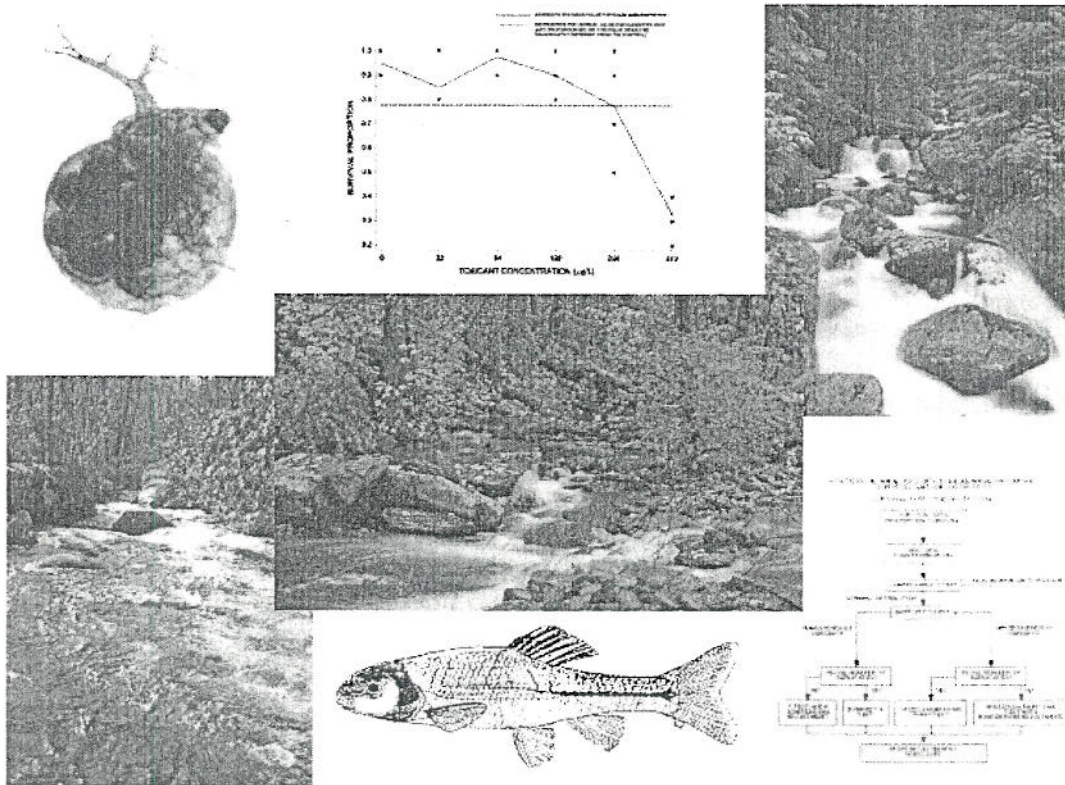EXHIBIT 8
ADMINISTRATIVE RECORD # 80

EPA

United States
Environmental Protection
Agency

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms

Fourth Edition

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8.5 EFFLUENT AND RECEIVING WATER SAMPLE HANDLING, PRESERVATION, AND SHIPPING

8.5.1   Unless the samples are used in an on-site toxicity test the day of collection (or hand delivered to the testing laboratory for use on the day of collection), they should be chilled and maintained at 0-6°C until used to inhibit microbial degradation, chemical transformations, and loss of highly volatile toxic substances.

8.5.2   Composite samples should be chilled as they are collected. Grab samples should be chilled immediately following collection.

8.5.3   If the effluent has been chlorinated, total residual chlorine must be measured immediately following sample collection.

8.5.4   Sample holding time begins when the last grab sample in a series is taken (i.e., when a series of four grab samples are taken over a 24-h period), or when a 24-h composite sampling period is completed. If the data from the samples are to be acceptable for use in the NPDES Program, the lapsed time (holding time) from sample collection to first use of each grab or composite sample must not exceed 36 h. EPA believes that 36 h is adequate time to deliver the samples to the laboratories performing the test in most cases. In the isolated cases, where the permittee can document that this delivery time cannot be met, the permitting authority can allow an option for on-site testing or a variance for an extension of shipped sample holding time. The request for a variance in sample holding time, directed to the USEPA Regional Administrator under 40 CFR 136.3(e) should include supportive data which show that the toxicity of the effluent sample is not reduced (e.g., because of volatilization and/or sorption of toxics on the sample container surfaces) by extending the holding time beyond more than 36 h. However, in no case should more than 72 h elapse between collection and first use of the sample. In static-renewal tests, each grab or composite sample may also be used to prepare test solutions for renewal at 24 h, 48 h, and/or 72 h after first use, if stored at 0-6°C, with minimum head space, as described in Subsection 8.5. If shipping problems (e.g., unsuccessful Saturday delivery) are encountered with renewal samples after a test has been initiated, the permitting authority may allow the continued use of the most recently used sample for test renewal. Guidance for determining the persistence of the sample is provided in Subsection 8.7.

8.5.5   To minimize the loss of toxicity due to volatilization of toxic constituents, all sample containers should be "completely" filled, leaving no air space between the contents and the lid.

8.5.6   SAMPLES USED IN ON-SITE TESTS

8.5.6.1   Samples collected for on-site tests should be used within 24 h.

8.5.7   SAMPLES SHIPPED TO OFF-SITE FACILITIES

8.5.7.1   Samples collected for off-site toxicity testing are to be chilled to 0-6°C during or immediately after collection, and shipped iced to the performing laboratory. Sufficient ice should be placed with the sample in the shipping container to ensure that ice will still be present when the sample arrives at the laboratory and is unpacked. Insulating material should not be placed between the ice and the sample in the shipping container unless required to prevent breakage of glass sample containers.

8.5.7.2   Samples may be shipped in one or more 4-L (1-gal) CUBITAINERS® or new plastic "milk" jugs. All sample containers should be rinsed with source water before being filled with sample. After use with receiving water or effluents, CUBITAINERS® and plastic jugs are punctured to prevent reuse.

8.5.7.3   Several sample shipping options are available, including Express Mail, air express, bus, and courier service. Express Mail is delivered seven days a week. Saturday and Sunday shipping and receiving schedules of private carriers vary with the carrier.

SECTION 9

CHRONIC TOXICITY TEST ENDPOINTS AND DATA ANALYSIS

9.1 ENDPOINTS

9.1.1 The objective of chronic aquatic toxicity tests with effluents and pure compounds is to estimate the highest "safe" or "no-effect concentration" of these substances. For practical reasons, the responses observed in these tests are usually limited to hatchability, gross morphological abnormalities, survival, growth, and reproduction, and the results of the tests are usually expressed in terms of the highest toxicant concentration that has no statistically significant observed effect on these responses, when compared to the controls. The terms currently used to define the endpoints employed in the rapid, chronic and sub-chronic toxicity tests have been derived from the terms previously used for full life-cycle tests. As shorter chronic tests were developed, it became common practice to apply the same terminology to the endpoints. The terms used in this manual are as follows:

9.1.1.1 Safe Concentration - The highest concentration of toxicant that will permit normal propagation of fish and other aquatic life in receiving waters. The concept of a "safe concentration" is a biological concept, whereas the "no-observed-effect concentration" (below) is a statistically defined concentration.

9.1.1.2 No-Observed-Effect-Concentration (NOEC) - The highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle (short-term) test, that causes no observable adverse effects on the test organisms (i.e., the highest concentration of toxicant in which the values for the observed responses are not statistically significantly different from the controls). This value is used, along with other factors, to determine toxicity limits in permits.

9.1.1.3 Lowest-Observed-Effect-Concentration (LOEC) - The lowest concentration of toxicant to which organisms are exposed in a life-cycle or partial life-cycle (short-term) test, which causes adverse effects on the test organisms (i.e., where the values for the observed responses are statistically significantly different from the controls).

9.1.1.4 Effective Concentration (EC) - A point estimate of the toxicant concentration that would cause an observable adverse affect on a quantal, "all or nothing," response (such as death, immobilization, or serious incapacitation) in a given percent of the organisms, calculated by point estimation techniques. If the observable effect is death or immobility, the term, Lethal Concentration (LC), should be used (see Subsection 9.1.1.5). A certain EC or LC value might be judged from a biological standpoint to represent a threshold concentration, or lowest concentration that would cause an adverse effect on the observed response.

9.1.1.5 Lethal Concentration (LC) - The toxicant concentration that would cause death in a given percent of the test population. Identical to EC when the observed adverse effect is death. For example, the LC50 is the concentration of toxicant that would cause death in 50% of the test population.

9.1.1.6 Inhibition Concentration (IC) - The toxicant concentration that would cause a given percent reduction in a non-quantal biological measurement for the test population. For example, the IC25 is the concentration of toxicant that would cause a 25% reduction in mean young per female or in growth for the test population, and the IC50 is the concentration of toxicant that would cause a 50% reduction.

9.2 RELATIONSHIP BETWEEN ENDPOINTS DETERMINED BY HYPOTHESIS TESTING AND POINT ESTIMATION TECHNIQUES

9.2.1 If the objective of chronic aquatic toxicity tests with effluents and pure compounds is to estimate the highest "safe or no-effect concentration" of these substances, it is imperative to understand how the statistical endpoints of these tests are related to the "safe" or "no-effect" concentration. NOECs and LOECs are determined by hypothesis testing (Dunnett's Test, a t test with the Bonferroni adjustment, Steel's Many-one Rank Test, or the Wilcoxon Rank

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9.4.1.2 The statistical methods recommended in this manual are not the only possible methods of statistical analysis. Many other methods have been proposed and considered. Certainly there are other reasonable and defensible methods of statistical analysis for this kind of toxicity data. Among alternative hypothesis tests some, like Williams' Test, require additional assumptions, while others, like the bootstrap methods, require computer-intensive computations. Alternative point estimation approaches most probably would require the services of a statistician to determine the appropriateness of the model (goodness of fit), higher order linear or nonlinear models, confidence intervals for estimates generated by inverse regression, etc. In addition, point estimation or regression approaches would require the specification by biologists or toxicologists of some low level of adverse effect that would be deemed acceptable or safe. The statistical methods contained in this manual have been chosen because they are (1) applicable to most of the different toxicity test data sets for which they are recommended, (2) powerful statistical tests, (3) hopefully "easily" understood by nonstatisticians, and (4) amenable to use without a computer, if necessary.

9.4.2 PLOTTING THE DATA

9.4.2.1 The data should be plotted, both as a preliminary step to help detect problems and unsuspected trends or patterns in the responses, and as an aid in interpretation of the results. Further discussion and plotted sets of data are included in the methods and the Appendices.

9.4.3 DATA TRANSFORMATIONS

9.4.3.1 Transformations of the data, (e.g., arc sine square root and logs), are used where necessary to meet assumptions of the proposed analyses, such as the requirement for normally distributed data.

9.4.4 INDEPENDENCE, RANDOMIZATION, AND OUTLIERS

9.4.4.1 Statistical independence among observations is a critical assumption in all statistical analysis of toxicity data. One of the best ways to insure independence is to properly follow rigorous randomization procedures. Randomization techniques should be employed at the start of the test, including the randomization of the placement of test organisms in the test chambers and randomization of the test chamber location within the array of chambers. Discussions of statistical independence, outliers and randomization, and a sample randomization scheme, are included in Appendix A.

9.4.5 REPLICATION AND SENSITIVITY

9.4.5.1 The number of replicates employed for each toxicant concentration is an important factor in determining the sensitivity of chronic toxicity tests. Test sensitivity generally increases as the number of replicates is increased, but the point of diminishing returns in sensitivity may be reached rather quickly. The level of sensitivity required by a hypothesis test or the confidence interval for a point estimate will determine the number of replicates, and should be based on the objectives for obtaining the toxicity data.

9.4.5.2 In a statistical analysis of toxicity data, the choice of a particular analysis and the ability to detect departures from the assumptions of the analysis, such as the normal distribution of the data and homogeneity of variance, is also dependent on the number of replicates. More than the minimum number of replicates may be required in situations where it is imperative to obtain optimal statistical results, such as with tests used in enforcement cases or when it is not possible to repeat the tests. For example, when the data are analyzed by hypothesis testing, the nonparametric alternatives cannot be used unless there are at least four replicates at each toxicant concentration.

9.4.6 RECOMMENDED ALPHA LEVELS

9.4.6.1 The data analysis examples included in the manual specify an alpha level of 0.01 for testing the assumptions of hypothesis tests and an alpha level of 0.05 for the hypothesis tests themselves. These levels are

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