



## MURPHY'S LAW AS APPLIED TO THE WET INTERLABORATORY STUDY

In October, 2000 EPA published preliminary results from their whole effluent toxicity interlaboratory study. The Agency also presented summary overviews of the results at the WEFTEC convention and the SETAC conference. Thereafter, the Agency submitted all preliminary results to formal scientific peer review.

As with any comprehensive research program, there were elements of EPA's interlaboratory WET study that did not go according to plan. The key question will be whether the deviations from protocol materially affected the integrity of the study design or the Agency's ability to draw valid scientific conclusions from the results. Below are some examples of the important questions raised within the document:

- 1) Almost all of the laboratories deviated from the standard methods required by EPA (see attached tables). How should such deviations be treated when calculating the percent of tests "successfully completed?" When should a deviation be considered significant (requiring retest) and when should a deviation be considered trivial? If some deviations are trivial, should the method procedures be revised to eliminate the non-essential requirements to avoid invalidating and repeating tests unnecessarily?
- 2) Two-thirds of the laboratories failed to observe a toxic response for the reference toxicant samples during the chronic *Ceriodaphnia dubia* tests. Given that the most sensitive *c. dubia* tests indicated no toxicity and the least sensitive *c. dubia* tests showed toxicity, how should the true nature of the original sample be classified: toxic or non-toxic? In addition, where the laboratories failed the reference toxicant tests, should the results from any of the other samples (blanks, effluent, receiving water) analyzed at the same lab during the same period should be excluded from consideration as is presently done during compliance monitoring?
- 3) When reference toxicants are analyzed as part of EPA's DMR QA/QC program, fewer than 1% of all laboratories report that the sample is "not toxic." In the interlaboratory study supervised by DynCorp, up to 70% of the reference toxicant samples, using *c. dubia*, were found to be non-toxic. What should be inferred from the difference in reference toxicant results between EPA's previous studies and the most recent study?

# “Preliminary Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods”<sup>1</sup>

## Deviations from Official Test Method for Ceriodaphnia dubia<sup>2</sup>

<b>ACUTE<sup>3</sup></b>	<b>Tests Initiated</b>	<b>Failed TAC<sup>5</sup></b>		<b>Method Deviation<sup>6</sup></b>		<b>Total Invalid<sup>7</sup></b>	
<b>Non-toxic Blank</b>	34	1	3%	10	29%	11	32%
<b>Reference Toxicant</b>	32	0	0%	21	66%	21	66%
<b>Municipal Effluent</b>	28	3	11%	10	36%	13	46%
<b>Receiving Stream</b>	13	1	8%	8	62%	9	69%
<b>Combined Results<sup>8</sup></b>	107	5	5%	49	46%	54	50%

<b>CHRONIC<sup>4</sup></b>	<b>Tests Initiated</b>	<b>Failed TAC<sup>5</sup></b>		<b>Method Deviation<sup>6</sup></b>		<b>Total Invalid<sup>7</sup></b>	
<b>Non-toxic Blank</b>	35	7	20%	18	51%	25	71%
<b>Reference Toxicant</b>	49	11	22%	18	37%	29	59%
<b>Municipal Effluent</b>	28	1	4%	16	57%	17	61%
<b>Receiving Stream</b>	13	3	23%	8	62%	11	85%
<b>Combined Results<sup>8</sup></b>	125	22	18%	60	48%	82	66%

Notes:

- 1) EPA 821-R-00-028A (October, 2000) “Preliminary Report”
- 2) Ceriodaphnia dubia is a fresh water flea EPA designated as a standard test species.
- 3) Data derived from Tables 9.2 - 9.5 in Vol. 1 of “Preliminary Report” (Acute method per EPA-600-4-90-027F; Aug., 1993)
- 4) Data derived from Tables 9.7 - 9.10 in Vol. 1 of “Preliminary Report” (Chronic method per EPA-600-4-91-002; July, 1994)
- 5) Number and percentage of tests that failed Test Acceptance Criteria (TAC) as calculated and reported by EPA.
- 6) Number and percentage of tests that deviated from mandatory procedures in the method but met the other TAC. Mandatory test procedures are defined in 40 CFR 136 (promulgated by EPA in 60 Fed. Reg. 53529 (Oct. 16, 1995). Deviations from study protocols that were not deviations from required methods were not included in the calculations.
- 7) Number and percentage of tests that deviated from mandatory test procedures in the method or failed to meet required Test Acceptance Criteria despite the fact that all participating laboratories were pre-qualified and all were notified, in writing, that such deviations were strictly prohibited.
- 8) Combined results for all four sample types: non-toxic blank, reference toxicant, municipal effluent, & receiving stream.
- 9) Many tests that failed TAC also deviated from mandatory methods; they were included only in the TAC column.

# “Preliminary Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods”<sup>1</sup>

## Deviations from Official Test Method for Pimephales promelas<sup>2</sup>

ACUTE <sup>3</sup>	Tests Initiated	Failed TAC <sup>5</sup>		Method Deviation <sup>6</sup>		Total Invalid <sup>7</sup>	
		Number	Percentage	Number	Percentage	Number	Percentage
<b>Non-toxic Blank</b>	28	0	0%	9	32%	9	32%
<b>Reference Toxicant</b>	39	1	3%	12	31%	13	33%
<b>Municipal Effluent</b>	30	1	3%	18	60%	18	60%
<b>Receiving Stream</b>	14	0	0%	5	36%	5	36%
<b>Combined Results<sup>8</sup></b>	111	2	2%	44	40%	45	41%

CHRONIC <sup>4</sup>	Tests Initiated	Failed TAC <sup>5</sup>		Method Deviation <sup>6</sup>		Total Invalid <sup>7</sup>	
		Number	Percentage	Number	Percentage	Number	Percentage
<b>Non-toxic Blank</b>	25	0	0%	15	60%	15	60%
<b>Reference Toxicant</b>	38	1	3%	27	71%	27	71%
<b>Municipal Effluent</b>	28	1	4%	20	71%	21	75%
<b>Receiving Stream</b>	14	0	0%	8	57%	8	57%
<b>Combined Results<sup>8</sup></b>	105	2	2%	70	67%	71	68%

Notes:

- 1) EPA 821-R-00-028A (October, 2000) “Preliminary Report”
- 2) Pimephales promelas (fathead minnow) is a fresh water fish EPA designated as a standard test species.
- 3) Data derived from Tables 9.13 - 9.16 in Vol. 1 of “Preliminary Report” (Acute method per EPA-600-4-90-027F; Aug., 1993)
- 4) Data derived from Tables 9.18 - 9.21 in Vol. 1 of “Preliminary Report” (Chronic method per EPA-600-4-91-002; July, 1994)
- 5) Number and percentage of tests that failed Test Acceptance Criteria (TAC) as calculated and reported by EPA.
- 6) Number and percentage of tests that deviated from mandatory procedures in the method but met the other TAC. Mandatory test procedures are defined in 40 CFR 136 (promulgated by EPA in 60 Fed. Reg. 53529 (Oct. 16, 1995). Deviations from study protocols that were not deviations from required methods were not included in the calculations.
- 7) Number and percentage of tests that deviated from mandatory test procedures in the method or failed to meet required Test Acceptance Criteria despite the fact that all participating laboratories were pre-qualified and all were notified, in writing, that such deviations were strictly prohibited.
- 8) Combined results for all four sample types: non-toxic blank, reference toxicant, municipal effluent, & receiving stream.