

than 2 TU_c [chronic toxicity units]. The NOEC endpoint did somewhat better: seven failures in 23 valid tests, [and] three of the seven were greater than 2 TU_c. Legally, all nine WET test failures may constitute potential permit violations regardless of the magnitude of failure. Copies of the study results and methodology can be downloaded at <http://www.toxicity.com>.

The most critical question is whether the SFW used in the study might be toxic. Two of three labs testing the sample said it was not. If the sample water was "marginally toxic," as Dr. De Lisle claims, then the most sensitive tests would identify it first. Quite the opposite occurred. There was a statistically significant positive correlation between test sensitivity and the probability of passing the test ($p=.03$, $r^2=60\%$, $n=23$).

Dr. De Lisle suggests that our sample water was "slightly toxic" because it did not conform to ASTM Type-I standards. EPA guidance recommends Milli-Q "or equivalent" water. Our production process did produce equivalent (or better) water. We used activated carbon, deionization, and microfiltration to produce presumptively nontoxic water. Subsequent testing by the chemistry lab, using more sensitive instrumentation, confirmed that its treatment system produces 18 megaohm water that meets ASTM Type-I standards.

EPA guidance strongly recommends relying on dilution water that is "routinely used with success in the laboratory" (EPA/600/4-91/002 @ p. 11). We chose the sample water based on our long history of using it to culture *Ceriodaphnia* and meet EPA's test acceptance criteria for control performance. Our lab has consistently passed EPA's DMR-QA [Discharge Monitoring Report Quality Assurance] tests each of the last 7 years. If our dilution water was "marginally toxic," there would be a tendency to underestimate the true toxicity of EPA's check samples. That hasn't happened.

Dr. De Lisle theorizes that trace levels of pesticides or heavy metals may have caused the observed "toxicity." In pre-study testing, we identified these potential problems and revised our procedures to reduce the risk by pre-rinsing sample bottles. There was no evidence of "toxics in toxic amounts" in the water that was shipped to the bioassay labs. There is a

statistically significant concentration-response relationship when all of the valid data is pooled ($p=0.008$; $r^2<0.5\%$; $n=1361$). This means 99.5% of all the observed differences in reproduction were caused by factors unrelated to our sample even if we assume it was "slightly toxic." That is not nearly a strong enough relationship to explain the large number of false positives reported.

Finally, Dr. De Lisle suggests that bioassay technicians may be subconsciously biased toward finding toxicity when sample bottles are labeled "reference toxicant." How does the technician's opinion influence *Ceriodaphnia* reproduction or the mathematics of statistical analysis? The possibility of "subconscious bias" makes it essential to evaluate laboratory performance in the blind.

Scientific objectivity depends on the premise that the labs are not aware that they are being evaluated. In our study, we also did as Dr. De Lisle suggested. The samples were labeled with a wide variety of different identifiers, including: "reference toxicant," "effluent," "process control sample," and other less meaningful alphanumeric descriptors. Systematic bias is unlikely.

Dr. De Lisle was right about two things. He stated that if our study results were true, then half the dischargers around the country would be performing TREs [toxicity reduction evaluations]. They are. Random low level chronic test failures have triggered many costly and unproductive investigations. Two new Water Environment Research Foundation studies also document an unexpectedly high rate of inconclusive TREs and statistical anomalies in WET testing throughout the nation.

Second, Dr. De Lisle stated that it is "difficult to prepare good blanks." Yet, dischargers whom he claims lack the "equipment, supplies, and expertise to do so" are legally required to produce hundreds of millions of gallons of nontoxic wastewater every day. Dr. De Lisle is correct in concluding that this leads "to a lot of frustration and anger for all involved." If water which has been deionized, treated with activated carbon, and microfiltered cannot be expected to pass EPA's toxicity tests, it is no wonder angry and frustrated [publicly owned treatment works] filed

suit challenging the standard methods for WET.

To settle the lawsuit, EPA agreed to conduct a large-scale study of interlaboratory variability in WET testing. This will provide an excellent opportunity to replicate our results. In addition, we have asked the Society of Environmental Toxicology and Chemistry (SETAC) to initiate an independent peer-review of the WESTCAS WET Method Blank Study. Their recommendations, along with Dr. De Lisle's, will provide invaluable assistance as EPA prepares to conduct its own study.