This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-96SR18500 with the U. S. Department of Energy.

DISCLAIMER

Update on Aquatic Toxicity/Whole Effluent Toxicity (WET) Issues, 2005

by
WINONA L. SPECHT
SAVANNAH RIVER NATIONAL LABORATORY

Approved by: _____
John B. Gladden, Section Manager
Environmental Analysis Section
Savannah River National Laboratory

Publication Date: July 2005

WESTINGHOUSE SAVANNAH RIVER COMPANY
SAVANNAH RIVER SITE
AIKEN, SC 29808

TABLE OF CONTENTS

		Page
EXECUTIVE SUMMARY		1
1.0	REGULATORY BACKGROUND	1
2.0	STATUS OF LEGAL CHALLENGES	2
2.1	U.S. Environmental Protection Agency	2
2.2	South Carolina Department of Health and Environmental Control	3
3.0	TECHNICAL ISSUES	3
3.1	Whole Effluent Toxicity Implementation Guidance	3
3.2	Estimation of Acute or Chronic Toxicity	4
3.3	Ion Imbalance	4
4.0	ECOTOX DATABASE	4
5.0	CURRENT RESEARCH AREAS	5
5.1	Risks of Heavy Metals Exposure Through Multiple Routes	5
5.2	Risk-Based Criteria for persistent Bioaccumulative Toxicants	5
5.3	Computational Toxicology	5
6.0	REFERENCES	6

EXECUTIVE SUMMARY

This paper summarizes recent changes in the field of aquatic toxicity/Whole Effluent Toxicity (WET) testing.

There are been numerous legal challenges to the validity of WET testing, both at the federal and state levels, but to date, the regulators have prevailed and WET testing is used as a regulatory tool to ensure that the biota of receiving streams are protected. The most recent ruling at the federal level was on December 10, 2004, when a federal appeals court in the District of Columbia upheld the validity of WET testing. At the state level, at the urging of the South Carolina Manufacturers Alliance, the state legislature passed a law (the South Carolina Aquatic Life Protection Act) in 2004 that requires the South Carolina Department of Health and Environmental Control (DHEC) to evaluate the accuracy and precision of the WET test. As a result, SCDHEC removed WET test limits from several NPDES permits. EPA took issue with the impact of the legislation and SCDHEC's actions, and as a result, EPA has taken over several NPDES permits from SCDHEC and threatened to revoke the state's delegated NPDES permit program. A new Act was signed into law in March 2005, which does not exclude the use of chronic toxicity testing for regulatory compliance. As a result, EPA has turned over the issuance of NPDES permits back to SCDHEC.

In December 2004, the U.S. EPA issued the Draft National WET Implementation Guidance document for review and comment. The guidance contains recommendations on the determination of "reasonable potential" for toxicity.

The EPA's ECOTOX database is a valuable resource of toxicity data for many chemicals. For those cases in which there are no toxicity data or very limited data available, the EPA has developed two models, the Interspecies Correlation Estimation (ICE) and the Acute to Chronic Estimation (ACE), for predicting toxicity.

Active areas of research include assessing the uptake of heavy metals via multiple routes of exposure, the development of risk-based criteria for persistent bioaccumulative toxicants, and the new field of computational toxicology, which utilizes modeling tools to predict toxic responses.

1.0 REGULATORY BACKGROUND

In 1972, The Clean Water Act (CWA) was enacted with the objective of "restoring the chemical, physical, and biological integrity of the Nation's waters. Along with other goals, CWA section 101(a)(3) states that .it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited. The U.S. Environmental Protection Agency (EPA) pursued this goal through implementation of the water quality standards program and the National Pollutant Discharge Elimination System (NPDES) permitting program. These programs have adopted an integrated strategy of water quality-based toxics control that includes three approaches: chemical-specific control, whole effluent toxicity (WET) control, and biological criteria/bioassessment (USEPA, 1991).

In 1995, EPA approved 17 WET test methods for use in NPDES permit monitoring [60 FR 53529; October 16, 1995]. The EPA-approved WET test methods resulted from many years of development and testing by EPA, States, municipalities, academia, and the regulated community. These WET test methods measure the acute and short-term

chronic toxicity of effluents and receiving waters to aquatic plants, invertebrates, and vertebrates from freshwater and marine environments. WET test methods approved for use in NPDES monitoring are listed in 40 CFR §136.3, Table IA.

2.0 STATUS OF LEGAL CHALLENGES

2.1 U.S. Environmental Protection Agency

Following the promulgation of WET test methods in 1995, various parties filed suit to challenge the rulemaking. Challengers argued that test results are variable and that the number of false positives is unacceptably high. To resolve that litigation, EPA entered into settlement agreements with the various parties. In a 1998 settlement agreement (Edison Electric Institute *et al.* v. USEPA, Settlement Agreement, July 24, 1998), EPA agreed to conduct an interlaboratory variability study of 12 EPA short-term chronic and acute whole effluent toxicity test methods (the WET Variability Study). EPA conducted the WET Variability Study from September 1999 to April 2000 and published preliminary results from the study in October 2000 (USEPA, 2000b; USEPA, 2000c. In total, the WET Variability Study generated interlaboratory precision data from testing more than 700 blind samples among 55 participant laboratories. In 2001, EPA submitted the preliminary results of the study for expert peer review. False positive rates were less than 5% for all WET test methods except the *Selenastrum* chronic test method conducted without EDTA (33.3%). Interlaboratory variability was described by the coefficient of variation (CV) calculated for point estimates. Interlaboratory CVs of LC50s (median lethal effect concentrations) ranged from 20.0% to 38.5% for acute test methods. Interlaboratory CVs of IC25s (25% inhibition concentrations) ranged from 10.5% to 58.5% for chronic test methods. The overall conclusion of the reviewers was that the variability of WET results was acceptable.

In December 2002, the EPA published its Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods. In this regulation, the EPA ratified approval of several test procedures for measuring the toxicity of effluents and receiving waters, but also withdrew two WET test methods from the list of nationally-approved biological test procedures for the analysis of pollutants. This action also revised some of the WET test methods to improve performance and increase confidence in the reliability of the results. This action was intended to satisfy settlement agreement obligations designed to resolve litigation over an earlier rulemaking that originally approved WET test methods.

The original litigants appealed the decision upholding WET testing. The case was argued on October 15, 2004. On December 10, 2004, a federal appeals court upheld an EPA test that the agency and states used to limit the cumulative toxicity of discharges from 6,500 facilities with the potential to harm sensitive species. The question before the US Circuit Court of Appeals focused on EPA's tests for nonlethal effects of a discharge, which the plaintiffs maintain are fallible and derived from flawed data. But the court held that the plaintiffs failed to prove EPA's use of the WET tests are "arbitrary, an abuse of discretion or otherwise not in accordance with the law." Among the plaintiffs' contentions rejected by the court is that WET tests produce an unacceptably high number of false positives. The plaintiffs define false positives more broadly than EPA, which claims that between 1% and 5% of WET tests detect toxicity when there is none. This second victory for the EPA increases the probability that WET testing will be used for compliance purposes for the foreseeable future.

2.2 South Carolina Department of Health and Environmental Control

In June 2004, the South Carolina General Assembly passed the Aquatic Life Protection Act (SC General Assembly Act 258, 2004), which effectively excluded the use of chronic toxicity testing for regulatory compliance in South Carolina. However, the Act contained a provision for the Act to be suspended if at any time the U.S. EPA publishes a Notice of Intent in the Federal Register to commence withdrawal of the South Carolina NPDES program as a direct result of the act. In July 2004, EPA Region 4 challenged the Act and responded by assuming the role of issuing all NPDES permits that contained requirements for chronic toxicity testing. Between July 2004 and March 2005, any NPDES permits that required chronic toxicity testing were issued by EPA Region 4, rather than SCDHEC. In March, 2005, the South Carolina General Assembly passed Act 25, which does not exclude the use of chronic toxicity testing for regulatory compliance. However, the bill does allow for the use of ambient receiving waters as control and dilution water in WET tests, exempts once-through non-contact cooling water which contains no additives from toxicity requirements, and allows dischargers to use WET testing protocols that utilize alternate species for toxicity testing. As with the earlier Act, Act 25 contains a provision for the Act to be suspended if at any time the U.S. EPA publishes a Notice of Intent in the Federal Register to commence withdrawal of the South Carolina NPDES program as a direct result of the act. Had the use of ambient receiving waters for control and dilution water been allowed by SCDHEC in the 1990s, SRS would probably had no need to develop the Alternate Species toxicity test, which would have saved a considerable amount of money and avoided the NOVs that the site received for toxicity failures, and ultimately the Consent Order that SRS entered into with EPA Region 4.

3.0 TECHNICAL ISSUES

3.1 Whole Effluent Toxicity (Wet) Implementation Guidance

On December 28, 2004, the U.S. EPA released the Draft National Whole Effluent Toxicity (WET) Implementation Guidance Under the NPDES Program (EPA-832-B-04-003) for public review and comment. The guidance was developed: to promote national consistency for NPDES WET testing, to reinforce compliance with existing NPDES regulations, including the reasonable potential determination regulations, and to clarify the EPA's position on WET testing by referring the reader to appropriate WET guidance documents. As discussed below, the guidance addresses a number of issues and questions that have been raised by states, regions and stakeholders. The guidance also outlines options that states may use to provide flexibility within the existing regulations.

The EPA guidance document recommends a step-wise approach that involves collecting high-quality WET data before or during the NPDES permit development process. This approach involves "reasonable potential" determinations based on small data sets. If no reasonable potential for toxicity exists, WET testing will not be required by the NPDES permit. For acute toxicity, the EPA recommends 0.3 acute toxic units (TU_a) as an acute criterion. An acute toxic unit is defined as the reciprocal of the effluent concentration that results in 50% mortality. For chronic toxicity, the EPA recommends 1.0 chronic toxic units (TU_c) as a chronic criterion, where a chronic toxic unit is defined as the reciprocal of the effluent concentration that causes no observable effect (NOEC).

3.2 Estimation Of Acute Or Chronic Toxicity

For many chemicals, and particularly for organic chemicals, there are no toxicity data available, or at best, very limited data. In order to provide an estimate of acute and chronic toxicity when data are lacking, the U.S. EPA has developed two predictive toxicological models, which include estimates of uncertainty. These models were developed primarily for use in probability-based ecological risk assessments. The Interspecies Correlation Estimation (ICE) model estimates acute toxicity values for numerous species using data for only one or a few surrogate test species (Asfaw et al., 2003). The Acute to Chronic Estimation (ACE) model predicts chronic toxicity from raw acute toxicity data (Ellersieck et al., 2003). These publications, including software, can be ordered free of charge by contacting Dr. Mark Ellersieck, University of Missouri (EllersieckM@missouri.edu or contacting the Librarian at the U.S. EPA Gulf Breeze Laboratory (pinnell.liz@epa.gov).

3.3 Ion Imbalance

The EPA has finally acknowledged that ion imbalance (either high or low) can cause toxicity, due to problems with osmoregulation. The most crucial ions are Ca, Mg, Na, K, Cl, Br, and SO_4. Total dissolved solids (TDS) is a good overall indicator of ion concentrations. If TDS >1340 mg/l, toxicity due to ion imbalance is likely. No low TDS has been specified (SETAC, 2004). If none of the TIE treatments remove toxicity from an effluent, an ion imbalance should be suspected. Approaches that can be used to deal with identifying toxicity due to ion imbalance include modeling; using a synthetic effluent that matches the ionic composition of the actual effluent (for Ca, Mg, Na, K, Cl, Br, and SO_4), or adding or removing ions to restore the ion balance. The EPA acknowledges that low ionic concentration (i.e. effluents that contain primarily well water) can result in toxicity, but they have not acknowledged that *Ceriodaphnia dubia* is inappropriate for use in very soft waters.

4.0 ECOTOX DATABASE

The EPA continues to update and add to the ECOTOX database. The ECOTOX database provides single chemical toxicity information for aquatic and terrestrial life. ECOTOX is an invaluable tool for examining impacts of chemicals on the environment. Peer-reviewed literature is the primary source of information encoded in the database. Pertinent information on the species, chemical, test methods, and results are abstracted and entered into the database. As of January 2004, there were listings for 10,325 chemicals, 6026 species, 19,171 references and a total of 472,705 records in the database. Of these almost 232,000 were for aquatic species and almost 241,000 were for terrestrial species. ECOTOX also contains a pesticide database that contains almost 5600 records for aquatic species and almost 4400 records for terrestrial species. ECOTOX can be accessed at: http://www.epa.gov/ecotox/.

5.0 CURRENT RESEARCH AREAS

5.1 Risks Of Heavy Metals Exposure Through Multiple Routes

A shortcoming in current assessments of the risk of exposure to metals in aquatic systems is an incomplete understanding of the importance of multiple exposure routes. To date, most studies have assumed that water is the most significant source of exposure; however, recent studies suggest that dietary exposure to metals could be important especially in areas where metal concentrations in the water column are low but are high in the food chain due to past contamination of sediments. The EPA is in the process of reviewing existing and ongoing work, as well as conducting research to assess how juvenile fish and other organisms are affected by exposure to metals their diet and the sediment.

5.2 Risk-Based Criteria For Persistent Bioaccumulative Toxicants

The EPA is currently developing a framework that will facilitate a more accurate determination of where and to what extent loadings of persistent bioaccumulative toxicants (PBTs) pose unacceptable ecological risks to aquatic ecosystems. Three major groups of PBTs will be addressed: halogenated organics, polycyclic aromatic hydrocarbons (PAHs), and organometallic compounds. The result of this research will be a scientific basis for the setting of risk-based water quality criteria to protect fish and wildlife populations from the effects of bioaccumulative toxicants.

5.3 Computational Toxicology

The EPA's Office of Research and Development (ORD) has initiated a research program on computational toxicology to better understand the relationships between sources of environmental pollutant exposure and adverse outcomes (U.S. EPA 2003). Computational toxicology is defined as the integration of modern computing and information technology with the technology of molecular biology and chemistry to improve EPA's prioritization of data requirements and risk assessments for toxic chemicals. Three strategic objectives of the initiative are to:
(1) improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes;
(2) provide predictive models for screening and testing; and
(3) improve quantitative risk assessment.
Computational toxicology includes several computational disciplines including: computational chemistry, which refers to physical-chemical mathematical modeling at the molecular level and includes such topics as quantum chemistry, force fields, molecular mechanics, molecular simulations, molecular modeling, molecular design, and cheminformatics; computational biology or bioinformatics, which refers to development of molecular biology databases and the analysis of the data; and systems biology, which refers to the application of mathematical modeling and reasoning to the understanding of biological systems and the explanation of biological phenomena.

6.0 REFERENCES

Asfaw, Amha, Mark R. Ellersieck and Foster L. Mayer. 2003. Interspecies Correlation Estimations (ICE) for Acute Toxicity to Aquatic Organisms and Wildlife. II. User Manual and Software. EPA/600/R-03/106. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. 14 p.

Ellersieck, Mark R., Amha Asfaw, Foster L. Mayer, Gary F. Krause, Kai Sun and Gunhee Lee. 2003. Acute-to-Chronic Estimation (ACE v2.0) with Time-Concentration-Effect Models: User Manual and Software. EPA/600/R-03/107. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. 20 p.

Society of Environmental Toxicology and Chemistry. 2004. Technical issue paper: Whole effluent toxicity testing: Ion imbalance. Pensacola FL, USA

South Carolina General Assembly. 2004. Aquatic Life Protection Act (Act 258).

South Carolina General Assembly. 2005. Aquatic Life Protection Act (Act 25)

U.S. EPA. 2000. Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136). EPA 821-B-00-004

U.S. EPA. 2001. Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods. EPA 821-B-01-004.

U.S. EPA. 2002a. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.

U.S. EPA. 2002b. Short-Term Methods For Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013.

U.S. EPA. 2002c. Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods; Final Rule Federal Register: November 19, 2002.

U.S. EPA. 2003. A Framework for a Computational Toxicology Research Program in ORD. EPA/600/R-03/065.

United States Court of Appeals For The District Of Columbia Circuit Court, Argued October 15, 2004, Decided December 10, 2004 No. 96-1062.