**Date:** May 4, 2018

**To:** Surface Water Ambient Monitoring Program (SWAMP) Round Table

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**Subject:** Toxicity data determination and categorization

## **Determining Significant SAMPLE Toxicity with Statistical Analysis**

The SWAMP database currently utilizes a two-tiered approach to determining toxicity. This hypothesis-testing approach first compares the organism responses from ambient samples to the responses from appropriate controls using a student's t-test statistical comparison. This is followed by a comparison to a toxicity threshold value that is 20% less than the control response. There are four possible outcomes of this approach:

| Qualifier                                       | Definition  | Explanation  |
|---|---|--|
| Not Significant,<br>Greater Similarity<br>(NSG) | Sample response is not significantly different from control response based on statistical test.  Sample response is similar to control response (less than 20% difference).         | The result indicates that the sample is not toxic. This data can be used with confidence.                  |
| Not Significant,<br>Less Similarity<br>(NSL)    | Sample response is not significantly different from control response based on statistical test.  Sample response is less similar to control response (greater than 20% difference). | The result indicates that the sample may or may not be toxic, and that further investigation is necessary. |
| Significant,<br>Greater Similarity<br>(SG)      | Sample response is significantly different from control response based on statistical test.  Sample response is similar to control response (less than 20% difference).             | The result indicates that the sample may or may not be toxic, and that further investigation is necessary. |
| Significant,<br>Less Similarity (SL)            | Sample response is significantly different from control response based on statistical test.  Sample response is less similar to control response (greater than 20% difference).     | The result indicates that the sample is toxic. This data can be used with confidence.                      |

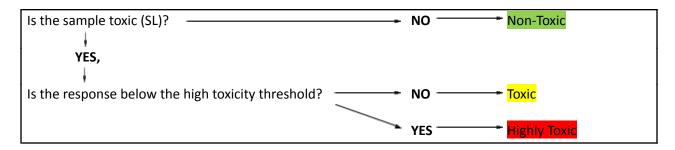
Of the four possible outcomes outlined above, only the SL qualifier is considered "toxic."



The current statistical approach could be replaced by other hypothesis-testing approaches such as the US EPA's Test for Significant Toxicity (U.S. EPA, 2010; Denton et al., 2011; Diamond et al., 2011). The TST uses the more robust Welch's t-test and incorporates a bioequivalence value in the formulation of the hypothesis. This process strengthens the overall power of the analysis. TST results are reported as either a "pass" (i.e., non-toxic) or "fail" (i.e., toxic), and the percent effect is reported to provide an indication of magnitude.

## **Categorization of SAMPLE Toxicity**

Each sample tested can be categorized as non-toxic or toxic based on statistics, but toxic sample can be further categorized as highly toxic using the flow chart below.



The high toxicity threshold is different from the toxicity threshold previously discussed, and was included to provide additional information regarding the magnitude of toxicity. The species specific thresholds were derived using methods based on Bay et al. (2007). The high toxicity threshold was derived for each endpoint as the mean between the most toxic 25<sup>th</sup> percentile of all toxic samples and the point of 99% confidence that the sample was toxic. The following table summarizes high toxicity thresholds for SWAMP toxicity test organisms.

| Toxicity Test Species     | Common Name         | Matrix       | Duratio<br>n | Endpoin<br>t | Threshold<br>(% of<br>Control) |
|---------------------------|---------------------|--------------|--------------|--------------|--------------------------------|
| Atherinops affinis        | Topsmelt            | Water        | 7 days       | Survival     | 22.2                           |
| Cariadanhnia duhia        | Daphnid             | Water        | 4 days       | Survival     | 33.2                           |
| Ceriodaphnia dubia        |                     | Water        | 7 days       | Survival     | 33.7                           |
| Eohaustorius estuarius    | Estuarine Amphipod  | Sedimen<br>t | 10 days      | Survival     | 37.8                           |
|                           | Freshwater Amphipod | Water        | 4 days       | Survival     | 66.1                           |
| Hyalella azteca           |                     | Sedimen<br>t | 10 days      | Survival     | 38.6                           |
|                           |                     | Sedimen<br>t | 10 days      | Growth       | 44.8                           |
| Mytilus galloprovincialis | Mussel              | Water        | 2 days       | Survival     | 77.2                           |
|                           | Fathead Minnow      | Water        | 4 days       | Survival     | 53.4                           |
| Pimephales promelas       |                     | Water        | 7 days       | Survival     | 57.4                           |
|                           |                     | Water        | 7 days       | Growth       | 57.2                           |

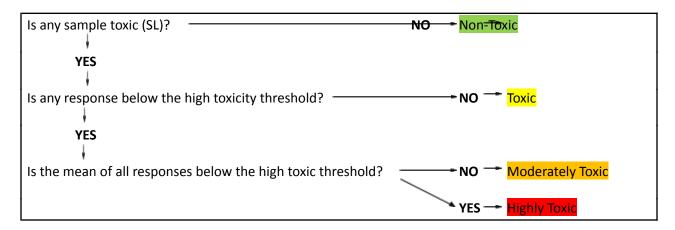


| Selenastrum capricornutum        | Green Alga     | Water  | 4 davs | Growth  | 43.8 |
|----------------------------------|----------------|--------|--------|---------|------|
| 3 Scienasti anii capinconnatanii | Gi CCII / liga | vvacci | T auys | GIOWLII | 73.0 |



## **Categorization of SITE Toxicity**

The process used to characterize the magnitude of toxicity at each site was designed to take into consideration the widely varying number of samples and test endpoints (such as amphipod survival or fish growth) among sites. All sites are categorized based on the most sensitive endpoint to produce a worst-case scenario result. This process considers both individual sample results and the mean results for sites with multiple samples. A fourth category, the moderate toxicity category, was added to the site characterization process to capture the magnitude of toxicity among multiple samples by comparing the mean response to the high toxicity threshold. Essentially, the moderate toxicity category was added to further characterize the site beyond the descriptors used for individual samples. The definition of moderate toxicity (having at least one highly toxic sample from the site) prohibits it from being used to characterize an individual sample.



## References

Bay, S.M., Greenstein, D., Young, D., 2007. Evaluation of methods for measuring sediment toxicity in California bays and estuaries. Technical Report 503. Southern California Coastal Water Research Project. Costa Mesa, CA.

Denton, D.L., Diamond, J., Zheng, L., 2011. Test of Significant Toxicity: A statistical application of assessing whether an effluent or site water is truly toxic. Environ Toxicol Chem 30, 1117-1126.

Diamond, J., Denton, D.L., Anderson, B.A., Phillips, B.M., 2011. It is time for changes in the analysis of whole effluent toxicity data. Integrated environmental assessment and management 8, 351-358.

U.S. EPA, 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document. EPA 833-R-10-004. Office of Wastewater Management. Washington DC.

