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Interlaboratory Comparison – Acute Toxicity Tests Using Estuarine Animals

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Environmental Research
Laboratory
Gulf Breeze FL 32561

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RESULTS: INTERLABORATORY COMPARISON--ACUTE TOXICITY TESTS USING ESTUARINE ANIMALS

by

Steven C. Schimmel
Environmental Research Laboratory
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FLORIDA 32561

INTRODUCTION

Under Section 4 of the Toxic Substances Control Act (TSCA), the administrator for the Environmental Protection Agency (EPA) can require environmental effects testing of chemical substances if (1) the manufacture, use, distribution, or disposal of that substance may present an unreasonable risk of injury to the environment; or (2) the substance will be produced in substantial quantities and is expected to enter the environment, and there are insufficient data to predict the effects of the chemical substance on the environment.

When the administrator issues a test rule for the performance of environmental effects testing, he must also provide test standards to be used in the development of test data. Before test standards are proposed, steps should be taken by the Agency to insure that data developed by each test standard are adequate and reliable.

This report summarizes the results of "round-robin" or precision tests to validate proposed test standards and to determine the degree of variability between data developed by different researchers using the same methodology. The tests were performed by 2 of EPA's Office of Research and Development laboratories and 4 laboratories under contract.

Contractors were instructed to follow the American Society for Testing and Materials (ASTM) "Proposed Standard Practice for Conducting Basic Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" (Draft 6) because EPA's Office of Toxic Substances (OTS) Fish Acute Toxicity Test Standard and the Mysid Shrimp Static and Flow-through Acute Toxicity Test Standard were not completed at the time of the contract award. Notebooks, progress reports, and final reports of the contract laboratories were examined to determine laboratory adherence to the ASTM test methods, which are similar to the now completed OTS Test Standards.

The ASTM document specifies certain required test methods or conditions in order for a test to be considered satisfactory. These conditions are always associated with the word "must." For example, Section 11.1.1 states (in part) that "for static tests at least 10 organisms must be exposed to each treatment." Suggestions for good test practices are generally phrased in words such as "should," rather than "must." For example, Section 10.3.1 states (in part): "In any single test all fish should be from the same year class and the standard length of the longest fish should be no more than twice that of the shortest fish." Each laboratory's final report was carefully scrutinized to determine if the "musts" in the ASTM method were fulfilled. If not, an attempt was made to determine if the test results were affected.

Test chemicals used in the "Round Robin" were silver nitrate and endosulfan. Selection of these chemicals depended on a number of factors, including:

(1) toxicity to selected species at or below water solubility; (2) chemical type (an organic and an inorganic); (3) scarcity of toxicity data in the literature on tests with these chemicals and species; (4) ease of chemical analysis; and (5) relatively low mammalian toxicity.

The test species selected for the "Round Robin" were the copepod, Acartia tonsa, the mysid shrimp, Mysidopsis bahia, and the sheepshead minnow, Cyprinodon variegatus. One static toxicity test was required from each laboratory for each species exposed to both endosulfan and silver nitrate. The LC50 values (concentration estimated to kill 50% of the test animals) required for these static tests were to be based on nominal concentrations. In addition, each laboratory was required to conduct flow-through tests on M. bahia and C. variegatus, using endosulfan and silver nitrate. The LC50 values obtained from the flow-through tests were to be based on both measured and nominal concentrations.

TOXICITY TEST RESULTS

Toxicity test data from each of the laboratories were analyzed at the Environmental Research Laboratory, Gulf Breeze, using programs for probit analysis, moving average and the binomial method. Results of statistical analyses of all test data are listed in Tables 1, 2, and 3.

Acartia tonsa Toxicity Tests

Eleven of the twelve A. tonsa toxicity tests produced data that were amenable to statistical analysis (Tables 1 and 2). Lab 5 did not produce a successful test with A. tonsa in an exposure to silver nitrate. This laboratory, as well as Lab 6, was totally inexperienced in handling and testing the species. Eleven attempts made by Lab 5 to collect field populations of this copepod and hold them in the laboratory for at least four days resulted in inadequate control survival. An LC50 in the endosulfan test was obtained by Lab 5 only when salinity was maintained at ambient ($\sim$8 $^{o}/_{oo}$) salinity conditions. Any attempt to raise the salinity gradually over a long period of time caused excessive (>15%) control mortality. Lab 6 attempted at least 30 toxicity tests with field-captured animals; all were unsuccessful. After obtaining lab-cultured individuals ($\sim$14 days old), two successful tests were conducted in five trials. Of the six laboratories, three maintained continuous A. tonsa cultures. No problems of control mortality were mentioned by any of the three. Only one of the three laboratories (Lab 3) that tested field-captured copepods conducted successful tests. The Environmental Research Laboratory, Gulf Breeze wished to address the problems of high control mortality in field-captured A. tonsa by contracting Lab 3 (outside the scope of the present contract) to conduct control survival experiments with us. Two trials were attempted, using Lab 3's personnel and methods of collection, holding, acclimation, and testing. Neither trial was successful.

The problem of excessive control mortality is two-fold: (1) The life expectancy for A. tonsa is approximately 30 days, and a 96-hour test is approximately 13% of that life expectancy; and (2) The chance is great that a significant number of field-collected animals are senescent and will die

during a test, whether in the control or experimental group. Thus, the problems of a long test period (relative to the life expectancy) and that of senescence in field-collected stocks produce excessive control mortality. The same argument might be made for field captured M. bahia, but in these tests each laboratory used lab-cultured juveniles (≤48 hours old).

We recommend that either of two changes be required when A. tonsa are tested: (1) that the test duration be reduced to 48 hours; or (2) lab cultures be maintained and adults ∿14 days old be used for toxicity tests. (Adults are suggested because they are more visible, thus easier to handle.) We believe that the second recommendation is more acceptable since in the first, the possibility of using senescent adults still exists; therefore, the likelihood for excessive control mortality still exists. Laboratory cultures of A. tonsa are easily maintained. (Labs 1, 2, 4 and [now] Lab 6 maintain cultures with little difficulty.)

Because of the problems associated with A. tonsa, the copepods will not be discussed further in the test results section.

Mysidopsis bahia and Cyprinodon variegatus Toxicity Tests

Sixty-six LC50 values of a required 72 (92%) were calculated in the "Round-Robin" procedure (Tables 1, 2, and 3). The condition for an acceptable LC50 value was that either probit analysis, moving average or a binomial LC50 value could be calculated. Two mysid tests were not acceptable. In the first, the mysids exposed to endosulfan in a static test produced by Lab 4 did not elicit mortality >50% (Table 2). In the second test (Lab 3; Table 3), the measured concentration of endosulfan was not amenable for use in the calculation of an LC50, i.e., most concentrations were non-detectable or less than 15% of nominal concentrations. The test was not repeated.

In order to estimate the variability of the LC50 values generated by each laboratory for a test type (i.e., mysid, endosulfan, static test, nominal concentration), we determined the ratio of the highest LC50 the lowest LC50 (H/L ratio) for that particular test type. The H/L ratios for M. bahia exposed to silver nitrate was 2.2 in static tests and 1.9 in flow-through tests based on nominal concentrations (Table 1); the H/L ratio for flowthrough tests based on measured silver concentrations was 4.8 (Table 3). In the endosulfan mysid tests, the H/L ratios were 6.1 for static tests and 5.2 for flow-through tests based on nominal concentrations (Table 2). The H/L ratios for mysid flow-through tests based on measured endosulfan concentrations was 3.4 (Table 3).

Thirty-two of the required 36 tests (89%) with C. variegatus produced data that were acceptable for the calculation of an LC50 (Tables 1, 2, and 3). Lab 5 failed to produce silver nitrate LC50 values for C. variegatus in both static and flow-through tests (Tables 1 and 3). The reason given for this failure was that the solubility of silver nitrate in 28 °/oo seawater was approximately 2,000 to 3,000 µg/l. Precipitation of the test chemical occurred at or above 3,000 µg/l. Therefore, C. variegatus placed in these nominal concentrations may not have been actually exposed to those concentrations due to the precipitation resulting in mortality less than the 50%. The silver nitrate LC50

values generated by other laboratories using this species ranged from 640 to 1,584 µg/l in static tests, and from 818 to 2,684 µg/l in flow-through tests. Lab 4 indicated that they observed precipitation of silver nitrate in the sheepshead minnow static test, and no LC50 could be calculated. The H/L ratio for the C. variegatus silver nitrate static test was 2.5; that for the flow-through tests based on nominal concentrations was 3.3 (Table 1). The H/L ratio for the C. variegatus silver nitrate tests based on measured concentration was 4.2 (Table 3).

Additional statistical analyses of the data in Tables 1, 2, and 3 were attempted by the ERL, Gulf Breeze consultant Dr. Jerry Oglesby but were frustrated by the lack of intra-laboratory replication. Analyses of the slopes of the mortality-concentration curves were made, but results were not amenable to interpretation. More observations on the problems associated with these test data and recommendations on the statistical design of future "Round-Robins" will be presented in the discussion and conclusions section of this report.

Analysis of the Adherence of Laboratories to the ASTM Method and Scope of Work.

Final reports from each laboratory involved in the acute "Round-Robin" were carefully reviewed to determine if the "must" requirements of the ASTM document and the Contract's Scope of Work were implemented. Tables 4, 5, 6, and 7 address these requirements and their implementation.

Reports from each laboratory were reviewed to determine the quality of seawater used in their toxicity tests. The ASTM method requires that diluent water (water from marine or estuarine sources that is pumped or otherwise delivered into the laboratory) be analyzed at least monthly for the following parameters: salinity, temperature, pH, dissolved oxygen (D.O.), particulates, total organic carbon (TOC) or chemical oxygen demand (COD), total organochlorine pesticides plus PCB's. In addition, diluent water used in the toxicity tests must be adjusted to levels specified in the Contract's Scope of Work. The Scope of Work parameters and their requirements were: salinity, 28 °/oo + 1.5 °/oo; temperature, 22° C + 0.5° C. Dissolved oxygen requirements for water at the start of the tests must be between 90 and 100% saturation; TOC, <2 mg/l; COD, <5 mg/l; particulates, <20 mg/l. Laboratories varied in how closely they followed the diluent and test water quality requirements of the ASTM draft (Table 4). Most laboratories failed to measure particulates in their diluent water, did not measure total organic carbon (TOC), or chemical oxygen demand (COD), and only one laboratory (Lab 6) analyzed diluent water for total organochlorine pesticides. Test water quality, however, was maintained at the required values and measurements of salinity, temperature, pH, and D.O. were made according to the ASTM method (Table 4). One exception was the A. tonsa endosulfan test conducted by Lab 5. In that test, salinity was maintained at 8 °/oo rather than the required 28 + 1.5 °/oo. Acceptable control survival could not be attained by that laboratory if the salinity was altered substantially from ambient conditions.

It is our judgment that although the above deviations of ASTM and Scope of Work were made, the tests concerned (other than the A. tonsa study) should

be considered valid. In all cases, diluent water was filtered before it was used as test water, and D.O. values during the tests were acceptable.

The ASTM method defines the maximum amount of loading (grams of exposure animal/liter of exposure water), carrier concentration in the test water for static and flow-through tests, and minimum turnover rates of test water required in aquaria used in flow-through tests. All laboratories met the loading requirements in both static and flow-through tests (Table 5). Lab 3 used 1.0 ml carrier/l seawater in both the A. tonsa and M. bahia endosulfan tests; a maximum of 0.5 ml/l is allowed. Lab 4 provided only two turnovers per day in their silver nitrate and endosulfan flow-through tests; a minimum of five turnovers per day is required. In addition, Lab 4 did not use a conventional delivery apparatus, but mixed the toxicant and diluent water in Mariott bottles and allowed the water to flow to the exposure chambers at a prescribed rate. Although not technically in violation of the ASTM method, this procedure is not recommended because of the potential for hydrolysis of test chemicals, adsorption of hydrophobic compounds to the glass of the bottles, potential for significant decrease in dissolved oxygen and increase in microflora that may affect the test results. Lab 4 indicated that the measured endosulfan was significantly below nominal in their flow-through tests; therefore, the delivery apparatus may have affected the measured concentration.

Test Animals

In contrast, six laboratories adhered more closely to the ASTM test method and Scope of Work requirements of using: ≤2 day old mysids; 28-day-old sheepshead minnows; 14-day acclimation time for sheepshead minnows; and acceptable control mortality (≤15% for A. tonsa, ≤10% for M. bahia, and ≤5% for C. variegatus) (Table 6). There were two exceptions: Lab 3 did not test juvenile M. bahia <2 days old, and Lab 2 did not acclimate C. variegatus for the required 14-day period. Personnel at Lab 3 were questioned on the age discrepancy and agreed to repeat the static studies with 2-day-old mysids. The 96-hour LC50 values generated for silver nitrate with the younger mysids (181 µg/l) compared favorably with those 6- to 8-days-old (203 µg/l); that for the 2-day-old mysids exposed to endosulfan was 0.29 µg/l, compared to that of the older animals (96-hr LC50 = 0.24 µg/l). The flow-through tests were not repeated with 2-day-old mysids, but should be a close estimate of those with the older individuals. Lab 2 never complied with the necessary acclimation time for sheepshead minnows (14 days for fish). Lab 2 purchased 21-day-old fish from a supplier and tested the fish after seven days acclimation. Justification for the reduced acclimation period was that younger fish would be adversely affected in shipment from the supplier. We disagree with the contention, but do not believe that the inadequate acclimation time would invalidate the test.

Each laboratory was evaluated to determine how it performed in analytical chemistry relative to the requirements in the ASTM methods document (Table 7). Criteria for silver nitrate and endosulfan were: validation of analytical methods (including acceptable percentage recoveries), use of reagent blanks, and percentage acceptable measured water concentrations.

The ASTM method requires that the measured water concentrations "must be no more than 30% higher or lower than the concentration calculated from the composition of the stock solution and the calibration of the toxicant-delivery system." Labs 4 and 5 were not in compliance with the validation of the analytical method and use of reagent blanks for both endosulfan and silver nitrate (Table 7). In both cases, no mention was made of these requirements in the final reports; therefore, it was assumed that the requirements were not met.

Performance of the laboratories in meeting the requirements for acceptable water concentrations were varied (Table 7). Labs 1 and 2 performed extremely well for both chemicals, whereas Labs 3 and 4 performed poorly. We believe that the 30% ASTM requirement is somewhat arbitrary since some chemicals (such as silver nitrate) are very water soluble in the parts per billion range, where endosulfan is very insoluble. Poor performance in analysis can result for endosulfan in most cases and may also cause problems for silver nitrate at the parts per million level, which were encountered in the sheepshead minnow tests. Therefore, poor performance in the measured water categories in this "Round-Robin" may not be as serious as it appears, and the problem may be that of too stringent requirements stipulated in the ASTM method.

DISCUSSION AND CONCLUSIONS

The results of the Acute Toxicity "Round-Robin" with estuarine animals indicate that the mean H/L ratio for all mysid and sheepshead minnow tests based on nominal concentrations was 3.5 (Tables 1 and 2); that for both species based on measured concentrations was 4.0 (Table 3). Therefore, LC50 values produced by different laboratories using these species and the ASTM method should fall within a factor of 4.0. If the Acartia tonsa tests are included, the mean H/L ratio for tests based on nominal concentrations would be 4.8. We were somewhat disappointed in the results of this study. In acute tests at ERL, Gulf Breeze, LC50 values in repeat tests seldom vary by more than a factor of two; other laboratories indicate similar variability.

Several factors might explain the variability we report here in the "Round-Robin." One factor is that the participating laboratories are widely separated geographically. Labs 1, 2, and 4 are situated in New England and 3, 5, and 6 are located on the Gulf Coast. One might expect racial differences in the species and this could translate to differing sensitivities. Also, different solvents were used (e.g., acetone, triethylene glycol, and methanol) in the endosulfan tests and different exposure apparatuses were used. More important, laboratories had differing degrees of test experience with the selected species and with flow-through tests. Labs 5 and 6 had never tested A. tonsa before this "Round-Robin," and Labs 2, 4, and 5 are relatively new laboratories in the field of aquatic toxicology. All factors considered, the H/L ratio of 4.0 derived for the M. bahia and C. variegatus studies is not surprising. Additional experience accrued by the participants and other facilities capable of conducting these tests will, undoubtedly, increase the precision of the test.

Based on the results of these studies and the probability of funding more acute "Round Robin" tests in the future, we believe some changes are warranted: (1) that a minimum of four laboratories participate and a minimum of three replicate tests be required from each laboratory. In this way, intra-laboratory, as well as inter-laboratory, variability can be estimated. Obviously, funding of the "Round-Robin" was a major obstacle that prevented replication. Over 50% of the allotted extramural monies were spent on the four contract laboratories. Therefore, two replicates may have been generated, but would not provide the three replicates necessary for the desired statistical analyses; and (2) only laboratory-cultured animals should be used. When M. bahia and A. tonsa are used, only juveniles or young adults are suitable for acute tests.

Table 1. Results of silver nitrate static and flow-through Acute Toxicity Tests using estuarine animals. Results are LC50 values (probit analysis based on nominal concentrations in µg/l); numbers in parentheses are the 95% confidence intervals.

	SILVER NITRATE				
	Acartia tonsa	Mysidopsis bahia		Cyprinodon variegatus	
LABORATORY	Static	Static	Flow-through	Static	Flow-through
1	30.9 (22.3-46.7)	264 (229-307)	274 (239-326)	1584 (1423-1835)	1524 (1368-1753)
2	66.0 (59-74)	251 (207-303)	282 (235-330)	1182 (1028-1354)	860[a] (769-991)
3	35.8 (30.5-41.6)	203 (158-263)	168 (138-209)	640[a] (360-1140)	818 (700-957)
4	23.5 (17.2-29.6)	248[a] (219-283)	325 (277-426)	Non-calculable[b]	1980 (1868-2089)
5	—[c]	178 (163-190)	248[a] (219-282)	—[c]	—[c]
6	36.4 (29.4-45.8)	117 (98-140)	211 (178-289)	1082 (1006-1169)	2684[a] (2258-3419)
$\bar{x}$	38.5	210	251	1122	1573
Std. dev.	16.2	56	56	388	788
Ratio high/low	2.8	2.2	1.9	2.5	3.3

[a]Moving average LC50 calculation
[b]Mortality <50% in highest concentration
[c]Unable to complete the test

Table 2. Results of endosulfan static and flow-through Acute Toxicity Tests using estuarine animals. Results are LC50 values (probit analysis based on nominal concentrations in µg/l); numbers in parentheses are the 95% confidence intervals.

	ENDOSULFAN				
	Acartia tonsa	Mysidopsis bahia		Cyprinodon variegatus	
LABORATORY	Static	Static	Flow-through	Static	Flow-through
1	0.45[a] (0.31-0.70)	1.12 (0.85-1.52)	1.77[a] (1.39-2.36)	2.87 (2.35-4.15)	1.61[b] (1.0-2.0)
2	0.12[a] (0.11-0.14)	0.46 (0.38-0.56)	0.34 (0.27-0.40)	2.7 (2.4-3.1)	2.5 (2.3-2.7)
3	0.05 (0.04-0.06)	0.24 (0.16-0.54)	0.36 (0.28-0.53)	1.4[a] (1.08-1.92)	0.71[a] (0.5-0.9)
4	0.28 (0.18-0.41)	Non-calculable[c]	1.04 (0.82-1.33)	1.2[b] (0.8-2.0)	1.4 (1.31-1.55)
5	0.40 (0.30-0.58)	1.47 (1.25-1.72)	1.52[a] (1.24-1.88)	2.81 (2.61-3.02)	2.74[a] (2.56-2.90)
6	0.03[a] (0.0-0.56)	0.73 (.58-.95)	1.04 (0.73-1.49)	3.45 (3.22-3.69)	1.19 (1.04-1.37)
$\bar{x}$	0.24	0.84	1.02	2.41	1.69
Std. dev.	0.25	0.5	0.53	0.91	0.78
Ratio high/low	15.	6.1	5.2	2.9	3.8

[a]Moving average LC50 calculation
[b]Binomial LC50 calculation
[c]Mortality <50% in highest concentration

Table 3. Results of flow-through Acute Toxicity Tests using estuarine animals. Test results are LC50 values (probit analysis, measured concentrations in µg/l); the number in parentheses are the 95% confidence intervals.

LABORATORY	SILVER NITRATE		ENDOSULFAN	
	Mysidopsis bahia	Cyprinodon variegatus	Mysidopsis bahia	Cyprinodon variegatus
1	256 (224-301)	1,356 (1,213-1,566)	12.9[a] (1.01-1.75)	1.15[c] (0.72-1.42)
2	300 (256-346)	898 (804-1,035)	0.38 (0.32-0.44)	1.1 (1.09-1.12)
3	86 (68-112)	441 (393-485)	Non-calculable[b]	0.34[c] (0.25-0.42)
4	313[a] (267-377)	1,510[a] (1,413-1,615)	0.94 (0.82-1.10)	0.60 (0.58-0.62)
5	65[a] (51.6-87.0)	—[d]	1.16[a] (0.95-1.45)	0.88[a] (0.82-0.93)
6	132 (109-184)	1,876 (1,692-2,023)	0.75 (0.48-1.19)	0.83 (0.70-1.03)
$\bar{x}$	192	1,216	0.94	0.81
Std. Dev.	111	558	0.36	0.31
Ratio High/Low	4.8	4.2	3.4	3.4

[a]Moving average LC50 calculation
[b]Measured concentrations were unacceptable
[c]Binomial LC50 calculation
[d]Test unsuccessful

Table 4. Performance of each laboratory in adhering to diluent and test water condition requirements specified in ASTM acute toxicity test procedures. A "Yes" designates compliance with the requirements; "No" designates that the required observations were not made.

LABORATORY	DILUENT WATER QUALITY					
	Salinity	pH	D.O.	Particulates	TOC or COD	Total Organochlorine Pesticides
1	Yes	Yes	Yes	No	No	No
2	Yes	Yes	Yes	Yes	Yes	No
3	Yes	Yes	Yes	No	No	No
4	Yes	Yes	Yes	No	No	No
5	Yes	Yes	Yes	No	No	No
6	Yes	Yes	Yes	Yes	No	Yes

	TEST WATER CONDITIONS			
	Salinity (28 ± 1.5 °/oo)	Temperature (22 ± 0.5 C)	pH (<0.8 range)	D.O. (>50% saturation
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes
5	Yes[a]	Yes	Yes	Yes
6	Yes	Yes	Yes	Yes

[a]All tests acceptable, except *Acartia tonsa* endosulfan test, which was conducted at ambient (~8 °/oo) salinity.

Table 5. Performance of each laboratory in adhering to specific test condition requirements stipulated in the ASTM acute toxicity test procedures. A "Yes" designates compliance with the requirements; "No" indicates noncompliance.

LABORATORY	STATIC TESTS		FLOW-THROUGH TESTS			
	Loading (<0.8 g/l)	Carrier concentration (<0.5 ml/l in endosulfan tests)	Loading (<1.0 g/l)	Carrier concentration (<0.1 ml/l in endosulfan tests)	Water turnover rate (>5/day)	Acceptable Test Apparatus
1	Yes	Yes	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes	Yes	Yes
3	Yes	No[a]	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes	No[b]	No[c]
5	Yes	Yes	Yes	Yes	Yes	Yes
6	Yes	Yes	Yes	Yes	Yes	Yes

[a]Excessive carrier used in Acartia tonsa study (1 ml/l) and Mysidopsis bahia study (1 mg/l).

[b]Inadequate turnover rate, ~2/day in all flow-through studies.

[c]Although not in violation of ASTM requirements, apparatus used is not recommended because chemical was mixed with diluent water in a Mariotte bottle and then delivered.

Table 6. Performance of each laboratory in adhering to test animal acclimation, age, and control mortality requirements specified in ASTM acute toxicity test procedures and the contract Scope of Work. A "Yes" designates compliance with the "must" requirements; "No" indicates noncompliance.

LABORATORY	MYSIDOPSIS BAHIA		CYPRINODON VARIEGATUS		
	Age (≤2 days)	Control Mortality (<10%)	Age (28 days)	Acclimation time (14 days)	Control Mortality (<5%)
1	Yes	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	No[b]	Yes
3	No[a]	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes	Yes
5	Yes	Yes	Yes	Yes	Yes
6	Yes	Yes	Yes	Yes	Yes

[a]6-8-day-old mysids used in flow-through test.

[b]7-day acclimation used.

Table 7. Performance of each laboratory in adhering to analytical chemistry requirements in the ASTM acute toxicity test procedures and Scope of Work. A "Yes" designates compliance with the requirements; "No" indicates non-compliance.

LABORATORY	SILVER NITRATE		
	Validation of Analytical Method	Reagent blank used	Tests having acceptable measured water concentrations (%)[a]
1	Yes	Yes	100
2	Yes	Yes	100
3	Yes	Yes	0
4	No	No	0
5	No	No	0
6	Yes	Yes	40
	ENDOSULFAN		
1	Yes	Yes	80
2	Yes	Yes	67
3	Yes	Yes	0
4	No	No	0
5	No	No	40
6	Yes	Yes	50

[a]<30% variation.