

### The EPA Whole Effluent Toxicity Test Interlaboratory Variability Study

Whenever possible Coastal Bioanalysts, Inc. participates in interlaboratory comparison (“round-robin” or “ring”) studies to better gauge laboratory test precision. Coastal Bioanalysts, Inc. participated with 54 other laboratories in the EPA Whole Effluent Toxicity Test Interlaboratory Variability Study. CBI was sponsored in this study by EPA/Dyncorp (*C. variegatus* tests), the American Metropolitan Sewerage Association, AMSA (*P. promelas*, *C. dubia* tests) and the Utilities Water Act Group, UWAG (*M. bahia* tests).

In this study EPA evaluated not only test endpoint values but also test performance. Raw data and bench sheets were reviewed and assigned data qualifier flags (52 total) in the following categories: test acceptability, sample receipt, dilution water, water quality, test conditions, organisms and quality control. The tables below summarize our performance in the study. Because NOEC values cannot be evaluated for precision only point estimates (LC50 or IC25) are presented. Additional information about the study and our performance can be found in the text of the EPA report\* (CBI lab I.D. number is 73).

\*EPA 821-B-01-004 Final Report: Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Vol. 1. Office of Water, Sept. 2001. Note: The report can be downloaded in PDF format at [www.epa.gov/waterscience/WET/](http://www.epa.gov/waterscience/WET/)

Table 1. Summary of CBI Performance in EPA WET Variability Study – Acute Tests; Freshwater Species

Species	Sample Type <sup>1</sup>	No. Tests by CBI	Total No. Tests	Data Qualifier Flags <sup>2</sup>		LC50 (% Sample)		
				CBI	Avg. for all Labs	CBI	Study Median	Study Mean
<i>C. dubia</i>	Blank	3	33	0	0.6	>100	>100	>100
	Ref. Tox.	2	38	0	0.8	35.4	36.0	38.6
<i>P. promelas</i>	Blank	1	27	0	1.0	>100	>100	>100
	Ref. Tox.	2	38	0	0.8	34.2	36.0	38.6

<sup>1</sup>In the blind study labs received non-toxic blank samples, reference toxicant samples, or spiked effluent or receiving water samples. Not all labs received all sample types and some labs received multiples of the same samples (to establish intralaboratory coefficients of variation).

<sup>2</sup>Number of data qualifier flags assigned by EPA in review of raw data. Although many types of flags assigned indicated deviations in test conduct or quality control problems, other flags indicated minor circumstances which might affect data interpretation.

Table 2. Summary of CBI Performance in EPA WET Variability Study –  
 Acute Tests; Estuarine Species

Species	Sample Type	No. Tests by CBI	Total No. Tests	Data Qualifier Flags		LC50 (% Sample)		
				CBI	Avg. for all Labs	Study CBI	Study Median	Study Mean
<i>C. variegatus</i>	Blank	1	7	0	2.0	>100	>100	>100
	Ref. Tox.	1	7	0	2.9	37.2	37.6	42.5
	Effluent	1	7	0	2.4	35.4	35.4	32.5
	Rcv. Water	1	7	0	2.3	35.4	22.5	24.9

Table 3. Summary of CBI Performance in EPA WET Variability Study –  
 Chronic Tests; Freshwater Species

Species	Sample Type	No. Tests by CBI	Total No. Tests	Data Qualifier Flags		LC50 or IC25 (% Sample) <sup>3</sup>			
				CBI	Avg. for all Labs	End Point	Study CBI	Study Median	Study Mean
<i>C. dubia</i>	Blank	1	27	0	2.1	Surv	>100	>100	>100
						Repr	>100	>100	96.9
	Ref. Tox.	2	36	0	2.0	Surv	>100	>100	98.8
						Repr	>100	>100	86.3
<i>P. promelas</i>	Blank	1	24	0	1.5	Surv	>100	>100	>100
						Gro	>100	>100	99.7
	Ref. Tox.	2	36	0	1.5	Surv	64.0	66.6	65.0
						Gro	51.5	56.9	52.9

<sup>3</sup>The LC50 was determined for the survival (Surv) endpoint and the IC25 for the sublethal growth (Gro) or reproduction (Rep) endpoints.

Table 4. Summary of CBI Performance in EPA WET Variability Study –  
 Chronic Tests; Estuarine Species

Species	Sample Type	No. Tests by CBI	Total No. Tests	Data Qualifier Flags		LC50 or IC25 (% Sample)			
				CBI	Avg. for all Labs	End Point	Study CBI	Study Median	Study Mean
<i>M. bahia</i>	Blank	1	7	1 <sup>4</sup>	2.9	Surv	>100	>100	>100
						Fecn <sup>5</sup>	>100	>100	>100
						Gro	>100	>100	>100
	Effluent	2	15	1 <sup>4</sup>	2.8	Surv	34.7	27.5	27.1
						Fecn	>25	>25	19.8
						Gro	31.1	20.2	21.5
						Surv	30.8	27.3	24.6
						Fecn	>25	18.8	18.1
						Gro	25.3	19.2	19.9
Rcv. Water	1	8	1 <sup>4</sup>	2.2	Surv	>100	>100	>100	
					Gro	>100	>100	>100	
					Surv	68.9	61.6	57.0	
<i>C. variegatus</i>	Blank	1	7	0	2.3	Gro	44.9	40.6	39.4
						Surv	34.2	34.3	34.5
	Ref. Tox.	1	7	0	2.3	Gro	26.8	27.9	28.0
						Surv	35.3	35.3	35.0
	Effluent	1	7	0	2.4	Surv	27.9	27.8	27.3
						Gro	27.9	27.8	27.3
	Rcv. Water	1	7	0	2.5	Surv	>100	>100	>100
						Gro	>100	>100	>100

<sup>4</sup>Data qualifier flag noted accidental loss of organisms during test. This occurred because a few mysids jumped out of test solutions, becoming caught on walls of test chamber. This commonly occurs in chronic mysid tests, particularly as animals become larger towards the end of the test. Half of all labs received the same data quality flag.

<sup>5</sup>Fecn = fecundity. Although all CBI tests met the criterion, approximately half of all the tests reported had insufficient egg production in control females to allow for analysis of the fecundity endpoint. Consequently the data sets are relatively small (n = 4 to 8) for this endpoint, resulting in less confidence in the estimate of the true mean value.