PACIFIC ECORISK | ENVIRONMENTAL CONSULTING & TESTING

Charles R. Hoppin (Chairman) and Members
State Water Resources Control Board

August 21, 2012

29

c/o Jeanine Townsend, Clerk to the Board
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

Subject: Comment Letter – Policy for Toxicity Assessment and Control

Dear Chairman Hoppin and Members:

By way of introduction, Pacific EcoRisk is a nationally accredited, full-service aquatic toxicity-testing laboratory located in Fairfield, CA. We are among the larger toxicity testing laboratories in the nation, and perform an average of ~5,000 aquatic toxicity tests per year. We also have extensive experience performing testing with all of the freshwater, estuarine, and marine toxicity test species. We are pleased to have this opportunity to provide our technical input on the draft Policy for Toxicity Assessment and Control (TST Policy).

29.1

Issue 1. Initial Derivation of the Method: Did Setting the Sample CV Equal to the Control CV Skew the Determination of Alpha?

The key objective of the TST tool is to identify whether or not the magnitude of impairment of test response in a sample is sufficient that the sample should be considered to be toxic. Of particular concern is the tool's determination of "sample is toxic" when the magnitude of impairment relative to the Control is less than the RMD threshold of 25%, but still high enough to be considered an indication of toxicity based upon the inter-replicate variability observed for that test. By definition, reductions of this magnitude are in the "partial response" portion of the concentration-response curve. This is important, as it should be expected that, in general, the inter-replicate variability will be larger in the "partial response" part of the curve than it will be at either extreme of the curve (i.e., at the Control treatment part of the curve, or at the complete impairment part of the curve).

However, in the peer-reviewed article on the TST development and validation (Denton et al. 2011), the authors state that in their simulation analyses used to select alpha values, all analyses were based on "equal variances between the sample and control for each scenario examined". However, in the real world, CVs for samples with 10-25% effects should be expected to be higher than the control CVs. This means that the respective test alphas were generated using CVs for the sample that are overly conservative, and not representative of the CV that should be expected to occur at the 10-25% impairment levels for IWC samples (e.g. effluent, stormwater, etc.).

We are not statisticians, and are not presuming to fully understand how to select the most accurate alpha values. However, we are concerned that the existing approach may have resulted in a TST tool that is overly conservative in the indication of IWC samples being toxic, particularly as the magnitude of the sample response approaches 25%.

2250 Cordelia Road, Fairfield, CA 94534 *phone*: 707.207.7760 *fax*: 707.207.7916 *www.pacificecorisk.com*

29.2 → **Issue 2. Initial Derivation of the Method: Did the Use of Pre-2002 Test Data Skew the Determination of Alpha?**

In response to lawsuits regarding various aspects of the WET tests, the EPA's chronic toxicity test methods were revised in 2002. Amongst the more significant revisions was the change in the calculation of the sublethal reproduction and growth endpoints:

Test Species	Test Endpoint	Pre-2002 Calculation	Post-2002 Calculation
Fathead Minnow	Growth	Mean Dry Weight (weight of surviving fish/mysids divided by the number of surviving fish/mysids)	"Biomass" (weight of surviving fish/mysids divided by the number of fish/mysids originally loaded into the replicate (dead or live))
Americamysis bahia			
Menidia beryllina			

The objective of this change was to increase the sensitivity of the test endpoints by adding a survival component into the assessment of growth. While achieving that objective, we are concerned that the inevitable occurrence of random mortalities in the control treatments may effectively inflate the "biomass" CVs (relative to the pre-2002 mean dry weight CVs), such that the pre-2002 control CVs for the growth endpoints were lower than the post-2002 test CVs.

In the peer-reviewed article on the TST development and validation (Denton et al. 2011), the authors state that for their analyses, the "data for other WET test methods were obtained mostly after 2002, the year in which those test methods were substantially refined". The term "mostly" in that statement indicates that some pre-2002 test data were included. It is unclear whether any of the pre-2002 test data used by Denton et al. included the growth endpoints for these tests, and if so, whether the data had been re-analyzed to generate inter-replicate variability reflective of the post-2002 test analysis approach.

While again acknowledging that we are not statisticians, we are concerned the use of the potentially lower pre-2002 CVs in the analyses used to select test alpha values may have skewed the alphas relative to their application to post-2002 test data.

29.3 → **Issue 3. The Role of "Adjusting the Degrees of Freedom (df)" in Determining Whether a Sample is "Toxic" vs. "Non-Toxic"**

The EPA implementation document for the NPDES Test of Significant Toxicity (EPA 833-R-0-003) states: "For mean effect levels greater than 10 percent but less than the unacceptable toxicity RMD threshold (20 percent for acute and 25 percent for chronic WET tests), the TST approach will still declare the IWC non-toxic depending on within-test variability". This implies that as the reduction in test response (relative to the Control) moves from 25% down to 10%, there is a progressively moving set of 'within test' variabilities that will determine whether or not the reduction for a particular test will result in the sample being declared "toxic" vs. "non-toxic".

Based upon our review, it is not discussed in the EPA TST document, the peer-reviewed publication, nor the State's draft policy document just how this is achieved. We are left to conclude that this is being accomplished by use of the "adjustment of degrees of freedom (df)"

29.3 → step in the performance of the TST analysis (see Appendix A of the EPA TST document and the State's June 2012 draft policy document), as this adjustment includes measures of inter-replicate variability, such that increased variability results in a shift in the applicable critical t-value (from Table B1 in the EPA TST document and Table 2 in the State's June 2012 draft policy document) that make it easier to declare as sample as being toxic.

The absence of any discussion on how this "adjustment of df" was developed (indeed, the use of the "adjustment of df" step in the TST analysis is not even mentioned in Denton et al. 2011) is somewhat troubling as there is no readily apparent information available that discusses what levels of variability were deemed to be acceptable (i.e., resulting in a sample being declared non-toxic) vs. the variability that would result in a sample being "toxic".

29.4 → **Issue 4. Inability of TST to Evaluate *Ceriodaphnia* Chronic Test Survival Results**

We have already received several phone calls from discharger clients who have attempted to use the TST spreadsheet to analyze their data expressing concern that they were unable to analyze *Ceriodaphnia dubia* chronic toxicity test survival data. While their queries were easily resolved, they reflect that the current limitation of explaining this issue in the State's draft policy document as a footnote to Table 1 is inadequate. We recommend that this be more explicitly discussed in the main narrative body of the policy document.

Issue 5. Listed Tier 1 and Tier 2 Test Species

29.5 → Based on our extensive experience performing toxicity testing with the Tier 1 and Tier 2 species listed in Table 1 of the Policy for Toxicity Assessment and Control, we submitted comments to the State Water Board staff (dated January 21, 2011 – see Attachment A) indicating that there were ***very real challenges*** in obtaining many of the Tier 1 species used for the chronic "West Coast" marine methods. Although the State Board staff received our comments, they were not addressed in the June 2012 draft.

As noted in our previous submittal:

- Larger dischargers will be required to perform monthly toxicity testing;
- Many of the Tier 1 West Coast marine species ***are not available year round, or are only available from one vendor in the entire US***;
- The *a priori* prioritization of West Coast Tier 1 species for testing complicate the permit compliance process for the discharger since it will require the dischargers to open dialogue with the Water Board to receive approval to change to Tier 2 species during times of the year when the West Coast Tier 1 species tend to not be available for toxicity testing; and
- The prioritization of West Coast Tier 1 species will result in a necessary flip-flopping back and forth between test species, which simply complicates the permit compliance process for the discharger.

Succinctly put, we believe that the "tiered species" process that still exists in the draft policy should be removed, and the species should simply be listed as options for the discharger to use for their determination of reasonable potential and compliance monitoring.

29.6 **Issue 6. There is No Tier 2 Alternative Test Species for Kelp**.
An additional item not addressed in the current Tier 1 and Tier 2 process is that **there is no Tier 2 alternative for kelp**. If a discharger is expected to perform testing with a marine alga, they must have an alternative when the adult kelp fronds are not available or are producing poor quality test organisms. We recommended that the euryhaline diatom, *Thalassiosira pseudonana*, be included in the list of approved species because it is:

- a resident organism in both estuaries (e.g. San Francisco Bay) and along the coast of California;
- amenable for testing of both estuarine and marine effluents;
- an approved and fully-promulgated standard toxicity test method (i.e., ASTM);
- a methodological direct analog to the freshwater alga *Selenastrum capricornutum*;
- readily cultured in the laboratory;
- already being used for NPDES permit compliance testing in California.

We recommend that the policy be revised to include the estuarine/marine diatom, *Thalassiosira pseudonona,* as an approved test species.

Should the State Board staff have any questions or would like to discuss these issues in greater detail, please have them call us at their convenience.

Regards,

Digitally signed by Stephen L. Clark
Date: 2012.08.20 15:19:41 -07'00'

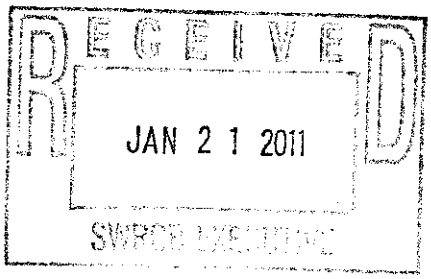
Stephen L. Clark
Vice President/Special Projects Director

Digitally signed by R. Scott Ogle
Date: 2012.08.20 14:25:02 -08'00'

R. Scott Ogle, Ph.D.
CEO/ Special Projects Director

Attachment A

PACIFIC ECORISK | ENVIRONMENTAL CONSULTING & TESTING



Jeanine Townsend
Clerk to the Board
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

January 20, 2011

Subject: Comment Letter – Policy for Toxicity Assessment and Control

Ms. Townsend:

By way of introduction, Pacific EcoRisk is a nationally accredited, full-service aquatic toxicity testing laboratory located in Fairfield, CA. We are among the larger toxicity testing laboratories in the nation, and perform an average of ~5,000 aquatic toxicity tests per year. We also have extensive experience performing testing with all of the freshwater, estuarine, and marine toxicity test species. We are pleased to have this opportunity to provide our technical input on the draft Policy for Toxicity Assessment and Control (TST Policy), with our comments focusing on some challenges related to performing testing with some of the species listed in the draft policy.

The list of test species that would be required for reasonable potential analyses and compliance monitoring is provided in Table 1 of the draft policy. This table lists the methods in two specific "tiers". Tier 1 methods are noted in the draft policy as being "preferred for reasonable potential analysis and routine monitoring". The applicable Water Board would have to approve the use of Tier 2 methods if the Tier 1 test species are unavailable. There is no justification in the draft policy for this hierarchical prioritization, but the policy clearly requires the selection of species from the EPA "West Coast" testing manual over the more routinely-used species from the EPA universal "Estuarine/Marine Chronic" manual. As the wide body of scientific literature has clearly established that no one species is consistently more sensitive to all contaminant classes than another test species, there is no justification for the tiered system for species selection in this policy. Furthermore, the current prioritization of test species does not address the limitations inherent to several of the West Coast species that are described in further detail below.

Giant Kelp, *Macrocystis pyrifera*

Giant kelp is a West Coast test species that requires the collection of reproductive kelp fronds from wild populations along the California coast. During summer periods, we have performed many rounds of kelp testing, and obtained poor germination rates for many (if not most) of these tests (this includes side-by-side testing of kelp obtained from different suppliers). In many of these cases, the kelp responses at the Lab Control treatment were below the EPA test acceptability criteria.

We researched the potential that environmental variables could effect the quality of the reproductive fronds to a point where they would not be viable for toxicity testing. A University

2250 Cordelia Road, Fairfield, CA 94534 *phone*: 707.207.7760 *fax*: 707.207.7916 *www.pacificecorisk.com*

of California report indicated that kelp reproduction becomes impaired at temperatures above 68°F. Seawater temperatures in the San Diego area where most of the kelp used in such tests are collected are often above 70°F for extended periods during the summer (a problem that might well become exacerbated as global warming becomes manifested). It seems evident that the higher seawater temperatures in the summer seriously compromises the ability of the kelp to meet the requirements for use in NPDES toxicity testing, a phenomenon that we have regularly observed.

We have similarly experienced challenges with obtaining viable reproductive kelp fronds during the winter periods immediately following storm events when the freshwater runoff from the rainfall flows out over the kelp beds, and again impairs the ability of the kelp to successfully reproduce.

With the current requirement for monthly testing in the proposed TST Policy, we believe that it will be inevitable that compromised kelp condition that can be expected to occur seasonally will similarly compromise the ability to successfully test with this species on a monthly basis.

Thalassiosira pseudonana

It is important to note that the draft policy does not include an alternative plant species for the giant kelp. Pacific EcoRisk highly recommends that the ASTM method using *Thalassiosira pseudonana* (an estuarine/marine diatom) should be added to the list of acceptable toxicity test methods for the TST Policy. This species can be cultured in the testing laboratory similarly to the routine lab culture of the freshwater algae *Selenastrum capricornutum* (effectively precluding seasonal complications). *Thalassiosira pseudonana* is also a species that is already included as a routine potential test species for chronic toxicity testing in NPDES permits in the San Francisco Bay region, and our lab's experience indicates that it is a routinely good test performer with low variability.

Mussels, *Mytilus sp.*

For many years, we have been performing the bivalve embryo development test for clients using EPA Test Method 1005.0, from the "West Coast" testing manual, which states that: "This method estimates the chronic toxicity of effluents and receiving waters to the embryos and larvae of several bivalve molluscs, the Pacific oyster (*Crassostrea gigas*) and the mussels (*Mytilus edulis*, *M. californianus*, *M. galloprovincialis*, *M. trossulus*)". We now understand that the tests that we have performed have been with *M. galloprovincialis*, the Mediterranean mussel (this is a non-native species that was introduced into the west coast waters many years ago, and which now dominates the *Mytilus* communities up and down the West Coast), which is now the only resident and readily available West Coast mussel species (more on this below). We have been pretty fortunate that this species has historically been amenable to being spawned year round. It is important to note, however, that the scientific literature does indicate that this species has been reported to occasionally fall out of reproductive condition in the summertime.

Organism availability during 2009 was an interesting case in point, as the *M. galloprovincialis* that we regularly obtain from our suppliers began not spawning in late June/July (this may have

been due to warmer than usual summertime water temperatures, as this was an El Nino year); we received communications from our suppliers documenting this problem. As a result, in preparation for the testing that we were to perform in July 2009, we had to obtain both *M. galloprovincialis* and oysters (*Crassostrea gigas*), with the intent to use the oysters should the *M. galloprovincialis* fail to spawn (oysters still being a common and readily available West Coast bivalve, whereas the alternative mussels, *M. trossulus* and *M. edulis*, are not [again, see more on this below]). The reason that the oyster is included in this set of potential test species is that it was the general situation that seasonal changes in reproductive condition by the oysters and *Mytilus* were often complementary. It was the practice of most labs back then to alternate between mussels and oysters on this seasonal basis. It is important to note that testing with multiple species to meet a compliance point for a single doubles the costs for the discharger. Unfortunately, neither species produced viable gametes, resulting in the lack of an acceptable compliance test.

So as to seek other options for our clients, we scouted all available bivalve sources and identified an East Coast company in New Hampshire that could provide *M. edulis* adults. Because of our concern regarding the scientific literature reports that *Mytilus* commonly stop spawning in the summer, we obtained a set of *M. edulis* and preceded to perform our client's toxicity test in August 2009, again resulting in a significant increase in test cost for our clients. The *M. edulis* spawned without incident, and the test was initiated, completed, and counted. Unfortunately, normal embryo development at the Control treatment was below the EPA's acceptability limit of 90% normal development, suggesting that the gametes were of suspect quality.

Subsequent studies of the seasonality of spawning condition in *M. edulis* indicate that the cause of the poor reproductive condition of the *M. edulis* at this time may have been due to the timing of their reproductive cycle. In their review of use of bivalves in toxicity testing, His et al. [1999] reported that for *M. edulis*, there is "a non-reproductive period during the summer" with spawning resuming in October or November. Newell et al. (1988) reported distinct seasonal trends in the reproductive condition of Atlantic Coast *M. edulis*, and specifically that *M. edulis* from New Hampshire (where our organisms were collected from) are at their worst reproductive condition at this time.

Again, it is to be noted that due to the need to collect adult organisms from wild (field) populations, seasonal influences can have a profound effect on the ability of the field organisms to perform successfully in lab testing.

Topsmelt, *Atherinops affinis*, and the Inland Silverside, *Menidia beryllina*

The chronic topsmelt, sheepshead minnow, and *Menidia* testing protocols are estuarine/marine fishes approved by the USEPA for the evaluation of effluent and receiving water toxicity. The EPA funded research for the development of "West Coast" species protocols in the early 1990s, and selected topsmelt as the native fish species for testing. However, it must be noted that *Menidia beryllina* are resident in embayments and estuaries in California (including the San Francisco estuary, where it should be considered to be an excellent surrogate for the sympatric endangered Delta smelt). With regards as to why *M. beryllina* should be listed as a Tier 1

species, there are some important issues to consider when selecting which species is best suited for use in complying with the proposed TST Policy.

Organism Availability

M. beryllina (and the sheepshead minnow) are readily available year-round at many culture facilities throughout the nation, which provides the toxicity testing laboratory with options as to which vendor provides the most healthy, and therefore reliable, organisms for use in the toxicity test. On the other hand, topsmelt are available from only one vendor nationwide. Although the quality of the topsmelt from this vendor is generally good, there are times when the organisms arrive at the laboratory in poor condition (i.e., due to shipping stress, unhealthy fish prior to shipping, etc.), resulting in several problems. There have also been times when the topsmelt culture facility has had spawning problems or other issues that resulted in no fish being available to purchase by testing laboratories. Testing with other species (*i.e., M. beryllina*) provides the added advantage that other vendors are available in this situation.

Organism Sensitivity

Pacific EcoRisk searched the USEPA toxicity database (ECOTOX) for the sensitivity of these species to metals (e.g., copper and zinc) and found that the sensitivity of these species was fairly similar. A cursory search of the literature for species comparison studies for pesticides resulted in the lack of a "most sensitive" species, as the most sensitive species was often compound specific. In summary, neither of the species can be considered the "most sensitive" to toxic stress. One clear finding of any search of toxicity databases is that there is far more information on the sensitivity of *M. beryllina* to toxicants than is the case for the topsmelt. Such data is critical for interpreting toxicity test data (i.e., for linking available analytical data to observed toxicity).

Supporting Toxicity and Biology Information

The *M. beryllina* testing has been around for decades. As a result, they have been used in a wide variety and number of biological and toxicological studies (i.e., you can think of *M. beryllina* as being the "white rat" of marine/estuarine fish studies). There are very few analogous studies with topsmelt. As a result, data for evaluating the test conditions or the toxicity of various contaminants to *Menidia* are much more comprehensive and available for use in evaluating the results of toxicity tests.

Test Response Variability

Having performed a large number of tests with both species, we feel qualified to state that the test responses of the topsmelt are markedly more variable to toxicant stress than is *M. beryllina*, as evidenced both in effluent tests and reference toxicant tests.

When flexibility exists as to which estuarine fish species to use for toxicity testing, Pacific EcoRisk recommend the use of *M. beryllina* since:

- they are readily available from multiple vendors year-round;
- there is far more environmental effects data in the literature for *Menidia* than for topsmelt and the sheepshead minnow; and

- they are commonly used in a variety of regulatory programs (e.g., NPDES permits, ambient monitoring studies, coastal monitoring, and special studies).

In summary, our considerable experience in testing with both species leads us to the professional conclusion that *M. beryllina* is the better test species, and should not be relegated to Tier 2.

Red Abalone, *Haliotis rufescens*
This test species was commonly used for compliance monitoring of marine discharges in the late 1990s when the abalone culture facilities invested the time to assure that the testing laboratories were receiving organisms that had "ripe" gonads such that they could be readily spawned in the laboratory and produce reliable (and acceptable) test results. However, this is no longer the case, and the few facilities that still supply adult abalone for toxicity testing purposes now 'pull' their organisms from the general culture population. This has resulted in considerable variability in the sexual maturity of the organisms that the testing labs can be expected to receive from these suppliers. Even as recently as within this past month, we received females from a trusted supplier that had no mature ovaries!

We have previously discussed this issue with Amy Wagner of the EPA's lab in Richmond, CA. She similarly reported that she had seen low quality "gravid" adults being provided by the commercial suppliers, and that her lab was forced to implement an "in-house" culture of abalone in order to ensure that they would have adequate quality adults. Given the relatively low frequency with which the abalone test is currently used by NPDES dischargers, it is simply not a viable option for non-coastal private sector labs, since we would have to establish a large culture facility requiring large amounts of seawater and copious amounts of kelp for food.

Mysid Shrimp, *Americamysis bahia*
The current list of Tier 1 species in the TST Policy does not include an estuarine/marine crustacean. This is a serious flaw, as crustaceans (and in particular *Americamysis bahia*) are typically among the most sensitive taxa (and often *the most sensitive*) to petroleum hydrocarbons, as well as being the most sensitive taxon to a wide variety of pesticides [which are targeted towards other closely-related arthropods (i.e., insects)], including organophosphate and pyrethroid pesticides.

Recommended Changes to the Draft Policy
The narrative in the draft policy providing the option to switch from the Tier 1 species to the more readily available Tier 2 species "if the Tier 1 species are unavailable" will complicate permit compliance by potentially causing regulated parties to flip-flop back and forth between Tier 1 species and Tier 2 species within a calendar year; the result would be the inconsistent use of test species for compliance monitoring during the course of a permit.

Furthermore, the current process for most Water Boards required to switch species is the submittal of a communication from the discharger to seek approval and the submittal of information/documentation supporting the request for the change in species. Since one of the State Board staffs objectives of the TST Policy is to simplify the permitting process for toxicity

testing, this goal will not be met in this circumstance and will require more case-by-case approvals to switch test species when the Tier 1 test species are not available.

More importantly, our company's 20+ year experience in performing these tests clearly indicates that the proposed Tier 2 species have many attributes that make them superior to some of the Tier 1 species. It is our best professional opinion that the tiered system for species selection should be eliminated from the policy, and the list of species selection should provide sufficient flexibility to the regulated parties to work with their testing laboratories to select species that are readily available from multiple vendors and that are of the highest quality for testing.

We also recommend that the policy be revised to include the estuarine/marine diatom, *Thalassiosira pseudonona,* as an approved test species.

We have a variety of scientific publications to support the conclusions and information above, so please don't hesitate to have the State Board staff contact us should they desire to learn more about these issues. Also, should the State Board staff have any questions or would like to discuss these issues in greater detail, please have them call us at their convenience.

Regards,

Stephen L. Clark
Vice President/Special Projects Director

R. Scott Ogle, Ph.D.
CEO/ Special Projects Director