manuals and include vertebrates,

invertebrates, and algae that are either laboratory-reared or wild-caught. Although ambient monitoring programs are not bound by these regulations, the toxicity test species utilized for the Water Board's Surface Water Ambient Monitoring Program (SWAMP) are all listed in these manuals.

Single and Multiple Concentrations

In multiple-concentration toxicity tests, a dilution series is created using laboratory water. A minimum of five concentrations and a control are required for compliance monitoring, and a dilution factor equal to, or greater than 0.5 is recommended by the U.S. EPA test methods. Single-concentration toxicity tests typically utilize a non-diluted sample (e.g. 100% ambient water) and a control. These tests are frequently used in ambient monitoring programs such as the Surface Water Ambient Monitoring Program (SWAMP).

STATISTICAL ANALYSIS

Once a toxicity test has concluded, the results are analyzed using a statistical approach. These analyses generally fall into one of two categories: hypothesis tests and point estimates. Hypothesis tests answer the question, "Does a critical concentration of the sample show a statistically significant decrease in organism response as compared to the control?" Hypothesis Tests are used to analyze data from both multiple- and single-concentration toxicity tests. For multiple concentration test, hypothesis tests such as Dunnett's and Steel's Many-One Rank Tests are be used to determine the "no observed effect concentration" (NOEC) and the "lowest observed effect concentration" (LOEC) endpoints. For single- concentration tests, the absence or presence of a statistically significant response is denoted with a "pass" or a "fail" endpoint, respectively. These endpoints are calculated using a t-test or the Test of Significant Toxicity (TST).

Figure 2. *H. azteca (amphipod)*

Point Estimates answer the question, "At what concentration is an effect observed, and is the critical concentration less than this value?" Point estimates utilize a dilution series to create a dose-response relationship that is analyzed usingthe Spearman-Karber Method, linear interpolations, and Probit analyses. These statistical approaches generate the effect concentration (EC), inhibition concentration (IC) and lethal concentration (LC) endpointsthat are typically represented as a percentage (e.g. the LC50 is the concentration that results in 50% mortality of the test organisms).

TOXICITY IDENTIFICATION AND CONTROL

If toxicity is demonstrated in a chronic or acute toxicity test, additional evaluations may be taken to identify the cause(s) of the toxicity. Typically, the first step involves the initiation of an accelerated toxicity testing schedule in order to determine whether or not the toxicity is ongoing. Upon confirmation, the discharger will be required to implement a Toxicity Reduction Evaluation (TRE) which is a stepwise process used to identify the causative toxicant(s). Although TREs vary widely in their complexity, they will frequently include a data acquisition phase, a facility evaluation, and a Toxicity Identification Evaluation (TIE). A TIE is a set of laboratory tests used to characterize the physical and chemical properties of the toxicity (e.g. solubility, volatility, decomposability, etc.) in an effort to identify the specific chemical(s) involved, and take the necessary steps to reduce or eliminate it.

QUALITY ASSURANCE

Comprehensive quality assurance programs for laboratories address every aspect of the toxicity testing process, from sample collection to data evaluation. Test review is an integral part of these programs and is conducted by both the testing laboratories and the Water Boards in order to ensure the accuracy of submitted data. For example, reference toxicant tests (i.e. positive controls) are a vital component as they are used to evaluate the health of test organisms and laboratory performance.

Acceptable toxicity test conditions, such as appropriate water temperature, photoperiod, and minimum number of replicates are determined by the program. Toxicity tests used to monitor discharges under the NPDES program (typically referred to as "whole effluent toxicity tests") must be conducted in accordance with the U.S. EPA's promulgated toxicity test manuals, which are incorporated by reference into Code of Federal Regulations, title 40, section 136.3. While the SWAMP program is not bound by these regulations, it utilizes nearly identical test requirements and recommendations in its toxicity Measurement Quality Objectives.

PROGRAMS AND APPLICATIONS

Toxicity testing is used in many Water Board programs as it is a versatile and reliable tool for detecting instream threats to aquatic biota (Grothe et al. 1996; De Vlaming and Norberg-King 1999). In addition to their extensive use in the NPDES program, toxicity tests are utilized in various Waste Discharge Requirements and by agricultural operations regulated under the Irrigated Lands Regulatory Program as well.

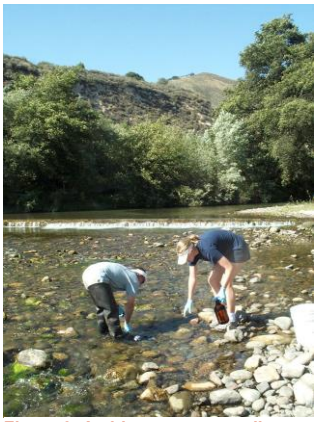

Figure 3. Ambient water sampling

Toxicity testing is also an integral component of ambient monitoring studies. The following is a selection of past and present SWAMP projects investigating toxicity in California's surface waters.

Toxicity in California Waters: A report that summarizes the findings of toxicity monitoring conducted by SWAMP and associated programs between 2001 and 2010.

Stream Pollution Trends (SPoT) Monitoring Program: This annual monitoring program analyzes trends in sediment toxicity in rivers throughout the state.

Acute Toxicity of Sacramento Area Urban Creeks to *Ceriodaphnia dubia*: The Central Valley Regional Water Board carried out this study of American River tributaries and urban creeks in 2007.

Toxicity Testing and Toxicity Identification Evaluation: This inter-agency project sought to identify compounds causing toxicity in Regions 3, 4, 7, and 9.

For a complete list of SWAMP's toxicity projects, please see the SWAMP websites listed in the reference section below.

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