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MEMORANDUM

SUBJECT: Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity (WET) Test Methods

FROM: Tudor T. Davies, Director
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TO: Water Management Division Directors, Regions I-X
Environmental Services Division Directors, Regions I-X

The purpose of this memorandum is to provide clarifications regarding the flexibility in the regulations promulgated at 60 Fed. Reg. 53529 (Oct. 16, 1995), which establish analytical test methods for the determination of whole effluent toxicity (WET). This information is important to the conduct of the WET test methods, and I ask that you share this memorandum with the States in your Region approved to administer the National Pollutant Discharge Elimination System (NPDES) permitting program. In addition, I would encourage States to make the information available to their NPDES permittees and appropriate test analysts.

The WET test methods are codified at 40 CFR part 136, including three test method manuals which are incorporated by reference. Because they are incorporated by reference, the test method manuals themselves constitute Federal regulations. The test method manuals do not, however, strictly prescribe every aspect of method conduct, and it is important to think carefully about how the WET test data will be used when making decisions on both the selection of the test species as well as the selection of individual test parameters where flexibility is allowed.

In many instances, the manuals use discretionary terms such as "may" or "should," with the understanding that the laboratory analyst must have flexibility to optimize successful test completion. In other instances, the manuals use stronger, compulsory terms such as "shall" or "must" to prescribe procedures necessary for nationwide standardization (specifically those procedures that assure the predictability of the methods to provide accurate results). When situations arise that are not addressed by the manuals, questions will become apparent -- where the method manuals neither authorize nor prohibit a procedure in question. In the majority of these instances, we anticipate that silence in the manual will indicate flexibility for the individual analyst. The remainder of this memorandum addresses issues that have already arisen in the areas of pH, ammonia, temperature, water hardness, test dilution concentrations, and a definition of "mean" number of cystocarps in the *Champia parvula* test.

1) pH and Ammonia Control

On p. 40 in the freshwater chronic manual (Sections 8.8.6 and 8.8.8) and p. 42 of the marine chronic manual (Sections 8.8.6), the manuals contain some discussion of the influence that pH and temperature have on ammonia toxicity. In this instance, the manuals do provide flexibility to the analyst to control artificial toxicity caused by pH drift provided that the analyst verifies the source of toxicity is, in fact, artificial. How to determine this "artificial toxicity" is described in Section 8.8.8. As the chronic manuals discuss the use of acids/bases to adjust the pH, likewise the use of CO2 in the headspace to control the pH is comparable.

The following are example techniques where pH can be controlled so that ammonia toxicity can be assessed. The pH can be controlled using appropriate procedures which do not significantly alter the nature of the effluent. For example, any procedure which removes ammonia, but does not remove other toxicants would be allowed. However, treatment with zeolite, would not routinely be allowed because it removes other toxicants. Controlling the carbon dioxide (CO2) environment, however, would be acceptable if carbon dioxide can be delivered directly to test chambers with airline tubing and a pipette or by using a complex solenoid system (on demand only). Another alternative is to maintain a closed carbon dioxide environment, delivering a solution of CO2 in oxygen to the closed system.

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2) Temperature

On p. 53 in the acute manual (Section 9.12.1) and in Tables 11-17, pages 57-70, the manual describes test temperatures for each acute test. The chronic test methods lists temperatures for each discrete species test protocol. A concern that has been raised is that, due to seasonal temperature fluctuations in receiving waters, the predictive capacity of the test methods does not reflect actual receiving water effects, and thus the methods should allow for seasonal variation in test temperatures. The acute manual does provide protocols for a wide variety of test species that encompass a broad range of test temperatures which could approximate the seasonal temperatures found in the receiving waters. Thus, to accommodate permittee concerns about the predictive capacity of the standardized test species to predict toxicity on a seasonal basis, permits may be written to include different approved test species for different seasons of the year. In this instance, however, the acute and chronic manuals do prescribe test temperatures in order to standardize the methods and limit test variability. Therefore, where variations in test temperature need to be taken into account, NPDES permits should include different approved test species, rather than authorizing seasonal variations in test temperatures for a given test species.

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3) Hardness

For the acute manual, the freshwater chronic manual, and the marine/estuarine chronic manual, Section 7 in each manual describes the use of dilution waters. In Appendix A of the acute manual, and in each specific test Section of the chronic manuals, the manuals describe how to culture or obtain the test organisms. Hardness of dilution water, as well as

hardness of water used to culture test organisms, may have an effect on successful completion of the tests. In some cases, the relative hardness of the dilution water compared to the organism culture water) may affect the expression of toxicity in the conduct of the tests, i.e., the accuracy of the tests at predicting toxicity. In this regard, the analyst has flexibility in performing the tests to optimize successful test completion. The type of the dilution water used in the effluent toxicity tests depends on the objective of the study. Tests can be conducted in the standard reconstituted dilution water to assess the absolute toxicity of the effluent. To evaluate whether or not the toxicity is present in the receiving water, the test can be conducted with a single grab sample of receiving water, or the hardness of the dilution water can be adjusted to match that of the receiving water (while taking care not to exceed the water hardness tolerance of the test organisms, which would cause stress to the test organisms, and affect the toxicity test results). In any case, if the dilution water is different than the culture water, then a second set of controls should be tested.

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4) Test Dilution Concentrations

The acute manual on p. 47 (Section 9.3.2), freshwater chronic manual on p. 42 (Section 8.10.2-3), and the marine manual on p. 45 (Section 8.10.2-3) describes whole effluent toxicity test dilution concentrations. Although permitting agencies might assume that a 100% effluent concentration must be included in the sample series, the manuals do not require this. The language "such as" is used to show an example of a concentration series. In some instances, such as marine tests, using 100% effluent as the highest effluent concentration is not realistic. In other instances 100% effluent may be unnecessary if the effluent is not highly variable; or the higher effluent concentrations are not of concern. The language in the manuals does, however, recommend bracketing the concentration of concern (such as the WET permit limit, or the receiving water concentration of concern). For example, if the effluent concentration in the receiving water is 12 percent, the dilution series could be 50 percent, 25 percent, 12.5 percent, 6.25 percent and 3.12 percent effluent.

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5) Acceptance Criteria for Champia parvula

The control acceptance criterion for the effluent sexual reproduction test using *Champia parvula* is an average of 10 or more cystocarps for the control treatment (p. 359 of the marine short-term chronic manual, #17). This means that some replicates may have fewer than 10 cystocarps, and some more than 10 cystocarps. This is acceptable as long as the overall mean number of cystocarps per replicate is at least 10 cystocarps.

The analyst may on occasion find that a single control replicate will have a greatly reduced or enhanced number of cystocarps relative to the remaining replicates. As with any toxicity test the analyst may have to determine the single "odd" replicate is an outlier. If the overall mean number of cystocarps is at least 10, with the low or high "odd" replicate excluded, then whether or not a single outlier is present does not effect the determination of control acceptability. If the remaining three replicates do not average 10 cystocarps, then the test should be repeated. An outlier, however, may effect the analyst's

conclusions concerning the level of toxicity in the samples tested along with that control.

In conclusion, I realize that additional questions may arise, and I encourage you to contact Teresa Norberg-King in the Duluth laboratory with these questions. She may be reached at (218) 720-5529. Over the summer EPA intends to publish a technical correction notice in the Federal Register for the three manuals and the part 136 rule. These corrections will not substantively change the content of the rule or manuals. During the next few months, EPA will be preparing that notice, and the list of corrections. Therefore, if you, or your staff are aware of mistakes or errors, please contact Teresa Norberg-King in Duluth on or before June 30, 1996. In addition, the Office of Wastewater Management is working with the Regions and labs to provide training on the conduct of these methods, as well as permit writing. For more information on this training effort, please contact Margaret Heber at (202) 260-7114. If you have any additional questions, or wish to discuss this further, please contact me at (202) 260-5400.

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URL: http://www.epa.gov/OST/WET/wetinfo.html
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