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# **Elimination of Whole Effluent Toxicity NPDES Permit Limits through the Use of an Alternative Testing Species and Reasonable Potential Analysis**

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## **ABSTRACT**

The cladoceran, *Ceriodaphnia dubia* (*C. dubia*), is required by the State of South Carolina to be used in whole effluent toxicity (WET) compliance tests in order to meet limits contained within National Pollutant Discharge Elimination System (NPDES) permits. Westinghouse Savannah River Company (WSRC) experienced WET test failures for no clear reason over a long period of time. Toxicity identification examinations on effluents did not indicate the presence of toxicants; therefore, the WET test itself was brought under suspicion. Research was undertaken with an alternate cladoceran, *Daphnia ambigua* (*D. ambigua*). It was determined that this species survives better in soft water, so approval was obtained from regulating authorities to use this “alternate” species in WET tests. The result was better test results and elimination of non-compliances. The successful use of *D. ambigua* allowed WSRC to gain approval from the South Carolina Department of Health and Environmental Control (SCDHEC) to remove WET limits from the NPDES permit.

## **KEYWORDS**

Toxicity, *Ceriodaphnia dubia*, *Daphnia ambigua*, NPDES, Reasonable Potential Analysis

## **INTRODUCTION**

The Savannah River Site (SRS) is a Department of Energy (DOE) facility located on a 310 square mile tract of land near Aiken, South Carolina. WSRC operates several facilities at SRS that discharge to surface waters under a National Pollutant Discharge Elimination System (NPDES) permit. Three of the outfalls regulated under this permit were required to meet compliance limits for whole effluent toxicity (WET). Two of the three outfalls consistently failed WET tests and WSRC was required to report permit limit violations to the regulating authority continually. Efforts at identifying the toxicant(s) were fruitless, so WSRC looked for other reasons why WET tests were failing. SRS effluents and receiving waters have very low hardness content. Research initiated by site personnel determined that the regulatory-required testing species, *C. dubia*, did not perform as well as another species, *D. ambigua*, in soft water (water hardness values less than twenty-five milligrams per liter). This fact led to additional research and ultimately to transmittal of a proposal to the South Carolina Department of Health and Environmental Control (SCDHEC) and region four of the Environmental Protection Agency (EPA-4) requesting approval to use this alternate species in SRS WET compliance tests.

## ALTERNATE SPECIES DEVELOPMENT

*C. dubia* is the predominant species used by the Environmental Protection Agency (EPA) for WET testing because it has been researched so extensively.



*D. ambigua*

It is easy to culture in the laboratory, making it amenable for use across the United States. However, it is not the only species that can provide adequate performance when determining toxicity in effluent discharges. *D. ambigua* was chosen by WSRC as a possible alternate testing species because it is commonly found in fresh waters of the southeastern United States. According to the literature, it is

fairly easy to culture in the laboratory. Most importantly, it can thrive in soft waters such as those found at SRS, making it a better species to use when determining toxic affects from effluents discharged into streams with low hardness.



*C. dubia*

Gaining regulatory approval to use *D. ambigua* in WSRC WET compliance tests required side-by-side testing against *C. dubia*. Research was conducted by personnel from WSRC and the University of South Carolina in which both species were cultured and tested for sensitivity to specific toxicants as well as to local surface waters. Results indicated that *D. ambigua* is a good replacement for *C. dubia* in soft waters and in streams that have naturally low pH values. Sensitivities between the two species were comparable. Full-scale tests using soft water from local streams resulted in reproduction problems in *C. dubia*, but seemed to enhance reproduction in *D. ambigua*. Study results were transmitted to SCDHEC and EPA-4 with a request to allow WSRC to use the alternate species in WET compliance tests.

EPA-4 and SCDHEC reviewed all data and, after a very careful and lengthy deliberation period, provided approval for WSRC to use *D. ambigua* instead of *C. dubia* in WET compliance tests. The approval process lasted over two years from start to finish, beginning with SCDHEC and ending with EPA-4. Additional data was provided to EPA-4 at their request during the two year period. Final approval was provided by EPA-4 only when the toxicologist at their Ecosystems Research Division in Athens, Georgia, was in agreement with all research data and conclusions.

The approval from EPA-4 was for *D. ambigua* only. This meant that WSRC could not use *C. dubia* in the event that problems arose while testing with *D. ambigua*. WSRC objected at first, but after healthy cultures of *D. ambigua* were generated quickly and WET testing was successful for several weeks, WSRC dropped their objection.

Even though EPA-4 did not approve the use of both species for compliance tests, tests were performed using both species initially just in case problems developed with *D. ambigua*. *D. ambigua* had been cultured and used only for the purpose of obtaining regulatory approval. It had not been used in actual compliance testing and there were unknowns regarding how consistently it would perform. The contract lab was asked to run side-by-side WET tests with both species using the exact same protocols that were required for compliance testing. The success rate was so high with *D. ambigua* that tests using *C. dubia* were eventually eliminated (Table 1).

**Table 1. Typical Results of Side-by-side WET Testing with *C. dubia* and *D. ambigua***

<i>Daphnia ambigua</i> Reproduction				<i>Ceriodaphnia dubia</i> Reproduction		
Outfall	Control	Effluent	Pass/Fail	Control	Effluent	Pass/Fail
A-01	20.1	28.4	Pass	31.2	15.2	Fail
A-11	20.1	24.6	Pass	32.3	24.1	Pass

## REASONABLE POTENTIAL ANALYSIS FOR NPDES PERMIT RENEWAL

Reasonable Potential is defined by EPA as “where an effluent is projected or calculated to cause an excursion above a water quality standard based on a number of factors....” Negotiation with SCDHEC for a new SRS NPDES permit was underway when EPA-4 approved the use of the alternate WET testing species. When it became clear that the use of *D. ambigua* would be successful, WSRC took the opportunity to ask SCDHEC what would be required to eliminate WET limits from the permit. SCDHEC determined that testing performed monthly for nine months would generate enough data for a valid reasonable potential analysis to determine whether or not water quality standards were being attained. Nine months of testing would cover three seasons and ensure that no problems developed during cold, warm and hot periods.

SCDHEC also indicated that, based upon EPA guidance, all tests would be required to exhibit no observed effect concentrations (NOEC) of 100 percent in order to eliminate WET limits from the permit. This requirement was based upon EPA guidance located in their “Technical Support Document for Water Quality-based Toxics Control” (known as the TSD) and the fact that the SRS effluents being tested discharge into ephemeral (zero natural flow) receiving streams. In order to show no reasonable potential, these effluents had to meet EPA ambient criteria concentrations of no more than one toxic unit (TU). A TU is defined by EPA as the reciprocal of the NOEC. For ephemeral streams where no blending is available, the equation is:

$$\text{Instream TU} = \text{Sample TU}_{\text{max}} \times \text{RPMF} \times \text{DF}$$

Where: Instream TU must be  $\leq 1$  to show no reasonable potential  
Sample TU<sub>max</sub> = maximum TUs from all toxicity analyses performed  
RPMF = reasonable potential multiplying factor (from Table 3-2 of TSD)  
DF = dilution factor from blending with receiving stream

To put it into simple terms, since there is no dilution provided within ephemeral streams, all nine monthly tests had to exhibit zero toxicity in order for SCDHEC to remove toxicity limits from the SRS NPDES permit.

WSRC had the tests performed from January through September 2002. The results were indisputable, resulting in NOECs of 100 percent in each outfall test (Table 2). The data was provided to SCDHEC who discussed it with EPA-4. Both regulating authorities concluded that there was no reasonable potential to exceed water quality standards. As a result, all WET limits were removed from the SRS NPDES permit that was reissued in November 2003.

**Table 2. Example WET Test Data Used for Reasonable Potential Analysis at A-01 Outfall**

Date	Reproduction versus % Sample Used						Result	IC25	NOEC	%Reduction
	Control	6.25%	12.5%	25%	50%	100%				
1/14/02	21.0	21.9	18.7	22.2	18.8	21.6	Pass	>100%	100%	0%
1/21/02	25.3	16.8	25.3	26.6	25.5	26.5	Pass	>100%	100%	0%
2/4/02	30.5	29.0	25.3	26.8	28.2	26.3	Pass	>100%	100%	0%
2/11/02	30.1	23.1	26.1	27.5	31.2	21.8	Invalid	-	-	-
2/25/02	26.3	25.7	24.8	26.7	27.2	26.8	Pass	>100%	100%	0%
3/4/02	25.6	22.6	24.5	25.6	24.5	11.2	Invalid	-	-	-
4/15/02	25.3	25.1	24.7	26.4	27.5	27.1	Pass	>100%	100%	0%
5/6/02	25.5	28.2	29.9	29.2	27.2	27.9	Pass	>100%	100%	0%
6/3/02	20.5	20.9	19.9	27.0	23.3	24.8	Pass	>100%	100%	0%
7/15/02	24.7	27.3	25.7	27.8	29.2	31.0	Pass	>100%	100%	0%
8/5/02	24.9	25.5	27.2	29.0	24.6	25.5	Pass	>100%	100%	0%
9/9/02	22.2	21.4	23.2	20.9	22.5	22.5	Pass	>100%	100%	0%

Note: Invalid tests were caused by laboratory problems and not by sample toxicity

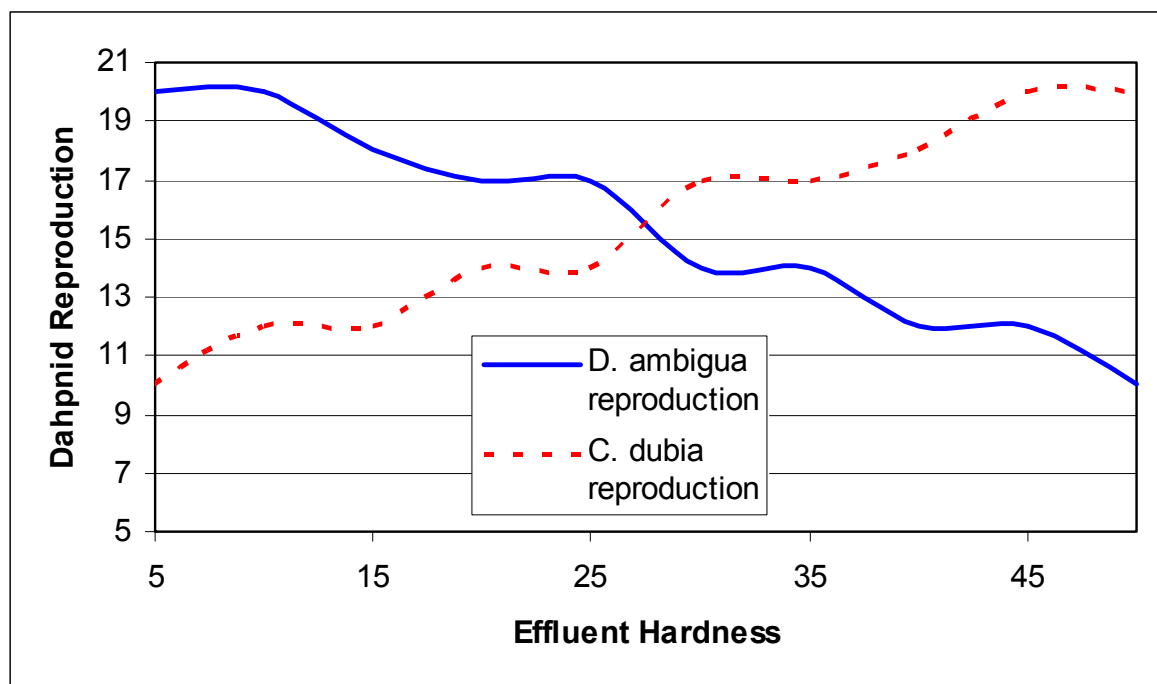
## APPLICABILITY AND DISCUSSION

NPDES permittees who are experiencing unsolvable WET test failures and permit limit violations when using *C. dubia* should give consideration to the use of an alternate testing species. WSRC was burdened with failures almost monthly. Laboratory analyses and toxicity identification evaluations could not uncover a definitive cause. Whenever a potential cause was considered, further evaluation lead to a dead end. It wasn't until the use of an indigenous species was initiated that WSRC discovered that there wasn't a toxicity problem. Rather, the problem was with the test or, more specifically, with the test organism required for use in low hardness effluents.

*D. ambigua* was determined to be a better species to use than *C. dubia* for WET tests in SRS effluents, mainly due to its relationship to water hardness. SRS surface waters and effluents typically have hardness values below ten milligrams per liter. Since research indicated that *D. ambigua* was indigenous to SRS, it was no surprise that this species performed better in WET tests analyzing low hardness waters.

Ephemeral streams also play a part in deciding which species to use in WET tests. Typically, a stream is considered ephemeral if it flows only in response to a rain event. *D. ambigua* is a good choice in low hardness situations. If an effluent that discharges into an ephemeral stream has low hardness, then the stream (which may be considered to be effluent-dependent) will also likely have low hardness. Conversely, effluents that discharge into perennial streams with higher hardness values (e.g., fifty milligrams per liter or greater) may not reap any benefit from the use of *D. ambigua*. NPDES permittees with WET limits should analyze their effluents and receiving streams for hardness content. It may be helpful to perform WET tests using both *D. ambigua* and *C. Dubia* at various hardness concentrations to determine which species is most suitable in a particular effluent/receiving stream situation. Figure 1 depicts what might result from such an evaluation.

**Figure 1. Possible effects of increased hardness on *D. ambigua* and *C. dubia* reproduction**



As shown, it is likely that there are hardness ranges for any given effluent where *D. ambigua* may perform better, where both may perform similarly, and where *C. dubia* may perform better. This information should help permittees to determine which species will work best under their conditions.

The use of *D. ambigua* has not been without its troubles. Laboratory cultures are healthy most of the time, but can experience set-backs. For example, during late 2003 problems developed with cultures of *D. ambigua* that caused all WET tests to be invalid due to mortality and lack of reproductivity in controls. The source of this “culture shock” was not obvious, so culture water, food, environmental conditions, disease, loss of genetic vigor and nutrition were each considered.

Laboratory culture water was eliminated as the probable cause since no new chemicals had been introduced and the process to generate it had not changed. Further, attempts to re-culture *D. ambigua* in other types of water known to have worked well in the past (moderately hard water, natural pond water, effluent with natural organics) were not significantly successful. Food (trout chow, alfalfa, *selenastrum*) was eliminated as the probable cause after examining it for pesticides and heavy metals to ensure that contaminants weren’t present. Environmental conditions (light, temperature, handling by technicians, etc.) were ruled out after determining that cultures of *C. dubia* were not experiencing any problems under identical conditions. Disease and genetic vigor were discounted when the results of a test that compared daphnid reproductivity within laboratory and natural pond water exhibited no significant difference. The blame was ultimately placed upon dietary issues (essential elements, fatty acids, vitamins, etc.), although this was never conclusively proven to be the cause.

Once cultures had recovered, it took some time for them to produce significant numbers of healthy neonates. This problem manifested itself during a month when a second round of WET analyses was necessary. In this situation, extra tests were performed with neonates that were apparently not as strong as usual and this resulted in some tests being invalid. It was concluded from this episode that cultures of *D. ambigua* must be provided an adequate recovery period after culture problems have been corrected.

To date, there is only one contract laboratory that is certified by the state of South Carolina to perform WET tests using *D. ambigua*. A second laboratory attempted to become certified, but was never able to complete the process due to difficulties maintaining viable cultures. Having only one laboratory certified to perform WET compliance tests is problematic. If troubles arise, there is no backup and permittees can find themselves out of compliance for not being able to report data that is required by their permit.

EPA's methodology for determining reasonable potential for WET is overly stringent. At the statistical confidence interval of ninety-five percent normally used in the test, WET failures may be expected to occur once in every 20 tests. Showing no reasonable potential for a discharge into an ephemeral stream over a period of several months when each test result must have an NOEC of 100 percent requires a level of luck. Just one NOEC less than 100 percent would exhibit reasonable potential and prevent elimination of permit limits. One solution to this dilemma might be to allow permittees to omit one test failure out of several months of testing as an outlier.

Another critical requirement when determining reasonable potential is the use of the appropriate species. SRS experienced continual monthly compliance test failures using the inappropriate species, *C. dubia*. It would have been impossible to have completed a successful analysis of reasonable potential without using *D. ambigua*. Permittees who are experiencing intermittent, unexplained, toxicity problems with *C. dubia*, especially if they discharge to an ephemeral stream, will never be capable of completing a successful analysis of no reasonable potential. It would be helpful if EPA would provide a written methodology describing how to complete an analysis of reasonable potential for WET. This methodology could include details about seasonal testing requirements and options for species that may be used.

The price for permittees to develop, test, and approve the use of an alternate WET testing species is high. SRS estimates that it cost approximately \$260,000 in laboratory fees and manpower before *D. ambigua* was approved for use, excluding laboratory and labor costs associated with the reasonable potential analysis. It also took expert support from a contract laboratory that was willing to put the required time and effort into a project that had just as much chance of failure as it did success. Although inquiries from other permittees about the possible use of *D. ambigua* on their effluents have been received by the laboratory, profitability for their efforts is not guaranteed. It would be helpful if EPA would simplify the process that permittees must use to develop and establish an alternate WET testing species.

It appears that state environmental regulators handle WET testing problems associated with hardness in varying ways. Some states may not have addressed the problem at all. One state allows hardness to be added to the WET test when very soft waters are analyzed. Another state may allow permittees to seek relief from WET permit limits under a regulatory variance

provision when toxicity test problems are caused by low hardness. Still another may allow side-by-side WET tests at differing hardness concentrations in order to show that soft water is the cause of toxicity. It is unfortunate that there is so much confusion about WET testing and how to deal with variability associated with hardness. Many of these issues could be resolved if EPA would perform the research necessary to better define the appropriate species that may be used in various regions of the United States. Without this effort, states and their permittees are likely to continue to struggle with WET test problems.

## CONCLUSIONS

WET test failures often result in violations of NPDES permit limits. In many cases, permittees cannot determine the cause even though they expend considerable dollars in the effort. SRS was able to determine that on-going WET test failures resulted from the use of an improper testing species and were able to convince EPA-4 and SCDHEC to approve the use of an alternate species. Success with the alternate species led to removal of WET limits by conducting a reasonable potential analysis which proved that SRS was not discharging any toxic effluents. Other NPDES permit holders with WET limits may also have success using *D. ambigua* if their effluents and associated surface waters exhibit low hardness.

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