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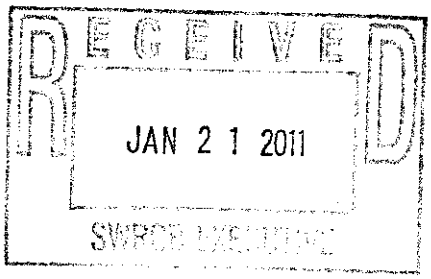

Jeanine Townsend
Clerk to the Board
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

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