

Office of Water EPA 821-R-02-012-ES

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Whole Effluent Toxicity Methods Errata Sheet

EPA has made corrections and clarifications to the following three Whole Effluent Toxicity (WET) methods manuals. These errata were proposed and promulgated as part of the 2017 Clean Water Act Methods Update Rule for the Analysis of Effluent (<u>82 FR 40836, August 28, 2017</u>; docket number EPA-HQ-OW-2014-0797).

- Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. <u>EPA 821-R-02-012</u>, October 2002. Short title: Acute Freshwater and Marine Manual.
- Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. <u>EPA 821-R-02-013</u>, October 2002. Short title: Chronic Freshwater Manual.
- Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. <u>EPA 821-R-02-014</u>, October 2002. Short title: Chronic Marine and Estuarine Manual.

Three tables are included in this errata document (one for each method manual, in the above order). Each table specifies:

- 1. Where in the document the change occurs
- 2. How the current text reads
- 3. What correction is being made
- 4. How the corrected text will read
- 5. The reason for the correction

Protection Agency, Offic	Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-012, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction	
Section 4, Quality Assurance, Section 4.4.1, Laboratory Water Used for Culturing and Test Dilution Water, Page 8	Water used for culturing and test dilution should be analyzed for toxic metals and organics at least annually or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth.	Add "test" between "minimum" and "acceptability criteria."	Water used for culturing and test dilution should be analyzed for toxic metals and organics at least annually or whenever difficulty is encountered in meeting minimum test acceptability criteria for control survival and reproduction or growth.	Clarification.	
Section 4, Quality Assurance, Section 4.8.3, Food Quality, Page 9	For foods (e.g., such as YCT) which are used to culture and test organisms, the quality of food should meet the requirements for the laboratory water used for culturing and test dilution water as described in Section 4.4 above.	Spell out acronym for YCT. Add "at the concentration in the culture or test solutions" at the end of the sentence.	For foods (<i>e.g.</i> , such as Yeast, Cereal leaves, and Trout chow (YCT)) which are used to culture and test organisms, the quality of food should meet the requirements for the laboratory water used for culturing and test dilution water as described in Section 4.4 above at the concentration in the culture or test solutions.	Clarification. Define the YCT components. Analysis should be at the concentration in the culture or in the test solutions so that the foods meet the requirements at the feeding concentrations.	
Section 4, Quality Assurance, Section 4.15.2, Documenting Ongoing Laboratory Performance, Page 18	A control chart should be prepared for each combination of reference toxicant, test species, test condition, and endpoint.	Change singular words "condition" and "endpoint" to plural.	A control chart should be prepared for each combination of reference toxicant, test species, test conditions, and endpoints.	Consistency correction with Section 4.16.2 in "Chronic Freshwater Manual" and "Chronic Marine and Estuarine Manual."	
Section 4, Quality Assurance, Section 4.15.2, Documenting Ongoing Laboratory Performance, Page 18	for establishing the control charts. In this technique, a running plot is maintained for the toxicity values (X _i) from successive tests with a given reference toxicant (Figure 1), and endpoints (LC50s) are examined to determine if they are within the prescribed limits. The types of control charts illustrated	Replace sentence "In this technique" with two sentences – "Successive toxicity endpoints (NOECs, IC25s, LC50s, etc.) should be plotted and examined to determine if the results (X ₁) are within prescribed limits (Figure 1). The chart should plot logarithm of concentration on the vertical axis against the date of the test or test number on the horizontal axis."	for establishing the control charts. Successive toxicity endpoints (NOECs, IC25s, LC50s, etc.) should be plotted and examined to determine if the results (X_1) are within prescribed limits (Figure 1). The chart should plot logarithm of concentration on the vertical axis against the date of the test or test number on the horizontal axis. The types of control charts illustrated	Consistency correction with Section 4.16.2 in "Chronic Freshwater Manual" and "Chronic Marine and Estuarine Manual."	



Errata for Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-012, October 2002				
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			average LC50 value $(\pm 2S)$ and plotted on the chart as horizontal lines serving as visual indicators of any trend of successive effect endpoints that are divergent results. Cusum charting can plot logarithm values for historical mean and warning limits on the graph with the y-axis as normal arithmetic scaling or plot LC50 values for historical mean and warning limits on the graph with the y-axis scaled as logarithm. Antilogarithm values can be used so that the original units (e.g., g/L) are displayed on the graph. The log values on a log scaled y-axis or antilog values on an arithmetic scale y-axis are not be mixed on a chart. (Data provided by the Environmental Services Division, U.S. Environmental Protection Agency Kansas City KS)	
Section 4, Quality Assurance, Section 4.16.1, Reference Toxicants, Page 21	requiring aquatic toxicity tests. EMSL-Cincinnati hopes to release EPA-certified solutions of cadmium and copper with accompanying toxicity data for the recommended test species, for use as reference toxicants through cooperative research and development agreements with commercial suppliers, and will continue to develop additional reference toxicants for future release. Standard reference materials	Delete sentence "EMSL- Cincinnati hopes to"	requiring aquatic toxicity tests. Standard reference materials	EMSL-Cincinnati no longer provides reference toxicants.
Section 5, Facilities and Equipment, Section 5.4, Reagents and Consumable Materials, Section 5.4.9.4, Dried, Powdered Leaves (CEROPHYLL ^{®)} , Page 25	Dried, Powdered Leaves (CEROPHYLL [®]). Dried, powdered, cereal leaves (e.g., CEROPHYLL [®] or equivalent) are available	Delete term CEROPHYLL [®] from section title. Delete "(e.g., CEROPHYLL [®] or equivalent)" from first sentence.	Dried, Powdered Leaves. Dried, powdered, cereal leaves are available	CEROPHYLL [®] , a commercial product, is no longer available.

Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-012, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 6, Test Organisms, Section 6.6.4, Transportation to the Test Site, Page 30	In static tests, marine organisms can be used at all concentrations of effluent by adjusting the salinity of the effluent to a standard salinity (such as 25‰) or to the salinity approximating that of the receiving water, by adding sufficient dry ocean salts, such as Forty Fathoms [®] , or equivalent, GP2 or hypersaline brine.	Replace "Forty Fathoms [®] " with "HW MARINEMIX [®] " in sentence.	by adding sufficient dry ocean salts, such as HW MARINEMIX [®] , or equivalent	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 7, Dilution Water, Section 7.2.1, Standard, Synthetic Dilution Water, Page 31	Standard, synthetic, dilution water is prepared with deionized water and reagent grade chemicals or mineral water (Tables 7 and 8) and commercial sea salts (FORTY FATHOMS [®] , HW MARINEMIX [®]) (Table 9).	Delete term "FORTY FATHOMS [®] " and add "or equivalent" to sentence.	and commercial salts (HW MARINEMIX [®] or equivalent) (Table 9).	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 7, Dilution Water, Section 7.2.4.2, Standard, Synthetic Seawater, Page 33	Synthetic seawater can also be prepared by adding commercial sea salts, such as FORTY FATHOMS [®] , HW MARINEMIX [®] or equivalent, to deionized water. For example, thirty-one parts per thousand (31‰) FORTY FATHOMS [®] can be prepared by dissolving 31 g of product per liter of deionized water. The salinity of the resulting solutions should be checked with a refractometer.	Delete "FORTY FATHOMS [®] " from the first sentence. Delete second sentence.	can also be prepared by adding commercial sea salts, such as HW MARINEMIX [®] or equivalent to deionized water. The salinity of the resulting solutions	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 7, Dilution Water, Section 7.3.4, Use of Receiving Water as Dilution Water, Page 33	Where necessary, the salinity of a surface water can be increased by the addition of artificial sea salts, such as FORTY FATHOMS [®] or equivalent, a natural seawater of higher salinity, or hypersaline brine.	Replace "Forty Fathoms [®] " with "HW MARINEMIX [®] " in sentence.	addition of artificial sea salts, HW MARINEMIX [®] , or equivalent	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.

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Section 9, Acute Toxicity Test Procedures, Section 9.5.3, Static Tests, Page 44	Saline dilution water can be prepared by adding dry salts (FORTY FATHOMS [®] or equivalent, or modified GP2) or hypersaline brine to de-ionized water, or a suitable surface freshwater, to adjust the salinity of the entire dilution series.	Replace "Forty Fathoms [®] " with "HW MARINEMIX [®] " and delete "modified" in sentence.	adding dry salts (HW MARINEMIX [®] or equivalent, or GP2) or	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 9, Acute Toxicity Test Procedures, Section 9.11.1, Feeding, Page 46	The organisms are fed at test renewal, 48 h after the test is initiated, if Regional or State policy requires a 96-h test duration.	Replace "fed at test renewal, 48 h after the test is initiated" with "are provided food 2 h prior to test renewal at 48h."	The organisms are provided food 2 h prior to test solution renewal at 48 h, if Regional or State policy requires a 96 h test duration.	Clarification of when foods can be added to match the text in the tables. The feeding regimes require that food(s) be made available while holding prior to the test; for some methods (Table 12, 13) for a minimum of 2 h prior renewing the test solutions in a test. Any feeding regimes require that the food is added 2 h prior to test solution renewal at 48 h (recommended); except for the mysid test, where food is added daily.
Section 9, Acute Toxicity Test Procedures, Section 9.11.2, Feeding, Page 46	Where <i>Artemia</i> nauplii are fed, the nauplii are first concentrated"	Add "the \leq 24 h old" after comma in sentence.	Where Artemia nauplii are fed, the ≤ 24 h old nauplii are first concentrated	Clarification of age of the brine shrimp to be fed.
Section 9, Acute Toxicity Test Procedures, Section 9.171, Summary of Test Conditions and Test Acceptability Criteria for Mysid, <i>Mysidopsis</i> <i>bahia</i> , Acute Toxicity Tests With Effluents and Receiving Waters (Test Method 2007.0) (Continued), Table 16, Line 17, Dilution Water, Page 60	5-30‰ ±10%; Uncontaminated source of seawater, deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX [®] , FORTY FATHOMS [®] , modified GP2, or equivalent) prepared with MILLI- Q [®] or equivalent deionized water (see Section 7, Dilution Water); or receiving water (available options)	Delete "FORTY FATHOMS [®] ," from sentence.	or artificial sea salts (HW MARINEMIX [®] , modified GP2, or equivalent)	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.

Errata for Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. U.S. Environmental

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 9, Acute	5-32‰ ±10%; Uncontaminated	Delete "FORTY	or artificial sea salts (HW MARINEMIX [®] , modified	FORTY FATHOMS [®] , a
Toxicity Test	source of seawater, deionized	FATHOMS [®] ," from	GP2, or equivalent)	commercial product, is no
Procedures, Section	water mixed with hypersaline	sentence.		longer available. Equivalent
9.171, Summary of Test	brine or artificial sea salts (HW			commercial products, such as
Conditions and Test	MARINEMIX [®] , FORTY			HW MARINEMIX [®] , are
Acceptability Criteria for	FATHOMS [®] , modified GP2, or			acceptable replacements.
Sheepshead Minnows,	equivalent) prepared with MILLI-			
Cyprinodon variegatus,	Q [®] or equivalent deionized water			
Acute Toxicity Tests	(see Section 7, Dilution Water); or			
With Effluents and	receiving water (available options)			
Receiving Waters (Test				
Method 2004.0)				
(Continued), Table 17,				
Line 17, Dilution Water,				
Page 62				
Section 9, Acute	5-32‰ ±10%; Uncontaminated	Delete "FORTY	or artificial sea salts (HW MARINEMIX [®] , modified	FORTY FATHOMS [®] , a
Toxicity Test	source of seawater, deionized	FATHOMS [®] " from	GP2, or equivalent)	commercial product, is no
Procedures, Section	water mixed with hypersaline	sentence.		longer available. Equivalent
9.171, Summary of Test	brine or artificial sea salts (HW			commercial products, such as
Conditions and Test	MARINEMIX [®] , FORTY			HW MARINEMIX [®] , are
Acceptability Criteria for	FATHOMS [®] , modified GP2, or			acceptable replacements.
Silverside, Menidia	equivalent) prepared with MILLI-			
beryllina, M. menidia,	Q [®] or equivalent deionized water			
and M. peninsulae,	(see Section 7, Dilution Water); or			
Acute Toxicity Tests	receiving water (available options)			
With Effluents and				
Receiving Waters (Test				
Method 2006.0)				
(Continued), Table 18,				
Line 17, Dilution Water,				
Page 64				

Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 11, Acute Toxicity Data Analysis, Section 11.2.4.3, Sub- bullet 6, The Trimmed- Karber Method, Page 79	A computer program which estimates the LC50 and associated 95% confidence interval using the Trimmed-Karber Method, can be obtained through the Environmental Monitoring and Support Laboratory (EMSL), 26 W. Martin Luther King Drive, Cincinnati, OH 45268. The program can be obtained from EMSL-Cincinnati by sending a diskette with a written request to the above address.	Correction Being Made Replace "through the Environmental Monitoring and Supportto the above address." with replacement software information.	Corrected Text can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the LC50 and associated 95% confidence interval using the Trimmed-Karber method can be used.	Reason For Correction Computer program is no longer available from Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Section 11, Acute Toxicity Data Analysis, Section 11.2.5.3, Sub- bullet 2, The Probit Method, Page 81	A machine-readable, compiled, version of a computer program to estimate the LC1 and LC50 and associated 95% confidence intervals using the Probit Method can be obtained from EMSL- Cincinnati by sending a diskette with a written request to the Environmental Monitoring Systems Laboratory, 26 W. Martin Luther King Drive, Cincinnati, OH 45268.	Replace "from EMSL- CincinnatiCincinnati, OH 45268" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the LC1 and LC50 and associated 95% confidence interval using the Probit method can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Appendix A, Distribution, Life Cycle, Taxonomy, and Culture Methods, Section 3.12.1, Culture Water, Page 218	Artificial seawater is prepared by dissolving FORTY-FATHOMS [®] or equivalent artificial sea salts in deionized water to a salinity of 20-30‰.	Replace "FORTY- FATHOMS [®] " with "HW MARINEMIX [®] " in sentence.	prepared by dissolving HW MARINEMIX [®] or equivalent artificial sea salts	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.

Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002					
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction	
Section 4, Quality Assurance, Section 4.4.1, Laboratory Water Used for Culturing and Test Dilution Water, Page 10	Water used for culturing and test dilution should be analyzed for toxic metals and organics at least annually or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth.	Add "test" between "minimum" and "acceptability criteria."	Water used for culturing and test dilution should be analyzed for toxic metals and organics at least annually or whenever difficulty is encountered in meeting minimum test acceptability criteria for control survival and reproduction or growth.	Clarification.	
Section 4, Quality Assurance, Section 4.8.3, Food Quality, Page 11	New batches of food used in culturing and testing should be analyzed for toxic organics and metals or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth.	Add "test" between "minimum" and "acceptability criteria."	New batches of food used in culturing and testing should be analyzed for toxic organics and metals or whenever difficulty is encountered in meeting minimum test acceptability criteria for control survival and reproduction or growth.	Consistency correction with Section 4.8.3 in "Acute Freshwater and Marine Manual" and Section 4.8.3 in "Chronic Marine and Estuarine Manual."	
Section 4, Quality Assurance Section 4.16, Documenting Ongoing Laboratory Performance, Page 17	<pre>upper contract limit A</pre>	Replace the graphs in Figure 1 with log scale graphs. Add explanatory text to Figure 1 legend.	A NOEC VALUES (X) NOEC VALUES (X) A NOEC VALUES (X) A COVER UNAT (Below the NDEC) A Toxicity Test with Reference Toxicant CENTRAL TENDENCY CENTRAL TENDENCY A CENTRAL TENDENCY CENTRAL TENDENCY A CENTRAL TENDENCY CENTRAL TEND	Correction and clarification. Cusum charting can either plot anti-logarithm values for historical mean and warning limits on the graph with the y-axis scaled as logarithm or plot logarithm values for historical mean and warning limits on the graph with the y-axis as normal arithmetic scaling. The figures A and B are revised to match the text in the manual and are shown using a log format. Cusum charts may be plotted using antilogarithm values so that the original units (e.g., g/L) are displayed on the graph. The log values on a log scaled y-axis or anti-log	

Errata for Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
			Figure 1. Control (cusum) charts: For NOEC results	values on an arithmetic scale
			(hypothesis estimates) (A): plot the value of the NOEC	y-axis are not be mixed on a
			and the values of the adjacent test concentrations of	chart. Many graphics
			NOEC as the upper and lower limits. For LC, EC or IC	programs do not allow
			results (point estimates) (B) plot the actual point	enlarging the log scale axis
			estimate, the cumulative mean (central tendency, middle	sufficiently (i.e., expanding
			line) and the upper and lower control limits ($\pm 2S$). The	within a log unit), and trends
			upper and lower control limits are calculated as the	can be difficult to view;
			average IC, EC, LC value $(\pm 2S)$ and plotted on the	therefore graphs using
			chart as horizontal lines serving as visual indicators of	antilogarithm values may be
			any trend of successive effect endpoints that are	preferable. Individual LC,
			divergent results. The cusum chart should plot logarithm	EC or IC data should be
			of concentration on the vertical axis against the date of	plotted on a graph. The graph
			the test or test number on the horizontal axis. Successive	should present the mean of
			toxicity endpoints (i.e., IC25) should be plotted and	the logarithmic LC, EC or
			examined to determine if the results are within	ICs for the central tendency.
			prescribed limits. Cusum charts can either plot the data	The upper and lower control
			with the x-axis scaled as logarithms as normal arithmetic	limits are calculated as the
			scaling or plot the anti-logarithm values on the graph	average LC, EC or IC value
			with the y-axis scaled as logarithm. The log values on a	plus-and-minus two standard
			log scaled y-axis or antilog values on an arithmetic scale	deviations and plotted on the
			y-axis are not be mixed on a chart.	chart as horizontal lines,
				serving as visual indicators of
				any trend of successive effect
				endpoints that are divergent
				results. For NOEC toxicity
				values, the control limits
				would consist of one
				concentration interval above
				and below the concentration
				tendency. NOEC volves
				connot be averaged (Section
				4 14 5); estual values and
				4.14.3), actual values and adjacent upper and lower
				concentrations to the NOEC
				value are plotted using the
				logarithm procedures
				described in Section 4 14 5
				and Section 9 Chronic
				Toyicity Test Endpoints and
				Data Analysis) the NOFC
				LOEC interval is used for the

Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002				
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				cusum chart. Reference: Environment Canada. 2009. Environment Canada's Guidance on Using Logarithms for Reference Toxicant Warning Chart. Biological Methods Section, September.
Section 11, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Larval Survival and Growth Test Method, Section 11.3.6.1.2, Interferences, Page 57	Total ammonia concentrations greater than 5 mg/L in the 100% effluent are an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Replace "the 100% effluent are" with "in any concentration tested may be"	Total ammonia concentrations greater than 5 mg/L in any concentration tested may be an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Clarification.
Section 11, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Larval Survival and Growth Test Method 1000.0, Section 11.10.1.2.3, Test Procedures, Page 65	Sufficient test solution (approximately 1500 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses at the high, medium, and low test concentrations.	Replace "at the high, medium and low test" with "for all" test concentrations.	Sufficient test solution (approximately 1500 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analysis for all test concentrations.	Clarification. All solutions need to have sufficient volume prepared, not just the high, medium, and low test concentrations.
Section 11, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Larval Survival and Growth Test Method 1000.0, Section 11.10.6.1.2, Observations During the Test, Page 67	Temperature and pH are measured at the end of each 24-h exposure period	Insert "beginning and" between "measured at the" and "end of each."	Temperature and pH are measured at the beginning and end of each 24-h exposure period	Clarification.
Section 11, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Larval Survival and Growth Test Method 1000.0, Section 11.10.6.1.4, Observations During the Test, Page 67	Conductivity, alkalinity, and hardness are measured in each new sample (100% effluent or receiving water) and in the control.	Delete "(100% effluent or receiving water)" from sentence. Insert "before they are dispensed to the test chambers" to the end of the sentence.	Conductivity, alkalinity, and hardness are measured in each new sample and in the control before they are dispensed to the test chambers.	Clarification.

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 12, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Embryo- Larval Survival and Teratogenicity Test Method 1001.0, Section 12.3.5.1.2, Interferences, Page 113	Total ammonia concentrations greater than 5 mg/L in the 100% effluent are an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Replace "the 100% effluent are" with "in any concentration tested may be"	Total ammonia concentrations greater than 5 mg/L in any concentration tested may be an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Clarification.
Section 12, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Embryo- Larval Survival and Teratogenicity Test Method 1001.0, Section 12.10.1.2.3, Test Solutions, Page 117	Sufficient test solution (approximately 1000 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses.	Change "1000 mL" to "1500 mL." Add "for all test concentrations" to end of sentence.	Sufficient test solution (approximately 1500 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses for all test concentrations.	Clarification. A volume of 1000 mL is not sufficient for the test. Consistency correction with Section 11.10.1.2.3 in "Chronic Freshwater Manual." All solutions need to have sufficient volume prepared, not just the high, medium and low test concentrations.
Section 12, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Embryo- Larval Survival and Teratogenicity Test Method 1001.0, Section 12.10.6.1.2, Observations During the Test, Page 119	Temperature and pH are measured at the end of each 24-h exposure period in at least one test chamber	Add "beginning and" between "the" and "end."	Temperature and pH are measured at the beginning and end of each 24-h exposure period in at least one test chamber	Clarification.
Section 12, Fathead Minnow, <i>Pimephales</i> <i>promelas</i> , Embryo- Larval Survival and Teratogenicity Test Method 1001.0, Section 12.10.6.1.4, Observations During the Test, Page 119	Conductivity, alkalinity, and hardness are measured in each new sample (100% effluent or receiving water) and in the control.	Delete "(100% effluent or receiving water)". Add "before they are dispensed to the test chambers" to the end of the sentence.	Conductivity, alkalinity, and hardness are measured in each new sample and in the control before they are dispensed to the test chambers.	Clarification.

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 13, Daphnid, <i>Ceriodaphnia dubia</i> , Survival and Reproduction Test Method 1002.0, Section 13.3.6.1.2 Interferences, Page 142	Total ammonia concentrations greater than 5 mg/L in the 100% effluent are an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Replace "the 100% effluent are" with "in any concentration tested may be."	Total ammonia concentrations greater than 5 mg/L in any concentration tested may be an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Clarification.
Section 13, Daphnid, <i>Ceriodaphnia dubia</i> , Survival and Reproduction Test Method 1002.0, Section 13.10.6.1.2, Observations During the Test, Page 157	Temperature and pH are measured at the end of each 24-h exposure period in at least one test chamber	Add "beginning and" between "at the" and "of each 24-h."	Temperature and pH are measured at the beginning and end of each 24-h exposure period in at least one test chamber	Clarification
Section 13, Daphnid, <i>Ceriodaphnia dubia</i> , Survival and Reproduction Test Method 1002.0, Section 13.10.6.1.4, Observations During the Test. Page 157	Conductivity, alkalinity, and hardness are measured in each new sample (100% effluent or receiving water) and in the control.	Delete "(100% effluent or receiving water)." Add "before they are dispensed to the test chambers" to end of the sentence.	An aliquot is samples for conductivity, alkalinity, and hardness in each new sample and in the control before they are dispensed to the test chambers.	Clarification.

Errata for Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. U.S. Environmental

Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 13, Daphnid, <i>Ceriodaphnia dubia</i> , Survival and Reproduction Test Method 1002.0, Section 13.13.3.1, Example of Analysis of the Daphnid Reproduction Data, Page 170	The response used in the statistical analysis is the number of young produced per adult female, which is determined by taking the total number of young produced until either the time of death of the adult or the end of the experiment, whichever comes first. In this three-brood test, offspring from fourth or higher broods should not be counted and should not be included in the total number of neonates produced during the test. An animal that dies before producing young, if it has not been identified as a male, would be included in the analysis with zero entered as the number of young produced. The subsequent calculation of the mean number of live young produced per adult female for each toxicant provides a combined measure of the toxicant's effect on both mortality and reproduction.	Add word "live" in the first (twice), second (once), and third (twice) sentences.	The response used in the statistical analysis is the number of live young produced per adult female, which is determined by taking the total number of live young produced until either the time of death of the adult or the end of the experiment, whichever comes first. In this three-brood test, offspring from fourth or higher broods should not be counted and should not be included in the total number of live neonates produced during the test. An animal that dies before producing live young, if it has not been identified as a male, would be included in the analysis with zero entered as the number of live young produced. The subsequent calculation of the mean number of live young produced per adult female for each toxicant provides a combined measure of the toxicant's effect on both mortality and reproduction.	Clarification.
Section 14, Green Alga, Selenastrum capricornutum, Growth Test Method 1003.0, Section 14.10.1.2.3, Test Procedures, Page 204	Sufficient test solution (approximately 900 or 1500 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses at the high, medium, and low test concentrations.	Replace "at the high, medium, and low" with "for all test."	Sufficient test solution (approximately 900 or 1500 mL) is prepared at each effluent concentration to provide 400 mL additional volume for chemical analyses for all test concentrations.	Clarification. All solutions need to have sufficient volume prepared, not just the high, medium, and low test concentrations.
Section 14, Green Alga, Selenastrum capricornutum, Growth Test Method 1003.0, Section 14.10.5.1.2, Observations During the Test. Page 206	Temperature and pH are measured at the end of each 24-h exposure period in at least one test flask	Add "beginning and" before "end."	Temperature and pH are measured at the beginning and end of each 24-h exposure period in at least one test flask	Clarification. The algae test does not have a daily renewal of test solution.

Errata for Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002

Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Appendix C, Dunnett's Procedure, Section 2.5, Computer Calculations, Page 275	Cincinnati, OH 45268. A compiled executable version of the program can be obtained from EMSL-Cincinnati by sending a written request to EMSL at 3411 Church Street, Cincinnati, OH 45244.	Replace "EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating an analysis of variance (ANOVA) and a multiple comparison of treatment means with the control mean (Dunnett's Procedure) can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Appendix I, Probit Analysis, 2, Page 309	A compiled, executable version of the program can be obtained from EMSL-Cincinnati by sending a written request to EMSL at 3411 Church Street, Cincinnati, OH 45244.	Replace "EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the EC1 and EC 50 (or LC1 and LC50) and the associated 95% confidence interval using the Probit method can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Appendix K, Trimmed Spearman-Karber Method, Section 10, Page 318	A computer program which estimates the LC50 and associated 95% confidence interval using the Trimmed Spearman-Karber Method, can be obtained from EMSL-Cincinnati by sending a written request to EMSL, 3411 Church Street, Cincinnati, OH 45244.	Replace "EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the LC50 and associated 95% confidence interval using the Trimmed Spearman-Karber Method can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Appendix M, Linear Interpolation Method, Section 7.1, Page 329	To obtain the program and supporting documentation, send a written request to EMSL- Cincinnati at 3411 Church Street, Cincinnati, OH 45244.	Replace "EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the mean and standard deviation of the bootstrapped ICp and associated 95% confidence intervals can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.

Errata for Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 821-R-02-013, October 2002

Protection Agency, Office of Water, Washington DC. EPA 821-K-02-014, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 4, Quality Assurance, Section 4.4.1, Laboratory Water Used For Culturing and Test Dilution Water, Page 10	The dilution water used in the toxicity tests may be natural seawater, hypersaline brine (100‰) prepared from natural seawater, or artificial seawater prepared from commercial sea salts, such as FORTY FATHOMS [®] or HW MARINEMIX [®] , if recommended in the method.	Delete "FORTY FATHOMS [®] or," from sentence. Add "or equivalent" after "MARINEMIX [®] " in sentence.	or artificial seawater prepared from commercial sea salts, such as HW MARINEMIX [®] or equivalent if recommended in the method.	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 4, Quality Assurance, Section 4.8.3, Food Quality, Page 11	the food should not be used (for analytical methods, see AOAC, 1990; and USDA, 1989).	Add sentence "For foods (e.g., flake food) which are used to culture and test organisms, the quality of the food should meet the requirements for laboratory water used for culturing and test dilution water as described in Section 4.4 above at the concentration in the culture or test solutions." after end of sentence.	the food should not be used (for analytical methods, see AOAC, 1990; and USDA, 1989). For foods (<i>e.g.</i> , flake food) which are used to culture and test organisms, the quality of the food should meet the requirements for laboratory water used for culturing and test dilution water as described in Section 4.4 above at the concentration in the culture or test solutions.	Analysis should be at the concentration in the culture or test waters; consistency correction with Section 4.8.3 "Acute Freshwater and Marine Manual" and Section 4.8.3 "Chronic Freshwater Manual"
Section 4, Quality Assurance, Section 4.8.4, Food Quality, Page 11	For foods (e.g., YCT) which are used to culture	Replace "YCT" with "flake food."	For foods (<i>e.g.</i> , flake food) which are used to culture	Correction. The food YCT is not used in any of the marine methods.

Protection Agency, Office	ce of water, washington DC. EPA	A 821-R-02-014, October 2	002	P
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 4, Quality Assurance, Section 4.16, Documenting Ongoing Laboratory Performance, Page 11	UPPER CONTROL LIMIT A CENTRAL TENDENCY Z LOWER CONTROL LIMIT	Replace the graphs in Figure 1 with log scale graphs. Add explanatory text to Figure 1 legend.	UPPER LIMIT (Above the NOEC) A	Correction and clarification. Cusum charting can either plot anti-logarithm values for historical mean and warning limits on the graph with the y-axis scaled as logarithm or
	0 5 10 15 20 UPPER CONTROLLIMIT (X+2.5) B Q CENTRAL TENDENCY		LOWER LIMIT (Below the NOEC) 0 5 10 15 20	plot logarithm values for historical mean and warning limits on the graph with the y-axis as normal arithmetic
			UPPER CONTROL LIMIT (X + 25)	scaling. The figures A and B are revised to match the text in the manual and are shown using a log format. Cusum charts may be plotted using
	r igue s. comos canos (n) esponens essag renns, (o) pom estantes (oc, oc oc).		CENTRAL TENDENCY	the original units (e.g., g/L) are displayed on the graph. The log values on a log scaled y-axis or antilog values on an arithmetic scale
			Figure 1. Control (cusum) charts: For NOEC results (hypothesis estimates) (A): plot the value of the NOEC and the values of the adjacent test concentrations of NOEC as the upper and lower limits. For LC, EC or IC results (point estimates) (B) plot the actual point estimate, the cumulative mean (central tendency, middle line) and the upper and lower control limits (\pm 2S). The upper and lower control limits are calculated as the average IC, EC, LC value (\pm 2S) and plotted on the chart as horizontal lines serving as visual indicators of any trend of successive effect endpoints that are divergent results. The cusum chart should plot logarithm of concentration on the vertical axis against the date of the test or test number on the horizontal axis. Successive toxicity endpoints (i.e., IC25) should be plotted and examined to determine if the results are within	y-axis are not be mixed on a chart. Many graphics programs do not allow enlarging the log scale axis sufficiently (i.e., expanding within a log unit), and trends can be difficult to view; therefore graphs using antilogarithm values may be preferable. Individual LC, EC or IC data should be plotted on a graph. The graph should present the mean of the logarithmic LC, EC or ICs for the central tendency. The upper and lower control limits are calculated as the average LC, EC or IC value plus-and-minus two standard
			with the x-axis scaled as logarithms as normal arithmetic	deviations and plotted on the chart as horizontal lines, serving as visual indicators of

Protection Agency, O	rotection Agency, Office of water, wasnington DC. EPA 821-R-02-014, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction	
			scaling or plot the anti-logarithm values on the graph with the y-axis scaled as logarithm. The log values on a log scaled y-axis or antilog values on an arithmetic scale y-axis are not be mixed on a chart.	any trend of successive effect endpoints that are divergent results. For NOEC toxicity values, the control limits would consist of one concentration interval above and below the concentration representing the central tendency. NOEC values cannot be averaged (Section 4.14.5); actual values and adjacent upper and lower concentrations to the NOEC value are plotted using the logarithm procedures described in Section 4.14.5 and Section 9, Chronic Toxicity Test Endpoints and Data Analysis) the NOEC- LOEC interval is used for the cusum chart. Reference: Environment Canada. 2009. Environment Canada's Guidance on Using Logarithms for Reference Toxicant Warning	
				Chart. Biological Methods Section, September.	
Section 4, Quality Assurance, Section 4.17.1, Reference Toxicants, Page 16	requiring aquatic toxicity tests. EMSL-Cincinnati hopes to release EPA-certified solutions of cadmium and copper for use as reference toxicants through cooperative research and development agreements with commercial suppliers, and will continue to develop additional reference toxicants for future release. Standard reference materials	Delete sentence "EMSL- Cincinnati hopes to"	requiring aquatic toxicity tests. Standard reference materials	EMSL-Cincinnati no longer provides reference toxicants.	

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 5, Facilities, Equipment, and Supplies, Section 5.1, General Requirements, Page 19	Water used for rearing, holding, acclimating, and testing organisms may be natural seawater or water made up from hypersaline brine derived from natural seawater, or water made up from reagent grade chemicals (GP2) or commercial (FORTY FATHOMS [®] or HW MARINEMIX [®]) artificial sea salts when specifically recommended in the method.	Delete "FORTY FATHOMS [®] " from sentence.	or water made up from reagent grade chemicals (GP2) or commercial (HW MARINEMIX [®]) artificial sea salts	FORTY FATHOMS [®] , a commercial product, is no longer available.
Section 6, Test Organisms, Section 6.6.4, Transportation to the Test Site, Page 25	by adding sufficient dry ocean salts, such as FORTY FATHOMS [®] , or equivalent, GP2, or hypersaline brine.	Delete "FORTY FATHOMS [®] , or equivalent," in sentence.	by adding sufficient dry ocean salts, such as GP2, or hypersaline brine.	FORTY FATHOMS [®] , a commercial product, is no longer available.
Section 7, Dilution Water, Section 7.2.1, Types of Dilution, Page 26	Standard, synthetic, dilution water is prepared with deionized water and reagent grade chemicals (GP2) or commercial sea salts (FORTY FATHOMS [®] , HW MARINEMIX [®]) (Table 3).	Delete "FORTY FATHOMS [®] ," and add "or equivalent" after "HW MARINEMIX [®] " in sentence.	or commercial artificial sea salts (HW MARINE MIX [®] or equivalent) (Table 3).	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 7, Dilution Water, Section 7.2.3.2, Standard Synthetic Sea Water, Page 27	Synthetic seawater can also be prepared by adding commercial sea salts, such as FORTY FATHOMS [®] , HW MARINEMIX [®] , or equivalent, to deionized water. For example, thirty-one parts per thousand (31‰) FORTY FATHOMS [®] can be prepared by dissolving 31 g of sea salts per liter of deionized water. The source water for the deionizer can be ground water or tap water.	Delete "FORTY FATHOMS [®] " from first sentence. Delete second sentence.	Synthetic seawater can be prepared by adding commercial sea salts, such as HW MARINEMIX [®] , or equivalent, to deionized water. The source water for the deionizer	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.

Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 7, Dilution Water, Section 7.3.3, Use of Receiving Water as Dilution Water, Page 28	Where necessary, the salinity of a surface water can be increased by the addition of artificial sea salts, such as FORTY FATHOMS [®] , HW MARINEMIX [®] , or equivalent, GP2, a natural seawater of higher salinity, or hypersaline brine.	Delete "FORTY FATHOMS [®] ," from sentence.	addition of artificial sea salts, such as HW MARINEMIX [®] , or equivalent, GP2, a natural seawater	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 11, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Larval Survival and Growth Test Method 1004.0, Section 11.3.6.1.2, Interferences, Page 56	Total ammonia concentrations greater than 5 mg/L in the 100% effluent are an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Replace "the 100% effluent are" with "any concentration tested may be."	Total ammonia concentrations greater than 5 mg/L in any concentration tested may be an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).	Clarification.
Section 11, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Larval Survival and Growth Test Method 1004.0, Section 11.10.1.3.1, Dilution Water, Page 68	Dilution water may be uncontaminated natural seawater (receiving water), HSB prepared from natural seawater, or artificial seawater prepared from FORTY FATHOMS [®] or GP2 sea salts (see Table 1 and Section 7, Dilution Water).	Replace "FORTY FATHOMS [®] " with "HW MARINEMIX [®] , or equivalent," and replace "1" with "3" in sentence.	or artificial seawater prepared from HW MARINEMIX [®] , or equivalent, or GP2 sea salts (see Table 3, Section 7	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements. Consistency with Section 11, Table 3, Summary of Test Conditions, Line 18, Dilution Water, Page 79
Section 11, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Larval Survival and Growth Test Method 1004.0, Section 11.10.1.2.3, Effluents, Table 2, Footnote 1, Page 69	The salinity of the initial 6,800 mL of 100% effluent is adjusted to 20‰ by adding 136 g of dry artificial sea salts (FORTY FATHOMS [®]).	Replace "136 g of dry artificial sea salts (FORTY FATHOMS [®])" with "an appropriate amount of dry artificial sea salts (e.g., HW MARINEMIX [®])" in sentence.	by adding an appropriate amount of dry artificial sea salts (e.g., HW MARINEMIX [®]).	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 11, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Larval Survival and Growth Test Method 1004.0, Section 11.10.7.1.2, Observations During the Test, Page 71	Temperature, pH, and salinity are measured at the end of each 24-h exposure period in one test chamber at each test concentration and in the control.	Add "beginning and" between "at the" and "end of." Add "at least one" between "period in" and "test chamber."	Temperature, pH, and salinity are measured at the beginning and end of each 24-hr exposure period in at least one test chamber at each test concentration and in the control.	Clarification.

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction	
Section 11, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Larval Survival and Growth Test Method 1004.0, Section 11.11, Summary of Test Conditions and Test Acceptability Criteria, Table 3, Line 18, Page 79	Uncontaminated source of natural seawater; deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX [®] , FORTY FATHOMS [®] , GP2 or equivalent) (available options)	Delete "FORTY FATHOMS [®] ," from sentence.	or artificial sea salts (HW MARINEMIX [®] , GP2 or equivalent)	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.	
Section 12, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Embryo Larval Survival and Tertatogenicity Test Method 1005.0, Section 12.6.10.3.8, Reagents and Consumable Materials, Table 1, Footnote 1, Page 126	The salinity of the initial 4000 mL of 100% effluent is adjusted to 20‰ by adding 80 g of dry artificial sea salts (HW MARINEMIX or FORTY FATHOMS [®]), and mixing for 1 h.	Delete "FORTY FATHOMS [®] ," from sentence, add " [®] " after "MARINEMIX", and add "equivalent" after "or" in sentence.	dry artificial sea salts (HW MARINEMIX [®] or equivalent) and mixing for 1 h.	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.	
Section 12, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Embryo Larval Survival and Tertatogenicity Test Method 1005.0, Section 12.10.1.3.1, Dilution Water, Page 131	Dilution water may be uncontaminated natural seawater (receiving water), HSB prepared from natural seawater, or artificial seawater prepared from FORTY FATHOMS [®] or GP2 sea salts (see Table 3 in Section 7, Dilution Water).	Replace "FORTY FATHOMS [®] " with "HW MARINEMIX [®] or equivalent" in sentence.	artificial seawater prepared from HW MARINEMIX [®] or equivalent or GP2 sea salts	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements. Consistency with Section 12, Table 2, Summary of Test Conditions, Line 16, Dilution Water, Page 137	
Section 12, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Embryo Larval Survival and Tertatogenicity Test Method 1005.0, Section 12.10.6.1.2, Observations During the Test, Page 132	Temperature, pH, and salinity are measured at the end of each 24-h exposure period in one test chamber at each test concentration and in the control.	Add "beginning and" between "measured at the" and "end of each." Add "at least" between "exposure period in" and "one test chamber."	Temperature, pH, and salinity are measured at the beginning and end of each 24-hr exposure period in at least one test chamber at each test concentration and in the control.	Clarification.	

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction	
Section 12, Sheepshead Minnow, <i>Cyprinodon</i> <i>variegatus</i> , Embryo Larval Survival and Tertatogenicity Test Method 1005.0, Section 12.12.1, Summary of Test Conditions and Test Acceptability Criteria, Table 2, Line 16, Dilution Water, Page 137	Uncontaminated source of natural seawater; deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX [®] , FORTY FATHOMS [®] , GP2, or equivalent) (available options)	Delete "FORTY FATHOMS [®] ," from sentence.	or artificial sea salts (HW MARINEMIX [®] , GP2, or equivalent)	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.	
Section 13, Inland Silverside, <i>Menidia</i> <i>beryllina</i> , Larval Survival And Growth Method 1006.0, Section 13.10.1.3.1, Dilution Water, Page 169	Dilution water may be uncontaminated natural seawater (receiving water), HSB prepared from natural seawater, or artificial seawater prepared from FORTY FATHOMS [®] or GP2 sea salts (see Table 3 in Section 7, Dilution Water).	Replace "FORTY FATHOMS [®] " with "HW MARINEMIX [®] " in sentence.	or artificial seawater prepared from HW MARINEMIX [®] or GP2	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements. Consistency with Section 13, Table 3, Summary of Test Conditions, Line 18, Dilution Water, Page 179.	
Section 13, Inland Silverside, <i>Menidia</i> <i>beryllina</i> , Larval Survival And Growth Method 1006.0, Section 13.10.7.1.2, Observations During the Test, Page 171	Temperature, pH, and salinity are measured at the end of each 24-hr exposure period in one test chamber at all test concentrations and in the control.	Add "beginning and" between "measured at the" and "end of each." Add "at least" between "exposure period in" and "one test chamber."	Temperature, pH, and salinity are measured at the beginning and end of each 24-hr exposure period in at least one test chamber at all test concentrations and the control.	Clarification.	

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 13, Inland Silverside, <i>Menidia</i> <i>beryllina</i> , Larval Survival And Growth Method 1006.0, Section 13.11, Summary of Test Conditions and Test Acceptability Criteria, Table 3, Line 18, Dilution Water, Page 179	Uncontaminated source of natural sea water, artificial seawater; deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX [®] , FORTY FATHOMS [®] , GP2 or equivalent) (available options)	Delete "FORTY FATHOMS [®] ," from sentence.	artificial sea salts (HW MARINEMIX [®] , GP2 or equivalent)	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 14, Mysid, <i>Mysidopsis bahia</i> , Survival, Growth, and Fecundity Test Method 1007.0, Section 14.5.16, Apparatus and Equipment, Page 217	Dissecting microscope (350-400X magnification)	Replace "350-400X" with "35-40X."	Dissecting microscope (35-40X magnification)	Typographical error.
Section 14, Mysid, <i>Mysidopsis bahia</i> , Survival, Growth, and Fecundity Test Method 1007.0, Section 14.10.2.3.1, Dilution Water, Page 228	Dilution water may be uncontaminated natural seawater (receiving water), HSB prepared from natural seawater, or artificial seawater prepared from FORTY FATHOMS [®] or GP2 sea salts (see Table 1 and Section 7, Dilution Water).	Replace "FORTY FATHOMS [®] " with "HW MARINEMIX [®] " in sentence.	or artificial seawater prepared from HW MARINEMIX [®] or GP2	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements. Consistency with Section 14, Table 3, Summary of Test Conditions, Line 20, Dilution Water, Page 242.
Section 14, Mysid, <i>Mysidopsis bahia</i> , Survival, Growth, and Fecundity Test Method 1007.0, Section 14.10.1.3, Test Procedures, Page 227	Eight replicate test vessels, each containing 5 to 7 day old animals, are used per effluent concentration and control.	Replace "5 to 7 day" with "5, 7-day."	Eight replicate test vessels, each containing 5, 7-day old animals, are used per effluent concentration and control.	Typographical error.

Protection Agency, Offi	Protection Agency, Office of Water, Washington DC. EPA 821-K-02-014, October 2002					
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction		
Section 14, Mysid, <i>Mysidopsis bahia</i> , Survival, Growth, and Fecundity Test Method 1007.0, Section 14.10.8.1.2, Observations During the Test, Page 229	Temperature, pH, and salinity are measured at the end of each 24-hr exposure period in one test chamber at each test concentration and control.	Add "beginning and" between "measured at the" and "end of each." Add "at least" between "exposure period in" and "one test chamber."	Temperature, pH, and salinity are measured at the beginning and end of each 24-hr exposure period in at least one test chamber at each test concentration and control.	Clarification.		
Section 14, Mysid, <i>Mysidopsis bahia</i> , Survival, Growth, and Fecundity Test Method 1007.0, Section 14.10.10.3.1, Termination of the Test, Page 230	Examine each replicate under a stereomicroscope (240X) to determine the number of immature animals	Replace "240X" with "24X."	Examine each replicate under a stereomicroscope (24X) to determine the number of immature animals	Typographical error.		
Section 14, Mysid , <i>Mysidopsis bahia</i> , Survival, Growth, and Fecundity Test Method 1007.0, Section 14.11.1, Summary of Test Conditions and Test Acceptability Criteria, Table 3, Line 18, Dilution Water, Page 242	Uncontaminated source of natural seawater, deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX [®] , FORTY FATHOMS [®] , GP2 or equivalent) (available options)	Delete "FORTY FATHOMS [®] ," from sentence.	artificial sea salts (HW MARINEMIX [®] , GP2 or equivalent)	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.		
Section 15 Sea Urchin, Fertilization Test Method, Section 15.6.18.3.8 Consumable Materials, Table 1, Footnote 1, Page 301	Stir solutions 1 h to ensure that the salts dissolve. The salinity of the initial 840 mL of 100% effluent is adjusted to 30‰ by adding 25.2 g of dry artificial sea salts (FORTY FATHOMS [®]).	Replace "25.2 g of dry artificial sea salts (FORTY FATHOMS [®])" with "an appropriate amount of dry artificial sea salts (e.g., HW MARINEMIX [®]) in sentence.	by adding an appropriate amount of dry artificial sea salts (e.g., HW MARINEMIX [®]).	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.		

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Section 15 Sea Urchin, <i>Arbacia punctulata</i> , Fertilization Test Method 1008.0, Section 15.6.19.7, Test Organisms, Sea Urchins, <i>Arbacia punctulata</i> , Page 303	To successfully maintain about 25 adult animals for 7 days at a field site, a screen-partitioned, 40-L glass aquarium using aerated, recirculating, clean saline water (30‰) and a gravel bed filtration system, is housed within a water bath, such as FORTY FATHOMS [®] or equivalent (15°C).	Replace "FORTY FATHOMS [®] " with "HW MARINEMIX [®] or equivalent" in sentence.	is housed within a water bath, such as HW MARINEMIX [®] or equivalent (15°C).	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.
Section 15 Sea Urchin, <i>Arbacia punctulata</i> , Fertilization Test Method 1008.0, Section 15.10.1.3.1, Dilution Water, Page 304	Dilution water may be uncontaminated natural seawater (receiving water), HSB prepared from natural seawater, or artificial seawater FORTY FATHOMS [®] or GP2 sea salts (see Table 2 and Section 7, Dilution Water).	Replace "FORTY FATHOMS [®] " with "HW MARINEMIX [®] or equivalent," in sentence.	or artificial seawater HW MARINEMIX [®] or equivalent, or GP2 sea salts	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements. Consistency with Section 15, Table 3, Summary of Test Conditions, Line 11, Dilution Water, Page 308.
Section 15 Sea Urchin, <i>Arbacia punctulata</i> , Fertilization Test Method 1008.0, Section 15.10.7.1.2, Observations During the Test, Page 306	Temperature, pH, and salinity are measured at the beginning of the exposure period in one test chamber at each concentration and in the control.	Replace "of the exposure period" with "and end of each 24-hr exposure period in at least one test chamber at each test concentration and control."	Temperature, pH, and salinity are measured at the beginning and end of each 24-hr exposure period in at least one test chamber at each test concentration and control.	Clarification.
Section 15, Sea Urchin, <i>Arbacia punctulata</i> , Fertilization Test Method 1008.0, Section 15.10.8.1, Start of the Test, Page 306	Sufficient test solution is prepared at each effluent concentration to provide additional volume for chemical analyses, at the high, medium, and low test concentrations.	Replace "at the high, medium and low" with "all test."	Sufficient test solution is prepared at each effluent concentration to provide additional volume for chemical analyses at all test concentrations.	Clarification.

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Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction	
Section 15 Sea Urchin, <i>Arbacia punctulata</i> , Fertilization Test Method 1008.0, Section 15.11.1, Summary of Test Conditions and Test Acceptability Criteria, Table 3, Line 11, Dilution Water, Page 308	Uncontaminated source of natural seawater; deionized water mixed with hypersaline brine or artificial sea salts (HW MARINEMIX [®] , FORTY FATHOMS [®] , GP2, or equivalent) (available options)	Delete "FORTY FATHOMS [®] ," from sentence.	artificial sea salts (HW MARINEMIX [®] , GP2 or equivalent)	FORTY FATHOMS [®] , a commercial product, is no longer available. Equivalent commercial products, such as HW MARINEMIX [®] , are acceptable replacements.	
Section 16, Red Macroalga, <i>Champia</i> <i>parvula</i> , Sexual Reproduction Test Method 1009.0, Section 16.10.1.3.1, Dilution Water, Page 343	The formula for the enrichment for natural seawater is listed in Table 1. Both EDTA and trace metals have been omitted. This formula should be used for the 2- day exposure period, but it is not critical for the recovery period. Since natural seawater quality can vary among laboratories, a more complete nutrient medium (e.g., the addition of EDTA) may result in faster growth (and therefore faster cystocarp development) during the recovery period.	Add text from Line 12, Dilution Water, Table 3, Summary of Test Conditions, Page 348	30‰ salinity natural seawater, or a combination of 50% of 30‰ salinity natural seawater and 50% of 30‰ salinity GP2 artificial seawater (see Section 7, Dilution Water). The formula for the enrichment for	Consistency with Section 16, Table 3, Summary of Test Conditions, Line 12, Dilution Water, Page 348	
Section 16, Red Macroalga, <i>Champia</i> <i>parvula</i> , Sexual Reproduction Test Method 1009.2, Section 16.10.6.1.2, Observations During the Test, Page 344	Temperature, pH, and salinity are measured at the end of each 24-hr exposure period in at least one test chamber at each test concentration and in the control.	Add "beginning and" between "at the" and "end of." Add "at least" between "period in" and "one test."	Temperature, pH, and salinity are measured at the beginning and end of each 24-hr exposure period in at least one test chamber at each test concentration and in the control.	Clarification.	
Appendix C, Dunnett's Procedure, Section 2.5, Computer Calculations, Page 408	The program was written in IBM- PC FORTRAN by Computer Sciences Corporation, 26 W. Martin Luther King Drive, Cincinnati, OH 45268. A compiled version of the program can be obtained from EMSL- Cincinnati by sending a diskette with a written request.	Replace "from EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating an analysis of variance (ANOVA) and a multiple of treatment means with the control mean (Dunnett's Procedure) can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.	

Frotection Agency, Office of Water, Washington DC. EFA 821-K-02-014, October 2002				
Text Location	Current Text	Correction Being Made	Corrected Text	Reason For Correction
Appendix H, Probit Analysis, Section 2, Page 436	A compiled, executable version of the program and supporting documentation can be obtained from EMSL-Cincinnati by sending a written request to EMSL at 3411 Church Street, Cincinnati, OH 45244.	Replace "from EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the EC1 and EC50 (or LC1 and LC50) and the associated 95% confidence intervals using Probit analysis can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Appendix J, Trimmed Spearman-Karber Method, Section 10, Page 445	A computer program which estimates the LC50 and associated 95% confidence interval using the Trimmed Spearman-Karber Method, can be obtained through the EMSL, 3411 Church Street, Cincinnati, OH 45244. The program can be obtained from EMSL-Cincinnati by sending a written request to the above address.	Replace "through the EMSL-Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the LC50 and associated 95% confidence interval using the Trimmed Spearman-Karber Method can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.
Appendix L, Linear Interpolation Method, Section 7.1, Page 458	A compiled, executable version of the program and supporting documentation can be obtained by sending a written request to EMSL-Cincinnati, 3411 Church Street, Cincinnati, OH 45244.	Replace "sending a written request to EMSL- Cincinnati" with replacement software information.	can be obtained by clicking on "contact us" at <u>http://www.epa.gov/med</u> . Any software capable of calculating the mean and standard deviation of the bootstrapped ICp and associated 95% confidence intervals can be used.	Computer program is no longer available from EMSL- Cincinnati. It can be obtained by sending a request to EPA using the link. Any software capable of completing the calculations is acceptable.

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. <u>EPA 821-R-02-012</u>, October 2002. Short title: Acute Freshwater and Marine Manual.

Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. <u>EPA 821-R-02-013</u>, October 2002. Short title: Chronic Freshwater Manual.

Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition. U.S. Environmental Protection Agency, Office of Water, Washington DC. <u>EPA 821-R-02-014</u>, October 2002. Short title: Chronic Marine and Estuarine Manual.