Division of Consolidated Laboratory Services- Richmond, VA
Adapted from VA-DEQ checklist Revision 3/25/09
Whole Effluent Toxicity Testing Method Checklist

ACUTE TEST DATA REVIEW
Referencing "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms", Fifth Edition, EPA 821-R-02-012, October 2002

Laboratory Name:_____VELAP ID_____

Inspection Date:_____ Assessor:_____

#	ACUTE DATA PARAMETER - (Some are organism specific)	YES	NO	Manual or Permit Req.
1.	Were pH, temp, Cl of sample checked at sample site (or within 15 minutes of sample retrieval)?			9.1.3
2.	If the sample was collected for off-site toxicity testing, was it held at 0-6° C during collection (composite) or chilled immediately following collection (grab)?			8.5.7.1
3.	Was the sample packed in ice and chilled to 0-6° C for transport to an off-site toxicity testing facility? NOTE: Frozen samples are not valid! NOTE: An exception to this would be for samples that are delivered for same day testing that may not have a chance to cool to this temperature range.			8.5.1, 8.5.7.1
4.	Were temperature and sample description recorded upon receipt by the lab?			8.6.1
5.	Was the test initiated within 36 hours of sample retrieval from sampler? NOTE: In isolated cases, an extension to this holding time can be allowed by VA DEQ (CO). Documentation of this permission must be presented with the test report and include the supportive data mentioned in 8.5.4 and 8.7.1			8.5.4, 8.6.2, and 8.7.1
6.	If filtration was necessary to remove debris or indigenous organisms, was a sieve with ≥60 µm mesh openings used?			7.3.5
7.	a. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 25° C prior to test initiation? (applies to *C. dubia, P. promelas*) b. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 25° C at 20 ppt salinity prior to test initiation? (applies to *A. bahia, C. variegatus)* c. Was the sample DO ≥ 6.0 mg/l and ≤ saturation at 12° C prior to test initiation? (applies to *O. mykiss*)			9.1.7 9.1.8
8.	If item 12. is "NO" for meeting the minimum DO levels for the organism used, was the DO adjusted up to the acceptable range (see a., b., and c. above) prior to test initiation?			9.1.8
9.	If the DO of the sample was greater than saturation at the test temperature, was the sample aerated to reduce it prior to test initiation?			9.1.8
10.	If the sample had a chlorine residual, was it dechlorinated?			9.1.6
11.	If the sample was dechlorinated, were controls treated with the same amount of dechlorination agent and run with untreated controls? (determines adverse effect of agent)			9.1.6
12.	Was the sample pH within the 6.0 - 9.0 range?			9.1.9
13.	If 18. is NO, and if the sample pH was adjusted, were parallel tests, one with an adjusted pH and one without an adjusted pH, run?			9.1.9
14.	If the pH was adjusted, was it adjusted to pH 7.0 (Freshwater tests) or pH 8.0 (Saltwater tests) by adding 1N NaOH or 1N HCl?			9.1.9
Notes/ Comments:				

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

#	ACUTE DATA PARAMETER - (Some are organism specific)	YES	NO	Manual or Permit Req.
15.	Was the age of the organisms in the correct range at test initiation? *a. P. promelas and C. variegatus* - 1-14 days old, within 24 hours of age of each other *b. O. mykiss* – 15 (swim-up or yolk sac adsorption)-30 days old *c. C. dubia* - <24 hours old *d. A. bahia* - 1-5 days old, within 24 hours of age of each other			Tables 11-16
16.	Were 5 geometric test concentrations (preferably 0.5 series) and 1 control (with the appropriate number of replicates) set up for LC_{50} or multi-dilution NOAEC tests?			2.3 9.3.2
17.	If the test organisms were obtained from an outside source, was a reference toxicant test run concurrently?			4.7.3
18.	If the concurrently run reference toxicant test should fail to meet acceptability criteria, was the reference toxicant test repeated?			4.7.5
19.	Was the test chamber size acceptable? *a. P. promelas, C. variegatus, A. bahia* - 250 ml minimum *b. O. mykiss* - 5000 ml minimum *c. C. dubia* - 30 ml minimum			Tables 12-19
20.	Was the test solution volume (sample aliquot) acceptable? *a. P. promelas, C. variegatus, A. bahia* - 200 ml minimum *b. O. mykiss* - 4000 ml minimum *c. C. dubia* - 15 ml minimum			Tables 12-19
21.	Was the minimum number of replicates per concentration represented? *a.* For effluent: 2 replicates (LC_{50} tests); For receiving waters: 4 replicates - *P. promelas, O. mykiss, C. variegatus, A. bahia* *Note: Some permits may specify 4 reps with 5 organisms in each for the NOAEC test, which is acceptable.* *b.* For effluent/ receiving waters: 4 replicates (LC_{50} tests) – *C. dubia*			Tables 12-19
22.	Was the minimum number of organisms in each replicate (the number of organisms times the number of replicates must equal 20 or more)? *a.* 10 organisms (LC_{50} tests) - *P. promelas, O. mykiss, C. variegatus, A. bahia* *Note: Some permits may specify 4 reps with 5 organisms in each for the NOAEC test, which is acceptable.* *b.* 5 organisms (LC_{50} tests) – *C. dubia*			Tables 12-19
23.	a. Was the dilution water synthetic moderately hard water or 20% DMW? (applies to freshwater species *P. promelas, O. mykiss, C. dubia)* *Note: Per 7.3.3 the regulatory authority may require a specific hardness (mod. hard).* b. Was the dilution water synthetic sea water made with deionized water and sea salts adjusted to 20 $\pm$ 2 ppt, or the same salinity as the receiving water? (applies to salt water species, *C. variegatus, A. bahia)*			7.1.1.1. 7.2.1. Table 7.
24.	Freshwater - Was the dilution water hardness within the range of 80-100 mg $CaCO_3$/L?			Tables 7, 8
25.	Freshwater - Was the dilution water alkalinity within the 57-64 mg $CaCO_3$/L?			Tables 7, 8
26.	Freshwater - Was the dilution water pH within the range of 7.4 – 7.8, or 7.9 – 8.3 for ıineral water?			Tables 7, 8
27.	a. The average test temperature for tests using *P. promelas, C. dubia C. variegatus, or A. bahia* should be 25 ± 1° C upon initiation and throughout the test. Was the test temperatures within 3° C (maximum minus minimum temperature) during the test? *More than a 3° deviation is a "No"* b. The average test temperature for tests using *O. mykiss* should be 12 ± 1° C upon initiation and throughout the test. Was the test temperatures within 3° C (maximum minus minimum temperature) during the test?			9.12.1, Tables 12-19, and VA DEQ guidance

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

#	ACUTE DATA PARAMETER - (Some are organism specific)	YES	NO	Manual or Permit Req.
28.	Was the temperature measured daily in one replicate of each concentration?			4.6.1 10.2.1.4
NOTE:	*If surrogate sample chambers were used for probe measurements, they MUST have contained the same number of organisms as the test chambers and have been subject to the same conditions as the test chambers; else, the data are not acceptable. This applies to pH, DO and conductivity readings.*			
29.	Was the DO measured daily in one replicate of each concentration? .			4.6.1, 10.2.1
30.	If the DO dropped to <4.0 mg/l, for warm water species, or <6 mg/L for *O. mykiss*, was aeration initiated? (Exceptions to this requirement are for tests *using C. dubia*, where aeration is impractical.)			9.14.1
31.	If aeration was necessary (and acceptable), were all test chambers aerated for the duration of the test, and the time at which aeration was initiated recorded?			9.14.2
32.	If aeration was necessary (and acceptable), was it applied at a maximum rate of 100 bubbles/minute so as not to cause injury to the organisms?			9.14.2
33.	Was pH measured at the 0, 24, and 48 hours for a 48-hour test, or at 0, 24, 48 hours, after renewal, 72 and at 96 hours for a 96-hour test in one replicate of each sample concentration?			4.6.1 10.2.1
34.	a. For a freshwater test, was conductivity measured at the beginning and end (also at renewal for 96-hour tests) of the test in the highest concentration and the control? (applies to freshwater species *P. promelas, O. mykiss, C. dubia) NOTE: It is recommended by VA DEQ that conductivity is measured in one replicate of each concentration at the beginning, renewal, and termination of a test.* b. For a saltwater test, was salinity measured at the beginning and end (also at renewal for 96-hour tests) of the test in the highest concentration and the control? (applies to salt water species, *C. variegatus, A. bahia) NOTE: It is recommended by VA DEQ that salinity is measured in one replicate of each concentration at the beginning, renewal, and termination of a test.*			10.2.1, 10.2.3 and VA DEQ guidance
35.	Was total ammonia measured in the effluent where toxicity may be contributed by unionized ammonia (i.e., where total ammonia ≥ 5 mg/l)?			9.1.5
36.	a. For a test using *Americamysis bahia*, were the mysids fed *Artemia* nauplii daily? b. For a 96-hour test using *Pimephales promelas*, or *Cyprinodon variegatus*, were the larvae fed prior to sample renewal at 48 hours?			9.11.1
37.	For a 96-hour test using *Pimephales promelas, Oncorhynchus mykiss*, or *Cyprinodon variegatus*, was the sample used for renewal the original sample?			8.5.4
38.	Was the daily photoperiod 16 hours light/8 hours dark?			9.10
39.	Were the surviving organisms counted daily in all test chambers?			10.1.4
40.	Was the test terminated at 48±1 hours (less than 47 hours invalidates the test) or 96±1 hours (less than 95 hours invalidates the test)?			9.15.1
41.	Was the percent survival in the controls ≥90%?			4.9.1, 9.16.1
42.	Was the LC_{50} correctly determined?			11.2

Notes/ Comments:

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

Comments on the Acute Data Review Form

Items in bold type (and shaded) are significant in that if they are answered "NO", the test is automatically deemed "not acceptable" and must be repeated to fulfill permit TMP requirements. Bold type items are numbers 2, 3, 7, 10, 13, 16, 21, 22, 23, 33, 34, 48 and 50.

RESPONSE GUIDE

1. Yes
2. Yes
3. Yes
4. Yes
5. Yes, preferably
6. Yes
7. Yes
8. Yes
9. Yes or N/A
10. Yes
11. Yes or N/A
12. Yes
13. If 12 "No", then Yes
14. Yes
15. Yes or No
16. Yes if 15. is "Yes", or No if 15. is "No"
17. Yes if 15. is "Yes", or N/A
18. Yes or No
19. to 35 Yes
36. Yes or N/A
37. to 52 Yes

RATING

ACCEPTABLE	NOT ACCEPTABLE

Comments:

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

CHRONIC TEST DATA REVIEW

Referencing:
Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Fourth Edition, EPA 821-R-02-013, October 2002 (Citations preceded by "F")
and
Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms, Third Edition, EPA 821-R-02-014, October 2002 (Citations preceded by "S")

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO	Manual or Permit Req.
1.	Was a minimum of 3 samples collected?			F-8.3.2
2.	Were pH, temp, Cl of sample checked at sample site (or within 15 minutes of sample retrieval) for each sample?			F-8.5.3
3.	If the samples were collected for off-site toxicity testing, were they held at 0-6° C during collection (composite) or chilled immediately following collection (grab)?			F-8.5.2 S-8.5.1
4.	Was each sample packed in ice and chilled to 0-6° C for transport? NOTE: Frozen samples are not valid! NOTE: An exception to this would be for samples that are delivered for same day testing that may not have a chance to cool to this temperature range.			F-8.5.7.1 S-8.5.7.1
5.	Were temperature and sample description recorded upon receipt of each sample?			S-8.6.1
6.	Was the test initiated within 36 hours of sample retrieval from sampler? NOTE: In isolated cases, an extension to this holding time can be allowed by VA DEQ (CO). Documentation of this permission must be presented with the test report and include the supportive data mentioned in 8.5.4 and 8.7.1			F-8.5.4 S-8.5.4
7.	Was the last use of the sample within 72 HOURS AFTER FIRST USE (sample age should not exceed 108 hours)?			F-8.5.4 S-8.5.4
8.	If filtration was necessary to remove debris or indigenous organisms, was a sieve with ≥60 µm mesh openings used?			F-8.8.2 S-7.3.4
9.	a. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 25° C prior to test initiation? (applies *to C. dubia* and *P. promelas)* b. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 25° C and 20 g/kg salinity prior to test initiation? (applies to *C. variegatus)* c. Was the sample DO ≥ 4.0 mg/l and ≤ saturation at 26° C and 20 g/kg salinity prior to test initiation? (applied to *A. bahia*)			F-8.8.3 S-8.8.4
10.	If item 13. is "NO" for meeting the minimum DO levels for the organism used, was the DO adjusted to the acceptable range (see a., b., and c. above) prior to test initiation?			F-8.8.3
11.	If the DO of the sample was greater than saturation at the test temperature, was the sample aerated to reduce it prior to test initiation?			F-8.8.3
12.	If the sample had a chlorine residual, was it dechlorinated?			F-8.8.7 S-8.8.7
13.	Did the permit allow for dechlorination of the sample? (Only if it contains a compliance schedule for a chlorine limit or for dechlorination)			Permit
14.	If the sample was dechlorinated, were controls treated with the same amount of dechlorination agent and run with untreated controls? (This determines any adverse effect of the dechlorination agent.)			F-8.8.7 S-8.8.7

Notes/ Comments:

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO	Manual or Permit Req.
15.	Was each sample pH within the 6.0 - 9.0 range?			F-8.8.8 S-8.8.9
16.	If 19. is NO, and if the sample pH was adjusted, were parallel tests, one with an adjusted pH and one without an adjusted pH, run? *NOTE: VA DEQ prefers that the effluent is used "as is", with regard to pH due to the problems associated with multiple samples.*			F-8.8.8 S-8.8.9
17.	If the pH was adjusted, was it adjusted to pH 7.0 (Freshwater tests) or pH 8.0 (Saltwater tests) by adding 1N NaOH or 1N HCl?			F-8.8.8 S 8.8.9
18.	Was the age of the organisms in the correct range at test initiation? *a.* *P. promelas* and *C. variegatus* - <24 hours old preferred (0-48 hours old is acceptable if the organisms are all within 24 hours in age of each other) *b.* *C. dubia* - <24 hours old, within 8 hours of age of each other? *c.* *A. bahia* - 7 days old, within 24 hours of age of each other			F-Tbl 11-1 S-Tbl 11-3 S-11.10.2.2 F-Tbl 13-3 S-Tbl 13-3
19.	If the test organisms were obtained from an outside source, was a reference toxicant test run concurrently? Alternatively, the supplier may provide control chart data from at least the last five monthly short-term chronic toxicity tests using the same reference toxicant and control conditions.			F-4.7.1 4.7.3 S-4.7.1
20.	If the concurrently run reference toxicant test should fail to meet acceptability criteria, was the reference toxicant test repeated?			F-4.7.4 S-4.7.4
21.	Was a minimum of 5 test concentrations and 1 control set up using concentrations appropriate for the limit or monitoring endpoint specified in the permit?			F-8.10. S-8.10
22.	Was the test chamber size acceptable? *a.* *P. promelas* - 500 ml minimum *b.* *C. variegatus* - 300-1000 ml *c.* *A. bahia* - 400 ml beaker or 8 oz cup (236 ml capacity) *d.* *C. dubia* - 30 ml minimum			F-Tbl 11-1 S-Tbl 11-3 F-Tbl 13-3 S-Tbl 13-3
23.	Was the test solution volume (sample aliquot) acceptable? *a.* *P. promelas* - 250 ml minimum *b.* *C. variegatus* - 250-750 ml *c.* *C. dubia* - 15 ml minimum *d.* *A. bahia* - 150 ml			F-Tbl 11-1 S-Tbl 11-3 F-Tbl 13-3 S-Tbl 13-3
24.	Was the minimum number of replicates per concentration represented? *a.* 4 replicates - *P. promelas, C. variegatus* *b.* 8 replicates - *A. bahia* *c.* 10 replicates - *C. dubia*			F-Tbl 11-1 S-Tbl 11-3 F-Tbl 13-3 S-Tbl 13-3
25.	Was the minimum number of organisms in each replicate? a. 10 organisms - *P. promelas, C. variegatus,* b. 5 organisms - *A. bahia* c. 1 organism - *C. dubia*			F-Tbl 11-1 S-Tbl 11-3 F-Tbl 13-3 S-Tbl 13-3

Notes/ Comments:

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO	Manual or Permit Req.
26.	a. Was the dilution water synthetic moderately hard water or 20% DMW? (applies to freshwater species *P. promelas, C. dubia)* *Note: The regulatory authority may require a specified hardness (mod. hard) for freshwater testing per F-7.3.5.* b. Was the salinity of the test water in the range of 20-30%? *(*applies to salt water species, *C. variegatus, A. bahia)*			F-7.1.1.1 S-14.6.11.1
27.	Freshwater - Was the dilution water hardness within the approximate range of 80-100 mg $CaCO_3$/L? (applies to freshwater species *P. promelas, C. dubia)*			F-Tables 3 & 4
28.	Freshwater - Was the dilution water alkalinity within the approximate range of 57- 64 mg $CaCO_3$/L? (applies to freshwater species *P. promelas, C. dubia)*			F-Tables 3 & 4
29.	Freshwater - Was the dilution water pH within the approximate range of 7.4 – 7.8; or 7.9 – 8.3 for mineral water? (applies to freshwater species *P. promelas, C. dubia)*			F-Tables 3 & 4
30.	a. The average test temperature for tests using *P. promelas, C. dubia, or C. variegatus* should be 25±1° C upon initiation and throughout the test. Did the test temperatures deviate by <u>not</u> more than 3° C (maximum minus minimum temperature) during the test? More than a 3° deviation is a "No" b. The average test temperature for tests using *A. bahia* should be 26±1° C upon initiation and throughout the test. Did the test temperatures deviate by <u>not</u> more than 3° C (maximum minus minimum temperature) during the test?			F-4.6.1 S-Table 3
31.	Was the temperature measured daily in one replicate of each concentration?			F-4.6.1 S-11.10.7.1.2
Note:	*If surrogate sample chambers were used for probe measurements, they MUST have contained the same number of organisms as the test chambers and have been subject to the same conditions as the test chambers; else, the data are not acceptable. This applies to pH, DO and conductivity readings.*			
32.	Was the DO measured daily, at the beginning and end of each 24 hour period, in one replicate of each concentration?			F-4.6.1 S-13.10.6.1.1
33.	If the DO dropped to <4.0 mg/l in a test using *P. promelas, C. variegatus*, or *A. bahia*, was aeration initiated? (For a test using *C. dubia*, a low DO sample should be aerated prior to test initiation or renewal, as aeration with the organisms present is impractical.)			F-8.8.4. S-11.10.4.1
34.	If aeration was necessary (and acceptable), were all test chambers aerated for the duration of the test, and the time at which aeration was initiated recorded? (Not applicable to tests using *C. dubia*)			F-8.8.4.2 S-11.10.4.1
35.	If aeration was necessary (and acceptable), was it applied at a maximum rate of 100 bubbles/minute so as not to cause injury to the organisms?			F-8.8.4.2 S-11.10.4.1
36.	Was pH measured at test initiation and at the end of each 24-hour period in one replicate of each concentration?			F-8.8.5 S-11.10.7.1.2
37.	Was the pH measured in the effluent sample each day before new test solutions are made?			F-8.8.6 S-11.10.7.1.3
38.	If toxicity may be caused by un-ionized ammonia (or where the ammonia is ≥ 5.0 mg/l), was total ammonia measured?			F-8.8.6

Notes/ Comments:

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO	Manual or Permit Req.
39.	a. For a freshwater test, was conductivity measured at the beginning of each 24-hour period in the 100% sample and the control? *(*applies to freshwater species *P. promelas, C. dubia) NOTE: It is recommended that conductivity is measured in one replicate of each dilution at the beginning of each 24-hour period.* b. For a saltwater test, was the salinity measured at the end of each 24-hour period in one replicate of each concentration? *(*applies to salt water species, *C. variegatus, A. bahia)*			F-8.8.5 VA DEQ guidance S-11.10.7.1.2
40.	a. For a test using *Americamysis bahia*, were the mysids fed *Artemia* nauplii (at a rate of 75/mysid) twice daily? b. For a test using *Pimephales promelas*, were the larvae fed 0.15 ml concentrated *Artemia* nauplii a minimum of twice daily? c. For a test using *Cyprinodon variegatus*, were the larvae fed *Artemia* nauplii once per day at a rate of 0.1 g (wet weight) for days 0-2, and 0.15 g (wet weight) for days 3-6? d. For a test using *Ceriodaphnia dubia*, were the organisms fed 0.1 ml YCT and 0.1 ml algae per day after renewal?			F-11.10.5.1 S-11.10.5 F-13.10.5.1
41.	Was the daily photoperiod 16 hours light/8 hours dark?			F-13.10.3.1 S-11.10.3
42.	Were the surviving organisms counted daily in all test chambers?			F-11.10.6.2.1 S-11.10.7.2.1
43.	Were the number of young produced recorded daily for the *C. dubia* test?			F-13.10.6.2.3
44.	Was the occurrence of males present noted in the *C. dubia* test? (Tests with no males noted may be indicative of no males present)			F-13.10.9.3
45.	Were individual treatments with males (1 or 2 replicates) and blocked rows containing $\geq$ 50% males (3 replicates or more) excluded from data analysis for the *C. dubia* reproduction endpoint? (The males are used for survival analysis)			F-13.13.1.4
46.	a. For tests using *P. promelas, C. variegatus*, or *A. bahia*, was the test terminated 7 days (this is interpreted as 7 24-hour periods) and within $\pm$ 1 hour of the time of day at which it was initiated? b. For tests using *C. dubia*, was the test terminated when 60% or more of the surviving females in the controls had produced their third brood within 8 days?			F-Table 1 and VA DEQ guidance S-11.10.9.1 F-13.10.9.1
47.	Was the percent survival in the controls $\geq$80%?			F-13.12.1 F-11.12.1 S-11.12.1 S-14.12.1
48.	Did the test meet the additional acceptability criteria? a. *P. promelas* - For tests initiated with larvae $\leq$ 24 hours old, was the average dry weight of the control larvae surviving at the end of the test $\geq$ 0.25 mg? b. *C. variegatus* - For tests initiated with larvae $\leq$ 24 hours old, was the average dry weight of control larvae $\geq$ 0.60 mg (unpreserved), or $\geq$ 0.50 mg (preserved)? c. *A. bahia* - Was the average weight of the controls $\geq$ 0.20 mg? d. *C. dubia* - Did reproduction in the controls average 15 or more young per surviving female? NOTE: Fourth brood neonates should not be counted. In addition to these test acceptability criteria, if fewer than eight replicates in the control remain after excluding males and blocks with 50% or more surviving organisms identified as males, the test is invalid and must be repeated with newly collected samples.			F-11.12.1 S-11.12.1 S-14.12.1 F-13.2.1 13.13.1.4
49.	Were the data Arcsin transformed prior to statistical analysis (*A. bahia, C. variegatus, P. promelas* – survival)?			S-Figure 5

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

#	CHRONIC DATA PARAMETERS - (Some are organism specific)	YES	NO	Manual or Permit Req.
50.	Was the NOEC correctly determined using the appropriate statistical method?			F-9.1
51.	Was the PMSD for the sublethal endpoint within upper bounds? (applicable for tests performed after 12/1/02) a. *P. promelas* growth - 30% b. *C. dubia* reproduction - 47% c. *A. bahia* growth - 37%			F,S-10.2.8
52.	If the PMSD exceeded the upper bound and no significant reduction was identified at the IWC, was the test repeated?			F,S-10.2.8.2.4.2
53.	Was the IC_{25} reported for the test?			F-9.1

Notes/ Comments:

Items in bold type (and shaded) are significant in that if they are answered "NO", the test is automatically invalidated and must be repeated to fulfill permit TMP requirements. Bold type items are numbers 2, 3, 4, 7, 10, 11, 14, 22, 23, 25, 34, 35, 52, 54, 56, 57, 60 and 61.

RESPONSE GUIDE

1. Yes
2. Yes
3. Yes
4. Yes
5. Yes, preferably
6. Yes
7. Yes
8. Yes, preferably
9. Yes, preferably; NA
10. Yes, unless granted variance
11. Yes, unless granted variance
12. Yes, or NA
13. Yes
14. If 13. is "No", then Yes; NA
15. Yes; No; NA
16. Yes; No; NA
17. If 16. is "Yes", then Yes
18. If 16. is "Yes", then Yes
19. Yes; No
20. Yes; NA
21. Yes; NA
22. Yes
23. Yes; NA
24. Yes; NA
25. Yes
26. Yes
27. Yes
28. Yes
29. Yes
30. Yes
31. Yes
32. Yes
33. Yes
34. Yes
35. Yes
36. Yes
37. Yes; NA
38. If 37. is "Yes", then Yes; NA
39. If 37. is "Yes", then Yes; NA
40 Yes
41. Yes
42. Yes; NA
43. Yes
44. Yes
45. Yes
46. Yes
47. Yes
48. Yes
49. Yes
50. Yes
51. Yes; NA
52. Yes
53. Yes, preferably
54. Yes
55. Yes
56. Yes
57. Yes
58. Yes
59. Yes
60. Yes
61. Yes
62-64. Yes

RESULTS

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

ACCEPTABLE	NOT ACCEPTABLE

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.