Reviewer Name: Gerald A. LeBlanc, PhD
Title: Professor and Head, Department of Environmental and Molecular Toxicology
Affiliation: North Carolina State University, Raleigh NC 27695-7633

1. Inclusion of the Test of Significant Toxicity in the draft Policy for Toxicity Assessment and Control.
The test of significant toxicity (TST) provides an efficient, cost-effective means of evaluating instream waste concentrations (IWC) for toxicity. The approach is statistically sound, reduces burden associated with the assays, and, by structuring the assay around a hypothesis of significant toxicity, provides incentive for precision in assay performance.

2. Use of effect level reporting in compliance determination.
The use of maximum daily effluent limitations (MDEL) and average monthly effluent limitations (AMEL), as described in the draft document, is an effective and appropriate approach to confirming toxicity associated with the IWC and triggering remediation activity. It is not evident why response levels of double those set for the TST are used in establishing compliance with these limitations. It seems that exceeding the TST, a statistically sound measure, should trigger exceedance of the MDEL. Furthermore, it is not clear why exceeding the MDEL ($\leq$0.4 effect level) requires a minimum of one follow-up test; while a lesser effect (>0.4<0.8) requires at least two follow-up tests. I would seem that any "fail" test should require at least two follow-up tests to ensure that the AMEL is not exceeded.

3. Comparative approaches to toxicity analyses.
Three design approaches to toxicity assessment are described: the NOEC approach, the point estimate approach, and the TST approach. The three approaches provide decidedly different descriptors of toxicity as is well described in the Draft Staff Report. Selection of an appropriate approach should be driven by the information needed from the toxicity assessment.

The NOEC approach seeks to define, or bracket, the threshold effect concentration of the toxicant as being between the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). This approach seeks to define the concentration of the toxicant below which the defined effect is not expected to occur.

The point estimate approach utilized a concentration-response curve, as defined though the testing of multiple concentrations of the toxicant, to interpolate a defined point on the curve (e.g., LC_{50} as the concentration that is expected to be lethal to 50% of the exposed organisms). The 50% response level is typically used as the endpoint in this approach as it has the greatest statistical strength. However, other endpoints also are used. For example, the EC_{05} (concentration of the toxicant that is expected at affect 5% of the exposed organisms) can be used as an estimator of the threshold effect concentration. The point estimate approach is most typically used to quantify the relative toxicity of a material, but also can be used to estimate threshold effect concentrations. The TST approach seeks simply to determine whether a defined exposure level of the test material causes a response that significantly deviates from controls. It provides no insight into the threshold effect concentration of the material, nor does it define

the relative toxicity of the test material. The purpose of routine and accelerated monitoring of wastewater is to test the hypothesis that the waster does exhibit measurable toxicity. All three test designs can be used to test this hypothesis. However, the TST provides the most direct, cost-effective, yet statistically sound means for establishing a lack of toxicity associated with the IWC.

4. Utility of the proposed accelerated monitoring schedule.

The proposed accelerated monitoring schedule, as described in the Draft Document, meets both federal requirements and state data needs. No concerns are noted.

5. General

The draft policy follows closely US EPA guidelines and no significant scientific concerns are noted. This reviewer is satisfied that the guideline will prove effective in the sagacious monitoring of wastewater for toxicity. The following are some general items that could improve clarity of the document.

Part I: Definitions, O. Replicate. Replicates are used to measure or quantify variability, but not to control variability.

Throughout the document, "response" and "effect" seem to be used interchangeable. The terms are not interchangeable since a "response" is associated with the test organism; while, "effect" is associated with the wastewater (e.g., the organisms respond to the effects of the wastewater). This misuse is most glaring in the equation on page 5 where response measures appear to be used to quantify an effect level. The units on both sides of the equation (response and effect) should be the same.

Part III.A.1 If all species exhibit no response to the IWC when establishing the most sensitive species for use in wastewater toxicity evaluations, is the discharger required to continue to use three species when evaluating reasonable potential? How does a discharger deal with a receiving water (with no waste water discharge) that is inhospitable to one or more of the species evaluated?

It would be helpful if all abbreviations used in the document are defined in Part I.