## Appendix A – Summary of historical data and laboratory techniques

The appendix is divided into six sections:

- 1) Inventory of available data
- 2) Biological response data
- 3) Water quality data
- 4) Reference toxicant data
- 5) Correlations among data variables
- 6) Potential sources of variance in test performance

Each section and/or graphic is appended with a caption to summarize important methodological information. No assessment or conclusion is provided.

### INVENTORY OF AVAILABLE DATA

Inventory updates include additional requests for lab specific information as well as additional quality assurance reviews for seemingly unusual data. The inventory includes some summary statistics requested by the Panel.

Table A1. Summary of the inventory of available test control and reference toxicant test data provided to SCCWRP from the accredited laboratories. Number of test controls are divided into how many replicates were utilized in each test.

		# of Test Controls		
	Total #	# of tests	# of tests	# of Ref Tox tests
Lab	Test Controls	with 10 reps	with 20 Reps	(incl. control)*
А	48	48	0	31
В	48	48	0	47
С	28	28	0	28
D	19	19	0	6
E	49	24	25	30
F	45	37	8	30
G	7	7	0	22
Н	0	0	0	17
1	30	30	0	30
J	7	7	0	21
К	19	19	0	15
L	27	27	0	30
М	59	59	0	34
Ν	30	30	0	30
0	30	30	0	30
Р	80	1	79	28
Q	25	25	0	23
Total	551	439	112	452

\* All reference toxicant controls had 10 replicates

Table A2. Inventory of lab techniques that was available to SCCWRP from the accredited laboratories at the time of historical data compilation.

		Brood Board	Chamber	Cham	ber	Chamber		Sample	į	
Lab	Light Intensity	WQ	Material	Volun	ne (ml)	Diameter (	cm)	Volum	e (ml)	Dilution Water Formula
А	Not measured	Not measured	glass		20		2.54		20	) MHW + Vitamins and Selenium
В	Sent	Not measured	glass		30		5.5		15	; MHW
С	Not measured	Not measured	polystyrene		29.57		4		1	5 MH + Vitamins and Selenium
D	Sent Ranges	Not measured	polystyrene		29.5		4		15	5 MHW + Selenium
E	Measure Quarterly	Not measured	polypropylene		29.5		3.8		30	) MHW
F	Measure every 6 months	Not measured	polypropylene		29.57		4.29		15	5 DMW; 80% DIW: 20% Perrier®
G	Not sent	Not measured	glass		26		2.9		15	5 DMW; 80% DIW: 20% Perrier®
Н	Unknown	Unknown	polystyrene		26		2.54	Not se	nt	DMW; 80% DIW: 20% Evian®
I	Measure annually	Sent	plastic		36.9		4.5		15	; Hoheisel
J	Unknown	Unknown	Unknown	Unkn	own	Unknown			15	; MHW
К	Sent Average	Sent	glass		20		2.8		15	5 L1650%, proprietary formula
L	Not measured	Not measured	plastic		29.57		4		15	5 MHW + Selenium
М	Have by test	Sent	polystyrene	Not se	ent		3.8		15	; MHW
Ν	Sent Ranges	Not measured	polystyrene		29.57		4		15	5 MHW + Selenium
0	Measure every 6 months	Sent	polystyrene		36.97		4.45		15	5 MHW + Selenium
Р	Have by test	Sent	polystyrene	N	ot sent		6		30	DMW; 80% DIW: 20% Perrier <sup>®</sup>
Q	Sent graphs	Not measured	polystyrene		30		4.32		15	DMW; 80% DIW: 20% Perrier®

MHW= EPA moderately hard water recipe using salts

MH= EPA hard water recipe using salts

DMW= EPA recipe for moderately hard water using diluted mineral water

DIW= Deionized water

Unclear= More than one recipe listed in SOP

# Table A3. Additional lab techniques that were available to SCCWRP from the accredited laboratories at the time of historical data compilation.

Lab	YCT Source	Cerophyl	Trout Chow	Algae Source	Termination Trigger	Termination Window
А	Purchased	NA	NA	In-house	≥60% having 3 broods	Strict window with single check
В	Purchased	NA	NA	Purchased	≥60% having 3 broods	Strict window with single check
		Fisher Science Cereal				No specific window with periodic
С	In-house	Grass Media	Aquamax 100	In-house	≥60% having 3 broods	checks
D	Purchased	NA	NA	Purchased	≥60% having 3 broods	Strict window with single check
E	Purchased	NA	NA	Purchased	NA	Test always runs seven days
F	In-house	Wheatgrass from ABS	Thomas Fish	Purchased	≥60% having 3 broods	No specific window with single check
G	In-house	Wheatgrass from ?	TetraMin Flakes	Purchased	≥80% having 3 broods	No specific window with periodic checks
		Starwest Botanicals				No specific window with periodic
Н	In-house	Organic Alfalfa Leaf	TetraMin Flakes	In-house	≥70% having 3 broods	checks
		Frontier Powdered Wheat	Silver Cup Trout Chow by			
Ι	In-house	Grass	Skretting	In-house	≥60% having 3 broods	Strict window with single check
К	In-house	Alfalfa from Co-Op	Skreeting Trout Chow	In-house	≥60% having 3 broods	Strict window with periodic checks
L	Purchased	NA	NA	Purchased	≥60% having 3 broods	Strict window with periodic checks
М	Purchased	NA	NA	Purchased	≥60% having 3 broods	Strict window with single check
			Purina Trout Chow + Carolina			
Ν	In-house	Carolina Cereal	Daphnia Food (50:50)	In-house	≥60% having 3 broods	Strict window with single check
0	In-house	Pines Wheat Grass	Zinfer Bros. Fin Fish Starter	In-house	≥60% having 3 broods	No specific window with single check
	In-house and	Cereal Grass Media from				No specific window with periodic
Р	ARO	Ward's	Zeigler Finfish Starter	Purchased	≥60% having 3 broods	checks
Q	Purchased	NA	NA	Purchased	≥60% having 3 broods	Strict window with single check

NA= Not applicable

Table A4. Summary status of biological data that is available to SCCWRP from the accredited laboratories. The data is divided into test controls vs reference toxicant test per laboratory.

		N	leonate	/Female	Tes	t Length	n (d)	Time t	o First B	Brood (d)	Number	of Males	per Test	Age	at Start	of Test (h)
Lab	Test Type	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range
А	Sample Control	29.9	48	18.9 - 44	6.5	48	6 - 7	3.6	48	3.0 - 4.2	0	48	0	NA	0	NA
А	Ref Tox Control	28.7	31	18.9 - 40.2	6.5	31	6 - 7	3.7	31	3 - 4.5	0	31	0	NA	0	NA
В	Sample Control	27.6	48	3.8 - 41.4	7.0	48	6 - 8	3.8	48	2.9 - 4.4	0	48	0	NA	0	NA
В	Ref Tox Control	26.7	47	0 - 46.1	6.6	47	2 - 8	3.5	47	0 - 5.1	0	47	0	NA	0	NA
С	Sample Control	35.8	28	5.3 - 50.2	7.0	28	6 - 7	4.1	28	3.6 - 5.4	0	28	0	5.5	16	3.2 - 7.1
С	Ref Tox Control	35.8	28	19.4 - 50.0	7.0	28	7 - 7	4.0	28	3.6 - 4.5	0.04	28	0 - 1	5.5	15	3.2 - 7.0
D	Sample Control	27.2	19	13.4 - 39.6	6.7	19	6 - 7	3.9	19	3.2 - 4.3	0	19	0	7.3	19	5.5 - 9.1
D	Ref Tox Control	28.4	6	17.2 - 35.1	7.0	6	6 - 7	3.9	6	3.0 - 4.3	0	6	0	6.3	6	4.8 - 7.8
Е	Sample Control	24.9	49	15 - 31.3	7.0	49	7 - 7	3.9	49	3.4 - 4.9	0	49	0	NA	0	NA
E	Ref Tox Control	25.1	30	17.6 - 35.3	7.0	30	7 - 7	3.9	30	3.6 - 4.3	0	30	0	NA	0	NA
F	Sample Control	26.0	45	19.2 - 40.3	6.5	45	6 - 7	3.8	45	3.0 - 4.2	0	45	0	12.2	44	6.2 - 23.8
F	Ref Tox Control	25.5	30	20.1 - 39.3	6.4	30	6 - 7	3.9	30	2.7 - 4.0	0	30	0	11.5	30	6.0 - 23.8
G	Sample Control	26.2	7	19.6 - 33.9	6.7	7	6 - 7	3.4	7	2.9 - 4.2	0	7	0	17.6	5	8.0 - 24.0
G	Ref Tox Control	23.1	22	15.7 - 28.4	6.7	22	6 - 8	3.8	22	2.7 - 5.1	0.09	22	0 - 1	16	21	8.0 - 24.0
н	Sample Control	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA	0	NA
н	Ref Tox Control	18.7	17	12.5 - 23.4	6.2	17	6 - 7	4.0	17	3.6 - 5.0	0	17	0	NA	0	NA
I	Sample Control	32.0	30	20.7 - 43.3	6.2	30	6 - 8	3.4	30	2.9 - 4.1	0.03	30	0 - 1	19.5	30	8.0 - 24.0
I	Ref Tox Control	31.4	30	23.1 - 44.0	6.0	30	5 - 6	3.3	30	2.7 - 4.0	0.03	30	0 - 1	19.7	30	8.0 - 24.0
J	Sample Control	17.2	7	11.2 - 22.1	6.9	7	6 - 8	4.1	7	3.3 - 4.7	0	7	0	NA	0	NA
J	Ref Tox Control	20.1	21	13.4 - 31.6	6.5	21	6 - 8	3.7	21	3.0 - 4.5	0.05	21	0 - 1	NA	0	NA
к	Sample Control	27.2	19	12.7 - 38.4	6.5	19	5 - 8	3.9	19	2.7 - 5.3	0	19	0	19.0	19	10 - 23.9
к	Ref Tox Control	27.6	15	15.6 - 41.9	6.5	15	6 - 7	3.5	15	1.8 - 4.2	0	15	0	20.0	14	14.2 - 23.9
L	Sample Control	25.5	27	16.6 - 35.7	7.1	27	7 - 8	4.0	27	3.1 - 4.6	0.04	27	0 - 1	3.3	25	2.8 - 7.5
L	Ref Tox Control	25.8	30	17 - 41.3	7.1	30	7 - 8	4.0	30	3.3 - 4.7	0.07	30	0 - 1	3.3	28	2.8 - 7.5
М	Sample Control	37.5	59	25.4 - 52.1	6.5	59	6 - 8	3.4	59	2.7 - 4.6	0	59	0	20.6	58	7.4 - 24.0
м	Ref Tox Control	35.0	34	3.4 -43.6	6.3	34	5 - 8	3.3	34	1.1 - 4.4	0	34	0	21.8	31	7.9 - 24.0
N	Sample Control	24.3	30	21.6 - 27	7.0	30	7 - 7	3.6	30	3.1 - 4.2	0	30	0	7.8	30	7.0 - 8.5
N	Ref Tox Control	23.8	30	21.1 - 26.9	7.0	30	7 - 7	3.6	30	3.0 - 3.9	0	30	0	7.8	30	7.0 - 8.5

		Ν	leonate	/Female	Tes	st Lengtł	n (d)	Time	to First I	Brood (d)	Number	of Males	per Test	Age	at Start	of Test (h)
Lab	Test Type	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range
0	Sample Control	35.3	30	21.9 - 47.5	6.2	30	6 - 7	3.3	30	2.4 - 4.1	0.33	30	0 - 2	18.5	30	7.5 - 24.0
0	Ref Tox Control	36.4	30	21.6 - 46.9	6.2	30	6 - 7	3.4	30	2.4 - 4.0	0.17	30	0 - 2	18.8	30	9.0 - 24.0
Р	Sample Control	33.1	80	21.1 - 41.8	6.3	80	6 - 8	3.4	80	3.0 - 4.0	0	80	0	18.9	79	3.3 - 23.9
Р	Ref Tox Control	33.1	28	20.7 - 43.8	6.1	28	6 - 8	3.1	28	3.0 - 4.1	0	28	0	20.6	28	2.6 - 24.6
Q	Sample Control	24.5	25	16.0 - 31.9	6.0	25	5 - 7	3.4	25	2.9 - 4.2	0	25	0	6.1	18	2.9 - 8.9
0	Ref Tox Control	24.4	23	12.8 - 31.0	6.1	23	5-8	3.4	23	3.0 - 4.5	0	23	0	6.2	16	2.9 - 8.9

Table A4 Continued. Summary status of biological data that is available to SCCWRP from the accredited laboratories.



### Hardness (mg/L CaCO<sub>3</sub>) Alkalinity (mg/L CaCO<sub>3</sub>) Conductivity (µS/cm) Dissolved Oxygen (mg/L) pН Temperature (°C) Range Ν Mean Lab Test Type Mean Ν Range Mean Ν Mean Ν Range Mean Range Mean Ν Range Ν Range 48 48 7.8 - 8.1 Sample Control 89.6 48 81.9 - 96 62.1 48 57 - 64 315 291 - 331 7.9 8.0 48 7.7 - 8.2 24.0 48 24.0 - 24.0 А А **Ref Tox Control** 90.0 31 81.9 - 96 62.1 31 57 - 64 316 31 291 - 330 8.0 31 7.8 - 8.1 8.0 31 7.7 - 8.2 24.0 31 24.0 - 24.1 В Sample Control 97.3 35 88.9 - 107.7 35 56 - 90.5 48 310 - 420 48 7.1 - 8.1 48 6.4 - 9.4 48 24.2 - 25.6 68.1 358 7.6 8.1 24.8 В **Ref Tox Control** 95.0 35 89 - 106.3 65.9 35 60 - 75.2 368 47 330 - 415 7.5 47 7.1 - 8.1 7.8 47 6.0 - 9.4 24.9 47 24.0 - 26.1 С Sample Control 152 26 80 - 190 110 28 60 - 148 557 28 330 - 711 8.1 28 7.9 - 8.6 7.7 28 7.1 - 8.3 25.1 28 24.5 - 25.9 С 26 8.2 **Ref Tox Control** 171 105 - 190 119 28 112 - 126 616 28 588 - 713 28 7.8 - 8.6 7.7 28 7.1 - 8.2 25.0 28 24.6 - 25.6 8.0 D 87.3 19 80 - 96 19 37 - 64 335 19 284 - 399 19 7.7 - 8.2 7.8 - 8.2 24.3 - 25.6 Sample Control 55.7 8.0 19 25.0 19 6 D **Ref Tox Control** 87.8 80 - 94 57.7 6 52 - 63 333 6 322 - 346 7.8 6 7.4 - 8.0 8.1 6 7.8 - 8.4 24.8 6 24.1 - 25.4 49 49 49 7.9 49 49 Е Sample Control 95.0 60 - 220 67.2 60 - 198 348 262 - 574 7.5 - 8.2 7.4 6.9 - 8.2 24.0 49 24.0 - 24.1 Е 61.2 **Ref Tox Control** 30 60 - 100 30 60 - 65 343 30 332 - 356 7.9 30 7.5 - 8.2 7.5 30 6.9 - 7.9 91.7 24.0 30 24.0 - 24.0 F 7.9 - 8.5 Sample Control 87.9 45 81 -99 81.5 45 64 - 98 377 45 185 - 1072 8.2 45 8.1 45 7.7 - 8.4 24.7 45 24.0 - 25.5 F 30 **Ref Tox Control** 88.5 81 - 99 83.3 30 67 - 98 190 30 186 - 195 8.2 30 8.0 - 8.3 8.1 30 7.9 - 8.4 30 23.8 - 25.3 24.7 5 7.7 - 8.1 G Sample Control 87.4 81 - 94 NA 0 NA 198 7 174 - 204 7.9 7 7.9 7 7.7 - 8.2 24.8 7 24.2 - 25.1 21 G 22 22 7.8 - 8.2 8.1 22 22 Ref Tox Control 86.1 81 - 98 NA 0 NA 195 171 - 214 8.0 7.8 - 8.4 24.8 24.3 - 25.4 0 0 NA 0 NA 0 NA NA 0 0 н Sample Control NA NA NA NA NA NA NA NA 0 17 8.1 17 Н **Ref Tox Control** 82.4 9 51.8 - 103.6 83.7 14 72 - 98 NA NA 8.1 7.9 - 8.2 8.0 - 8.3 25.6 17 24.0 -26.6 Sample Control 90.9 30 85 - 95 73.3 30 68 - 76 358 30 269 - 415 7.9 30 7.7 - 8.1 7.7 30 6.6 - 8.5 24.8 30 24.3 - 25.5 **Ref Tox Control** 90.9 30 85 - 95 73.3 30 68 - 76 411 30 362 - 743 8.0 30 7.8 - 8.1 7.7 30 6.1 - 8.5 30 24.1 - 25.5 24.8 Sample Control 0 0 7 311 - 338 7 7.8 - 8.1 7.7 7 7.2 - 8.0 24.6 - 25.7 1 NA NA NA NA 328 7.9 25.2 7 **Ref Tox Control** NA 0 NA NA 0 NA 324 21 308 - 333 7.9 21 7.5 - 8.1 7.7 21 7.2 - 8.0 25.0 21 24.1 - 25.6 1 17 Κ Sample Control 81.9 8 - 108 59.9 17 54 - 84 299 19 258 - 442 7.8 19 7.3 - 8.1 7.8 19 7.4 - 8.0 24.4 19 23.5 - 25.1 К **Ref Tox Control** 92 1 1 15 15 6.7 - 8.0 7.8 15 7.2 - 8.3 15 23.4 - 25.6 NA 56.0 NA 299 258 - 319 7.7 24.4 27 Sample Control 98.8 86 - 100 61.5 27 57 - 64 413 27 383 - 442 8.0 27 7.4 - 8.3 8.1 27 7.3 - 8.7 24.4 27 24.0 - 25.1 **Ref Tox Control** 30 30 30 7.8 30 7.4 - 8.2 7.3 - 8.7 24.0 - 25.0 98.9 86 - 100 61.5 57 - 64 416 381 - 447 8.2 30 24.3 30 М Sample Control 96.7 58 80 - 170 69.3 58 60 - 116 351 59 299 - 582 8.3 59 8.0 - 8.5 6.8 59 6.2 - 7.2 24.9 59 23.0 - 27.6 0 **Ref Tox Control** 0 NA 34 332 - 372 34 8.0 - 8.6 34 6.4 - 7.2 23.8 - 26.1 Μ NA NA NA 346 8.2 6.8 24.9 34 212 - 330 Ν Sample Control 87.7 30 81 - 91 58.3 30 24 - 61 299 30 8.2 30 8.1 - 8.5 8.6 30 8.3 - 8.8 24.7 30 24.3 - 25.1 30 81 - 91 30 302 30 30 8.0 - 8.2 8.6 30 30 Ν **Ref Tox Control** 87.5 59.5 57 - 61 271 - 327 8.1 8.4 - 8.8 24.7 24.3 - 25.3

## Table A5. Summary status of water quality data that is available to SCCWRP from the accredited laboratories. The data is divided into test sample controls and reference toxicant test controls per laboratory.

		Hardnes	s (mg	/L CaCO₃)	Alkalinit	y (mg	/L CaCO₃)	Conduct	ivity (į	uS/cm)	рН			Dissolve	d Oxy	gen (mg/L)	Tempera	ature	°C)
Lab	Test Type	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range	Mean	N	Range
о	Sample Control	94.9	30	89 - 99	61.9	30	60 - 40	365	30	356 - 374	7.9	30	7.7 - 8.1	8.3	30	8.0 - 8.8	24.9	30	24.3 - 25.2
0	Ref Tox Control	94.9	30	89 - 99	61.9	30	60 - 40	365	30	357 - 372	7.9	30	7.6 - 8.0	8.3	30	7.8 - 8.7	24.9	30	24.4 - 25.4
Р	Sample Control	94.6	80	87 - 99	88.7	75	75 - 84	205	80	193 - 212	8.1	80	7.8 - 8.4	8.4	80	8.2 - 8.7	24.9	80	24.3 - 25.4
Р	Ref Tox Control	93.9	28	87 - 99	89.0	27	84 - 97	205	28	199 - 210	8.1	28	7.7 - 8.4	8.4	28	8.2 - 8.7	24.8	28	24.4 - 25.3
Q	Sample Control	87.7	19	84 - 112	86.9	19	78 - 101	203	25	191 - 275	8.2	25	7.9 - 8.5	8.0	25	7.9 - 8.3	25.1	25	24.1 - 26.4
0	Ref Tox Control	87.7	17	83 - 111	87.7	17	78 - 101	206	23	194 - 269	8.2	23	7.8 - 8.4	8.0	23	7.7 - 8.3	25.1	23	24.0 - 26.3

Table A5 continued. Summary status of water quality data that is available to SCCWRP from the accredited laboratories.



### **BIOLOGICAL RESPONSE DATA**

This section summarizes the basic biological response data from the accredited laboratories. The left half of each lab's distribution is a box plot with box hinges equivalent to the 75<sup>th</sup>,50<sup>th</sup> (median), and 25<sup>th</sup> percentiles. The whiskers are 1.5x the quartile ranges. The right half of each labs distribution is a violin plot. The circle symbol is the lab mean.



Figure A1. Mean number of neonates per female per test for controls for each laboratory. This was created by averaging the number neonates per female across all replicates in each test. The test acceptability criterion for controls is a mean of 15 neonates per female per test.



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Figure A2. Coefficient of variation (CV) for number of neonates per female per test for controls for each laboratory. CVs are calculated as the SD/mean for each test. See the next plot for a zoom in on a reduced scale from 0.0 - 1.0.



Figure A3. Coefficient of variation (CV) for number of neonates per female per test for controls for each laboratory. CVs are calculated as the SD/mean for each test. This plot is a zoom in on a reduced scale from 0.0 - 1.0. See the previous graph for the full scale from 0 - 8. Current unofficial EPA guidance is that long-term average CVs (the circle symbol) should be equal to or less than 0.15 for each laboratory.



Figure A4. Example daily production – Lab C. Neonates per female per control replicate per day for three tests with different reproduction rates.





Figure A5. Example daily production – Lab E. Neonates per female per control replicate per day for three tests with different reproduction rates.





Figure A6. Example daily production – Lab M. Neonates per female per control replicate per day for three tests with different reproduction rates.





Figure A7. Test length by laboratory. The test termination was identified by each lab for each test.

Figure A8. Mean survival of control replicates per test per laboratory. Each symbol is the mean survival of control replicates per test. Test acceptability criteria is 80% mean survival.



Figure A9. Age of females at test initiation per test per laboratory. Only 12 of the 17 labs provided age at test initiation. Max age was used because not all labs track the range of times of individual replicates. Test guidance requires replicates be within 8 hours of each other and no replicate greater than 24 hours old.



### WATER QUALITY DATA

Water quality is a requirement of every test.

However, not all labs measured or reported water quality in the same fashion. For example, pH, DO, and temperature were measured daily while hardness, alkalinity, and conductivity were measured between once per test and everyday depending on the lab. Some labs measured from test chambers while others utilized surrogates. For the following analyses, we averaged data to once per test, which is the most common time step across the most labs. All units were standardized for the following analyses.

















### Figure A13. Mean Dissolved oxygen for each test per laboratory.







Figure A15. Mean temperature for each test per laboratory.

### **REFERENCE TOXICANT DATA**

All of the laboratories provided reference toxicant data, but not all laboratories use the same reference toxicant. Here we present both survival and reproduction endpoints.



Figure A16. Reference toxicant LC50 by test per laboratory. Not all laboratories used the same reference toxicant. LC50 values were provided by each laboratory.



Figure A17. Reference toxicant IC50 by test per laboratory. Not all laboratories used the same reference toxicant. IC50 values were provided by each laboratory



Lab	Mean PMSD	CV of Mean PMSD	Range of Mean PMSD
А	16.73	0.534	6.95 - 44.23
В	31.83	0.468	12.02 - 102
С	24.11	0.574	7.65 – 79.66
D	23.88	0.544	11.44 - 46.54
E	19.62	0.246	11.36 - 31.19
F	22.16	0.437	8.64 - 57.11
G	31.24	0.262	18.52 - 45.86
Н	28.62	1.299	6.66 - 159.05
I	18.78	0.828	7.88 - 80.06
J	32.65	0.795	14.22 - 121
К	29.40	0.858	9.47 - 109
L	22.33	0.254	10.95 - 34.35
М	22.68	0.493	11.22 - 53.63
Ν	15.64	0.268	9.46 - 24.21
0	17.86	0.599	7.31 - 47.63
Р	15.61	0.392	7.56 - 40.79
Q	18.77	0.394	8.37 - 33.39

Table A6. Percent minimum significant difference (PMSD) for reference toxicants in the historical dataset.



### CORRELATIONS

The following plots are heat maps of Spearmen Rank Correlations among variables. Biological variables, lab technique, and water quality variables are all combined to give a first impression picture of relationships. Data are analyzed on a per test basis (so test means or single values, whichever was available). The correlation coefficient values that created the heat map can be found <u>here</u>. As noted in the data summary tables, not all labs provided all of the data, so the individual parameters evaluated will vary among different labs.



Figure A18. Heat map of spearman rank correlations between all variables with all labs and all tests combined. Not the best way to do this, but a big picture start.



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### Figure A19. Spearman Rank Correlations by laboratory

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### Laboratory B Rank Correlations





![](_page_39_Figure_0.jpeg)

### Laboratory E Rank Correlations

![](_page_40_Figure_0.jpeg)

![](_page_41_Figure_0.jpeg)

![](_page_42_Figure_0.jpeg)

### Laboratory H Rank Correlations

![](_page_43_Figure_0.jpeg)

![](_page_44_Figure_0.jpeg)

### Laboratory J Rank Correlations

![](_page_45_Figure_0.jpeg)

![](_page_46_Figure_0.jpeg)

# Laboratory L Rank Correlations

![](_page_47_Figure_0.jpeg)

![](_page_48_Figure_0.jpeg)

![](_page_49_Figure_0.jpeg)

## Laboratory O Rank Correlations

![](_page_50_Figure_0.jpeg)

## Laboratory P Rank Correlations

![](_page_51_Figure_0.jpeg)

# Potential Sources of Variance in Test Performance – Historical Data

Table B29. Variable importance values from random forest regression models of mean of control neonate production as predicted by the reported lab techniques and test conditions in the historical test data. Predictor variables are ranked by their importance within a given lab. Importance is measured by the % increase in Mean Square Error when the variable was omitted from model runs. N is the number of tests used to create the model.

Lab	Response Variable	Predictor Variable	Variable Importance	n	Variable Importance
			(%MSE Change)		Rank
А	Mean Neonates	Alkalinity (mg/L)-Mean	95.9	78	1
А	Mean Neonates	Conductivity (uS/cm)-CV	48.0	78	2
А	Mean Neonates	Conductivity (uS/cm)-StDev	47.7	78	3
А	Mean Neonates	Conductivity (uS/cm)-Mean	36.7	78	4
А	Mean Neonates	pH-Mean	30.5	78	5
А	Mean Neonates	Hardness (mg/L)-Mean	29.0	78	6
А	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	23.9	78	7
А	Mean Neonates	Hardness (mg/L)-CV	20.3	78	8
А	Mean Neonates	Hardness (mg/L)-StDev	18.9	78	9
А	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	14.7	78	10
А	Mean Neonates	Dissolved Oxygen (mg/L)-CV	14.4	78	11
А	Mean Neonates	Year	13.9	78	12
А	Mean Neonates	pH-CV	13.3	78	13
А	Mean Neonates	Alkalinity (mg/L)-StDev	12.0	78	14
А	Mean Neonates	Alkalinity (mg/L)-CV	11.7	78	15
А	Mean Neonates	pH-StDev	9.1	78	16
А	Mean Neonates	Temperature (C)-CV	0.0	78	18.5
А	Mean Neonates	Test Replicates	0.0	78	18.5
А	Mean Neonates	Temperature (C)-StDev	0.0	78	18.5
А	Mean Neonates	Temperature (C)-Mean	0.0	78	18.5
В	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	51.2	95	1
В	Mean Neonates	Temperature (C)-Mean	23.6	95	2

В	Mean Neonates	Dissolved Oxygen (mg/L)-CV	23.4	95	3
В	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	21.2	95	4
В	Mean Neonates	Year	20.9	95	5
В	Mean Neonates	Conductivity (uS/cm)-CV	20.3	95	6
В	Mean Neonates	Conductivity (uS/cm)-StDev	17.7	95	7
В	Mean Neonates	pH-Mean	10.6	95	8
В	Mean Neonates	pH-StDev	7.7	95	9
В	Mean Neonates	pH-CV	6.2	95	10
В	Mean Neonates	Conductivity (uS/cm)-Mean	4.3	95	11
В	Mean Neonates	Temperature (C)-StDev	2.2	95	12
В	Mean Neonates	Temperature (C)-CV	2.0	95	13
В	Mean Neonates	Test Replicates	0.0	95	14
С	Mean Neonates	Temperature (C)-Mean	25.6	56	1
С	Mean Neonates	Year	24.9	56	2
С	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	23.8	56	3
С	Mean Neonates	Conductivity (uS/cm)-Mean	17.3	56	4
С	Mean Neonates	Hardness (mg/L)-Mean	16.3	56	5
С	Mean Neonates	pH-Mean	16.1	56	6
С	Mean Neonates	Alkalinity (mg/L)-Mean	12.0	56	7
С	Mean Neonates	pH-StDev	11.9	56	8
С	Mean Neonates	pH-CV	11.9	56	9
С	Mean Neonates	Conductivity (uS/cm)-CV	10.8	56	10
С	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	9.7	56	11
С	Mean Neonates	Dissolved Oxygen (mg/L)-CV	6.6	56	12
С	Mean Neonates	Temperature (C)-CV	1.8	56	13
С	Mean Neonates	Conductivity (uS/cm)-StDev	1.3	56	14
С	Mean Neonates	Temperature (C)-StDev	0.5	56	15
С	Mean Neonates	Test Replicates	0.0	56	16
D	Mean Neonates	Age at Start (hrs)	30.5	25	1
D	Mean Neonates	Average Age	30.4	25	2
D	Mean Neonates	Temperature (C)-StDev	20.3	25	3
D	Mean Neonates	Temperature (C)-CV	19.3	25	4

D	Mean Neonates	Hardness (mg/L)-Mean	13.0	25	5
D	Mean Neonates	Conductivity (uS/cm)-Mean	7.6	25	6
D	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	6.1	25	7
D	Mean Neonates	Dissolved Oxygen (mg/L)-CV	2.7	25	8
D	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	0.8	25	9
D	Mean Neonates	Alkalinity (mg/L)-Mean	0.6	25	10
D	Mean Neonates	Temperature (C)-Mean	0.2	25	11
D	Mean Neonates	pH-StDev	0.1	25	12
D	Mean Neonates	Test Replicates	0.0	25	13
D	Mean Neonates	pH-CV	0.0	25	14
D	Mean Neonates	Year	-0.8	25	15
D	Mean Neonates	pH-Mean	-3.7	25	16
D	Mean Neonates	Conductivity (uS/cm)-StDev	-4.4	25	17
D	Mean Neonates	Conductivity (uS/cm)-CV	-6.3	25	18
Е	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	95.4	139	1
Е	Mean Neonates	pH-StDev	75.9	139	2
Е	Mean Neonates	pH-CV	73.3	139	3
Е	Mean Neonates	Hardness (mg/L)-Mean	65.6	139	4
Е	Mean Neonates	Dissolved Oxygen (mg/L)-CV	64.6	139	5
Е	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	63.4	139	6
Е	Mean Neonates	pH-Mean	62.2	139	7
Е	Mean Neonates	Alkalinity (mg/L)-Mean	62.0	139	8
Е	Mean Neonates	Alkalinity (mg/L)-CV	60.4	139	9
Е	Mean Neonates	Conductivity (uS/cm)-CV	58.5	139	10
Е	Mean Neonates	Conductivity (uS/cm)-Mean	58.3	139	11
Е	Mean Neonates	Alkalinity (mg/L)-StDev	56.0	139	12
Е	Mean Neonates	Conductivity (uS/cm)-StDev	54.3	139	13
Е	Mean Neonates	Hardness (mg/L)-CV	46.0	139	14
Е	Mean Neonates	Hardness (mg/L)-StDev	44.0	139	15
Е	Mean Neonates	Year	43.4	139	16
Е	Mean Neonates	Test Replicates	30.6	139	17
Е	Mean Neonates	Temperature (C)-Mean	14.6	139	18

Е	Mean Neonates	Temperature (C)-CV	14.4	139	19
Е	Mean Neonates	Temperature (C)-StDev	14.3	139	20
F	Mean Neonates	Age at Start (hrs)	30.8	75	1
F	Mean Neonates	Average Age	30.2	75	2
F	Mean Neonates	pH-CV	24.0	75	3
F	Mean Neonates	pH-StDev	22.3	75	4
F	Mean Neonates	Alkalinity (mg/L)-Mean	17.9	75	5
F	Mean Neonates	Hardness (mg/L)-Mean	14.5	75	6
F	Mean Neonates	Year	10.6	75	7
F	Mean Neonates	pH-Mean	7.0	75	8
F	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	7.0	75	9
F	Mean Neonates	Conductivity (uS/cm)-Mean	2.6	75	10
F	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	2.6	75	11
F	Mean Neonates	Dissolved Oxygen (mg/L)-CV	2.4	75	12
F	Mean Neonates	Temperature (C)-StDev	0.4	75	13
F	Mean Neonates	Conductivity (uS/cm)-StDev	0.4	75	14
F	Mean Neonates	Test Replicates	-1.0	75	15
F	Mean Neonates	Temperature (C)-CV	-1.1	75	16
F	Mean Neonates	Conductivity (uS/cm)-CV	-6.0	75	17
F	Mean Neonates	Temperature (C)-Mean	-7.4	75	18
G	Mean Neonates	Conductivity (uS/cm)-Mean	36.9	29	1
G	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	30.6	29	2
G	Mean Neonates	Dissolved Oxygen (mg/L)-CV	26.6	29	3
G	Mean Neonates	Average Age	7.5	29	4
G	Mean Neonates	Age at Start (hrs)	5.5	29	5
G	Mean Neonates	Temperature (C)-Mean	3.5	29	6
G	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	0.1	29	7
G	Mean Neonates	Test Replicates	0.0	29	8
G	Mean Neonates	Hardness (mg/L)-Mean	-0.5	29	9
G	Mean Neonates	Year	-0.7	29	10
G	Mean Neonates	Conductivity (uS/cm)-CV	-2.5	29	11
G	Mean Neonates	pH-CV	-3.1	29	12

G	Mean Neonates	Temperature (C)-CV	-3.3	29	13
G	Mean Neonates	Temperature (C)-StDev	-3.4	29	14
G	Mean Neonates	pH-StDev	-3.5	29	15
G	Mean Neonates	Conductivity (uS/cm)-StDev	-3.6	29	16
G	Mean Neonates	pH-Mean	-9.5	29	17
Н	Mean Neonates	Dissolved Oxygen (mg/L)-CV	17.0	17	1
Н	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	16.7	17	2
Н	Mean Neonates	pH-CV	13.7	17	3
Н	Mean Neonates	pH-StDev	11.3	17	4
Н	Mean Neonates	pH-Mean	11.2	17	5
Н	Mean Neonates	Test Replicates	0.0	17	6
Н	Mean Neonates	Temperature (C)-StDev	-3.0	17	7
Н	Mean Neonates	Temperature (C)-CV	-3.0	17	8
Н	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	-3.5	17	9
Н	Mean Neonates	Temperature (C)-Mean	-5.9	17	10
Н	Mean Neonates	Alkalinity (mg/L)-Mean	-6.0	17	11
Н	Mean Neonates	Year	-7.7	17	 12
H I	Mean Neonates Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean	-7.7 32.7	17 60	12 1
H   	Mean Neonates Mean Neonates Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean	-7.7 32.7 26.5	17 60 60	12 1 2
H     	Mean Neonates Mean Neonates Mean Neonates Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean	-7.7 32.7 26.5 24.6	17 60 60 60	12 1 2 3
H       	Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean	-7.7 32.7 26.5 24.6 22.1	17 60 60 60 60	12 1 2 3 4
H         	Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev	-7.7 32.7 26.5 24.6 22.1 16.4	17 60 60 60 60 60	12 1 2 3 4 5
H           	Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV	-7.7 32.7 26.5 24.6 22.1 16.4 16.3	17 60 60 60 60 60 60	12 1 2 3 4 5 6
H             	Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates Mean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8	17 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7
H               	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4	17 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8
H 	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev Dissolved Oxygen (mg/L)-StDev	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4 10.3	17 60 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8 9
H                 	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev Dissolved Oxygen (mg/L)-StDev pH-CV	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4 10.3 9.1	17 60 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8 9 10
H 	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev Dissolved Oxygen (mg/L)-StDev pH-CV Year	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4 10.3 9.1 3.8	17 60 60 60 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8 9 10 11
H 	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev Dissolved Oxygen (mg/L)-StDev pH-CV Year Conductivity (uS/cm)-CV	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4 10.3 9.1 3.8 2.3	17 60 60 60 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8 9 10 11 11 12
H 	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev Dissolved Oxygen (mg/L)-StDev pH-StDev Vear Conductivity (uS/cm)-CV Age at Start (hrs)	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4 10.3 9.1 3.8 2.3 1.8	17 60 60 60 60 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8 9 10 11 12 12
H 	Mean NeonatesMean Neonates	Year Dissolved Oxygen (mg/L)-Mean Alkalinity (mg/L)-Mean Temperature (C)-Mean Hardness (mg/L)-Mean Temperature (C)-StDev Dissolved Oxygen (mg/L)-CV Temperature (C)-CV pH-StDev Dissolved Oxygen (mg/L)-StDev pH-CV Year Conductivity (uS/cm)-CV Age at Start (hrs) Conductivity (uS/cm)-Mean	-7.7 32.7 26.5 24.6 22.1 16.4 16.3 12.8 10.4 10.3 9.1 3.8 2.3 1.8 1.5	17 60 60 60 60 60 60 60 60 60 60 60 60 60	12 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Ι	Mean Neonates	Test Replicates	0.0	60	16
Ι	Mean Neonates	Average Age	-0.4	60	17
Ι	Mean Neonates	pH-Mean	-4.5	60	18
К	Mean Neonates	Temperature (C)-Mean	29.1	34	1
К	Mean Neonates	Temperature (C)-CV	23.2	34	2
К	Mean Neonates	Temperature (C)-StDev	23.1	34	3
К	Mean Neonates	pH-Mean	18.6	34	4
К	Mean Neonates	pH-CV	15.4	34	5
К	Mean Neonates	pH-StDev	12.8	34	6
К	Mean Neonates	Year	12.3	34	7
К	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	4.8	34	8
К	Mean Neonates	Age at Start (hrs)	3.8	34	9
К	Mean Neonates	Average Age	3.5	34	10
К	Mean Neonates	Dissolved Oxygen (mg/L)-CV	2.8	34	11
К	Mean Neonates	Test Replicates	0.0	34	12
К	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	-2.7	34	13
К	Mean Neonates	Conductivity (uS/cm)-Mean	-5.4	34	14
L	Mean Neonates	Average Age	31.5	57	1
L	Mean Neonates	Age at Start (hrs)	31.5	57	2
L	Mean Neonates	pH-Mean	22.1	57	3
L	Mean Neonates	Year	18.1	57	4
L	Mean Neonates	Dissolved Oxygen (mg/L)-CV	16.3	57	5
L	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	8.3	57	6
L	Mean Neonates	Conductivity (uS/cm)-Mean	7.0	57	7
L	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	1.6	57	8
L	Mean Neonates	Test Replicates	0.0	57	9
L	Mean Neonates	pH-StDev	-1.9	57	10
L	Mean Neonates	pH-CV	-3.2	57	11
L	Mean Neonates	Temperature (C)-StDev	-3.2	57	12
L	Mean Neonates	Temperature (C)-CV	-3.7	57	13
L	Mean Neonates	Temperature (C)-Mean	-3.8	57	14
L	Mean Neonates	Conductivity (uS/cm)-StDev	-5.7	57	15

L	Mean Neonates	Conductivity (uS/cm)-CV	-7.0	57	16
Μ	Mean Neonates	Year	56.3	95	1
Μ	Mean Neonates	Conductivity (uS/cm)-StDev	18.3	95	2
Μ	Mean Neonates	Conductivity (uS/cm)-CV	15.6	95	3
Μ	Mean Neonates	Conductivity (uS/cm)-Mean	7.2	95	4
Μ	Mean Neonates	Temperature (C)-StDev	6.0	95	5
Μ	Mean Neonates	pH-StDev	5.8	95	6
Μ	Mean Neonates	pH-CV	5.3	95	7
Μ	Mean Neonates	Dissolved Oxygen (mg/L)-CV	4.3	95	8
Μ	Mean Neonates	Temperature (C)-CV	3.1	95	9
Μ	Mean Neonates	Light Intensity (fc)-Mean	2.7	95	10
Μ	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	2.0	95	11
Μ	Mean Neonates	Test Replicates	0.0	95	12
Μ	Mean Neonates	Temperature (C)-Mean	-1.1	95	13
Μ	Mean Neonates	Age at Start (hrs)	-1.8	95	14
Μ	Mean Neonates	Average Age	-3.3	95	15
Μ	Mean Neonates	pH-Mean	-3.4	95	16
Μ	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	-4.5	95	17
Ν	Mean Neonates	Hardness (mg/L)-Mean	55.9	60	1
Ν	Mean Neonates	Year	24.6	60	2
Ν	Mean Neonates	Hardness (mg/L)-CV	16.1	60	3
Ν	Mean Neonates	Alkalinity (mg/L)-StDev	14.5	60	4
Ν	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	12.6	60	5
Ν	Mean Neonates	Alkalinity (mg/L)-Mean	12.1	60	6
Ν	Mean Neonates	Conductivity (uS/cm)-CV	8.7	60	7
Ν	Mean Neonates	Age at Start (hrs)	8.7	60	8
Ν	Mean Neonates	Conductivity (uS/cm)-StDev	7.8	60	9
Ν	Mean Neonates	Hardness (mg/L)-StDev	6.7	60	10
Ν	Mean Neonates	Conductivity (uS/cm)-Mean	6.6	60	11
Ν	Mean Neonates	Average Age	6.3	60	12
Ν	Mean Neonates	Alkalinity (mg/L)-CV	4.9	60	13
N	Mean Neonates	Dissolved Oxygen (mg/L)-CV	3.1	60	14

Ν	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	2.8	60	15
Ν	Mean Neonates	Test Replicates	0.0	60	16
Ν	Mean Neonates	pH-Mean	0.0	60	17
Ν	Mean Neonates	pH-CV	-0.1	60	18
Ν	Mean Neonates	Temperature (C)-Mean	-0.7	60	19
Ν	Mean Neonates	pH-StDev	-1.0	60	20
Ν	Mean Neonates	Temperature (C)-StDev	-15.2	60	21
Ν	Mean Neonates	Temperature (C)-CV	-16.9	60	22
0	Mean Neonates	Hardness (mg/L)-Mean	34.9	60	1
0	Mean Neonates	pH-Mean	30.0	60	2
0	Mean Neonates	Temperature (C)-Mean	29.3	60	3
0	Mean Neonates	Year	25.9	60	4
0	Mean Neonates	Conductivity (uS/cm)-Mean	21.6	60	5
0	Mean Neonates	Alkalinity (mg/L)-Mean	15.6	60	6
0	Mean Neonates	Average Age	12.4	60	7
0	Mean Neonates	Age at Start (hrs)	11.0	60	8
0	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	7.0	60	9
0	Mean Neonates	Temperature (C)-CV	7.0	60	10
0	Mean Neonates	pH-CV	5.0	60	11
0	Mean Neonates	Temperature (C)-StDev	4.3	60	12
0	Mean Neonates	Dissolved Oxygen (mg/L)-CV	3.4	60	13
0	Mean Neonates	pH-StDev	2.2	60	14
0	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	1.8	60	15
0	Mean Neonates	Conductivity (uS/cm)-StDev	1.8	60	16
0	Mean Neonates	Conductivity (uS/cm)-CV	0.4	60	17
0	Mean Neonates	Test Replicates	0.0	60	18
Р	Mean Neonates	Alkalinity (mg/L)-Mean	69.2	108	1
Р	Mean Neonates	pH-StDev	36.4	108	2
Р	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	33.2	108	3
Р	Mean Neonates	Dissolved Oxygen (mg/L)-CV	32.4	108	4
Р	Mean Neonates	Hardness (mg/L)-Mean	30.3	108	5
Р	Mean Neonates	Year	29.6	108	6

Р	Mean Neonates	pH-CV	25.2	108	7
Р	Mean Neonates	pH-Mean	24.6	108	8
Р	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	22.9	108	9
Р	Mean Neonates	Temperature (C)-Mean	22.4	108	10
Р	Mean Neonates	Light Intensity (fc)-Mean	17.0	108	11
Р	Mean Neonates	Conductivity (uS/cm)-Mean	16.2	108	12
Р	Mean Neonates	Temperature (C)-StDev	13.2	108	13
Р	Mean Neonates	Temperature (C)-CV	13.1	108	14
Р	Mean Neonates	Age at Start (hrs)	12.0	108	15
Р	Mean Neonates	Average Age	11.6	108	16
Р	Mean Neonates	Conductivity (uS/cm)-CV	10.5	108	17
Р	Mean Neonates	Conductivity (uS/cm)-StDev	9.0	108	18
Р	Mean Neonates	Test Replicates	2.6	108	19
Q	Mean Neonates	Dissolved Oxygen (mg/L)-Mean	45.1	48	1
Q	Mean Neonates	Year	33.1	48	2
Q	Mean Neonates	Temperature (C)-Mean	26.2	48	3
Q	Mean Neonates	Dissolved Oxygen (mg/L)-CV	15.3	48	4
Q	Mean Neonates	Dissolved Oxygen (mg/L)-StDev	13.4	48	5
Q	Mean Neonates	Conductivity (uS/cm)-CV	10.1	48	6
Q	Mean Neonates	Conductivity (uS/cm)-StDev	6.0	48	7
Q	Mean Neonates	pH-Mean	3.8	48	8
Q	Mean Neonates	pH-StDev	1.7	48	9
Q	Mean Neonates	Test Replicates	0.0	48	10
Q	Mean Neonates	pH-CV	-1.2	48	11
Q	Mean Neonates	Conductivity (uS/cm)-Mean	-2.5	48	12
Q	Mean Neonates	Temperature (C)-StDev	-7.7	48	13
Q	Mean Neonates	Temperature (C)-CV	-8.0	48	14

Table B 30. Variable importance values from random forest regression models of mean of control neonate production as predicted by the reported lab techniques and test conditions in the historical test data. Predictor variables are ranked by their importance within a given lab. Importance is measured by the % increase in Mean Square Error when the variable was omitted from model runs. N is the number of tests used to create the model.

Lab	Response Variable	Predictor Variable	Variable Importance (%MSE Change)	n	Variable Importance Rank
А	CV of Neonates	Conductivity (uS/cm)-CV	9.6	78	1
А	CV of Neonates	Conductivity (uS/cm)-StDev	9.1	78	2
А	CV of Neonates	pH-StDev	6.6	78	3
А	CV of Neonates	pH-CV	6.3	78	4
А	CV of Neonates	Dissolved Oxygen (mg/L)-CV	5.9	78	5
А	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	5.8	78	6
А	CV of Neonates	Alkalinity (mg/L)-Mean	5.5	78	7
А	CV of Neonates	Hardness (mg/L)-Mean	4.5	78	8
А	CV of Neonates	Alkalinity (mg/L)-CV	3.8	78	9
А	CV of Neonates	Conductivity (uS/cm)-Mean	2.5	78	10
А	CV of Neonates	Alkalinity (mg/L)-StDev	2.1	78	11
А	CV of Neonates	pH-Mean	1.4	78	12
А	CV of Neonates	Temperature (C)-CV	0.0	78	14.5
А	CV of Neonates	Test Replicates	0.0	78	14.5
А	CV of Neonates	Temperature (C)-StDev	0.0	78	14.5
А	CV of Neonates	Temperature (C)-Mean	0.0	78	14.5
А	CV of Neonates	Year	-0.4	78	17
А	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-3.4	78	18
А	CV of Neonates	Hardness (mg/L)-StDev	-5.0	78	19
А	CV of Neonates	Hardness (mg/L)-CV	-5.0	78	20
В	CV of Neonates	Temperature (C)-StDev	4.4	95	1
В	CV of Neonates	Conductivity (uS/cm)-CV	4.3	95	2
В	CV of Neonates	Conductivity (uS/cm)-StDev	3.9	95	3
В	CV of Neonates	Temperature (C)-CV	3.6	95	4

В	CV of Neonates	pH-StDev	2.6	95	5
В	CV of Neonates	pH-CV	2.6	95	6
В	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	2.5	95	7
В	CV of Neonates	Dissolved Oxygen (mg/L)-CV	2.0	95	8
В	CV of Neonates	Temperature (C)-Mean	1.8	95	9
В	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	1.5	95	10
В	CV of Neonates	Year	0.5	95	11
В	CV of Neonates	Test Replicates	0.0	95	12
В	CV of Neonates	pH-Mean	-0.4	95	13
В	CV of Neonates	Conductivity (uS/cm)-Mean	-0.5	95	14
С	CV of Neonates	pH-Mean	17.6	56	1
С	CV of Neonates	Conductivity (uS/cm)-Mean	16.0	56	2
С	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	14.1	56	3
С	CV of Neonates	pH-StDev	10.7	56	4
С	CV of Neonates	pH-CV	10.6	56	5
С	CV of Neonates	Year	8.0	56	6
С	CV of Neonates	Hardness (mg/L)-Mean	7.5	56	7
С	CV of Neonates	Temperature (C)-CV	6.6	56	8
С	CV of Neonates	Temperature (C)-StDev	6.2	56	9
С	CV of Neonates	Temperature (C)-Mean	5.9	56	10
С	CV of Neonates	Alkalinity (mg/L)-Mean	5.1	56	11
С	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	4.8	56	12
С	CV of Neonates	Dissolved Oxygen (mg/L)-CV	3.5	56	13
С	CV of Neonates	Conductivity (uS/cm)-CV	0.5	56	14
С	CV of Neonates	Test Replicates	0.0	56	15
С	CV of Neonates	Conductivity (uS/cm)-StDev	-2.0	56	16
D	CV of Neonates	Year	16.0	25	1
D	CV of Neonates	Dissolved Oxygen (mg/L)-CV	2.4	25	2
D	CV of Neonates	Test Replicates	0.0	25	3
D	CV of Neonates	Average Age	-0.3	25	4
D	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	-0.6	25	5
D	CV of Neonates	Age at Start (hrs)	-1.2	25	6

DCV of NeonatespH-CV-2.425DCV of NeonatespH-StDev-2.425DCV of NeonatesDissolved Oxygen (mg/L)-Mean-4.525	7 8 9
DCV of NeonatespH-StDev-2.425DCV of NeonatesDissolved Oxygen (mg/L)-Mean-4.525	8
D CV of Neonates Dissolved Oxygen (mg/L)-Mean -4.5 25	9
	10
D CV of Neonates pH-Mean -6.2 25	10
D CV of Neonates Hardness (mg/L)-Mean -6.9 25	11
D CV of Neonates Alkalinity (mg/L)-Mean -7.7 25	12
D CV of Neonates Temperature (C)-CV -9.0 25	13
D CV of Neonates Temperature (C)-StDev -9.3 25	14
D CV of Neonates Conductivity (uS/cm)-CV -10.2 25	15
D CV of Neonates Conductivity (uS/cm)-StDev -12.1 25	16
D CV of Neonates Conductivity (uS/cm)-Mean -12.6 25	17
D CV of Neonates Temperature (C)-Mean -12.8 25	18
E CV of Neonates Conductivity (uS/cm)-Mean 78.9 139	1
E CV of Neonates pH-Mean 75.4 139	2
E CV of Neonates pH-CV 75.3 139	3
E CV of Neonates Alkalinity (mg/L)-Mean 73.5 139	4
E CV of Neonates pH-StDev 72.9 139	5
E CV of Neonates Dissolved Oxygen (mg/L)-Mean 70.7 139	6
E CV of Neonates Hardness (mg/L)-Mean 69.0 139	7
E CV of Neonates Dissolved Oxygen (mg/L)-CV 64.3 139	8
E CV of Neonates Conductivity (uS/cm)-StDev 63.7 139	9
E CV of Neonates Conductivity (uS/cm)-CV 62.4 139	10
ECV of NeonatesDissolved Oxygen (mg/L)-StDev61.6139	11
E CV of Neonates Year 51.9 139	12
E CV of Neonates Hardness (mg/L)-CV 46.9 139	13
E CV of Neonates Hardness (mg/L)-StDev 44.5 139	14
E CV of Neonates Alkalinity (mg/L)-CV 28.4 139	15
E CV of Neonates Alkalinity (mg/L)-StDev 25.7 139	16
E CV of Neonates Test Replicates 19.4 139	17
E CV of Neonates Temperature (C)-StDev 8.6 139	18
E CV of Neonates Temperature (C)-Mean 6.7 139	19
E CV of Neonates Temperature (C)-CV 6.2 139	20

F	CV of Neonates	Age at Start (hrs)	51.2	75	1	
F	CV of Neonates	Average Age	50.4	75	2	
F	CV of Neonates	Alkalinity (mg/L)-Mean	36.7	75	3	
F	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	18.4	75	4	
F	CV of Neonates	Conductivity (uS/cm)-Mean	7.5	75	5	
F	CV of Neonates	Conductivity (uS/cm)-StDev	7.2	75	6	
F	CV of Neonates	Conductivity (uS/cm)-CV	7.0	75	7	
F	CV of Neonates	pH-Mean	3.4	75	8	
F	CV of Neonates	Hardness (mg/L)-Mean	1.9	75	9	
F	CV of Neonates	Test Replicates	0.5	75	10	
F	CV of Neonates	Temperature (C)-Mean	-2.0	75	11	
F	CV of Neonates	pH-StDev	-3.4	75	12	
F	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	-3.7	75	13	
F	CV of Neonates	pH-CV	-3.8	75	14	
F	CV of Neonates	Year	-4.0	75	15	
F	CV of Neonates	Dissolved Oxygen (mg/L)-CV	-6.2	75	16	
F	CV of Neonates	Temperature (C)-CV	-8.5	75	17	
F	CV of Neonates	Temperature (C)-StDev	-13.1	75	18	
G	CV of Neonates	Conductivity (uS/cm)-Mean	21.1	29	1	
G	CV of Neonates	pH-Mean	9.3	29	2	
G	CV of Neonates	pH-CV	7.4	29	3	
G	CV of Neonates	pH-StDev	6.1	29	4	
G	CV of Neonates	Test Replicates	0.0	29	5	
G	CV of Neonates	Temperature (C)-CV	-2.8	29	6	
G	CV of Neonates	Year	-3.0	29	7	
G	CV of Neonates	Temperature (C)-StDev	-3.5	29	8	
G	CV of Neonates	Conductivity (uS/cm)-CV	-3.7	29	9	
G	CV of Neonates	Hardness (mg/L)-Mean	-4.4	29	10	
G	CV of Neonates	Conductivity (uS/cm)-StDev	-5.0	29	11	
G	CV of Neonates	Age at Start (hrs)	-5.5	29	12	
G	CV of Neonates	Average Age	-5.7	29	13	
G	CV of Neonates	Dissolved Oxygen (mg/L)-CV	-6.3	29	14	

G	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	-6.3	29	15
G	CV of Neonates	Temperature (C)-Mean	-9.2	29	16
G	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-11.8	29	17
Н	CV of Neonates	pH-Mean	23.9	17	1
Н	CV of Neonates	pH-CV	20.0	17	2
Н	CV of Neonates	pH-StDev	19.2	17	3
Н	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	14.4	17	4
Н	CV of Neonates	Dissolved Oxygen (mg/L)-CV	12.2	17	5
Н	CV of Neonates	Temperature (C)-StDev	7.1	17	6
Н	CV of Neonates	Temperature (C)-CV	5.0	17	7
Н	CV of Neonates	Year	2.2	17	8
Н	CV of Neonates	Test Replicates	0.0	17	9
Н	CV of Neonates	Temperature (C)-Mean	-2.2	17	10
Н	CV of Neonates	Alkalinity (mg/L)-Mean	-7.2	17	11
Н	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-8.8	17	12
Ι	CV of Neonates	pH-StDev	7.8	60	1
Ι	CV of Neonates	Age at Start (hrs)	6.4	60	2
Ι	CV of Neonates	pH-CV	6.3	60	3
Ι	CV of Neonates	Alkalinity (mg/L)-Mean	6.2	60	4
Ι	CV of Neonates	Average Age	5.7	60	5
Ι	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	5.1	60	6
Ι	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	2.2	60	7
Ι	CV of Neonates	pH-Mean	2.0	60	8
Ι	CV of Neonates	Temperature (C)-StDev	1.3	60	9
Ι	CV of Neonates	Temperature (C)-CV	1.0	60	10
Ι	CV of Neonates	Test Replicates	0.0	60	11
I	CV of Neonates	Dissolved Oxygen (mg/L)-CV	-1.2	60	12
I	CV of Neonates	Hardness (mg/L)-Mean	-1.6	60	13
Ι	CV of Neonates	Conductivity (uS/cm)-CV	-2.2	60	14
Ι	CV of Neonates	Conductivity (uS/cm)-StDev	-2.5	60	15
Ι	CV of Neonates	Year	-5.5	60	16
Ι	CV of Neonates	Temperature (C)-Mean	-5.7	60	17

Ι	CV of Neonates	Conductivity (uS/cm)-Mean	-6.7	60	18
К	CV of Neonates	Temperature (C)-CV	18.7	34	1
К	CV of Neonates	Temperature (C)-StDev	16.7	34	2
К	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	10.0	34	3
К	CV of Neonates	Year	5.7	34	4
К	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	5.7	34	5
К	CV of Neonates	pH-Mean	4.2	34	6
К	CV of Neonates	pH-StDev	3.8	34	7
К	CV of Neonates	pH-CV	3.1	34	8
К	CV of Neonates	Temperature (C)-Mean	3.0	34	9
К	CV of Neonates	Dissolved Oxygen (mg/L)-CV	2.6	34	10
К	CV of Neonates	Test Replicates	0.0	34	11
К	CV of Neonates	Average Age	0.0	34	12
К	CV of Neonates	Age at Start (hrs)	-2.3	34	13
К	CV of Neonates	Conductivity (uS/cm)-Mean	-5.0	34	14
L	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	4.8	57	1
L	CV of Neonates	Dissolved Oxygen (mg/L)-CV	1.3	57	2
L	CV of Neonates	Average Age	1.0	57	3
L	CV of Neonates	Age at Start (hrs)	0.7	57	4
L	CV of Neonates	Test Replicates	0.0	57	5
L	CV of Neonates	pH-CV	-4.9	57	6
L	CV of Neonates	pH-Mean	-7.1	57	7
L	CV of Neonates	pH-StDev	-7.2	57	8
L	CV of Neonates	Temperature (C)-StDev	-8.9	57	9
L	CV of Neonates	Temperature (C)-Mean	-9.0	57	10
L	CV of Neonates	Conductivity (uS/cm)-Mean	-9.1	57	11
L	CV of Neonates	Year	-9.1	57	12
L	CV of Neonates	Temperature (C)-CV	-10.0	57	13
L	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-11.5	57	14
L	CV of Neonates	Conductivity (uS/cm)-CV	-14.0	57	15
L	CV of Neonates	Conductivity (uS/cm)-StDev	-15.5	57	16
Μ	CV of Neonates	Dissolved Oxygen (mg/L)-CV	6.6	95	1

Μ	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	5.6	95	2	
Μ	CV of Neonates	Temperature (C)-StDev	5.6	95	3	
Μ	CV of Neonates	Temperature (C)-CV	5.0	95	4	
Μ	CV of Neonates	Conductivity (uS/cm)-StDev	3.8	95	5	
Μ	CV of Neonates	Conductivity (uS/cm)-Mean	2.9	95	6	
Μ	CV of Neonates	pH-CV	2.9	95	7	
Μ	CV of Neonates	pH-StDev	2.4	95	8	
Μ	CV of Neonates	Conductivity (uS/cm)-CV	1.8	95	9	
Μ	CV of Neonates	Temperature (C)-Mean	1.1	95	10	
Μ	CV of Neonates	pH-Mean	0.6	95	11	
Μ	CV of Neonates	Test Replicates	0.0	95	12	
Μ	CV of Neonates	Year	-0.6	95	13	
Μ	CV of Neonates	Age at Start (hrs)	-1.2	95	14	
Μ	CV of Neonates	Average Age	-1.3	95	15	
Μ	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-2.3	95	16	
Μ	CV of Neonates	Light Intensity (fc)-Mean	-6.4	95	17	
Ν	CV of Neonates	Hardness (mg/L)-CV	39.1	60	1	
Ν	CV of Neonates	Hardness (mg/L)-Mean	31.3	60	2	
Ν	CV of Neonates	Year	23.5	60	3	
Ν	CV of Neonates	Conductivity (uS/cm)-Mean	20.6	60	4	
Ν	CV of Neonates	Hardness (mg/L)-StDev	19.9	60	5	
N	CV of Neonates	pH-CV	11.6	60	6	
Ν	CV of Neonates	pH-StDev	10.9	60	7	
Ν	CV of Neonates	Conductivity (uS/cm)-CV	9.4	60	8	
Ν	CV of Neonates	Age at Start (hrs)	9.4	60	9	
Ν	CV of Neonates	Average Age	8.8	60	10	
Ν	CV of Neonates	Alkalinity (mg/L)-Mean	8.2	60	11	
Ν	CV of Neonates	Temperature (C)-Mean	7.3	60	12	
Ν	CV of Neonates	Conductivity (uS/cm)-StDev	6.4	60	13	
N	CV of Neonates	Alkalinity (mg/L)-StDev	5.6	60	14	
N	CV of Neonates	Alkalinity (mg/L)-CV	2.8	60	15	
N	CV of Neonates	pH-Mean	0.6	60	16	

Ν	CV of Neonates	Test Replicates	0.0	60	17
Ν	CV of Neonates	Temperature (C)-CV	0.0	60	18
Ν	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-0.4	60	19
Ν	CV of Neonates	Temperature (C)-StDev	-1.9	60	20
Ν	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	-3.2	60	21
Ν	CV of Neonates	Dissolved Oxygen (mg/L)-CV	-3.7	60	22
0	CV of Neonates	Conductivity (uS/cm)-CV	11.9	60	1
0	CV of Neonates	Conductivity (uS/cm)-StDev	11.0	60	2
0	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	5.8	60	3
0	CV of Neonates	Dissolved Oxygen (mg/L)-CV	5.0	60	4
0	CV of Neonates	Average Age	4.2	60	5
0	CV of Neonates	Age at Start (hrs)	4.2	60	6
0	CV of Neonates	Alkalinity (mg/L)-Mean	4.2	60	7
0	CV of Neonates	Temperature (C)-CV	2.9	60	8
0	CV of Neonates	Test Replicates	0.0	60	9
0	CV of Neonates	Temperature (C)-StDev	-0.2	60	10
0	CV of Neonates	Year	-2.5	60	11
0	CV of Neonates	Conductivity (uS/cm)-Mean	-2.6	60	12
0	CV of Neonates	pH-StDev	-3.1	60	13
0	CV of Neonates	Temperature (C)-Mean	-3.5	60	14
0	CV of Neonates	pH-Mean	-4.1	60	15
0	CV of Neonates	Hardness (mg/L)-Mean	-4.6	60	16
0	CV of Neonates	pH-CV	-5.2	60	17
0	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	-14.8	60	18
Р	CV of Neonates	pH-StDev	43.4	108	1
Р	CV of Neonates	Conductivity (uS/cm)-CV	37.0	108	2
Р	CV of Neonates	pH-CV	32.8	108	3
Р	CV of Neonates	Conductivity (uS/cm)-StDev	32.5	108	4
Р	CV of Neonates	Year	30.8	108	5
Р	CV of Neonates	Alkalinity (mg/L)-Mean	22.3	108	6
Р	CV of Neonates	Hardness (mg/L)-Mean	13.8	108	7
Р	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	10.0	108	8

Р	CV of Neonates	Dissolved Oxygen (mg/L)-CV	9.3	108	9
Р	CV of Neonates	Age at Start (hrs)	8.9	108	10
Р	CV of Neonates	Average Age	8.7	108	11
Р	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	7.9	108	12
Р	CV of Neonates	Temperature (C)-StDev	7.3	108	13
Р	CV of Neonates	Temperature (C)-CV	6.9	108	14
Р	CV of Neonates	Light Intensity (fc)-Mean	4.8	108	15
Р	CV of Neonates	Conductivity (uS/cm)-Mean	2.4	108	16
Р	CV of Neonates	Temperature (C)-Mean	2.4	108	17
Р	CV of Neonates	Test Replicates	-1.6	108	18
Р	CV of Neonates	pH-Mean	-2.0	108	19
Q	CV of Neonates	Dissolved Oxygen (mg/L)-Mean	27.6	48	1
Q	CV of Neonates	Conductivity (uS/cm)-StDev	15.4	48	2
Q	CV of Neonates	Conductivity (uS/cm)-CV	14.3	48	3
Q	CV of Neonates	pH-CV	4.3	48	4
Q	CV of Neonates	pH-StDev	3.9	48	5
Q	CV of Neonates	Year	1.4	48	6
Q	CV of Neonates	Test Replicates	0.0	48	7
Q	CV of Neonates	Temperature (C)-Mean	-2.9	48	8
Q	CV of Neonates	Conductivity (uS/cm)-Mean	-4.1	48	9
Q	CV of Neonates	pH-Mean	-6.3	48	10
Q	CV of Neonates	Temperature (C)-StDev	-9.6	48	11
Q	CV of Neonates	Dissolved Oxygen (mg/L)-CV	-10.4	48	12
Q	CV of Neonates	Temperature (C)-CV	-11.8	48	13
Q	CV of Neonates	Dissolved Oxygen (mg/L)-StDev	-16.4	48	14

Figure A20. A heat map summarizing the frequency at which different lab technique or test condition variables were selected within the top 5 most important variables in explaining the pattern in mean control neonate production in the historical test data from each lab by the random forest models detailed in Tables B29 and 30. The cooler/darker the color the more frequently that variable was assigned that particular rank. Frequencies are expressed as a percentage of the number of labs that reported a given technique/condition variable to account for differences in reporting rate among labs for different variables.

![](_page_70_Figure_1.jpeg)

Figure A21. A heat map summarizing the frequency at which different lab technique or test condition variables were selected within the top 5 most important variables in explaining the pattern in CV of control neonate production in the historical test data from each lab by the random forest models detailed in Tables B29 and 30. The cooler/darker the color the more frequently that variable was assigned that particular rank. Frequencies are expressed as a percentage of the number of labs that reported a given technique/condition variable to account for differences in reporting rate among labs for different variables.

![](_page_71_Figure_1.jpeg)
# DRAFT

# Appendix B Baseline ILS Study Plan and Data

## **OVERVIEW OF BASELINE TESTING PROCEDURE**

The specific objective of the baseline testing is to collect additional *C. dubia* chronic toxicity data and a more complete/consistent lab technique dataset across California-accredited laboratories. Twelve (12) laboratories participated in an intercomparison exercise consisting of several split samples tested in three separate testing batches. This testing design is proposed to generate a minimum of seven (7) control datasets per participating laboratory. This was statistically determined based on analyses of the width of the confidence interval to assess intra-laboratory precision. Our analyses indicated that the grand mean for control neonate production from 7 separate tests (each test performed with 10 replicates) would increase our confidence that such mean would fall within the historical control grand mean +/- 5 neonates.

Split samples to be tested include:

- Sample 1: Moderately hard water recipe #1 (EPA MHW-salts) to be tested at full strength (i.e., 100%). This sample was tested along with one (1) laboratory control consisting of the lab's own dilution water recipe.
- Sample 2A: Moderately hard water recipe #2 (EPA DMW); Perrier<sup>®</sup>) to be tested at full strength (i.e., 100%). This sample was tested along with one (1) laboratory control consisting of the lab's own dilution water recipe.
- Sample 2B-F: 5 concentrations of sodium chloride (NaCl) diluted in MHW recipe #2 (i.e., Perrier<sup>®</sup>). All samples were prepared at SCCWRP according to the procedure described earlier in the QAPP. These samples were tested as is (i.e., no additional sample dilution allowed) along with one (1) laboratory control consisting of the lab's own dilution water recipe.
- Sample 3: NaCl was provided (as a solid) to each lab with detailed instructions to prepare 5 dilutions using the lab's own dilution water. This serial dilution was tested along with one (1) laboratory control consisting of the lab's own dilution water recipe. *Note that Sample 3 is now replacing the requirement for each lab to test their routine reference toxicant with each testing batch.*

### SUMMARY OF STANDARD OPERATING PROCEDURES

Participating laboratories (n= 12) analyzed three separate test batches within a  $\sim$  8-week window, using their own standard operating procedures for the *C. dubia* chronic toxicity test. A summary of standard operating procedures (SOPs), test acceptability criteria (TAC) and measurement expectations are provided in **Table 1** and in the QAPP. However, all laboratories were required to meet the following specifications:

- All tests were carried out to 8 days (i.e., 192 hours).
- All samples, including lab controls, were performed with 10 replicate chambers.
- Assignment of neonates at test set-up must use the randomized blocking by known parentage, using only brood board chambers with a minimum of 8 neonates from the adult on test initiation day. Each test (i.e., sample and associated laboratory control) was treated as independent for blocking and randomization, except for samples 2A and 2B-F and the two associated controls that must be blocked by the same known parentage.
- A 500 mL-sample of their own dilution water was collected at test initiation using the container provided by SCCWRP and shipped back to SCCWRP within 24 hours. This sample was used for analysis of ion composition.

- Test solutions were renewed daily within a 24 +/- 1 hour window to enhance the comparability of neonate counts among laboratories. Specific time of renewal (hours and minutes) were recorded and initialed.

Additionally, participating laboratories were required to report data that may not be currently documented/reported including (note that the specifics for taking these measurements are provided in the QAPP):

- Number of males, unhealthy and dead adults, and dead neonates in the brood board. This data is to be collected for all days from every chamber within any brood boards that are used to initiate the test. The expectation is that this would be about 6 to 10 days of data depending on the age of the brood board at test initiation
- Specific beginning and end time window for age of neonates at test initiation
- Water quality parameters (air and water temperature, pH, DO, conductivity) at test initiation, termination, and before and after daily renewal, to the decimal place specified in the QAPP. If possible, water temperature was also continuously monitored at the test location.
- Light intensity and twice daily air temperature within the testing area at the time of the experiments and reported in the units specified in the QAPP.

Parameter <sup>1</sup>	Description						
Test organism	Ceriodaphnia dubia						
Protocol(s)	EPA/821/R-02-013; EPA 821-R-02012-ES <sup>1</sup>						
Exposure	Static, daily renewal						
No. replicate test chambers	10 replicates per sample/dilution						
Sample holding time <sup>2</sup>	Up to 48 hours before test initiation						
Test duration	8 days, i.e., 192 hours						
Endpoints	Survival and reproduction (number of neonates per female)						
Laboratory control	One laboratory dilution water control per test sample						
Water quality measurements	Daily: air and water temperature in °C, pH and dissolved oxygen in mg/L reported with 0.1 precision; conductivity in $\mu$ S/cm. Continuous monitoring of water temperature, if possible. Upon receipt and test termination: hardness and alkalinity in mg/L CaCO <sub>3</sub>						

**Table 1.** Summary of test conditions and test acceptability criteria (TAC) for the *Ceriodaphnia dubia* survival and reproduction test.

<sup>&</sup>lt;sup>1</sup> USEPA. 2016. Whole Effluent Toxicity Methods Errata Sheet. 28 p. Office of Water EPA 821-R-02012-ES. December 2016 <u>https://www.epa.gov/cwa-methods</u>; USEPA. 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013.US. Environmental Protection Agency, Office of Water, Washington, DC.

Parameter <sup>1</sup>	Description
	Once during test in testing area: light intensity in foot-candles; air temperature in °C (0.1 °C precision)
Test Acceptability Criteria (TAC)	80% or greater survival and an average of 15 or more live neonates per surviving female in the controls at test termination (i.e., 8 days)

<sup>1</sup> Parameters and test conditions used in this study are suitable for investigative/non-compliance testing but may be different than those required for NPDES permit testing. <sup>2</sup> This is a deviation from the promulgated method.

### **OVERVIIEW OF SPLIT SAMPLE PREPARATION AND DISTRIBUTION**

Bulk test water samples were prepared in the SCCWRP laboratories as described in the QAPP using large sample containers with spigots and thoroughly mixed on a large-capacity stirrer to ensure that the samples are homogeneous. The number of samples to be tested by the participating laboratories are presented in **Table 2**. Bulk samples were allowed to equilibrate for up to 48 hours before preparing the split-samples that were shipped to the laboratories. Subsampling of the bulk test samples were conducted using 3.8 L cubitainers filled to the top. All cubitainers were randomly filled in two steps. First, each cubitainer was filled halfway. Then the cubitainers were filled the rest of the way in no particular order. Each cubitainer was labeled with a unique sample ID and stored in the dark in the walk-in fridge at 4 °C less than 48 hours before shipping them to the participating laboratories.

Sample ID	Number of samples per lab per round	Number of sample dilutions to test	Number of lab control to include per sample
1	1	_*	1
2A	1	_*	1
2B-G	5	_*	1
3	1 <sup>¥</sup>	5	1

**Table 2.** Number of split-samples to be tested by the 12 participating laboratories for each round. Threetesting rounds were completed for this study.

\*Water samples DO NOT require further dilution before testing.

<sup>4</sup> Sample 3 was shipped as a powder with instructions to prepare the serial dilution for testing.

To evaluate their preparation method and prevent unexpected toxicity, SCCWRP prepared bulk water samples and sent them to one laboratory for a *C. dubia* chronic toxicity test. If unspiked samples are not toxic and a **Concentration**-response appears normal for the dilution series, the preparation method was deemed suitable for the ILS. SCCWRP prepared fresh bulk samples and split them in individual cubitainers as described above. Since all methods and equipment were the same for subsequent rounds, this preliminary testing was only carried out for round one.

To ensure that all subsamples are representative of the original bulk test samples, two subsamples were collected in separate vessels from each cubitainer before shipment. The first set (50 mL) was used to measure

conductivity, alkalinity, and hardness. The subsample was discarded after the measurements are completed. The second set (500 mL) was collected for ion composition analysis. Due to sample volume requirements, SCCWRP collected ion composition analysis from each cubitainer before shipping. These subsamples were collected in 500 mL HDPE bottles, filled to the top, and shipped to the analytical laboratory (Physis) to measure bicarbonate, carbonate, chloride, fluoride, nitrate, sulfate, selenium, and major cations (calcium, phosphate, magnesium, sodium). The analyses were completed within 21 days of sampling to meet holding time requirements.

Split samples were shipped to each laboratory starting August 22, 2022, according to the schedule presented below. Samples were shipped on wet ice using priority overnight (OnTrac or FedEx) service to the laboratories to the addresses in Appendix A of the QAPP. The shipments included chain-of-custody (COC) forms completed by SCCWRP, and a copy of the study plan and testing instructions.

Upon delivery, temperature, conductivity, pH, dissolved oxygen, hardness, and alkalinity were measured and recorded for each sample to document their stability before testing is initiated. These measurements were made from a small subsample poured into a clean secondary vessel. Probes and any other measuring equipment cannot be used in the cubitainer, and the subsample used for water quality were discarded after use (subsample cannot be used for testing or as a chemistry or archived sample). Additionally, a 125-mL sample was collected from each cubitainer at the time of test initiation and archived. Once all chemistry and water quality samples were collected by both SCCWRP and the laboratories, there was more than 3 L remaining in each cubitainer to conduct the 8-day *C. dubia* test.

For sample 3, 14.00 g of NaCl was weighed and placed in 100 mL HDPE containers. Each laboratory received one container with instructions to prepare the serial dilution using their own lab dilution water (i.e., dilute the supplied NaCl in 7.0 L of their own dilution water and perform a 50% dilution series to generate a total of 5 dilutions). Similar to the split-water samples, once the dilutions were prepared, the laboratories recorded temperature, conductivity, pH, dissolved oxygen, hardness, and alkalinity for each dilution at test initiation. A 125-mL sample was collected and archived from each dilution at test initiation.

Note that approximately one (1) hour prior to test initiation and water changes, the volume of water needed to renew the test solutions should be adjusted/maintained at test temperature.

### DATA SUBMISSION

SCCWRP provided an Excel data submittal form and culture/bench sheet templates to the participating laboratories. All test data in electronic format and scanned copies of the culture/bench sheets were submitted to the SCCWRP data portal. Data required include:

- Laboratory information
- Sample information upon receipt (time, temperature, condition, and more as described above)
- Testing conditions including dilution water and food recipe
- Brood board health data
- Bench water quality data for testing, survival and reproduction counts
- Control charts for reference toxicant tests for the last 12 months

### Figure B1: Overview of the *C. dubia* baseline study design.



Test batch per lab per testing round (x 3 rounds)

# INVENTORY OF AVAILABLE DATA

Summary of lab participation and data collected:

- Eleven labs participated in testing.
  - A 12<sup>th</sup> lab (Lab I) suffered a ciliate infection in their culture and test early in Round 1 and had to abort the test. They could not get their cultures healthy to participate in the subsequent rounds
- For Round 1, nine laboratories tested samples.
  - Labs B and M had their samples arrive too late for testing (3 days after shipping)
  - Lab N had culture problems and was unable to test Sample 3
- For Round 2, eleven laboratories tested samples.
  - Samples for the same two labs, B and M, with shipping issues in Round 1 arrived one day late but were tested within holding time.
- For Round 3, eleven laboratories tested samples, all on time.
  - Lab B had a problem with their dilution water, which led to the deaths of all organisms within a day.
     Therefore, all their lab controls and the Sample 3 series were unsuccessful. They did carry out the Sample 1 and 2A-F series to completion.
- Lab L tested only three sets of controls in each round instead of the requested four (they tested one control for the 2A-F series instead of having a separate control for 2A)
- Out of 132 expected laboratory controls from 11 labs, 117 were tested to completion

## **BIOLOGICAL RESPONSE DATA**

Table B1. Summary of biological data for Sample 1 collected from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. For each lab, the data is presented as mean of 3 rounds (except for labs B and M who could not participate in round 1). N values refer to the number of tests included in the mean and CV.

		Neonates/Adult Female			Number of Broods (8 days)				Time to First Brood				
Lab	Sample Type	Mean	Ν	Range	CV	Mean	N	Range	CV	Mean	N	Range	CV
А	EPA MHW (1) (salts)	38.6	3	35-44	0.13	4.1	3	4.0-4.1	0.014	4.0	3	4.0-4.0	0
В	EPA MHW (1) (salts)	35.0	2	34-36	0.024	3.0	2	3.1-3.4	0.065	4.0	2	4.0-4.1	0.017
E	EPA MHW (1) (salts)	16.0	3	13-21	0.25	4.7	3	4.3-5.0	0.075	4.2	3	4.0-4.6	0.076
F	EPA MHW (1) (salts)	20.1	3	18-21	0.072	4.5	3	4.4-4.6	0.022	3.4	3	3.1-3.5	0.069
G	EPA MHW (1) (salts)	31.6	3	27-35	0.13	4.1	3	4.0-4.1	0.014	3.3	3	3.0-3.9	0.16
L	EPA MHW (1) (salts)	24.3	3	22-26	0.092	3.2	3	2.8-3.6	0.12	4.2	3	4.1-4.8	0.032
М	EPA MHW (1) (salts)	32.4	2	27-38	0.24	3.0	2	2.3-3.8	0.35	3.6	2	3.1-4.0	0.18
Ν	EPA MHW (1) (salts)	7.1	3	3-11	0.59	1.8	3	0.9-2.8	0.51	4.6	3	4.3-5.0	0.075
0	EPA MHW (1) (salts)	31.0	3	24-35	0.19	4.2	3	4.0-4.5	0.069	3.0	3	3.0-3.0	0
Р	EPA MHW (1) (salts)	38.6	3	36-41	0.065	4.0	3	3.9-4.0	0.015	4.0	3	4.0-4.0	0
Q	EPA MHW (1) (salts)	36.2	3	30-41	0.14	3.6	3	3.4-3.7	0.043	3.9	3	3.6-4.0	0.060

Table B2. Summary of biological data for Sample 2A collected from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. For each lab, the data is presented as mean of 3 rounds (except for labs B and M who could not participate in round 1). N values refer to the number of tests included in the mean and CV.

		Neonates/Adult Female			Number of Broods (8 days)				Time to First Brood				
Lab	Sample Type	Mean	Ν	Range	CV	Mean	Ν	Range	CV	Mean	Ν	Range	CV
А	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	39.0	3	34-43	0.12	4.0	3	3.9-4.0	0.015	4.0	3	4.0-4.0	0
В	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	26.2	2	21-31	0.27	3.0	2	2.8-3.1	0.072	4.0	2	4.0-4.0	0
Е	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	15.4	3	11-21	0.34	4.7	3	4.4-4.8	0.049	4.2	3	4.0-4.4	0.050
F	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	21.2	3	20-22	0.047	4.7	3	4.5-5.0	0.053	3.2	3	3.0-3.5	0.091
G	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	33.1	3	32-35	0.043	4.0	3	4.0-4.1	0.014	3.7	3	3.0-4.1	0.16
L	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	25.6	3	22-30	0.16	3.0	3	2.3-3.4	0.20	4.2	3	4.1-4.3	0.032
Μ	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	35.2	2	35-35	0.008	3.8	2	3.5-4.0	0.094	3.6	2	3.3-4.0	0.14
Ν	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	9.2	3	4-17	0.73	1.8	3	1.2-2.9	0.51	5.1	3	4.0-6.6	0.27
0	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	29.9	3	24-36	0.21	4.0	3	3.9-4.1	0.029	3.0	3	3.0-3.0	0
Р	EPA MHW (DMW); Perrier <sup>®</sup> Water (2A)	37.0	3	35-38	0.056	3.8	3	3.7-4.1	0.060	4.0	3	4.0-4.0	0
Q	Perrier Water (2A)	36.6	3	35-40	0.070	3.9	3	3.9-4.0	0.015	4.0	3	4.0-4.0	0

Table B3. Summary of biological data for laboratory dilution water collected from the eleven laboratories participating in the baseline C. dubia interlaboratory study. For each lab, the data is presented as mean of 3 rounds (except for labs B and M who could not participate in round 1). N values refer to the number of tests included in the mean and CV.

		Neonates/Adult Female				Number of Broods (8 days)				Time to First Brood			
Lab	Sample Type	Mean	Ν	Range	CV	Mean	N	Range	CV	Mean	N	Range	CV
А	Lab Water	40.2	12	35-44	0.072	4.0	12	3.7-4.1	0.025	4.0	12	4.0-4.0	0
В	Lab Water	29.8	4	21-36	0.22	3.2	4	2.4-3.8	0.19	4.3	4	4.0-5.1	0.13
E	Lab Water	14.9	12	8-22	0.32	4.5	12	4.1-5.0	0.067	4.3	12	3.8-4.8	0.075
F	Lab Water	17.6	12	16-20	0.072	4.4	12	4.0-4.9	0.071	3.4	12	3.0-3.9	0.096
G	Lab Water	30.8	12	29-35	0.060	4.0	12	3.2-4.2	0.070	3.5	12	3.0-4.0	0.13
L	Lab Water	28.9	9	22-33	0.13	3.1	9	2.6-3.8	0.12	4.3	9	4.0-5.0	0.088
Μ	Lab Water	26.0	8	8-34	0.33	2.7	8	1.1-3.4	0.26	3.7	8	3.0-4.2	0.13
Ν	Lab Water	19.6	11	12-32	0.30	3.4	11	2.9-4.2	0.12	4.0	11	3.3-4.4	0.094
0	Lab Water	31.4	12	26-38	0.106	4.3	12	4.0-4.7	0.045	3.0	12	3.0-3.2	0.019
Р	Lab Water	36.6	12	33-38	0.054	3.9	12	3.7-4.0	0.036	4.0	12	4.0-4.1	0.007
Q	Lab Water	35.5	12	29-42	0.13	3.7	12	3.1-4.0	0.094	4.0	12	4.0-4.0	0

Table B4. Additional biological data collected by the participating laboratories across all samples. N values refer to the number of tests included in the mean and CV.

	Mir	n. Age	@ Test Start	(h)	Max. Age @ Test Start (h)			Number o per Test	f Males	Calculated Test Duration (Days to 60% of females having 3 broods)			
Lab	Mean	Ν	Range	CV	Mean	N	Range	CV	Mean	N	Mean	N	Range
А	6.7	12	6-8	0.098	14.6	12	14-15	0.035	0	12	7.0	12	7-7
В	0	4	0-0	0	6.8	4	6.8-6.8	0	0	4	7.2	4	7-8
E	4.3	12	4-5	0.11	8.0	12	8-8	0	0	12	6.5	12	6-7
F	10.8	12	8-16	0.34	18.5	12	15-24	0.20	0	12	6.1	12	6-7
G	12.2	12	6-20	0.53	18.7	12	14-25	0.26	0	12	6.2	12	6-7
L	4.0	9	1-8	0.53	15.5	9	5-24	0.49	0	9	7.2	9	7-8
М	12.7	8	6-18	0.45	19.5	8	14-24	0.23	0	8	6.6	8	6-8
Ν	6.4	11	4-13	0.67	14.4	11	12-21	0.30	0	11	6.7	11	6-8
0	14.3	12	7-22	0.36	20.8	12	15-24	0.18	0	12	6.0	12	6-6
Р	1.8	12	1-3	0.42	6.3	12	5-8	0.13	0	12	6.7	12	6-7
Q	2.1	12	0.5-5	0.59	6.6	12	5-10	0.20	0	12	7.0	12	7-7

Table B5. Summary of number of neonates per surviving female in EPA Moderately Hard Water (Sample 1) from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. The mean was calculated by taking the total number of neonates produced for all females and dividing by the number of surviving females. For each lab, the data is presented as mean of 3 rounds (except for labs B and M who could not participate in round 1). N values refer to the number of tests included in the mean and CV.

		Neonates/ Surviving Female							
Lab	Sample Type	Mean	N	Mean Range	CV				
А	EPA MHW (1)	39.9	3	35-44	0.16				
В	EPA MHW (1)	39.5	2	34-44	0.12				
E	EPA MHW (1)	15.1	3	11-20	0.27				
F	EPA MHW (1)	19.5	3	18-21	0.16				
G	EPA MHW (1)	31.6	3	27-35	0.15				
L	EPA MHW (1)	29.3	3	28-31	0.18				
М	EPA MHW (1)	36.0	2	30-42	0.27				
Ν	EPA MHW (1)	13.8	3	10-17	0.62				
0	EPA MHW (1)	31.0	3	24-35	0.20				
Р	EPA MHW (1)	38.5	3	36-41	0.15				
Q	EPA MHW (1)	37.3	3	34-41	0.062				



Table B6. Summary of number of neonates per surviving female in Perrier based Moderately Hard Water (Sample 2A) from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. The mean was calculated by taking the total number of neonates produced for all females and dividing by the number of surviving females. For each lab, the data is presented as mean of 3 rounds (except for labs B and M who could not participate in round 1). N values refer to the number of tests included in the mean and CV.

Lab	Sample Type	Mean	Ν	Mean Range	CV
А	DMW) Perrier <sup>®</sup> Water (2A)	39.0	3	34-43	0.13
В	DMW) Perrier <sup>®</sup> Water (2A)	27.9	2	21-35	0.45
E	DMW) Perrier <sup>®</sup> Water (2A)	14.0	3	11-17	0.43
F	DMW) Perrier <sup>®</sup> Water (2A)	21.2	3	20-22	0.089
G	DMW) Perrier <sup>®</sup> Water (2A)	33.1	3	32-35	0.11
L	DMW) Perrier <sup>®</sup> Water (2A)	30.8	3	27-34	0.22
М	DMW) Perrier <sup>®</sup> Water (2A)	38.2	2	37-39	0.28
Ν	DMW) Perrier <sup>®</sup> Water (2A)	17.6	3	7-25	0.61
0	DMW) Perrier <sup>®</sup> Water (2A)	29.9	3	24-36	0.18
Р	DMW) Perrier <sup>®</sup> Water (2A)	39.1	3	38-41	0.082
Q	DMW) Perrier <sup>®</sup> Water (2A)	36.6	3	35-40	0.12

Table B7. Summary of number of neonates per surviving female in laboratory control water from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. The mean was calculated by taking the total number of neonates produced for all females and dividing by the number of surviving females. For each lab, the data is presented as mean of 3 rounds (except for labs B and M who could not participate in round 1). N values refer to the number of tests included in the mean and CV.

		Neonates/ Surviving Female							
Lab	Sample Type	Mean	Ν	Mean Range	CV				
А	Lab Water	40.5	12	37-44	0.099				
В	Lab Water	32.1	4	23-36	0.23				
E	Lab Water	14.8	12	8-22	0.28				
F	Lab Water	17.8	12	16-21	0.13				
G	Lab Water	33.0	12	29-42	0.11				
L	Lab Water	30.4	9	27-35	0.21				
М	Lab Water	29.2	8	11-38	0.37				
Ν	Lab Water	23.1	11	15-35	0.34				
0	Lab Water	32.0	12	29-38	0.20				
Р	Lab Water	38.3	12	35-47	0.14				
Q	Lab Water	36.1	12	31-42	0.11				



		Neonates/Ad	lult Fe	male (60%	Trigger)	Neonates/Adult Female (80% Trigger)				
Lab	Sample Type	Mean	Ν	Range	CV	Mean	N	Range	CV	
А	Lab Water	40.2	12	35-44	0.072	40.2	12	35-44	0.072	
В	Lab Water	29.8	4	21-36	0.22	29.8	4	21-36	0.22	
E	Lab Water	14.9	12	8-22	0.32	15.6	12	12-22	0.30	
F	Lab Water	17.6	12	16-20	0.072	17.6	12	16-20	0.072	
G	Lab Water	30.8	12	29-35	0.060	30.8	12	29-34	0.060	
L	Lab Water	28.9	9	22-33	0.13	29.5	9	26-33	0.077	
М	Lab Water	26.0	8	8-34	0.33	26.2	8	8-34	0.32	
Ν	Lab Water	19.6	11	12-32	0.30	20.7	11	12-32	0.31	
0	Lab Water	31.4	12	26-38	0.106	31.4	12	26-38	0.106	
Р	Lab Water	36.6	12	33-38	0.054	36.6	12	33-38	0.052	
Q	Lab Water	35.5	12	29-42	0.13	35.5	12	29-42	0.13	

Table B8. Number neonates per female calculated at the protocol trigger (60% of surviving females reaching 3 broods) and at an alternate trigger used by some labs (80% of surviving females reaching 3 broods), in the lab's dilutions water. N values refer to the number of tests included in the mean and CV.

Table B9. Age of females in brood boards on the day their neonates were used to initiate testing during the baseline *C. dubia* ILS. Note that the N value is variable between laboratories, depending on how many brood boards they used to initiate testing. Some labs do single, large brood boards, while others do multiple smaller boards.

	Mean Age of Female at Test		
Lab	Initiation (Days)	N	Range
Α	6.3	3	6 - 7
В	9.0	2	9 - 9
E	10.7	3	10 - 11
F	9.7	9	9 - 11
G	7.7	6	7 - 8
L	12.4	14	12 - 14
М	8.0	3	7 - 9
N	7.6	7	7 -11
0	8.0	4	8 - 8
Р	6.8	5	6 - 7

Q 9.3 3 9-10	
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Table B10. Information on the algae used for feeding *Ceriodaphnia* during ILS testing.

Lab	Algae Source	Algae Concentration (cells/mL)	Algae Concentration Measurement					
А	In-house	250,000	By lab for each batch					
В	ABS	695,600	By the supplier					
E	ABS	233,333	By the supplier					
F	ARO	233,333	By lab for each batch					
G	ABS	200,000	By the supplier					
L	ABS	220,000	By the supplier					
М	ABS	210,000	By the supplier					
N	In-house	213,000	By lab for each batch					
0	In-house	245,000	By lab for each batch					
Р	ABS	300,000	By the supplier					
Q	ABS	215,000	By lab for each batch					

NA= Information not supplied by lab.



				YCT Concentration	Feeding rate (ml
				in Test Chamber	
Lab	YCT Source	YCT Recipe	Feeding Method	(µl/ml)	
		Fleishman's Yeast+Blue Seal Alfalfa+Zeigler #1 Finfish Crumble Trout			0.113
А	ARO	Chow	In test solution	0.0075	
В	ABS	NA	In test solution	0.0168*	2.52
Е	ABS	NA	Direct addition	0.0067	0.101
F	In-house	Fleischmann's Yeast + Pines Wheatgrass + Thomas Fish Co Trout Chow	Direct addition	0.0067	0.101
G	ABS	NA	Direct addition	0.0067	0.101
L	ABS	NA	Direct addition	0.0067	0.101
М	ARO	NA	Direct addition	0.0067	0.101
		Trout Chow (Purina Aquamax Fry Starter 100) / Carolina Daphnia Food			0.101
N	In-house	(Wards Cereal Grass Media)	Direct addition	0.005*	
		Fleishman's active dry yeast + Pines Wheatgrass + Purina Trout Chow			0.105
0	In-house	(supplied by ABS)	In test solution	0.007	
Р	ARO	NA	In test solution	0.0067	0.101
Q	ABS	NA	Direct addition	0.0067	0.101

## Table B11. Information on the YCT used for feeding *Ceriodaphnia* during ILS testing.

NA= Not applicable, lab purchases YCT.

• Note: Lab B was 2.5X more than the amount of YCT described by EPA; and Lab N was 0.75X less than the amount described by EPA.

Table B12. Individual test batches not meeting test acceptability criterion for reproduction (≥ 15 neonates/surviving female).

Lab	Test Round	Mean Neonates/Surviving Female
E	Round 2	12.3
E	Round 2	12.7
E	Round 3	8.9
E	Round 3	10.4
E	Round 3	9.3
E	Round 3	8.5
М	Round 2	10.9

Note: All four Round 3 tests for Lab B did not meet test acceptability due to zero survival.

Table B13. Individual test batches not meeting acceptable brood board mortality (< 20%). Note that all labs had at least 8 neonates per brood board female used to initiate tests and no females were older than 14 days.

Lab	Test Round	Brood Board Percent	Mean Neonates per Female				
		Mortality	in Lab Control				
Ν	Round 1	42	15.5				
N	Round 1	42	12.7				
Ν	Round 1	42	11.9				
L	Round 1	23	28.8				
L	Round 2	36*	32.8				
L	Round 2	36*	27.7				
L	Round 2	36*	31.9				

\* Average mortality of four broods used to initiate the three test batches.

Table B14. Brood board health parameters recorded in boards used to initiate the tests during the baseline *C. dubia* interlaboratory study. Data is expressed as percentage of brood board cups exhibiting a health issue category per brood board. N values refer to the number of brood boards used by each lab to initiate all of their tests.

	Unhealthy Adult			C	Dead Adult			Male		Unhealthy Neonates			Dead Neonates		
Lab	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range
А	0	5	NA	0	5	NA	0	5	NA	0	5	0-0	0	5	NA
В	0	2	NA	11.2	2	7.5-15.0	0	2	NA	0	2	0-0	0	2	NA
E	0	3	NA	0	3	NA	0	3	NA	0	3	0-0	0	3	NA
F	1.1	9	0-10	1.5	9	0-6.7	0	9	NA	0.2	9	0-1.7	0.2	9	0-1.7
G	3.3	6	0-20	0.6	6	0-3.3	1.1	6	0-6.7	0	6	0-0	0	6	NA
L	3.3	14	0-10	17.6	14	0-46.7	1.9	14	0-10.0	0	14	0-0	0	14	NA
Μ	0	3	NA	5.6	3	0-11.7	0	3	NA	9.5	3	1.7-21.7	20.0	3	11.7-33.3
Ν	0	7	NA	11.0	7	0-41.7	0	7	NA	0	7	0-0	0	7	NA
0	0	4	NA	1.2	4	0-3.3	0	4	NA	0	4	0-0	0	4	NA
Р	0.2	5	0-3.3	2.9	5	0-13.3	0	5	NA	8.0	5	3.3-13.3	17.3	5	6.7-23.3
Q	0	3	0-0	2.8	3	0-5.0	0	3	NA	0.6	3	0-1.7	1.1	3	0-1.7

NA= not applicable

Figure B2. Box plot of number of neonates per adult female for the lab's own dilution water in the ILS. The plot shows the results of each control sample within a round by laboratory (N= 3 or 4). The black line within each box represents the median and the green is the mean. The lines extending horizontally for each lab is the mean neonates per adult female from the historical data previously submitted by the laboratories.



## Lab's Own Dilution Water: Mean Number Neonates

Figure B3. Box plot of coefficient of variation for the mean number of neonates per adult female in the lab's own dilution water in the ILS. The plot shows the results of each control sample within a round by laboratory (N= 3 or 4). The black line within each box represents the median and the green is the mean. The lines extending horizontally for each lab is the mean neonates per adult female from the historical data previously submitted by the laboratories.



## Lab's Own Dilution Water: Mean CV of Neonates per Female

# DRAFT

Figure B4. Box plot of number of neonates per adult female for Sample 1 (EPA MHW) in the ILS. Since there is only one sample per round, the plot is of individual replicates (N=10). The black line within each box represents the median and the green is the mean. The dots are the high and low outlier, if any. The lines extending horizontally for each lab is the mean neonates per adult female from the historical data previously submitted by the laboratories. Laboratories are ordered based on the water type typically used in their cultures.





# Sample 1 (EPA MHW): Mean Number Neonates per Female

Figure B5. Box plot of number of neonates per adult female for Sample 2A (DMW Perrier®) in the ILS. Since there is only one sample per round, the plot is of individual replicates (N=10). The black line within each box represents the median and the green is the mean. The dots are the high and low outlier, if any. The lines extending horizontally for each lab is the mean neonates per adult female from the historical data previously submitted by the laboratories. Laboratories are ordered based on the water type typically used in their cultures.



Sample 2A (Perrier): Mean Number Neonates per Female

# DRAFT



# Average Age of Brood Board Females vs Mean # Test Neonates for Controls

Figure B7. Plot of the age of the females whose neonates were used to initiate a test batch versus the number of neonates produced in Sample 1 (EPA MHW).



Average Age of Brood Board Females vs Mean # Test Neonates for Sample 1

Figure B8. Plot of the age of the females whose neonates were used to initiate a test batch versus the number of neonates produced in Sample 2A (DMW with Perrier<sup>®</sup>).



Average Age of Brood Board Females vs Mean # Test Neonates for Sample 2A

# DRAFT

Figure B9. Plot of the age of the females whose neonates were used to initiate a test batch versus the IC25 for the Sample 2 series (DMW with Perrier®).



Average Age of Brood Board Females vs IC25 for Sample Series 2

Figure B10. Plot of the age of the females whose neonates were used to initiate a test batch versus the number the IC25 for the Sample 3 series (lab's own dilution water).



Average Age of Brood Board Females vs IC25 for Sample Series 3

# DRAFT

# **CONCENTRATION-**RESPONSE DATA

This section summarizes the concentration-response data from samples consisting of dilution water spiked with different concentrations of sodium chloride. SCCWRP prepared Samples 2A-F in DMW (Perrier<sup>®</sup>) water, and split samples were shipped to the labs participating in the ILS. Sample 3 was shipped as a solid to the labs, and each lab prepared concentrations using their own lab water. Data are plotted as mean neonates per sample type per round against the measured conductivity and against the nominal sodium chloride concentration. Point estimates (IC and LC) were calculated in Python according to the EPA manual



Figure B11. Concentration-response plot of all three rounds of testing for Lab A series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.



Figure B12. Concentration-response plot of all three rounds of testing for Lab A series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend.



Round 1 Lab Round 2 Lab

Round 3 Lab

IC25 - 1020

IC25 - 1113
Figure B13. Concentration-response plot of all three rounds of testing for Lab B series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend. Note that for Lab B, Round 1 samples were not tested, and for Round 3, the Sample 3 series all had complete mortality on Day 1.



NC = Not Calulable

Figure B14. Concentration-response plot of all three rounds of testing for Lab B series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend. Note that for Lab B, Round 1 samples were not tested and for Round 3, the Sample 3 series all had complete mortality at Day 1.



Figure B15. Concentration-response plot of all three rounds of testing for Lab E series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.



Figure B16. Concentration-response plot of all three rounds of testing for Lab E series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend.



Figure B17. Concentration-response plot of all three rounds of testing for Lab F series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.







Figure B19. Concentration-response plot of all three rounds of testing for Lab G series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.







Figure B21. Concentration-response plot of all three rounds of testing for Lab L series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.





Figure B22. Concentration-response plot of all three rounds of testing for Lab L series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend.

**Figure B23.** Concentration-response plot of all three rounds of testing for Lab M series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend. Note that Lab M did not participate in Round 1 due to sample delivery issues.



**Figure B24.** Concentration-response plot of all three rounds of testing for Lab M series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend. Note that Lab M did not participate in Round 1 due to sample delivery issues.



**Figure B25.** Concentration-response plot of all three rounds of testing for Lab N series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend. Note that Lab N did not analyze the Sample 3 series in Round 1 due to insufficient neonates.



**Figure B26.** Concentration-response plot of all three rounds of testing for Lab N series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend. Note that Lab N did not analyze the Sample 3 series in Round 1 due to insufficient neonates.



Figure B27. Concentration-response plot of all three rounds of testing for Lab O series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.





Figure B28. Concentration-response plot of all three rounds of testing for Lab O series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend.



Figure B29. Concentration-response plot of all three rounds of testing for Lab P series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.



Figure B30. Concentration-response plot of all three rounds of testing for Lab P series 2 and 3 dilutions based on the nominal sodium chloride concentration. The IC25 for each sample can be found in the legend.



Figure B31. Concentration-response plot of all three rounds of testing for Lab Q series 2 and 3 dilutions based on the laboratories measured conductivity. The IC25 for each sample can be found in the legend.







## DRAFT

Lab Cada	Devied #	SCCWI	RP Perrier (Sample	es 2A-F)	Lab Dilution Water (Sample 3 series)					
Lab Code	Round #	IC25	IC50	LC50	IC25	IC50	LC50			
А	1	1772	2404	3459	2288	2867	3481			
A	2	1907	2436	3257	2135	2841	NC			
A	3	2392	2921	3242	2301	2917	3552			
В	2	414.2	631.0	727.9	1822	2465	2272			
В	3	506.7	614.4	1450	NC	NC	NC			
E	1	654.3	798.0	889.2	1004	2707	NC			
E	2	1006	1826	3257	1277	2157	NC			
E	3	1362	1894	3117	1623	2347	4096			
F	1	1868	2454	2971	2417	2964	3433			
F	2	1846	2513	NC	2312	3054	NC			
F	3	2118	2824	3617	2470	3165	3747			
G	1	1390	2048	2510	1803	2293	2447			
G	2	1751	2554	NC	2063	2792	NC			
G	3	2273	3112	NC	1963	2790	NC			
L	1	1694	2497	NC	1584	2446	NC			
L	2	2030	2439	2201	2214	2804	3045			
L	3	1694	2459	3197	2332	2869	3071			
М	2	1783	2274	2231	2354	2605	2878			
М	3	1988	2651	3467	1817	2473	3585			
Ν	1	723.1	931.6	2320	NC	NC	NC			
Ν	2	249.9	982.9	NC	925.2	1298	1455			
N	3	1635	2362	3917	871.6	1071	2285			
0	1	323.5	1166	2434	1410	1845	2807			
0	2	1295	1986	2231	1604	2242	3134			
0	3	1572	2067	2568	920.8	1772	3578			
Р	1	1336	1748	2711	2475	2965	3274			
Р	2	1630	2180	2671	2468	3005	3934			
Р	3	2302	2866	3917	2420	2955	3136			
Q	1	1251	1801	2699	2376	2931	3012			
Q	2	1444	2074	1889	2251	2862	3020			
Q	3	2316	2850	2918	1890	2586	NC			

Table B15. Point estimate values based on measured conductivity (µS/cm) in samples 2 and 3 tested as dilution series during the baseline *C. dubia* ILS.

\*NC = Not Calculable

# DRAFT

Lab Cada	Downal #	SCCWF	RP Perrier (Sample	s 2A-F)	Lab Dilution Water (Sample 3 series)				
Lab Code	Rouna #	IC25	IC50	LC50	IC25	IC50	LC50		
А	1	1013	1383	2000	1113	1447	1800		
A	2	1178	1500	2000	1020	1426	NC		
А	3	1152	1447	1625	1113	1467	1833		
В	2	194.0	333.9	392.9	844.3	1214	1100		
В	3	187.0	249.1	650.0	NC	NC	NC		
E	1	329.3	408.2	458.3	335.2	1324	NC		
E	2	572.0	1129	2000	412.2	877.6	NC		
E	3	603.8	882.5	1556	576.8	931.4	1833		
F	1	1069	1412	1714	1153	1455	1714		
F	2	1141	1547	NC	1124	1560	NC		
F	3	1000	1392	1833	1242	1655	2000		
G	1	770.5	1174	1444	826.5	1227	1444		
G	2	1083	1572	NC	998.6	1404	NC		
G	3	1168	1760	NC	1045	1532	NC		
L	1	964.6	1437	NC	777.8	1276	NC		
L	2	1253	1502	1357	1028	1363	1500		
L	3	777.5	1190	1600	1065	1381	1500		
М	2	1102	1401	1375	1265	1522	1800		
М	3	932.0	1296	1750	755.9	1095	1667		
N	1	367.1	481.7	1333	NC	NC	NC		
N	2	69.7	555.5	NC	417.9	652.0	750.0		
N	3	746.8	1136	2000	367.7	485.0	1200		
0	1	88.7	627.7	1400	559.3	801.3	1333		
0	2	775.0	1226	1375	668.5	1015	1500		
0	3	713.8	973.1	1250	297.0	756.4	1750		
Р	1	736.6	998.9	1562	1201.4	1472.3	1642.9		
Р	2	1009	1344	1643	1191.2	1487.6	2000.0		
Р	3	1102	1416	2000	1174	1471	1571		
Q	1	682.4	1030	1556	1101	1401	1444		
Q	2	880.1	1279	1167	1175	1477	1556		
Q	3	1110	1407	1444	1204	1544	NC		

Table B16. Point estimate values based on nominal NaCl (mg/L) concentrations for samples 2 and 3 tested as dilution series during the baseline C. dubia ILS.

\*NC = Not Calculable

# DRAFT

Table B17. Minimum significant difference (MSD) and percent minimum significant difference (PMSD) values for dilution series sample reproduction endpoint. Values exceeding EPA (2000) 90<sup>th</sup> percentile value of 37% are highlighted in *italics*.

	Round 1			Rou	nd 2	Round 3			
Lab ID	Sample Series	MSD	PMSD	MSD	PMSD	MSD	PMSD		
A	2A-2F	9.88	29.1	3.83	8.82	3.35	8.43		
A	3 Series	4.38	11.7	4.34	10.0	5.52	14.5		
В	2A-2F	NA	NA	9.35	44.1	10.9	52.8		
В	3 Series	NA	NA	15.4	49.7	NA	NA		
E	2A-2F	5.55	32.7	5.54	38.7	3.91	36.6		
E	3 Series	4.54	22.1	5.31	26.5	2.58	30.3		
F	2A-2F	7.84	35.2	6.49	31.2	5.86	28.7		
F	3 Series	2.82	16.9	3.81	20.0	2.75	16.6		
G	2A-2F	4.26	13.4	5.00	15.2	7.09	20.5		
G	3 Series	6.14	21.5	5.04	17.4	11.6	39.2		
L	2A-2F	6.27	20.8	15.6	63.1	14.2	64.4		
L	3 Series	7.78	25.7	7.63	23.9	5.44	18.6		
M	2A-2F	NA	NA	15.7	42.3	16.8	47.5		
M	3 Series	NA	NA	6.45	84.8	8.62	25.4		
N	2A-2F	3.59	102	8.48	113	7.00	41.9		
N	3 Series	NA	NA	8.11	25.5	5.06	29.9		
0	2A-2F	6.73	22.4	6.16	17.1	7.21	30.4		
0	3 Series	7.23	23.9	8.40	26.3	10.2	34.3		
Р	2A-2F	9.52	26.1	9.18	26.0	4.20	11.0		
Р	3 Series	6.33	19.0	6.05	15.8	5.15	14.0		
Q	2A-2F	5.89	14.9	7.24	20.5	6.01	17.2		
Q	3 Series	5.73	14.0	3.47	10.1	3.63	11.0		

NA=Not available either due to sample not being tested or sample being extremely toxic.

### WATER QUALITY DATA

Table B18. Summary of conductivity and pH data collected in control chambers from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

		Condu	uctivity	(µS/cm)	Conductivity (µS/cm)		pH- before			pH- after			
			befor	e		afte	r						
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	N	Range
А	Lab Water	313	12	310-317	296	12	295-298	7.52	12	7.42-7.62	7.86	12	7.82-7.92
А	Perrier Water (2A)	196	3	190-199	181	3	176-183	7.64	3	7.60-7.70	7.76	3	7.74-7.79
А	EPA MHW (1)	386	3	385-390	369	3	365-371	7.53	3	7.44-7.59	7.72	3	7.71-7.73
В	Lab Water	338	8	292-387	335	8	312-360	7.57	8	7.38-7.80	7.43	8	7.16-7.70
В	Perrier Water (2A)	212	2	211-212	206	2	192-219	7.45	2	7.38-7.52	7.14	2	7.07-7.21
В	EPA MHW (1)	338	2	327-349	378	2	360-395	7.45	2	7.38-7.52	7.14	2	7.10-7.17
Е	Lab Water	376	12	366-382	376	12	367-381	8.06	12	7.96-8.09	8.14	12	8.13-8.19
Е	Perrier Water (2A)	207	3	203-210	209	3	206-212	8.07	3	8.02-8.14	8.15	3	8.09-8.21
Е	EPA MHW (1)	413	3	406-421	417	3	412-423	8.04	3	8.01-8.10	8.12	3	8.03-8.24
F	Lab Water	207	12	200-218	189	12	186-191	8.17	12	8.15-8.19	8.15	12	8.10-8.17
F	Perrier Water (2A)	195	3	184-204	174	3	171-176	8.17	3	8.15-8.18	7.96	3	7.91-8.03
F	EPA MHW (1)	386	3	380-395	358	3	353-362	8.07	3	8.05-8.09	7.95	3	7.92-8.00
G	Lab Water	190	12	184-196	174	12	172-176	8.10	12	7.97-8.20	8.10	12	7.98-8.20
G	Perrier Water (2A)	192	3	188-194	177	3	173-181	8.10	3	7.98-8.19	7.87	3	7.80-7.98
G	EPA MHW (1)	372	3	362-386	364	3	357-371	8.05	3	7.92-8.13	7.89	3	7.81-8.02
L	Lab Water	351	9	340-360	345	9	341-350	8.13	9	7.99-8.21	8.12	9	8.10-8.17
L	Perrier Water (2A)	167	3	160-170	164	3	158-168	8.22	3	8.20-8.25	7.94	3	7.77-8.14
L	EPA MHW (1)	350	3	340-360	343	3	337-348	8.18	3	8.17-8.20	8.04	3	7.93-8.10
М	Lab Water	386	8	364-399	348	8	344-350	8.30	8	8.24-8.35	8.17	8	8.16-8.17
М	Perrier Water (2A)	210	2	202-218	188	2	187-188	8.44	2	8.43-8.45	8.00	2	7.84-8.16
М	EPA MHW (1)	421	2	418-425	378	2	376-380	8.25	2	8.25-8.25	7.99	2	7.93-8.05
Ν	Lab Water	254	11	248-262	244	11	239-251	7.82	11	7.02-8.08	8.11	11	8.08-8.15
Ν	Perrier Water (2A)	149	3	147-151	143	3	139-145	8.01	3	7.87-8.12	8.09	3	7.98-8.16
Ν	EPA MHW (1)	302	3	294-313	296	3	283-310	7.88	3	7.73-8.00	7.97	3	7.85-8.06
0	Lab Water	344	12	337-351	344	12	323-351	7.85	12	7.78-7.95	7.89	12	7.79-7.96
0	Perrier Water (2A)	183	3	179-186	176	3	174-180	7.98	3	7.91-8.04	7.80	3	7.70-7.87
0	EPA MHW (1)	380	3	377-384	360	3	359-362	7.86	3	7.78-7.94	7.77	3	7.70-7.88

Table B18 cont.	Summary of conduct	tivity and pH data colle	ected in control cr	nambers from the	e eleven laboratorio	es participating in t	the baseline C. C	Jubia
interlaboratory	study. Water quality	parameters are preser	nted in two catego	ories (before and	after renewal of te	est solutions).		

		Condu	Conductivity (µS/cm)		Condu	Conductivity (µS/cm)			pH- before			pH- after		
			befor	e		after								
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	N	Range	
Р	Lab Water	219	12	216-223	218	12	215-224	8.02	12	7.88-8.27	7.89	12	7.74-7.99	
Р	Perrier Water (2A)	196	3	195-198	196	3	195-196	8.18	3	8.10-8.30	8.01	3	7.97-8.06	
Р	EPA MHW (1)	384	3	380-387	379	3	374-382	8.15	3	8.04-8.31	7.93	3	7.86-8.00	
Q	Lab Water	180	12	177-186	202	12	193-250	8.08	12	7.98-8.17	8.19	12	8.16-8.24	
Q	Perrier Water (2A)	173	3	168-175	185	3	181-187	7.76	3	7.72-7.79	8.20	3	8.18-8.23	
Q	EPA MHW (1)	355	3	352-358	376	3	374-377	7.77	3	7.74-7.82	8.10	3	8.07-8.14	

Table B19. Summary of dissolved oxygen (DO) and water temperature (water temp) data collected in control chambers from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

		DO	(mg/L)	before	DO (mg/L) after		Water	er temp (°C) before		Water temp (°C) after			
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range
Α	Lab Water	6.44	12	5.76-6.93	7.65	12	7.29-7.84	24.1	12	24.0-24.3	24.2	12	24.1-24.4
А	Perrier Water (2A)	6.48	3	5.88-6.79	8.01	3	7.96-8.04	24.1	3	24.1-24.2	24.2	3	24.1-24.3
А	EPA MHW (1)	6.32	3	5.79-6.68	7.88	3	7.77-7.97	24.2	3	24.0-24.3	24.5	3	24.4-24.6
В	Lab Water	8.67	8	8.20-8.40	9.12	8	8.51-9.80	24.8	8	24.7-24.9	24.7	8	24.5-24.9
В	Perrier Water (2A)	8.62	2	8.40-8.85	8.91	2	8.51-9.31	24.8	2	24.7-24.9	24.7	2	24.6-24.9
В	EPA MHW (1)	8.34	2	7.99-8.68	8.88	2	8.51-9.26	24.8	2	24.7-24.9	24.7	2	24.6-24.9
Е	Lab Water	7.44	12	7.22-7.71	7.76	12	7.71-7.93	25.0	12	25.0-25.0	24.9	12	24.8-25.0
Е	Perrier Water (2A)	7.51	3	7.36-7.66	7.89	3	7.79-7.96	25.0	3	24.9-25.0	25.0	3	25.0-25.0
Е	EPA MHW (1)	7.40	3	7.29-7.56	7.92	3	7.73-8.15	25.0	3	24.9-25.0	25.0	3	25.0-25.0
F	Lab Water	8.10	12	7.90-8.29	8.38	12	7.81-8.79	25.0	12	24.7-25.4	25.2	12	24.9-25.5
F	Perrier Water (2A)	7.99	3	7.89-8.09	8.74	3	8.70-8.81	25.2	3	25.1-25.4	25.3	3	25.1-25.4
F	EPA MHW (1)	7.98	3	7.85-8.09	8.80	3	8.77-8.83	25.1	3	25.0-25.2	25.3	3	25.0-25.5
G	Lab Water	8.32	12	8.28-8.40	8.35	12	8.30-8.39	25.0	12	24.5-25.2	24.6	12	24.4-24.8
G	Perrier Water (2A)	8.30	3	8.26-8.35	9.07	3	8.91-9.20	25.1	3	25.0-25.2	24.8	3	24.7-24.9
G	EPA MHW (1)	8.30	3	8.26-8.35	8.96	2	8.76-9.10	25.0	3	25.0-25.2	24.9	3	24.9-25.0
L	Lab Water	8.71	9	8.61-8.83	9.10	9	8.96-9.31	24.2	9	24.0-24.6	24.1	9	24.0-24.3
L	Perrier Water (2A)	8.68	3	8.65-8.72	9.63	3	9.17-10.1	24.3	3	24.0-24.6	24.1	3	24.0-24.2
L	EPA MHW (1)	8.71	3	8.70-8.71	9.61	3	9.20-10.2	24.3	3	24.0-24.6	24.2	3	24.0-24.3
Μ	Lab Water	6.69	8	6.41-6.89	7.50	9	7.21-9.07	24.9	8	24.3-25.6	24.4	8	24.0-24.9
Μ	Perrier Water (2A)	6.70	2	6.62-6.79	8.04	2	7.84-8.25	24.9	2	24.8-25.1	24.7	2	24.5-24.9
Μ	EPA MHW (1)	6.62	2	6.50-6.74	8.10	2	7.80-8.39	24.9	2	24.8-25.0	24.6	2	24.4-24.7
Ν	Lab Water	7.46	11	6.56-8.01	8.32	11	8.31-8.36	25.1	11	24.7-25.4	24.9	11	24.7-25.1
Ν	Perrier Water (2A)	7.46	3	6.71-8.08	8.32	3	8.27-8.36	25.1	3	25.0-25.4	24.6	3	24.4-24.7
Ν	EPA MHW (1)	7.46	3	6.69-8.04	8.30	3	8.26-8.33	25.1	3	24.9-25.3	24.6	3	24.5-24.6
0	Lab Water	7.80	12	7.61-7.96	8.45	12	8.10-8.77	25.1	12	24.9-25.6	24.9	11	24.7-25.2
0	Perrier Water (2A)	7.87	3	7.78-7.94	10.2	3	9.79-10.5	25.2	3	25.0-25.4	24.6	3	24.4-24.7
0	EPA MHW (1)	7.89	3	7.75-8.02	10.1	3	9.93-10.3	25.3	3	25.2-25.4	24.6	3	24.5-24.6

Table B19 cont. Summary of dissolved oxygen (DO) and water temperature (water temp) data collected in control chambers from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions)

		DO	(mg/L)	before	DO (mg/L) after			Water	Water temp (°C) before			Water temp (°C) after		
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	N	Range	
Р	Lab Water	8.30	12	8.22-8.38	8.27	12	8.14-8.34	25.0	12	24.7-25.4	25.2	12	25.0-25.4	
Р	Perrier Water (2A)	8.44	3	8.38-8.54	8.19	3	8.14-8.21	25.1	3	25.0-25.4	25.3	3	25.3-25.4	
Р	EPA MHW (1)	8.47	3	8.41-8.51	8.16	3	8.03-8.23	25.1	3	24.8-25.3	25.3	3	25.2-25.5	
Q	Lab Water	7.93	12	7.84-8.01	8.33	12	8.25-8.43	25.1	12	24.9-25.6	23.5	12	22.8-24.0	
Q	Perrier Water (2A)	8.63	3	8.59-8.69	8.27	3	8.22-8.35	25.2	3	25.0-25.4	22.8	3	22.0-23.3	
Q	EPA MHW (1)	8.66	3	8.48-8.89	8.36	3	8.32-8.44	25.3	3	25.2-25.4	22.7	3	22.0-23.2	

Table B20. Summary of air temperature data collected in control chambers from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

			(°C) Be	fore	(°C) After			
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	
А	Lab Water	24.7	12	24.6-24.7	24.5	12	24.4-24.7	
А	Perrier Water (2A)	24.7	3	24.7-24.7	24.5	3	24.4-24.7	
А	EPA MHW (1)	24.7	3	24.7-24.7	24.5	3	24.4-24.6	
В	Lab Water	24.8	8	24.1-25.1	24.7	8	24.4-25.0	
В	Perrier Water (2A)	24.8	2	24.6-25.0	24.8	2	24.5-25.0	
В	EPA MHW (1)	24.8	2	24.6-25.0	24.8	2	24.5-25.0	
Е	Lab Water	25.0	12	25.0-25.0	24.9	12	24.9-25.0	
Е	Perrier Water (2A)	24.9	3	24.9-25	25.0	3	24.9-25.0	
Е	EPA MHW (1)	25.0	3	25.0-25.0	25.0	3	24.9-25.0	
F	Lab Water	26.6	12	26.4-26.8	26.6	12	26.5-26.8	
F	Perrier Water (2A)	26.6	3	26.4-26.8	26.6	3	26.5-26.7	
F	EPA MHW (1)	26.6	3	26.4-26.8	26.6	3	26.5-26.7	
G	Lab Water	25.5	12	25.2-25.7	25.4	6	25.2-25.7	
G	Perrier Water (2A)	25.5	3	25.2-25.7	25.4	3	25.2-25.7	
G	EPA MHW (1)	25.5	3	25.2-25.7	25.5	3	25.2-25.7	
L	Lab Water	22.7	9	21.8-24.0	23.0	9	21.9-24.3	
L	Perrier Water (2A)	22.7	3	21.8-24.1	23.0	3	22.0-24.3	
L	EPA MHW (1)	22.7	3	21.8-24.0	23.1	3	22.0-24.3	
М	Lab Water	25.8	8	25.6-26.3	26.0	8	25.3-26.5	
М	Perrier Water (2A)	25.6	2	25.6-25.6	26.1	2	25.7-26.5	
М	EPA MHW (1)	25.6	2	25.6-25.6	26.2	2	25.9-26.5	
Ν	Lab Water	25.4	11	25.2-25.6	25.4	11	25.3-25.5	
Ν	Perrier Water (2A)	25.5	3	25.4-25.6	25.3	3	25.2-25.4	
Ν	EPA MHW (1)	25.4	3	25.3-25.6	25.4	3	25.2-25.5	
0	Lab Water	25.6	12	25.4-25.7	25.6	12	25.4-25.9	
0	Perrier Water (2A)	NA	NA	NA	NA	NA	NA	
0	EPA MHW (1)	NA	NA	NA	NA	NA	NA	



NA: Not available

Table B20 cont. Summary of air temperature data collected in control chambers from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions)

			(°C) Bef	ore	(°C) After			
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	
Р	Lab Water	25.4	12	24.9-25.6	25.3	12	25.1-25.5	
Р	Perrier Water (2A)	25.4	3	25.2-25.5	25.4	3	25.2-25.5	
Р	EPA MHW (1)	25.4	3	25.1-25.6	25.3	3	25.1-25.5	
Q	Lab Water	25.9	12	25.9-26.1	26.0	12	25.8-26.1	
Q	Perrier Water (2A)	25.9	3	25.9-26.0	26.0	3	25.9-26.0	
Q	EPA MHW (1)	25.9	3	25.9-26.0	26.0	3	25.9-26.0	



Table B21. Summary of hardness and alkalinity data collected in control chambers from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study. These water quality parameters were only measured after renewal of test solutions. N values refer to the number of tests conducted and included in the means.

		Hardne	′L CaCO₃)	Alkalinity (mg/L CaCO₃)			
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range
А	Lab Water	82.7	3	82-84	64.0	3	64-64
А	Perrier Water (2A)	86.3	3	85-89	84.3	3	82-86
А	EPA MHW (1)	119	3	110-127	66.3	3	60-73
В	Lab Water	93.0	2	90-96	65.0	2	64-66
В	Perrier Water (2A)	82	2	74-90	76.0	2	74-78
В	EPA MHW (1)	121	2	118-124	57.0	2	52-62
Е	Lab Water	95.5	2	95-96	61.0	2	60-62
E	Perrier Water (2A)	80.7	3	75-84	67.0	3	60-71
E	EPA MHW (1)	109	3	107-109	61.3	3	60-62
F	Lab Water	89.3	3	89-90	86.7	3	82-90
F	Perrier Water (2A)	85.7	3	84-87	76.7	3	74-79
F	EPA MHW (1)	120	3	116-126	58.7	3	49-70
G	Lab Water	81.7	3	80-84	78.8	4	76-81
G	Perrier Water (2A)	88.0	3	87-90	75.3	3	72-80
G	EPA MHW (1)	130	3	115-144	63.7	3	60-68
L	Lab Water	88.7	6	70-132	67.0	6	50-100
L	Perrier Water (2A)	100	3	80-144	80.3	3	62-108
L	EPA MHW (1)	104	3	82-138	65.3	3	54-84
М	Lab Water	100	2	100-100	67.0	3	66-68
Μ	Perrier Water (2A)	89	2	88-90	80.0	2	80-80
М	EPA MHW (1)	121	2	120-122	59.0	2	58-60
Ν	Lab Water	87.5	3	84-90	57.3	3	57-59
Ν	Perrier Water (2A)	86.3	3	81-90	74.3	3	73-75
Ν	EPA MHW (1)	122	3	118-125	56.0	3	55-57
0	Lab Water	88.8	12	54-98	66.5	3	61-83
0	Perrier Water (2A)	90.8	6	79-120	76.3	6	63-85
0	EPA MHW (1)	116	4	114-117	59.0	4	57-60



Table B21 cont. Summary of hardness and alkalinity data collected in control chambers from the eleven laboratories participating in the baseline C. dubia
interlaboratory study. These water quality parameters were only measured after renewal of test solutions.

		Hardne	ss (mg/	/L CaCO₃)	Alkalinity (mg/L CaCO₃)			
Lab	Sample Type	Mean	Ν	Range	Mean	N	Range	
Р	Lab Water	94.3	12	92-97	91.7	12	90-93	
Р	Perrier Water (2A)	81.7	3	79-84	79.3	3	83-76	
Р	EPA MHW (1)	115	3	112-117	61.7	3	59-64	
Q	Lab Water	89.8	12	86-97	86.2	12	83-89	
Q	Perrier Water (2A)	85.3	3	81-89	80.7	3	77-83	
Q	EPA MHW (1)	116	3	114-118	60.0	3	58-62	



Table B22. Summary of water quality data collected from the brood boards used to initiate the tests during the baseline *C. dubia* interlaboratory study. The data is divided into two categories: 'before' defined as water in test chambers for 24 hours, and 'after' defined as water quality measurements recorded after renewal in the test chambers. N values refer to the number of water quality measurements of the brood boards reported by the laboratories.

		Hardness		Alkalinity		Conductivity			рН				
		(mg/L CaCO <sub>3</sub> )			(mg/L CaCO₃)		(µS/cm)						
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range
А	Before	NM	-	-	NM	-	-	312	5	310-313	7.6	5	7.5-7.7
А	After	85	5	82-87	61	5	59-64	304	5	302-306	7.7	5	7.6-7.8
В	Before	NM	-	-	NM	-	-	350	2	341-358	7.3	2	7.2-7.5
В	After	92	1	-	64	1	-	355	2	343-367	7.2	2	7.0-7.4
Е	Before	NM	-	-	NM	-	-	370	3	363-376	8.0	3	7.8-8.1
Е	After	95	3	95-95	60	3	60-60	369	3	363-374	8.0	3	7.8-8.1
F	Before	NM	-	-	NM	-	-	209	9	206-212	8.2	9	8.2-8.2
F	After	88	9	84-91	86	9	76-92	190	9	189-191	8.1	9	8.1-8.2
G	Before	NM	-	-	NM	-	-	188	6	183-191	8.2	6	8.1-8.2
G	After	80	6	79-81	76	6	75-78	172	6	171-173	8.0	6	8.0-8.1
L	Before	NM	-	-	NM	-	-	348	10	340-358	8.1	10	8.1-8.2
L	After	82.8	5	71-90	60.0	5	58-64	341	7	330-346	8.1	7	8.1-8.2
М	Before	NM	-	-	NM	-	-	391	3	386-397	8.3	3	8.1-8.4
М	After	99.7	2	99-100	66.3	2	65-67	344	3	342-346	8.2	3	8.1-8.2
Ν	Before	NM	-	-	NM	-	-	260	7	251-306	8.0	7	8.0-8.2
Ν	After	85.4	7	83-88	57.3	7	57-59	254	7	243-273	8.1	7	8.0-8.1
0	Before	NM	-	-	NM	-	-	339	4	335-347	7.7	4	7.7-7.9
0	After	91.0	4	88-92	61.8	4	61-64	327	4	322-336	7.8	4	7.8-7.9
Р	Before	NM	-	-	NM	-	-	218	3	216-219	8.0	3	7.9-8.0
Р	After	92.8	5	91-94	91.4	5	90-94	210	5	202-216	7.7	5	7.6-7.8
Q	Before	NM	-	-	NM	-	-	174	3	168-181	8.0	3	8.0-8.0
Q	After	89.3	3	86-93	85.9	3	84-89	201	3	197-207	8.1	3	8.1-8.1
Table B22 cont. Summary of water quality data collected from the brood boards used to initiate the tests during the baseline *C. dubia* interlaboratory study. The data is divided into two categories: 'before' defined as water in test chambers for 24 hours, and 'after' defined as water quality measurements recorded after renewal in the test chambers. N values refer to the number of water quality measurements of the brood boards reported by the laboratories.

		Dissolv	ed Ox	ygen (mg/L)	Water	Temp	erature (°C)	Air T	empe	erature (°C)
Lab	Sample Type	Mean	Ν	Range	Mean	Ν	Range	Mean	Ν	Range
А	Before	6.33	5	6.16-6.70	24.6	5	24.2-25.0	25.4	5	24.8-26.2
А	After	7.21	5	7.04-7.66	24.8	5	24.2-24.5	25.7	5	25.2-26.4
В	Before	8.76	2	8.71-8.80	24.8	2	24.8-24.9	24.8	2	24.7-24.9
В	After	9.49	2	8.68-10.3	24.9	2	24.8-25.0	24.8	2	24.7-24.9
Е	Before	7.70	3	7.42-8.08	24.9	3	24.9-25.0	25.1	3	25.0-25.1
Е	After	7.76	3	7.47-8.14	25.0	3	24.9-25.0	25.1	3	25.0-25.1
F	Before	7.99	9	7.82-8.18	25.0	9	24.8-25.1	26.4	9	26.2-26.6
F	After	8.00	9	7.82-8.62	24.9	9	24.8-25.2	26.5	9	26.4-26.6
G	Before	8.30	6	8.20-8.50	25.2	6	25.1-25.6	25.4	6	25.0-25.7
G	After	8.49	6	8.44-8.54	25.0	6	24.7-25.3	25.8	4	25.5-26.0
L	Before	8.70	10	8.58-8.81	24.4	10	24.1-24.8	23.4	10	22.0-25.2
L	After	8.92	7	8.81-9.17	24.1	7	23.6-24.6	23.7	7	22.1-25.7
М	Before	6.52	3	6.41-6.73	24.2	3	23.8-24.8	24.2	3	23.7-24.8
М	After	6.95	3	6.89-7.06	24.4	3	23.7-24.9	24.1	3	23.6-24.6
Ν	Before	7.93	7	7.78-8.22	24.9	6	24.7-25.2	25.0	7	24.8-25.3
Ν	After	8.08	7	8.02-8.22	24.9	6	24.8-24.9	25.0	7	24.8-25.3
0	Before	7.77	4	7.45-8.07	24.9	4	24.8-24.9	25.6	3	25.3-26.1
0	After	7.74	4	7.53-8.04	24.7	2	24.5-24.9	25.7	2	25.5-25.9
Р	Before	8.26	3	8.26-8.26	25.2	3	25.2-25.3	25.7	3	25.5-26.1
Р	After	8.49	5	8.27-8.84	25.2	5	25.1-25.2	25.5	3	25.4-25.8
Q	Before	7.98	3	7.97-7.99	24.6	3	23.6-25.3	25.8	3	25.8-25.9
Q	After	8.40	3	8.36-8.43	22.9	3	22.1-23.2	25.8	3	25.7-25.9

Lab	Sample	Hardness	Total	Chloride	Nitrate	Sulfate	Bicarbonate	Se	Ca	Mg	К	Na
		(mg/L	Alk.	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(ug/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
		CaCO3)	(mg/L									
Α	EPA MHW (1)	87	55	1.96	0.498	111	55	0.0243	33.8	0.71	6.6	68.6
E	EPA MHW (1)	93	56	1.92	0.498	109	56	ND	36.1	0.75	6.34	66.5
F	EPA MHW (1)	89	55	1.96	0.501	120	55	0.031	34.3	0.71	6.12	67.8
G	EPA MHW (1)	91	55	1.91	0.501	111	55	0.023	35.4	0.73	ND	26.1
L	EPA MHW (1)	87	55	1.95	0.494	108	55	0.026	33.6	0.71	ND	33
Ν	EPA MHW (1)	69	55	1.9	0.499	113	55	0.0302	26.5	0.80	ND	25.3
0	EPA MHW (1)	72	55	1.97	0.498	113	55	0.0345	27.4	0.83	ND	25.6
Р	EPA MHW (1)	72	56	1.97	0.497	112	56	0.0355	27.5	0.76	ND	25.6
Q	EPA MHW (1)	90	54	1.97	0.497	108	54	ND	34.7	0.70	6.48	67.2
А	Lab Water	103	55	1.95	0.493	74.7	55	0.027	16.3	15.1	ND	29.6
Е	Lab Water	112	60	4.21	ND	83.4	60	ND	17.9	16.4	5.59	33
F	Lab Water	98	83	4.27	0.763	4.52	83	0.13	37.8	0.81	ND	ND
G	Lab Water	86	74	3.88	0.728	4.12	74	0.092	33.4	0.72	ND	ND
L	Lab Water	148	55	1.88	ND	111	55	1.67	33.2	15.7	ND	30.5
Ν	Lab Water	103	56	1.95	0.495	74.2	56	3.06	16	15.2	ND	30.6
0	Lab Water	116	56	4.52	ND	84.1	56	1.46	18.4	17.0	ND	33.3
Р	Lab Water	91	85	4.66	0.715	4.68	85	0.14	35.3	0.80	ND	ND
Q	Lab Water	91	80	4.18	0.756	4.48	80	0.133	35.2	0.73	ND	6.31
А	Perrier (2A)	90	73	3.94	0.747	4.14	73	0.116	34.8	0.72	ND	5.39
Е	Perrier (2A)	121	74	3.92	0.746	4.22	74	0.112	28.4	12.2	ND	5.43
F	Perrier (2A)	90	73	3.9	0.75	4.14	73	0.114	34.9	0.70	ND	5.66
G	Perrier (2A)	82	73	3.89	0.75	4.1	73	0.109	31.5	0.80	ND	ND
L	Perrier (2A)	87	74	3.91	0.75	4.11	74	0.149	33.6	0.72	ND	ND
Ν	Perrier (2A)	82	71	3.91	0.75	4.09	71	0.126	31.5	0.81	ND	ND
0	Perrier (2A)	82	72	3.93	0.75	4.11	72	0.165	31.4	0.78	ND	ND
Р	Perrier (2A)	83	72	3.93	0.752	4.12	72	0.164	31.9	0.79	ND	ND

Table B23. Water chemistry data for lab dilution waters and split samples (samples 1 and 2A) used in Round 1.

Q Perrier (2A) 75 73 3.93 0.745 4.14 73 0.116 28.9 0.69 ND 5.58	QI	Perrier (2A)	75	73	3.93	0.745	4.14	73	0.116	28.9	0.69	ND	5.58
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ND= Not detected.



Lab Sample Hardness Total Alk. Chloride Nitrate Sulfate Bicarbonate Se (ug/L) Ca Mg К Na (mg/L CaCO3) (mg/L (mg/L)(mg/L)(mg/L)(mg/L)(mg/L)(mg/L)(mg/L)(mg/L)CaCO<sub>3</sub>) EPA MHW (1) А ND 135 56 1.88 ND 111 56 0.0421 31.4 13.8 28.7 EPA MHW (1) В 57 128 1.89 ND 111 57 ND 30.0 13 ND 27.8 Е EPA MHW (1) 145 55 1.88 ND 112 55 0.025 32.5 15.4 ND 30.1 EPA MHW (1) F 136 58 112 58 28.6 1.88 ND ND 31.6 13.8 ND G EPA MHW (1) 126 56 1.85 56 0.0867 12.9 ND 111 29.1 ND 27.5 EPA MHW (1) L 129 57 1.89 112 57 30.0 13.2 27.8 ND ND ND EPA MHW (1) Μ 134 56 1.88 ND 113 56 0.022 31.1 13.7 ND 28.7 56 Ν EPA MHW (1) 148 112 15.6 56 1.83 ND ND 33.4 ND 30.5 EPA MHW (1) 0 128 57 1.89 112 57 30.0 12.8 27.7 ND ND ND EPA MHW (1) Ρ 130 56 113 56 30.3 1.90 ND 0.030 13.1 ND 27.9 EPA MHW (1) Q ND 134 57 1.87 113 57 0.037 13.6 28.8 ND 31.4 Lab Water А 88.2 59 3.11 ND 76.2 59 0.0373 14.7 12.5 ND 27.3 В Lab Water 15.4 10 0.595 12.8 10 2.78 2.05 ND ND ND ND Е Lab Water 62 ND 84.7 62 16.0 13.9 30.5 97.0 4.19 ND ND F Lab Water ND ND 97.8 83 4.29 0.76 4.59 83 0.117 37.8 0.834 Lab Water G 3.84 ND 74 74 84.6 0.729 4.08 0.118 32.7 0.700 ND Lab Water L ND 127 57 1.87 111 57 1.92 29.7 12.8 27 ND Lab Water Μ NA Ν Lab Water ND 89.9 58 1.94 ND 78.4 58 15.1 12.7 27.0 ND 0 Lab Water 97.0 60 3.02 ND 83.2 60 1.46 16.4 13.6 ND 29.5 Ρ Lab Water 104 4.72 0.057 87 0.733 4.93 87 40.1 0.851 ND ND Lab Water Q 92 79 4.06 0.743 4.33 79 0.150 35.6 0.768 ND ND А Perrier (2A) 97.7 77 3.99 0.737 4.32 77 0.0968 37.8 0.835 ND ND В Perrier (2A) 90.5 77 4.02 0.736 4.34 77 0.103 35.0 0.769 ND ND Е Perrier (2A) 103 3.98 0.737 77 77 4.31 0.122 39.8 0.988 ND ND

Table B24. Water chemistry data for lab dilution waters and split samples (samples 1 and 2A) used in Round 2.

# DRAFT

## Table B24. Water chemistry from Round 2 continued.

Lab	Sample	Hardness	Total	Chloride	Nitrate	Sulfate	Bicarbonate	Se (ug/L)	Ca	Mg (mg/L)	К	Na
		(mg/L	Alk.	(mg/L)	(mg/L)	(mg/L)	(mg/L)		(mg/L)		(mg/L)	(mg/L)
		CaCO3)	(mg/L									
			CaCO3)									
F	Perrier (2A)	96.9	76	4.05	0.738	4.42	76	0.141	37.4	0.856	ND	ND
G	Perrier (2A)	89.1	77	4.01	0.735	4.35	77	0.165	34.5	0.749	ND	ND
L	Perrier (2A)	92.8	78	4.01	0.736	4.36	78	0.122	35.9	0.773	ND	ND
Μ	Perrier (2A)	95.3	76	3.97	0.735	4.30	76	0.137	36.8	0.812	ND	ND
Ν	Perrier (2A)	102	78	3.98	0.737	4.31	78	0.106	39.2	0.944	ND	ND
Р	Perrier (2A)	90.5	77	4.01	0.736	4.33	77	0.123	35.0	0.761	ND	ND
Q	Perrier (2A)	95.9	77	3.97	0.734	4.32	77	0.168	37.1	0.820	ND	ND
0	Perrier (2A)	92.8	78	4.03	0.736	4.35	78	0.145	35.8	0.794	ND	ND

NA= Not analyzed due to labeling error.

ND= Not detected.

Sulfate Lab Sample Hardness Total Chloride Nitrate Bicarbonate Se Ca К Na Mg (mg/L (mg/L)(mg/L) (mg/L)(mg/L) (mg/L)(mg/L) (ug/L) (mg/L)(mg/L)Alk. CaCO3) (mg/L CaCO3) Lab Water А 75.7 56 84.0 56 3.03 ND ND 14.7 11.5 26.1 ND Perrier (2A) А 3.92 93.1 77 0.740 4.16 77 0.0985 35.9 0.861 ND ND EPA MHW (1) А 29.2 12.0 26.3 122 56 2.12 0.499 104 56 ND ND В Lab Water 65 65 111 2.17 ND 98.3 ND 0.175 26.8 ND 29.8 Perrier (2A) В 92.5 77 3.96 0.738 4.17 77 0.154 35.5 0.923 ND ND В EPA MHW (1) 122 104 55 0.0643 29.4 11.8 ND 26.1 55 2.14 ND Lab Water Е 95.3 61 61 4.00 ND 84.6 ND 16.8 13.0 ND 29.2 Е Perrier (2A) 3.88 75 91.2 75 0.740 4.16 0.128 35.1 0.829 ND ND Е EPA MHW (1) 109 54 121 2.07 0.030 29.0 11.9 ND 25.7 54 ND F Lab Water 97.8 84 4.11 0.756 4.42 84 0.0975 37.7 0.869 ND ND F Perrier (2A) 89.5 77 3.86 0.737 4.12 77 0.130 34.6 0.764 ND ND F EPA MHW (1) 106 122 55 2.09 ND 55 ND 29.0 12.0 ND 26.7 Lab Water G 90.1 74 3.82 0.728 4.07 74 0.133 34.7 0.859 ND ND G Perrier (2A) 92.8 76 3.97 0.741 4.18 76 0.124 35.8 0.847 ND ND G EPA MHW (1) ND 124 105 55 29.9 55 2.15 ND 0.0638 11.9 25.7 L Lab Water 124 2.06 ND 105 57 1.92 30.0 12.0 ND 26.3 57 L Perrier (2A) 92.6 3.79 0.736 4.09 0.128 35.7 0.855 ND 76 76 ND L EPA MHW (1) 102 55 2.08 0.500 103 55 0.0699 24.2 10.1 ND 22.5 Μ Lab Water 29.6 1.94 66 102 66 ND 91.3 1.95 18.4 13.7 ND Perrier (2A) Μ 93.2 77 3.82 0.737 4.08 77 0.164 35.9 0.885 ND ND EPA MHW (1) Μ 120 56 2.08 ND 105 56 0.0511 28.3 12.0 ND 26.6 Ν Lab Water 26.5 92.0 58 2.28 ND 79.9 58 3.15 16.5 12.3 ND Ν Perrier (2A) 93.8 76 3.92 0.741 4.16 76 0.0993 36.1 0.923 ND ND Ν EPA MHW (1) 124 55 2.14 ND 105 55 29.6 12.1 ND 26.3 ND 0 Lab Water 57 97.1 57 5.76 ND 87.9 1.46 17.1 13.2 ND 29.7 0 Perrier (2A) 90.8 75 3.82 0.735 4.09 75 0.112 35.1 0.781 ND ND 0 EPA MHW (1) 55 25.8 120 55 2.05 ND 104 0.0224 28.6 11.8 ND

Table B25. Water chemistry data for lab dilution waters and split samples (samples 1 and 2A) used in Round 3.

# DRAFT

Lab	Sample	Hardness (mg/L CaCO3)	Total Alk. (mg/L CaCO3)	Chloride (mg/L)	Nitrate (mg/L)	Sulfate (mg/L)	Bicarbonate (mg/L)	Se (ug/L)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	Na (mg/L)
Р	Lab Water	106	86	4.74	0.739	4.85	86	0.168	40.7	0.922	ND	ND
Р	Perrier (2A)	75.4	76	3.84	0.736	4.11	76	0.158	29.2	0.628	ND	ND
Р	EPA MHW (1)	102	55	2.09	ND	105	55	0.062	24.2	10.1	ND	22.0
Q	Lab Water	96.9	81	4.14	0.773	4.09	81	0.121	37.4	0.838	ND	ND
Q	Perrier (2A)	91	76	3.82	0.736	4.08	76	0.161	35.1	0.801	ND	ND
Q	EPA MHW (1)	122	54	2.08	ND	105	54	0.089	29.0	12.0	ND	25.6

Table B25. Water chemistry from Round 3 continued

ND= Not detected.

Table B26. Neonate production and adult female survival data for Sample 1 (EPA Moderately Hard Water) collected from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study presented by test. Each test had 10 replicates.

Lab	Round	Mean	CV of	# Surviving	
		Neonates/Adult	Neonates/Female	Females	
		Female			
Α	1	35.2	0.160	10	
Α	2	44.4	0.058	10	
Α	3	36.1	0.271	9	
В	2	34.4	0.171	10	
В	3	35.6	0.466	8	
E	1	20.5	0.339	10	
E	2	13.4	0.183	10	
E	3	11.3	0.277	10	
F	1	17.6	0.208	10	
F	2	20.8	0.142	10	
F	3	20	0.130	10	
G	1	27.2	0.306	10	
G	2	32.3	0.062	10	
G	3	35.2	0.082	10	
L	1	21.8	0.410	7	
L	2	25	0.386	9	
L	3	26.1	0.267	9	
М	2	26.9	0.574	9	
М	3	37.9	0.314	9	
Ν	1	6.9	1.26	4	
Ν	2	3.0	1.90	3	
Ν	3	11.3	0.515	8	
0	1	33.1	0.100	10	
0	2	35.4	0.075	10	
0	3	24.4	0.417	10	

Table B26 continued. Neonate production and adult female survival data for Sample 1 (EPA Moderately Hard Water) collected from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study presented by test. Each test had 10 replicates.

Lab	Round	Mean Neonates/Adult	CV of Neonates/Female	# Surviving Females
		Female		
Р	1	35.8	0.261	10
Р	2	40.6	0.061	10
Р	3	39.2	0.118	10
Q	1	40.6	0.052	10
Q	2	37.5	0.075	10
Q	3	30.4	0.339	9

Table B27. Neonate production and survival data for Sample 2A (Perrier based Moderately Hard Water) collected from the eleven laboratories participating in the baseline *C. dubia* interlaboratory study presented by test. Each test had 10 replicates.

Lab	Round	Mean	CV of	# Surviving
		Neonates/Adult	Neonates/Female	Females
		Female		
А	1	34.0	0.195	10
А	2	43.4	0.086	10
А	3	39.7	0.108	10
В	2	21.2	0.521	10
В	3	31.1	0.522	9
E	1	17.0	0.331	10
E	2	14.3	0.556	10
E	3	10.7	0.399	10
F	1	22.3	0.075	10
F	2	20.8	0.091	10
F	3	20.4	0.101	10
G	1	31.8	0.052	10
G	2	32.9	0.196	10
G	3	34.6	0.084	10
L	1	30.2	0.285	9
L	2	24.7	0.436	9
L	3	22.0	0.588	7

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Lab	Round	Mean	CV of	# Surviving
		Neonates/Adult	Neonates/Female	Females
		Female		
Μ	2	37.1	0.207	10
Μ	3	35.4	0.390	9
Ν	1	3.5	1.21	5
Ν	2	7.5	1.77	3
Ν	3	16.7	0.517	8
0	1	30.1	0.147	10
0	2	36.0	0.053	10
0	3	23.7	0.330	10
Р	1	36.5	0.289	9
Р	2	34.6	0.274	9
Р	3	38.3	0.078	10
Q	1	39.6	0.102	10
Q	2	35.3	0.088	10
Q	3	35.0	0.169	10



Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females
А	1	37.1	0.117	10
А	1	35.1	0.279	9
А	1	38.3	0.129	10
А	1	37.5	0.137	10
А	2	44.1	0.067	10
А	2	43.2	0.060	10
А	2	40.7	0.103	10
А	2	43.5	0.084	10
А	3	40.8	0.054	10
Α	3	41.5	0.086	10
Α	3	42.1	0.059	10
Α	3	38.0	0.215	10
B	2	35.9	0.108	10

0.135

0.173

0.625

В

В

В

В

В

B B 31.9

30.8

20.6

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
E	1	17.3	0.289	10	
E	1	21.7	0.154	10	
Е	1	17.0	0.277	10	
Е	1	20.5	0.240	10	
Е	2	12.3	0.325	10	
E	2	18.7	0.356	10	
E	2	12.7	0.236	10	
E	2	20.0	0.351	10	
E	3	8.9	0.238	10	
E	3	10.4	0.276	10	
E	3	9.3	0.360	10	
E	3	8.5	0.264	10	
F	1	16.4	0.191	10	
F	1	17.1	0.061	10	
F	1	17.2	0.135	10	
F	1	16.7	0.170	10	
F	2	19.1	0.166	10	
F	2	18.8	0.100	9	
F	2	19.9	0.128	10	
F	2	19.0	0.135	10	
F	3	17.8	0.075	10	
F	3	16.2	0.110	10	
F	3	16.6	0.158	10	
F	3	16.5	0.087	10	

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
G	1	31.3	0.054	10	
G	1	30.0	0.146	9	
G	1	29.8	0.063	10	
G	1	28.6	0.095	10	
G	2	28.7	0.309	9	
G	2	31.8	0.213	9	
G	2	32.5	0.054	10	
G	2	29.0	0.230	10	
G	3	32.8	0.124	10	
G	3	34.5	0.045	10	
G	3	31.1	0.187	9	
G	3	29.6	0.463	7	
L	1	28.8	0.143	10	
L	1	31.6	0.155	9	
L	1	30.3	0.301	10	
L	2	32.8	0.272	10	
L	2	27.7	0.319	9	
L	2	31.9	0.169	10	
L	3	24.9	0.387	9	
L	3	21.9	0.387	8	
L	3	29.2	0.115	10	
М	2	25.0	0.393	10	
Μ	2	26.1	0.483	9	
М	2	21.5	0.632	8	
Μ	2	7.6	1.01	7	
М	3	33.9	0.258	9	
М	3	29.6	0.521	8	
М	3	30.0	0.438	9	
М	3	33.9	0.209	10	

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
Ν	1	15.5	0.601	8	
Ν	1	12.7	0.575	8	
Ν	1	11.9	0.531	8	
Ν	2	21.4	0.462	8	
Ν	2	23.9	0.557	9	
Ν	2	25.0	0.415	8	
Ν	2	31.8	0.248	9	
Ν	3	20.8	0.280	9	
N	3	16.2	0.476	8	
Ν	3	19.9	0.292	10	
Ν	3	16.9	0.340	8	
0	1	31.1	0.225	10	
0	1	31.3	0.199	10	
0	1	33.3	0.167	10	
0	1	30.2	0.194	10	
0	2	31.4	0.226	10	
0	2	38.2	0.073	10	
0	2	36.6	0.087	10	
0	2	31.9	0.194	9	
0	3	26.3	0.32	9	
0	3	28.7	0.257	10	
0	3	28.6	0.233	10	
0	3	29.6	0.195	10	

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
Р	1	37.5	37.5 0.133		
Р	1	37.2	0.203	8	
Р	1	38.3	0.296	9	
Р	1	33.3	0.341	9	
Р	2	37.1	0.143	10	
Р	2	36.9	0.219	10	
Р	2	32.8	0.297	9	
Р	2	38.3	0.165	10	
Р	3	38.1	0.163	10	
Р	3	37.8	0.100	10	
Р	3	34.8	0.196	10	
Р	3	36.7	0.088	10	
Q	1	41.1	0.058	10	
Q	1	39.0	0.103	10	
Q	1	42.2	0.055	10	
Q	1	41.0	0.076	10	
Q	2	33.8	0.094	10	
Q	2	36.0	0.053	10	
Q	2	34.3	.3 0.064 10		
Q	2	35.8	0.077	10	
Q	3	30.2	30.2 0.300 9		
Q	3	31.3	0.136	10	
Q	3	28.7	0.372	9	
Q	3	33.1	0.096	10	

Figure B33. Plot of the age of the females whose neonates were used to initiate a test batch versus the IC50 value expressed as measured conductivity for the Sample 2 series (Perrier based Moderately Hard Water). Symbols represent the laboratory code.



Average Age of Brood Board Females vs IC50 for Sample Series 2

Age of Brood Board Females at Test Initiation (Days)

Figure B34. Plot of the age of the females whose neonates were used to initiate a test batch versus the IC50 value expressed as measured conductivity for the Sample 3 series (lab's own dilution water). Symbols represent the laboratory code.





Figure B35. Plot of the age of the females whose neonates were used to start the test versus mean neonate production for labs that culture in EPA Moderately Hard Water for Sample 1 (EPA Moderately Hard Water. Symbols represent the laboratory code.

Average Age of Adult Used to Start the Test vs Mean Neonate Production for Labs that Culture in EPA MHW for Sample 1



Figure B36. Plot of the age of the females whose neonates were used to start the test versus mean neonate production for labs that culture in Perrier based Moderately Hard Water vs. neonate production for Sample 2A (Perrier based Moderately Hard Water). Symbols represent the laboratory code.

Average Age of Adult Used to Start the Test vs Mean Neonate Production for Labs that Culture in Perrier for Sample 2A



# DRAFT

Figure B37. Box plots of IC25 by laboratory for nominal NaCl concentrations during the baseline ILS using combined SCCWRP-supplied and laboratory-supplied dose response samples in rounds 1 through 3. Letters at 0 on the y-axis denotes post-hoc Tukey tests for significant differences between laboratories.



## POTENTIAL SOURCES OF VARIANCE IN TEST PERFORMANCE

Figure B38. A plot showing the importance of each test, brood board, and feeding variable to explain the patterns in mean neonate production per living female across all labs in Samples 1 and 2A from the Baseline Intercalibration tests (rounds 1, 2, and 3). Mean neonate production was modeled using random forest regression with each of the metric on the y-axis as a predictor variable. Importance is expressed as the % increase in model Mean Square Error when the variable



### was removed from the model.

### Random Forest Regression Variable Importance

Mean Neonate Production per Live Female v2 in EPA and Perrier (xrepro.live~metric)



Variable Importance (% Increase in MSE When Ommitted)

Figure B39. A plot showing the importance of each test, brood board, and feeding variable to explain the patterns in mean neonate production per living female across all labs in Samples 1 and 2A from the Baseline Intercalibration tests (rounds 1, 2, and 3). CV of neonate production was modeled using random forest

regression with each of the metric on the y-axis as a predictor variable. Importance is expressed as the % increase in model Mean Square Error when the variable was removed from the model.

### CV Neonate Production per Live Female in EPA and Perrier (cvrepro.live~metric) Brood Board Mean Alkalinity -- - 0 Age of Alge Food -Brood Board Mean Conductivity --0 $\bigcirc$ Test Max pH --0 Test Mean Water Temp Test Min Air Temp Test Max Air Temp Mean Age of Initial Female Test Ca:Mg ratio Brood Board Mean Hardness . Test Mean pH · Test Calcium Test Mean Air Temp Test Max DO. Test Sulfate · Test Mean DO · Test Hardness -Test Min DO-Test Max Water Temp Test Alkalinity Test Selenium (ug/L) -Age of YCT Food -Brood Board Mean DO. Test Magnesium Brood Board Mean Water Temp Test Bicarbonate nitrate\_as\_n -Test Flouride -Test Min Water Temp Test Min pH · Test Ammonia · Test Sodium · Max Age of Initial Female -Brood Board Mean pH -Test Chloride - $\cap$ Median Age of Initial Female --0 Test Postassium 10 15 0 5 Variable Importance (% Increase in MSE When Ommitted)

# DRAFT

## Appendix C Second ILS Study Plan and Summary Data

## OVERVIEW OF BASELINE TESTING PROCEDURE

The specific objective of the second ILS was to collect additional C. dubia chronic toxicity data and a more complete/consistent lab technique dataset across California-accredited laboratories. Ten (10) laboratories participated in an intercomparison exercise consisting of several split samples tested in three separate testing batches within a ~ 8-week window. Testing design (Figure C1), sample type, preparation and distribution was done as described in the baseline ILS study plan (**Appendix B**). However, laboratories followed a more consistent set of laboratory techniques (see below).

### Figure C1: Overview of the C. dubia baseline study design.



## SUMMARY OF STANDARD OPERATING PROCEDURES

All laboratories were required to meet the following specifications:

- Limit the age of adults used to start the test to 6-10 days old.
- Use < 24 hr old neonates produced within an 8-hr window.
  - Record which brood number (e.g., 3rd, 4th, or 5th) is used to start the test.
  - Record the specific beginning and end time window for age of neonates at test initiation (hours and minutes).
- Evaluate brood board health for 2 weeks prior to testing using a common set of health criteria provided by SCCWRP.
- Use randomization by blocking of known parentage AND randomization of cups on the test board. Test set-up must use the randomized blocking by known parentage, using 10 randomly selected brood board chambers with a minimum of 8 neonates from the adult on test initiation day. Each test will be treated as independent for blocking by known parentage except for samples 2A and 2B-F. These two tests will be treated as one for the purposes of blocking by known parentage.
- Renew or terminate test boards daily at 24 hours within a 2-hr window from test initiation.
- Conduct the tests for 8 days (i.e., 192 hours). Record of when the tests would have ended if the lab was using their own SOP.
- Independently quantify initial food density (both algae and YCT, do not use numbers provided by the supplier) and describe feeding procedure (incl. quantification method and volume dispensed) to estimate food density in test cups and ensure that it meets EPA protocols requirements. Record all information and measurements. Use repeating pipettor or volumetric pipette for accurate volumes.
- Follow holding times of YCT ≤ 8 days after thawing and algae ≤ 21 days (from time of receipt if purchased or from production if cultured in-house).
- Document split broods on bench sheets at the time of observation including number and size of neonates, and observations of females' movement and presence of eggs in pouch.

## INVENTORY OF DATA COLLECTED

- Ten labs participated in testing.
- For Round 1 nine laboratories tested samples
  - Lab M could not participate in the first round due to staffing issues.
- For Round 2 ten laboratories tested samples
  - Half of the samples for Lab B were returned to SCCWRP by the shipping company, but were turned around the next day and all samples were tested together and within holding time
  - Lab G had a mix up of their concentrations for Series 3 samples leading to data for that test being unusable.
- For Round 3 ten laboratories tested samples, all on time
- Lab I consistently had high mortality and low reproduction in the samples sent them by SCCWRP, but generally had good success with their laboratory controls.
- Out of 120 expected laboratory controls from 10 labs, 115 were tested to completion.
- Of the 115 controls completed, 4 did not pass test acceptability criteria.

Lab ID	Lab controls	#1- MHW	#2A- DMW Perrier®	#2B-F- SCCWRP concentration- response	#3- Lab concentration- response
Α	12	3	3	3	3
В	12	3	3	3	3
Е	12	3	3	3	3
F	12	3	3	3	3
G	11	3	3	3	2
I	12	3	3	3	3
L	-	-	-	-	-
м	8	2	2	2	2
N	12	3	3	3	3
0	12	3	3	3	3
Р	-	-	-	-	-
Q	12	3	3	3	3
All Labs	115	29	29	29	28

Table C1. Inventory of data collected in the second ILS. Here, a dataset is defined as one sample tested with 10 replicates.

Table C2. Individual test batches not meeting test acceptability criteria for reproduction ( $\geq$  15 neonates/surviving female) or survival (>90% in controls) in the second ILS. Cells with dashes indicate that the endpoint met test acceptability criteria for that sample.

Lab	Test Round	Mean Neonates/Surviving Female	Survival
Ν	Round 2	1.2	-
Ν	Round 2	5.9	-
N	Round 2	0	-
N	Round 2	2.4	-
1	Round 2	-	70%

Table C3. Individual test batches not meeting acceptable brood board mortality (< 20%). Note that all labs had at least 8 neonates per female used to initiate tests and no females were older than 14 days.

Lab	Test Round	Brood Board Percent Mortality	Mean Neonates per Female in Lab Control	
I	Round 1	23	33.9ª	
1	Round 1	22	42.0 <sup>b</sup>	
1	Round 2	23	33.4°	
G	Round 1	_25	22.3 <sup>c</sup>	
<sup>a</sup> Mean c	of three controls that	t were initiated from that b	prood board.	

<sup>b</sup>Brood board used to initiate one test.

<sup>c</sup>Mean of two controls that were initiated from that brood board.

## **BIOLOGICAL RESPONSE DATA**

Figure C2. Schematic box plot of the mean number of neonates per female in laboratory control water for each test in both ILS. Dots represent the individual test values and the + symbol represents the mean of all the tests. Data represents protocol specified test termination of the day when 60% of control females achieved three broods.





Figure C3. Schematic box plot of the mean number of neonates per female in laboratory in the sample waters provided by SCCWRP for each test in both ILS. The dots represent the individual test values and the + symbol represents the mean of all the tests.


300 -250 -200. 150 **-**100 . 80 -**CV Neonates per Female** 60 • ILS 1 - Lab Control . ILS 2 - Lab Control 40 · . . . 1. +20 . 0 Ė Ň Ň B F Ĝ Ó P Q А L

Figure C4. Schematic plot of the coefficient of variation for the number of neonates per female in laboratory control water for each test in both ILS. The dots represent the individual test values and the + symbol represents the mean of all the tests.





Figure C5. Schematic box plot of the coefficient of variation for the number of neonates per female in laboratory in the sample waters provided by SCCWRP for each test in both ILS. The dots represent the individual test values and the + symbol represents the mean of all the tests.

Table C4. Summary of reproduction for Sample 1 from the laboratories participating in the baseline and second C. dubia interlaboratory studies, based on the number of neonates in three broods. For each lab, the data is presented as mean of 3 rounds (except for lab M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

	Neonates/Adult Female				Neonates/Adult Female			
	Baseline				Second			
Lab	Mean	N	Mean Range	CV of Mean	Mean	N	Mean Range	CV of Mean
А	38.6	3	35-44	0.13	38.1	3	36-41	0.058
В	35.0	2	34-36	0.024	43.8	3	40-47	0.075
E	16.0	3	13-21	0.25	31.3	3	30-34	0.088
F	20.1	3	18-21	0.072	33.7	3	33-35	0.030
G	31.6	3	27-35	0.13	36.9	3	25-45	0.285
1					2.6	3	0-6	1.23
L	24.3	3	22-26	0.092				
М	32.4	2	27-38	0.24	41.4	2	41-42	0.014
N	7.1	3	3-11	0.59	15.4	3	2-25	0.780
0	31.0	3	24-35	0.19	35.8	3	32-39	0.093
Р	38.6	3	36-41	0.065				
Q	36.2	3	30-41	0.14	35.1	3	32-40	0.132

Test acceptability criterion is a mean of 15 neonates/surviving female.

Table C5. Summary of biological data for Sample 2A (diluted mineral water with Perrier®) collected from the laboratories participating in the baseline and second *C. dubia* interlaboratory studies, based on the number of neonates in three broods. For each lab, the data is presented as mean of 3 rounds (except for lab M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

	Neonates	eonates/Adult Female				Neonates/Adult Female			
		Baseline			Second				
Lab	Mean	N	Mean Range	CV of Mean	Mean	N	Mean Range	CV of Mean	
А	39.0	3	34-43	0.12	37.1	3	32-40	0.112	
В	26.2	2	21-31	0.27	28.9	3	24-38	0.263	
E	15.4	3	11-21	0.34	32.4	3	30-36	0.093	
F	21.2	3	20-22	0.047	32.3	3	30-36	0.100	
G	33.1	3	32-35	0.043	32.5	3	25-43	0.290	
1					4.9	3	0-8	0.889	
L	25.6	3	22-30	0.16					
Μ	35.2	2	35-35	0.008	38.6	2	39-39	0.004	
N	9.2	3	4-17	0.73	16.9	3	7-23	0.502	
0	29.9	3	24-36	0.21	33.2	3	28-37	0.125	
Р	37.0	3	35-38	0.056					
Q	36.6	3	35-40	0.07	25.3	3	20-30	0.214	

Test acceptability criterion is a mean of 15 neonates/surviving female.

Table C6. Summary of biological data for laboratory dilution water from the laboratories participating in the baseline and second *C. dubia* interlaboratory studies, based on the number of neonates in three broods. For each lab, the data is presented as mean of 3 rounds (except for lab M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Neonates	s/Adult Fer	nale		Neonates/Adult Female				
			Baseline				Second			
Lab	Sample Type	Mean	N	Mean Range	CV of Mean	Mean	N	Mean Range	CV of Mean	
А	Lab Water	40.2	12	35-44	0.072	38.9	12	34-43	0.088	
В	Lab Water	29.8	4	21-36	0.22	43.8	12	35-50	0.105	
E	Lab Water	14.9	12	8-22	0.32	32.3	12	31-34	0.031	
F	Lab Water	17.6	12	16-20	0.072	31.4	12	26-36	0.087	
G	Lab Water	30.8	12	29-35	0.06	32.1	11 <sup>a</sup>	22-43	0.246	
I	Lab Water					36.7	12	27-43	0.131	
L	Lab Water	28.9	9	22-33	0.13					
Μ	Lab Water	26.0	8	8-34	0.33	39.3	8	34-45	0.102	
N	Lab Water	19.6	11	12-32	0.3	15.1	12	0-25	0.649	
0	Lab Water	31.4	12	26-38	0.106	35.1	12	27-41	0.120	
Р	Lab Water	36.6	12	33-38	0.054					
Q	Lab Water	35.5	12	29-42	0.13	28.7	12	17-42	0.258	

Test acceptability criterion is a mean of 15 neonates/surviving female.

<sup>a</sup>One test lost due to technical error.

Figure C6. Dot plot of the mean number of neonates per female expressed as a percentage of control response for Sample 1 (EPA Moderately Hard Water) for each test in both ILS.



Sample 1

Figure C7. Dot plot of the mean number of neonates per female expressed as a percentage of control response for Sample 2A (DMW Perrier®) for each test in both ILS.





Table C7. Summary of reproduction endpoint for Sample 1 (EPA MHW) from both ILS, expressed as a percentage of control reproduction. For each lab, the data is presented as mean of 3 rounds (except for labs B and M for the baseline and lab M for the second who could not participate in round 1 of testing). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Baselir	ne		Second			
Lab	Control Adjusted Mean (%)	N	Mean Range	CV of Mean	Control Adjusted Mean (%)	N	Mean Range	CV of Mean
А	94.6	3	88-101	0.064	98.6	3	93-106	0.072
В	95.8	1 <sup>a</sup>	-	-	100	3	96-105	0.049
E	118	3	109-127	0.076	101	3	96-108	0.065
F	110	3	107-112	0.024	104	3	101-106	0.029
G	102	3	87-113	0.133	118	3	113-125	0.054
I					9.0 <sup>b</sup>	3	0.3-23	1.32
L	85.6	3	77-105	0.195				
Μ	110	3	108-112	0.027	106	2	92-119	0.182
N	37.6	3	14-54	0.559	127	3	106-158	0.216
0	104	3	93-113	0.098	103	3	97-110	0.063
Р	103	3	95-109	0.068				
Q	102	3	96-106	0.052	123	3	103-151	0.203

<sup>a</sup>The laboratory control had zero reproduction for round 3, therefore a control adjusted mean could not be calculated.

<sup>b</sup>The laboratory had low reproduction and survival in Sample 1, but normal values in their laboratory controls.

Table C8. Summary of reproduction endpoint for Sample 2A (diluted mineral water with Perrier<sup>®</sup>) second ILS, expressed as a percentage of control reproduction. For each lab, the data is presented as mean of 3 rounds (except for lab M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

			Baseline				Second			
Lab	Sample Type	Control Adjusted Mean (%)	N	Mean Range	CV of Mean	Control Adjusted Mean (%)	N	Mean Range	CV of Mean	
А	DMW Perrier <sup>®</sup> (2A)	97.7	3	96-100	0.026	92.8	3	90-98	0.051	
В	DMW Perrier <sup>®</sup> (2A)	66.5	<b>1</b> ª	-	-	72.1	3	68-79	0.085	
E	DMW Perrier <sup>®</sup> (2A)	85.9	3	76-103	0.172	98.9	3	93-108	0.081	
F	DMW Perrier <sup>®</sup> (2A)	122	3	111-130	0.085	107	3	100-119	0.099	
G	DMW Perrier <sup>®</sup> (2A)	103	3	100-106	0.028	104	3	99-110	0.056	
I	DMW Perrier <sup>®</sup> (2A)					13.2 <sup>b</sup>	3	0-22	0.877	
L	DMW Perrier <sup>®</sup> (2A)	95.1	3	89-100	0.060					
М	DMW Perrier <sup>®</sup> (2A)	131	2	120-142	0.122	91.8	2	89-95	0.047	
Ν	DMW Perrier <sup>®</sup> (2A)	54.0	3	28-103	0.788	113	3	106-124	0.085	
0	DMW Perrier <sup>®</sup> (2A)	91.0	3	83-96	0.081	92.2	3	85-97	0.068	
Ρ	DMW Perrier <sup>®</sup> (2A)	97.7	3	94-101	0.038					
Q	DMW Perrier <sup>®</sup> (2A)	106	3	96-116	0.093	107	3	100-113	0.063	

<sup>a</sup>The laboratory control had zero reproduction for round 3, therefore a control adjusted mean could not be calculated.

<sup>b</sup>The laboratory had low reproduction and survival in Sample 1, but normal values in their laboratory controls.

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females
А	1	37.5	0.407	9
А	2	36.3	0.050	10
А	3	40.6	0.093	10
В	1	40.5	0.180	10
В	2	43.7	0.177	9
В	3	47.1	0.092	10
E	1	30.0	0.092	10
E	2	29.5	0.158	10
E	3	34.5	0.099	10
F	1	34.8	0.073	10
F	2	32.8	0.107	10
F	3	33.6	0.092	10
G	1	25.4	0.266	10
G	2	40.8	0.114	10
G	3	44.6	0.128	10
1	1	6.2	0.127	0
1	2	1.5	1.81	3
I	3	0.1	3.16	0
М	2	41.0	0.279	8
М	3	41.8	0.190	10
Ν	1	24.9	0.055	9
Ν	2	1.9	2.65	10
Ν	3	19.4	0.377	10
0	1	36.6	0.063	10
0	2	32.1	0.144	10
0	3	38.6	0.055	10
Q	1	32.3	0.152	9
Q	2	32.6	0.205	9
Q	3	40.5	0.375	10

Table C9. Neonate production and adult female survival data for Sample 1 (EPA Moderately Hard Water) collected from the ten laboratories participating in the second *C. dubia* interlaboratory study presented by test. Each test had 10 replicates.

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females
А	1	38.8	0.248	9
А	2	32.4	0.091	10
А	3	40.2	0.074	10
В	1	24.0	0.272	10
В	2	37.7	0.391	9
В	3	25.1	0.348	9
E	1	29.8	0.112	10
E	2	31.8	0.068	10
E	3	35.7	0.030	10
F	1	35.9	0.069	10
F	2	31.1	0.130	9
F	3	29.8	0.080	10
G	1	24.8	0.340	9
G	2	29.7	0.381	8
G	3	43.0	0.127	10
1	1	6.4	0.132	1
1	2	8.4	0.594	8
1	3	0	-	2
М	2	38.7	0.321	9
М	3	38.5	0.187	10
N	1	23.4	0.212	10
N	2	7.3	0.646	10
Ν	3	20.1	0.400	9
0	1	37.4	0.072	10
0	2	28.5	0.074	10
0	3	33.8	0.116	10
Q	1	19.5	0.831	8
Q	2	30.2	0.192	9
Q	3	26.3	0.542	9

Table C10. Neonate production and survival data for Sample 2A (diluted mineral water with Perrier<sup>®</sup>) collected from the ten laboratories participating in the second *C. dubia* interlaboratory study presented by test. Each test had 10 replicates.

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females
А	1	40.5	0.228	9
А	1	42.8	0.131	10
А	1	33.9	0.355	9
А	1	38.0	0.169	9
А	2	34.1	0.137	10
А	2	36.2	0.070	10
А	2	34.3	0.132	10
А	2	40.3	0.070	10
А	3	41.9	0.135	10
А	3	40.9	0.197	10
А	3	40.5	0.243	10
А	3	43.0	0.068	10
В	1	35.1	0.284	10
В	1	40.8	0.184	10
В	1	42.9	0.099	10
В	1	42.0	0.078	10
В	2	45.7	0.058	10
В	2	47.6	0.160	9
В	2	50.3	0.062	10
В	2	48.7	0.096	10
В	3	44.7	0.118	10
В	3	36.5	0.334	10
В	3	45.9	0.116	10
В	3	45.6	0.110	10

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
E	1	30.8	0.061	10	
E	1	31.9	0.101	10	
E	1	32.5	0.086	10	
Е	1	31.8	0.072	10	
E	2	30.6	0.178	10	
E	2	33.4	0.055	10	
E	2	33.8	0.041	10	
E	2	32.1	0.090	10	
E	3	31.9	0.140	10	
E	3	33.0	0.087	10	
E	3	33.3	0.062	10	
E	3	32.5	0.117	10	
F	1	34.6	0.061	10	
F	1	35.7	0.044	10	
F	1	33.7	0.050	10	
F	1	33.9	0.070	10	
F	2	31.1	0.100	10	
F	2	26.2	0.314	10	
F	2	29.9	0.128	10	
F	2	29.4	0.365	9	
F	3	31.7	0.172	9	
F	3	29.7	0.125	10	
F	3	29.1	0.114	10	
F	3	31.4	0.075	10	

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
G	1	21.9	0.382	8	
G	1	22.5	0.423	10	
G	1	22.1	0.325	10	
G	1	27.7	0.351	8	
G	2	32.6	0.300	8	
G	2	29.3	0.515	8	
G	2	39.6	0.128	10	
G	2	NT	NT	NT	
G	3	39.5	0.158	10	
G	3	43.3	0.096	10	
G	3	39.3	0.325	9	
G	3	35.0	0.453	8	
1	1	27.4	0.375	8	
1	1	35.6	0.242	9	
1	1	38.7	0.346	9	
1	1	42.0	0.106	9	
I	2	36.1	0.166	10	
I	2	38.9	0.143	10	
I	2	31.3	0.214	10	
I	2	30.6	0.460	7	
1	3	37.3	0.256	9	
1	3	43.4	0.116	9	
1	3	38.0	0.372	8	
1	3	40.8	0.347	9	

NT= Not tested. Technical error led to loss of sample.

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females
Μ	2	44.6	0.203	10
М	2	43.6	0.226	10
М	2	41.1	0.242	10
Μ	2	40.0	0.161	8
Μ	3	35.1	0.405	9
Μ	3	40.6	0.210	9
Μ	3	34.3	0.488	8
Μ	3	35.0	0.408	9
Ν	1	23.4	0.233	9
Ν	1	22.1	0.309	10
Ν	1	24.9	0.090	10
Ν	1	25.4	0.199	9
Ν	2	1.2	3.16	10
Ν	2	5.9	1.58	10
Ν	2	0	-	10
Ν	2	2.4	1.11	10
Ν	3	16.6	0.563	10
Ν	3	18.5	0.402	10
Ν	3	20.4	0.307	10
N	3	20	0.496	9

Lab	Round	Mean Neonates/Adult Female	CV of Neonates/Female	# Surviving Females	
0	1	37.6	0.054	10	
0	1	38.7	0.046	10	
0	1	35.9	0.081	10	
0	1	37.1	0.041	10	
0	2	29.2	0.173	10	
0	2	33.5	0.106	10	
0	2	30.4	0.208	10	
0	2	27.1	0.331	10	
0	3	38.3	0.076	10	
0	3	35.6	0.070	10	
0	3	37.3	0.133	10	
0	3	40.8	0.050	10	
Q	1	21.4	0.620	9	
Q	1	17.2	0.933	8	
Q	1	22.8	0.590	9	
Q	1	28.5	0.220	9	
Q	2	28.3	0.508	9	
Q	2	30.2	0.207	10	
Q	2	31.1	0.170	10	
Q	2	23.5	0.648	9	
Q	3	35.1	0.361	10	
Q	3	24.6	0.694	9	
Q	3	41.9	0.112	10	
Q	3	39.4	0.375	9	

Table C12. Age of female *Ceriodaphnia dubia* when their neonates were used to initiate testing in baseline and second ILS. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

Lab Mea	an Age of Femal t Initiation (Day	le at s)	Ν	Mean Range	Mean Age of Female at Test Initiation (Days)	Ν	Mean Range	
	Baselin				Second			
А	6.3		3	6 - 7	6.2	4	6 - 7	
В	9.0		2	9 - 9	7.0	3	7 - 7	
E	10.7		3	10 - 11	9.0	3	7 - 10	
F	9.7		9	9 - 11	9.0	6	7 - 10	
G	7.7		6	7 - 8	7.0	6	7 - 7	
L	12.4		14	12 - 14				
1					8.7	6	8 - 9	
М	8.0		3	7 - 9	8.6	5	8 - 9	
N	7.6		7	7 - 11	8.5	8	8 - 9	
0	8.0		4	8 - 8	7.8	6	7 - 9	
Р	6.8		5	6 - 7				
Q	9.3		3	9 - 10	9.0	5	9 - 9	

Table C13. Summary of the number of broods produced during eight days of testing in laboratory dilution water collected from the 12 laboratories participating in the baseline and second *C. dubia* interlaboratory study. For each lab, the data is presented as mean of 3 rounds (except for lab M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

				Nu	mber of B	roods (8 da	iys)		
			Baseline				S	econd	
Lab	Sample Type	Mean	Ν	Mean	CV of	Mean	N	Mean	CV of
				Range	Mean			Range	Mean
А	Lab Water	4.0	12	3.7-4.1	0.025	4.0	12	3.7-4.0	0.025
В	Lab Water	3.2	4	2.4-3.8	0.19	4.0	12	3.9-4.0	0.011
Е	Lab Water	4.5	12	4.1-5.0	0.067	4.3	12	3.8-5.0	0.10
F	Lab Water	4.4	12	4.0-4.9	0.071	4.5	12	4.0-5.0	0.093
G	Lab Water	4.0	12	3.2-4.2	0.070	3.8	11 <sup>a</sup>	3.4-4.0	0.052
1	Lab Water					3.8	11 <sup>b</sup>	3.6-4.0	0.040
L	Lab Water	3.1	9	2.6-3.8	0.12				
Μ	Lab Water	2.7	8	1.1-3.4	0.26	4.3	<b>8</b> <sup>c</sup>	4.0-4.6	0.046
Ν	Lab Water	3.4	11	2.9-4.2	0.12	2.9	8 <sup>b</sup>	2.6-3.2	0.066
0	Lab Water	4.3	12	4.0-4.7	0.045	4.2	12	4.0-5.0	0.069
Р	Lab Water	3.9	12	3.7-4.0	0.036				
Q	Lab Water	3.7	12	3.1-4.0	0.094	3.3	12	2.6-4.0	0.15

<sup>a</sup>Test lost due to technical error.

<sup>b</sup>Test(s) that did not meet test acceptability criteria not included.

<sup>c</sup>Unable to participate in Round 1 due to staffing issues.

Table C14. Summary of the number of days to the first brood produced in laboratory dilution water collected from the 12 laboratories participating in the baseline and second *C. dubia* interlaboratory study. For each lab, the data is presented as mean of 3 rounds (except for lab M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

					Time to F	irst Brood			
			Baseline				S	econd	
Lab	Sample Type	Mean	N	Mean	CV of	Mean	N	Mean	CV of
				Range	Mean			Range	Mean
А	Lab Water	4.0	12	4.0-4.0	0	3.6	12	3.0-4.0	0.13
В	Lab Water	4.3	4	4.0-5.1	0.13	3.9	12	3.6-4.0	0.044
Е	Lab Water	4.3	12	3.8-4.8	0.075	4.2	12	3.6-4.7	0.083
F	Lab Water	3.4	12	3.0-3.9	0.096	3.3	12	3.0-3.9	0.10
G	Lab Water	3.5	12	3.0-4.0	0.13	3.8	11 <sup>a</sup>	3.0-4.0	0.10
1	Lab Water					3.7	11 <sup>b</sup>	3.0-4.0	0.085
L	Lab Water	4.3	9	4.0-5.0	0.088				
Μ	Lab Water	3.7	8	3.0-4.2	0.13	3.2	8 <sup>c</sup>	3.0-3.6	0.068
Ν	Lab Water	4.0	11	3.3-4.4	0.094	4.1	8 <sup>b</sup>	3.3-5.0	0.18
0	Lab Water	3.0	12	3.0-3.2	0.019	3.4	12	3.0-4.0	0.095
Ρ	Lab Water	4.0	12	4.0-4.1	0.007				
Q	Lab Water	4.0	12	4.0-4.0	0	4.1	12	4.0-4.4	0.030

<sup>a</sup>Test lost due to technical error.

<sup>b</sup>Test(s) that did not meet test acceptability criteria not included.

<sup>c</sup>Unable to participate in Round 1 due to staffing issues.

Table C15. Summary of minimum age of neonates used to initiate testing from the baseline and second ILS. The N value represents the total number of samples tested. Note that Lab M has a lower number because they were unable to participate in Round 1 of the second ILS. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Minimum Neonate Age at Test Start (h)											
		Ba	seline		Second								
Lab	Mean	N	Mean	CV of	Mean	Mean N Mean CV of							
			Range	Mean			Range	Mean					
А	6.7	12	6-8	0.098	8.8	12	5-15	0.48					
В	0	4	0-0	0	7.7	12	8-8	0.031					
E	4.3	12	4-5	0.11	5.0	12	3-6	0.28					
F	10.8	12	8-16	0.34	10.0	12	7-15	0.34					
G	12.2	12	6-20	0.53	8.1	12	4-18	0.74					
1					10.2	12	4-17	0.51					
L	4.0	9	1-8	0.53									
Μ	12.7	8	6-18	0.45	17.5	8	14-20	0.14					
Ν	6.4	11	4-13	0.67	3.7	12	1-5	0.51					
0	14.3	12	7-22	0.36	12.8	12	8-21	0.35					
Р	1.8	12	1-3	0.42									
Q	2.1	12	0.5-5	0.59	2.0	12	0.4-3	0.40					

Table C16. Summary of maximum age of neonates used to initiate testing from the baseline and second ILS. The N value represents the total number of test performed. Note that Lab B has a lower number because they were unable to participate in Round 1. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Maximum Neonate Age at Test Start (h)												
		Ва	seline		Second									
Lab	Mean	Ν	Mean	CV of	Mean	Ν	Mean	CV of						
			Range	Mean			Range	Mean						
А	14.6	12	14-15	0.035	16.5	12	13-22	0.23						
В	6.8	4	6.8-6.8	0	22.3	12	21-24	0.046						
E	8.0	12	8-8	0	21.0	12	19-22	0.067						
F	18.5	12	15-24	0.20	17.6	12	15-22	0.19						
G	18.7	12	14-25	0.26	15.3	12	12-23	0.31						
1	15.5	9	5-24	0.49	17.4	12	12-23	0.25						
L	19.5	8	14-24	0.23										
Μ	14.4	11	12-21	0.30	23.3	8	22-24	0.024						
Ν	20.8	12	15-24	0.18	9.7	12	9-10	0.049						
0	6.3	12	5-8	0.13	19.6	12	16-24	0.17						
Р	6.6	12	5-10	0.20										
Q	14.6	12	14-15	0.035	5.1	12	3-7	0.27						

Table C17. Calculated day testing would have ended using the protocol trigger of 60% of control females having produced three broods for the baseline and second ILS. The N value refers to the number of tests performed. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

	Calculated	Fest Durat	ion (Days to	60% of female	s having 3 bro	ods)
		Baseline			Second	
Lab	Mean	N	Mean Range	Mean	N	Mean Range
А	7.0	12	7-7	6.2	12	6-7
В	7.2	4	7-8	6.8	12	6-7
E	6.5	12	6-7	6.7	12	6-7
F	6.1	12	6-7	6.0	12	6-6
G	6.2	12	6-7	6.6	11ª	6-7
1				6.3	11 <sup>b</sup>	6-7
L	7.2	9	7-8			
М	6.6	8	6-8	6.0	8 <sup>c</sup>	6-6
Ν	6.7	11	6-8	7.5	8 <sup>b</sup>	7-8
0	6.0	12	6-6	6.0	12	6-6
Р	6.7	12	6-7			
Q	7.0	12	7-7	7.1	12	7-8

<sup>a</sup>Test lost due to technical error.

<sup>b</sup>Test(s) that did not meet test acceptability criteria not included.

<sup>c</sup>Unable to participate in Round 1 due to staffing issues.

Table C18. Average number of males observed per test in the baseline and second ILS. The N value refers to the number of tests successfully performed. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Numbe	r of Males per	Test
	Baseli	ine	Se	econd
Lab	Mean	N	Mean	N
А	0	12	0	12
В	0	4	0	12
E	0	12	0	12
F	0	12	0	12
G	0	12	0.36	11 <sup>a</sup>
1			0	11 <sup>b</sup>
L	0	9		
М	0	8	0	8 <sup>c</sup>
Ν	0	11	0	8 <sup>b</sup>
0	0	12	0	12
Р	0	12		
Q	0	12	0.42	12

<sup>a</sup>Test lost due to technical error.

<sup>b</sup>Test(s) that did not meet test acceptability criteria not included.

<sup>c</sup>Unable to participate in Round 1 due to staffing issues.



Table C19. Summary of number of neonates per surviving female in EPA Moderately Hard Water (Sample 1) from the 12 laboratories participating in the baseline *C. dubia* interlaboratory study. The mean was calculated by taking the total number of neonates produced for all females and dividing by the number of surviving females. For each lab, the data is presented as mean of 3 rounds (except for labs M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Neonates/ Surviving Female											
		E	Baseline			S	econd						
Lab	Mean	Ν	Mean	CV of	Mean	N	Mean	CV of					
			Range	Mean			Range	Mean					
А	39.9	3	35-44	0.16	39.5	3	36-42	0.072					
В	39.5	2	34-44	0.12	45.4	3	40-49	0.095					
E	15.1	3	11-20	0.27	31.3	3	30-34	0.088					
F	19.5	3	18-21	0.16	33.7	3	33-35	0.030					
G	31.6	3	27-35	0.15	36.9	3	25-45	0.28					
1					*	*	*	*					
L	29.3	3	28-31	0.18									
М	36.0	2	30-42	0.27	46.5	2	42-51	0.14					
Ν	13.8	3	10-17	0.62	16.3	3	2-28	0.81					
0	31.0	3	24-35	0.20	35.8	3	32-39	0.093					
Р	38.5	3	36-41	0.15									
Q	37.3	3	34-41	0.062	37.5	3	36-40	0.069					

\*Very low or no surviving females.

Table C20. Summary of number of neonates per surviving female in diluted mineral water with Perrier<sup>®</sup> (Sample 2A) from the 12 laboratories participating in the baseline and second *C. dubia* interlaboratory studies. The mean was calculated by taking the total number of neonates produced for all females and dividing by the number of surviving females. For each lab, the data is presented as mean of 3 rounds (except for labs M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Neonates/ Surviving Female											
		E	Baseline			ç	Second						
Lab	Mean	Ν	Mean	CV of	Mean	Mean N Mean		CV of					
			Range	Mean			Range	Mean					
А	39.0	3	34-43	0.13	38.6	3	32-43	0.14					
В	27.9	2	21-35	0.45	31.3	3	24-42	0.30					
E	14.0	3	11-17	0.43	32.4	3	30-36	0.093					
F	21.2	3	20-22	0.089	33.4	3	30-36	0.096					
G	33.1	3	32-35	0.11	35.9	3	28-43	0.22					
I					*	*	*	*					
L	30.8	3	27-34	0.22									
М	38.2	2	37-39	0.28	40.8	2	38-43	0.078					
N	17.6	3	7-25	0.61	17.7	3	7-23	0.51					
0	29.9	3	24-36	0.18	33.2	3	28-37	0.13					
Р	39.1	3	38-41	0.082									
Q	36.6	3	35-40	0.12	29.1	3	24-34	0.16					

\*Very low or no surviving females.

Table C21. Summary of number of neonates per surviving female in laboratory dilution water from the 12 laboratories participating in the baseline and second *C. dubia* interlaboratory studies. The mean was calculated by taking the total number of neonates produced for all females and dividing by the number of surviving females. For each lab, the data is presented as mean of 3 rounds (except for labs M who could not participate in round 1). N values refer to the number of tests included in the mean and CV. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

			Neonates/ Surviving Female								
			E	Baseline			Second				
Lab	Sample Type	Mean	Ν	Mean	CV of	Mean	Ν	Mean	CV of		
				Range	Mean			Range	Mean		
А	Lab Water	40.5	12	37-44	0.099	39.9	12	34-45	0.089		
В	Lab Water	32.1	4	23-36	0.23	44.3	12	35-53	0.12		
E	Lab Water	14.8	12	8-22	0.28	32.3	12	31-34	0.031		
F	Lab Water	17.8	12	16-21	0.13	31.9	12	26-36	0.090		
G	Lab Water	33.0	12	29-42	0.11	35.8	11	22-44	0.23		
I	Lab Water					41.1	11	31-48	0.14		
L	Lab Water	30.4	9	27-35	0.21						
Μ	Lab Water	29.2	8	11-38	0.37	43.1	8	39-50	0.084		
Ν	Lab Water	23.1	11	15-35	0.34	15.7	12	0-28	0.66		
0	Lab Water	32.0	12	29-38	0.20	35.1	12	27-41	0.12		
Р	Lab Water	38.3	12	35-47	0.14						
Q	Lab Water	36.1	12	31-42	0.11	30.8	12	22-44	0.22		

Figure C8. Schematic box plot of the IC25 of NaCl spiked waters based on the nominal concentration in the second ILS. The dots represent the values of the individual tests and the + represents the mean of all the tests.





Figure C9. Schematic box plot of the IC50 of NaCl spiked waters based on the nominal concentration in the second ILS. The dots represent the values of the individual tests and the + represents the mean of all the tests.

Figure C10. Schematic box plot of the IC50:IC25 of NaCl spiked waters based on the nominal concentration in the second ILS. The dots represent the values of the individual tests and the + represents the mean of all the tests.



Lab Code	Round #	SCCV (	VRP DMW Samples 2	Perrier® A-F)	Lab Diluti	on Water (Sa	mple 3 series)
		IC25	IC50	LC50	IC25	IC50	LC50
А	1	2151	2788	NC	2312	2905	3274
А	2	2270	2832	NC	1725	2612	NC
А	3	2483	2975	3117	2282	2124	2080
В	1	2555	3194	NC	2021	2762	3342
В	2	2959	3794	NC	2445	3057	NC
В	3	2374	3042	NC	2282	3101	4014
Е	1	1205	1608	NC	1307	1753	NC
E	2	1080	1777	NC	1209	1690	NC
Е	3	901.3	1651	NC	1113	1795	NC
F	1	2314	2905	3242	2230	2782	2992
F	2	2498	3038	3274	2509	3091	NC
F	3	2327	2936	3557	2383	2994	NC
G	1	2628	3317	NC	2645	3427	NC
G	2	2173	2985	NC	NT	NT	NC
G	3	2333	3205	NC	2754	3728	NC
1	1	NC	NC	NC	NC	NC	NC
	2	NC	NC	NC	NC	NC	NC
1	3	NC	NC	NC	NC	NC	NC
М	2	2049	2703	3377	1960	2690	NC
М	3	1904	2714	2838	1738	2564	NC
Ν	1	1364	1761	NC	1298	1600	NC
Ν	2	NC	NC	NC	NC	NC	NC
Ν	3	541.8	1099	NC	376.9	720.4	NC
0	1	2308	2848	3917	1552	1886	2071
0	2	2563	3018	2918	1852	2112	1747
0	3	1433	1880	3617	2465	3027	4050
Q	1	1071	1760	1760	2573	3080	3549
Q	2	2323	2576	3130	615.3	870.8	1484
Q	3	803.7	1907	2868	2424	2987	3106

Table C22. IC25/50 and LC50 from NaCl spiked samples from the second ILS. Data are expressed as measured conductivity in µS/cm.

\*NC = Not Calculable

\*NT = Not tested

Lab Code	Lab Code Round #		IW Perrier®	(Samples 2A-F)	Lab Dilu	ition Water (San	nple 3 series)
Lab Coue	Kouna #	IC25	IC50	LC50	IC25	IC50	LC50
А	1	1018	1372	NC	1043	1363	1562
А	2	1085	1397	NC	748.6	1216	NC
А	3	1203	1476	1556	1128	1419	1500
В	1	1243	1598	NC	876.0	1267	1571
В	2	1468	1932	NC	1021	1347	NC
В	3	1142	1514	NC	1020	1484	2000
E	1	521.3	733.0	NC	473.4	681.6	NC
E	2	461.7	821.4	NC	393.4	682.0	NC
E	3	380.1	755.3	NC	411.1	726.2	NC
F	1	1109	1438	1625	1074	1382	1500
F	2	1211	1512	1643	1284	1606	NC
F	3	1116	1455	1800	1172	1506	NC
G	1	1284	1666	NC	1254	1681	NC
G	2	1031	1482	NC	NT	NT	NT
G	3	1119	1604	NC	1329	1845	NC
*	1	651.7	870	62.5	36.0	72.1	62.5
*	2	48.6	97.2	83.3	409.4	988.1	62.5
*	3	691.2	794.1	62.5	32.3	64.6	62.5
М	2	963.9	1325	1700	885.1	1290	NC
М	3	888.0	1331	1400	742.3	1186	NC
Ν	1	1364	1761	NC	1298	1599.72	NC
Ν	2	1234	1886	NC	508.7	572.4	NC
Ν	3	541.8	1099	NC	376.9	720.4	NC
0	1	2308	2848	3917	1552	1886	2071
0	2	2563	3018	2918	1852	2112	1747
0	3	1433	1880	3617	2465	3027	4050
Q	1	1071	1760	1760	2573	3080	3549
Q	2	2502	2973	3130	615.3	870.8	1484
Q	3	803.7	1907	2868	2424	2987	3106

Table C23. IC25/50 and LC50 results from NaCl spiked samples from the second ILS. Data are expressed as nominal NaCl concentrations in mg/L.

\*Values are presented for this laboratory, however the number of neonates was very low and the response plots are flat.

Figure C11. Schematic box plot of the PMSD values of NaCl spiked waters. The dots represent individual test values and the + symbol represents the mean value of all the tests. Note: Data have not been censored based on the shape of the concentration-response plot.



Table C24. Minimum significant difference (MSD) and percent minimum significant difference (PMSD) values for dilution series sample reproduction endpoint. Values exceeding USEPA (2000) 90<sup>th</sup> percentile value of 37 are highlighted in *italics*. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

		Ro	und 1	Round 2		Round 3		Round 1		Round 2		Round 3	
				Base	eline				Seco	nd			
Lab ID	Sample Series	MSD	PMSD	MSD	PMSD	MSD	PMSD	MSD	PMSD	MSD	PMSD	MSD	PMSD
A	2A-2F	9.88	29.1	3.83	8.82	3.35	8.43	9.49	24.5	3.37	10.3	3.74	9.31
A	3 Series	4.38	11.7	4.34	10.0	5.52	14.5	6.91	18.2	5.57	13.8	3.89	9.05
В	2A-2F	NA	NA	9.35	44.1	10.9	52.8	7.43	31.0	9.37	24.9	10.8	43.1
В	3 Series	NA	NA	15.4	49.7	NA	NA	5.45	13.0	7.41	15.2	4.88	10.7
E	2A-2F	5.55	32.7	5.54	38.7	3.91	36.6	2.75	9.24	3.60	11.3	3.09	8.66
E	3 Series	4.54	22.1	5.31	26.5	2.58	30.3	2.82	8.86	3.30	10.3	3.13	9.64
F	2A-2F	7.84	35.2	6.49	31.2	5.86	28.7	3.72	10.4	3.30	10.6	3.36	11.3
F	3 Series	2.82	16.9	3.81	20.0	2.75	16.6	5.04	14.9	5.63	19.2	4.54	14.5
G	2A-2F	4.26	13.4	5.00	15.2	7.09	20.5	9.37	37.8	11.0	37.0	8.83	20.5
G	3 Series	6.14	21.5	5.04	17.4	11.6	39.2	7.42	26.8	NT	NT	9.29	26.5
I	2A-2F							NA	NA	NA	NA	NA	NA
I	3 Series							NA	NA	8.12	26.6	NA	NA
L	2A-2F	6.27	20.8	15.6	63.1	14.2	64.4						
L	3 Series	7.78	25.7	7.63	23.9	5.44	18.6						
М	2A-2F	NA	NA	15.7	42.3	16.8	47.5	NT	NT	8.71	22.5	12.2	31.6
М	3 Series	NA	NA	6.45	84.8	8.62	25.4	NT	NT	10.3	25.7	17.7	50.6
N	2A-2F	3.59	102	8.48	113	7.00	41.9	4.50	19.2	3.84	52.6	8.58	42.7
N	3 Series	NA	NA	8.11	25.5	5.06	29.9	3.91	15.4	3.38	140	6.40	32.0
0	2A-2F	6.73	22.4	6.16	17.1	7.21	30.4	2.37	6.33	6.81	23.9	6.53	19.3
0	3 Series	7.23	23.9	8.40	26.3	10.2	34.3	5.35	14.4	6.04	22.3	4.16	10.2
Р	2A-2F	9.52	26.1	9.18	26.0	4.20	11.0						
Р	3 Series	6.33	19.0	6.05	15.8	5.15	14.0						
Q	2A-2F	5.89	14.9	7.24	20.5	6.01	17.2	13.0	66.6	3.9	12.8	12.5	47.4
Q	3 Series	5.73	14.0	3.47	10.1	3.63	11.0	4.1	14.3	11.9	50.6	8.2	19.5

NA=Not available either due to sample not being tested or sample being extremely toxic. NT=Not tested

Table C25. Brood board health parameters recorded in boards that were used to initiate the tests during the baseline and second *C. dubia* interlaboratory studies. Data is expressed as percentage of brood board cups exhibiting a health issue category per brood board. N values refers to the number of brood boards used by each lab to initiate all their tests. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

			Unhea	lthy Adul	t		Dead Adult						
	E	Baselin	e	Second			Baseline			Second			
Lab	Mean	N	Mean	Mean	N	Mean	Mean	N	Mean	Mean	N	Mean	
			Range			Range			Range			Range	
А	0	5	0-0	0	4	0-0	0	5	0-0	0	4	0-0	
В	0	2	0-0	10.8	3	5-20	11.2	2	7.5-15.0	2.5	3	0-7.5	
E	0	3	0-0	0	3	0-0	0	3	0-0	0	0	0-0	
F	1.1	9	0-10	0.24	7	0-1.7	1.5	9	0-6.7	6.7	7	0-13.3	
G	3.3	6	0-20	2.1	6	0-5	0.6	6	0-3.3	14.3	6	3.3-25.0	
I				4.2	6	1.7-8.3				17.5	6	6.7-23.3	
L	3.3	14	0-10				17.6	14	0-46.7				
М	0	3	0-0	0	5	0-0	5.6	3	0-11.7	7.7	5	1.7-15.0	
Ν	0	7	0-0	1.9	8	0-15.0	11.0	7	0-41.7	1.2	8	0-6.7	
0	0	4	0-0	0	5	0-0	1.2	4	0-3.3	2.0	5	0-6.7	
Р	0.2	5	0-3.3				2.9	5	0-13.3				
Q	0	3	0-0	2.7	5	0-11.7	2.8	3	0-5.0	7.0	5	3.3-15.0	

Table C26. Additional brood board health parameters recorded in boards that were used to initiate the tests during the baseline and second *C. dubia* interlaboratory studies. Data is expressed as percentage of brood board cups exhibiting a health issue category per brood board. N values refers to the number of brood boards used by each lab to initiate all their tests. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

			М	ale			Unhealthy Neonates						
		Baselir	ne	Second			Baseline			Second			
Lab	Mean	N	Mean Range	Mean	N	Mean Range	Mean	N	Mean Range	Mean	N	Mean Range	
А	0	5	0-0	0	4	0-0	0	5	0-0	0	4	0-0	
В	0	2	0-0	0	3	0-0	0	2	0-0	0	3	0-0	
E	0	3	0-0	0	3	0-0	0	3	0-0	0	3	0-0	
F	0	9	0-0	0	7	0-0	0.2	9	0-1.7	0	7	0-0	
G	1.1	6	0-6.7	0	6	0-0	0	6	0-0	0	6	0-0	
I				0	6	0-0				33.0	6	3.3-73.3	
L	1.9	14	0-10.0				0	14	0-0				
Μ	0	3	0-0	0	5	0-0	9.5	3	1.7-21.7	20.3	5	6.7-31.7	
N	0	7	0-0	0	8	0-0	0	7	0-0	0	8	0-0	
0	0	4	0-0	0	5	0-0	0	4	0-0	0	5	0-0	
Р	0	5	0-0		r		8.0	5	3.3-13.3				
Q	0	3	0-0	0	5	0-0	0.6	3	0-1.7	0	5	0-0	

Table C27. Additional brood board health parameters recorded in boards that were used to initiate the tests during the baseline and second *C. dubia* interlaboratory studies. Data is expressed as percentage of brood board cups exhibiting a health issue category per brood board. N values refers to the number of brood boards used by each lab to initiate all their tests. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

			Dead Ne	Other Abnormalities*						
		Basel	ine		Secon	d	Second			
Lab	Mean	1ean N Mea Rang		Mean N		Mean Range	Mean	N	Mean Range	
А	0	5	0-0	0	4	0-0	0	4	0-0	
В	0	2	0-0	0	3	0-0	0	3	0-0	
E	0	3	0-0	0	3	0-0	0	3	0-0	
F	0.2	9	0-1.7	0	7	0-0	0.2	7	0-1.7	
G	0	6	0-0	0	6	0-0	2.1	6	0-10	
I				13.6	6	0-36.7	1.1	6	0-3.3	
L	0	14	0-0							
М	20.0	3	11.7-33.3	34.3	5	15-51.7	0	5	0-0	
Ν	0	7	0-0	0	8	0-0	0	8	0-0	
0	0	4	0-0	0	5	0-0	0.3	5	0-1.7	
Р	17.3	5	6.7-23.3							
Q	1.1	3	0-1.7	0	5	0-0	0	5	0-0	

\*This was a new code for the Second ILS.
Table C28. Summary of water treatment methods from the ten laboratories participating in the second *C. dubia* interlaboratory study.

Lab	Source Water	Treatment Step 1	Treatment Step 2	Treatment Step 3	Treatment Step 4	Treatment Step 5	Treatment Step 6	Final DI Water
^	Municipal	Carbon cartridgo	lon ovchango	lon ovchango	Filtration	Disinfoction: UV	NI/A	туре
A	www.cipar	Carbon cartinge	IOITEXCHAIlge	Ion exchange Filtration I		Disiniection. 0v	IN/A	1
в	Municipal	Carbon cartridge	Ion exchange	Ion exchange	Other organic	Other organic	Ν/Δ	1
D	Wallepa	carbon cartriage	ion exchange	clean-up		clean-up	N/A	1
Е	Municipal	Filtration	lon exchange	Carbon cartridge N/A		N/A	N/A	1
F	Municipal	Filtration	Carbon cartridge	Reverse osmosis	Ion exchange	N/A	N/A	2
G	Municipal	Filtration	Carbon cartridge	Reverse osmosis	Ion exchange	N/A	N/A	2
I	Municipal	Carbon cartridge	Reverse osmosis	Ion exchange	Filtration	Disinfection: UV	N/A	1
	Municipal		Carbon contrideo	Filtration	Disinfontions UV	Disinfection: 0.2	NI / A	1
IVI	wunicipal	ion exchange	Carbon cartridge	Filtration	Disinfection: UV	μm filter	N/A	1
	Municipal	Cilture ti e u	Carbon cartridge	Filtunting	Devenue e e e e e e e e e		Disinfections 10/	2.
IN	iviunicipai	Flitration	x3	Filtration	Reverse osmosis	Ion exchange	Disinfection: UV	2+
0	Municipal	Carbon cartridge	Water softener	Filtration	Reverse osmosis	Ion exchange	N/A	1
0	Arrowhead					NI / A	NI / A	NI / A
Q	Distilled Water	IN/A	IN/A	IN/A	N/A	IN/A	IN/A	IN/A

Table C29. Information on the algae used for feeding Ceriodaphnia during ILS testing. Values for the baseline represent a single value reported by the laboratory that may have been measured by the laboratory or the vendor. Values for the second ILS represent the mean of values for the three rounds and were measured by the laboratories in all cases. Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

Lab	Algae Source		Algae Concentration (cells/mL)									
		Baseline	Second ILS (Mean)*	Second ILS (Range)								
А	In-house	250,000	277,308	249,709 - 332,388								
В	ABS	695,600	429,600	187,200 – 550,800								
E	ABS	233,333	211,000	200,000 - 233,000								
F	ARO	233,333	233,333	233,333 - 233,333								
G	ABS	200,000	219,667	216,000 – 226,333								
I			235,800	233,400 – 247,000								
L	ABS	220,000										
М	ABS	210,000	220,000	209,000 - 231,000								
Ν	In-house	213,000	214,000	213,000 - 216,000								
0	In-house	245,000	252,000	245,000 – 255,000								
Р	ABS	300,000										
Q	ABS	215,000	222,667	220,000 - 228,000								
*200,000	to 230,000 cells/m	nl are recommended	in the EPA manual.									

**Table C30. Information on the YCT used for feeding** *Ceriodaphnia* during ILS testing. Note that for the baseline, feeding data was provided only on a volume-to-volume basis (volume of YCT stock to volume in test chamber). Lab I did not participate in the Baseline and labs L and P did not participate in the Second ILS.

	VOT		Feeding	YCT Volume In Test Chamber	YCT Volume In Test Chamber	YCT Mass In Test Chambers (mg/l)
Lab	YCI Source		Method	(mi/mi)	(mi/mi)	
				Baseline	Second	Second
		Fleishman's Yeast+Blue Seal Alfalfa+Zeigler #1				
А	ARO	Finfish Crumble Trout Chow	In test solution	0.0075	0.0088	0.012
В	ABS	NA	In test solution	0.0168	0.012	0.014
Е	ABS	NA	Direct addition	0.0067	0.0067	0.012
		Fleischmann's Yeast + Pines Wheatgrass +				
F	In-house	Thomas Fish Co Trout Chow	Direct addition	0.0067	0.0067	0.010
G	ABS	NA	<b>Direct</b> addition	0.0067	0.0089	0.015
		Fleischmann's Yeast + Frontier Co-Op			0.007	
1	In-house	Wheeatgrass + Skretting Trout Chow	In test solution			0.013
L	ABS	NA	Direct addition	0.0067		
М	ARO	NA	<b>Direct addition</b>	0.0067	0.0067	0.012
		Trout Chow (Purina Aquamax Fry Starter 100) /				
		Carolina Daphnia Food (4 oz) + Fleischmann's				
		baker's yeast (one pouch 7 grams) + Cerophyl				
Ν	In-house	(Wards Cereal Grass Media)	Direct addition	0.005	0.0067	0.012
		Fleishman's active dry yeast + Pines Wheatgrass				
0	In-house	+ Purina Trout Chow (supplied by ABS)	In test solution	0.007	0.007	0.013
Р	ARO	NA	In test solution	0.0067		
Q	ABS	NA	Direct addition	0.0067	0.0067	0.012

NA= Not applicable, lab purchases YCT.

## WATER QUALITY DATA

Table C31. Summary of conductivity and pH data collected in control chambers from the ten laboratories participating in the second *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

		Conduc	tivity (μS	/cm) Before	Conductivity (µS/cm) After		pH: Before			pH: After			
Lab	Sample Type	Mean	N	Mean	Mean	N	Mean	Mean	N	Mean	Mean	N	Mean
				Range			Range			Range			Range
А	Lab Water	313	12	309-317	289	12	288-291	7.66	12	7.51-7.74	7.97	12	7.90-8.06
А	DMW Perrier <sup>®</sup> (2A)	205	3	203-208	184	3	182-186	7.83	3	7.78-7.88	7.79	3	7.62-7.92
А	MHW (1)	398	3	373-417	374	3	347-395	7.55	3	7.47-7.64	7.77	3	7.70-7.82
В	Lab Water	380	12	358-433	376	12	358-388	7.51	12	7.21-7.64	6.90	12	6.73-7.10
В	DMW Perrier <sup>®</sup> (2A)	235	3	234-237	218	3	220-230	7.58	3	7.51-7.69	6.75	3	6.53-6.96
В	MHW (1)	414	3	399-442	407	3	377-450	7.41	3	7.25-7.49	6.98	3	6.87-7.11
E	Lab Water	370	12	368-371	369	12	368-370	7.98	12	7.95-8.00	8.00	12	7.99-8.01
E	DMW Perrier <sup>®</sup> (2A)	236	3	224-250	227	3	213-243	7.95	3	7.89-8.00	7.95	3	7.91-7.99
E	MHW (1)	405	3	367-471	399	3	378-441	7.93	3	7.89-7.95	7.99	3	7.91-8.06
F	Lab Water	209	12	203-223	192	12	189-194	8.22	12	8.18-8.30	8.16	12	0.04-0.10
F	DMW Perrier <sup>®</sup> (2A)	198	3	194-203	177	3	174-181	8.23	3	8.18-8.30	7.87	3	7.67-8.11
F	MHW (1)	380	3	368-392	362	3	337-378	8.07	3	8.03-8.14	7.95	3	7.94-7.96
G	Lab Water	198	11	194-218	184	11	182-186	8.16	11	8.10-8.26	8.17	11	8.09-8.24
G	DMW Perrier <sup>®</sup> (2A)	199	3	198-199	187	3	184-190	8.17	3	8.10-8.28	7.70	3	7.56-7.94
G	MHW (1)	380	3	340-406	381	3	351-403	8.07	3	8.05-8.12	7.81	3	7.73-7.87
1	Lab Water	403	12	361-583	338	12	324-358	7.91	12	7.83-7.99	7.89	12	7.78-7.94
1	DMW Perrier <sup>®</sup> (2A)	204	3	198-217	179	3	177-181	8.01	3	7.96-8.04	7.67	3	7.46-7.90
1	MHW (1)	406	2	391-422	381	3	337-433	7.88	3	7.81-7.99	7.82	3	7.74-7.92
М	Lab Water	357	8	350-367	329	8	326-334	8.25	8	8.21-8.31	8.03	8	7.98-8.12
М	DMW Perrier <sup>®</sup> (2A)	200	2	197-203	182	2	179-186	8.35	2	8.34-8.36	7.74	2	7.74-7.74
М	MHW (1)	418	2	410-425	379	2	371-387	8.17	2	8.17-8.18	7.95	2	7.90-8.00
Ν	Lab Water	291	12	274-309	271	12	260-292	8.15	12	8.05-8.22	8.15	12	8.05-8.30
Ν	DMW Perrier <sup>®</sup> (2A)	179	3	170-185	161	3	155-169	8.21	3	8.18-8.27	8.11	3	8.01-8.24
Ν	MHW (1)	333	3	284-361	328	3	288-350	8.13	3	8.12-8.14	7.96	3	7.85-8.12
0	Lab Water	353	12	347-358	341	12	334-348	7.71	12	7.63-7.76	7.87	12	7.81-7.92
0	DMW Perrier <sup>®</sup> (2A)	198	3	195-203	188	3	186-190	7.91	3	7.89-7.92	7.65	3	7.46-7.93
0	MHW (1)	389	3	363-393	374	3	350-396	7.74	3	7.72-7.76	7.81	3	7.80-7.82
Q	Lab Water	213	12	209-221	191	12	191-191	8.31	12	8.14-8.44	8.22	12	8.07-8.34
Q	DMW Perrier <sup>®</sup> (2A)	203	3	202-204	187	3	185-189	8.34	3	8.21-8.42	7.73	3	7.59-7.94
Q	MHW (1)	410	3	366-441	381	3	353-403	8.17	3	8.04-8.23	7.87	3	7.82-7.90

Table C32. Summary of dissolved oxygen (DO) and water temperature (water temp) data collected in control chambers from the ten laboratories participating in the second *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

		DC	D (mg/L)	Before	DC	D (mg/L)	After	Water Temp (°C) Before		Water Temp (°C) After			
Lab	Sample Type	Mean	Ν	Mean	Mean	N	Mean	Mean	N	Mean	Mean	N	Mean
				Range			Range			Range			Range
А	Lab Water	6.95	12	6.62-7.15	7.63	12	7.37-7.90	24.3	12	24.1-25.0	24.3	12	24.0-24.5
А	DMW Perrier®(2A)	6.92	3	6.82-6.98	7.89	3	7.77-8.01	24.3	3	24.2-24.3	24.3	3	24.0-24.4
А	MHW (1)	6.44	3	6.21-6.56	7.85	3	7.64-8.01	24.7	3	24.1-25.1	24.5	3	24.2-24.8
В	Lab Water	8.59	12	8.38-8.91	8.57	12	8.29-8.79	25.5	12	25.3-25.6	25.5	12	25.2-25.6
В	DMW Perrier®(2A)	8.68	3	8.62-8.73	9.61	3	8.98-10.10	25.4	3	25.3-25.5	25.4	3	25.2-25.6
В	MHW (1)	8.63	3	8.46-8.79	9.41	3	9.10-9.94	25.5	3	25.3-25.6	25.5	3	25.2-25.5
E	Lab Water	7.37	12	7.34-7.41	7.93	12	7.89-8.00	24.7	12	24.5-24.7	24.8	12	24.8-24.9
E	DMW Perrier®(2A)	7.40	3	7.29-7.51	7.94	3	7.87-8.01	24.7	3	24.6-24.8	24.8	3	24.8-24.8
E	MHW (1)	7.41	3	7.31-7.51	7.98	3	7.91-8.04	24.7	3	24.6-24.7	24.8	3	24.8-24.8
F	Lab Water	8.25	12	8.05-8.43	8.05	12	7.83-8.14	24.8	12	24.3-25.6	24.9	12	24.7-25.0
F	DMW Perrier®(2A)	8.09	3	8.06-8.12	8.83	3	8.77-8.87	25.1	3	24.5-25.6	25.0	3	24.8-25.3
F	MHW (1)	8.15	3	8.09-8.21	8.86	3	8.77-8.94	24.9	3	24.6-25.1	25.1	3	24.9-25.3
G	Lab Water	7.96	11	7.66-8.31	8.18	11	8.11-8.26	24.8	11	24.6-25.0	24.4	11	24.2-24.6
G	DMW Perrier <sup>®</sup> (2A)	8.05	3	7.84-8.28	8.89	3	8.74-8.98	24.9	3	24.7-25.0	24.8	3	24.6-25.0
G	MHW (1)	7.91	3	7.61-8.16	8.95	3	8.85-9.05	24.9	3	24.8-25.0	24.6	3	24.6-24.8
1	Lab Water	6.74	12	6.07-7.60	8.32	12	7.63-9.10	24.8	12	24.5-25.4	24.9	12	24.4-25.2
1	DMW Perrier®(2A)	6.76	3	6.62-6.99	9.15	3	8.87-9.66	24.7	3	24.6-24.8	24.8	3	24.8-24.9
1	MHW (1)	6.51	2	6.22-7.05	9.33	3	8.81-9.60	25.0	3	24.5-25.5	24.9	3	24.6-25.2
М	Lab Water	6.64	8	6.50-6.74	7.27	8	7.11-7.40	22.9	8	22.1-24.1	24.8	8	24.3-25.5
М	DMW Perrier <sup>®</sup> (2A)	6.61	2	6.55-6.66	7.73	2	7.48-7.99	22.1	2	21.6-22.6	24.7	2	24.6-24.7
М	MHW (1)	6.58	2	6.54-6.62	7.69	2	7.36-8.02	22.8	2	22.8-22.9	24.6	2	24.4-24.7
Ν	Lab Water	8.42	12	8.12-8.59	8.44	12	8.33-8.61	24.9	12	24.5-25.3	25.2	12	24.6-25.6
Ν	DMW Perrier <sup>®</sup> (2A)	8.38	3	8.20-8.54	8.44	3	8.31-8.57	24.9	3	24.7-25.1	24.6	3	24.5-24.8
Ν	MHW (1)	8.39	3	8.22-8.49	8.41	3	8.34-8.51	24.9	3	24.7-25.1	24.8	3	24.5-25.0
0	Lab Water	7.74	12	7.54-7.86	8.92	12	8.46-9.33	24.7	12	24.4-25.0	24.7	12	24.3-25.0
0	DMW Perrier®(2A)	7.77	3	7.69-7.90	10.45	3	9.77-10.93	24.8	3	24.6-25.0	24.9	3	24.7-25.1
0	MHW (1)	7.77	3	7.56-7.90	10.05	3	9.86-10.26	24.8	3	24.6-24.9	24.7	3	24.4-24.9
Q	Lab Water	8.27	12	8.22-8.35	7.87	12	7.83-7.90	24.2	12	23.9-24.3	25.6	12	25.4-25.8
Q	DMW Perrier®(2A)	8.24	3	8.24-8.25	8.52	3	8.47-8.57	24.1	3	24.0-24.3	25.3	3	25.2-25.4
Q	MHW (1)	8.25	3	8.24-8.27	8.51	3	8.46-8.59	24.1	3	24.1-24.1	25.4	3	25.3-25.4

Table C33. Summary of air temperature data collected in control chambers from the ten laboratories participating in the second *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

			(°C) Bef	ore		(°C) Afte	er
Lab	Sample Type	Mean	N	Mean	Mean	N	Mean
				Range			Range
А	Lab Water	25.2	12	25.0-25.4	25.1	12	25.0-25.4
А	DMW Perrier <sup>®</sup> (2A)	25.2	3	25.1-25.3	25.2	3	25.1-25.2
Α	MHW (1)	25.3	3	25.2-25.5	25.2	3	25.1-25.2
В	Lab Water	24.2	12	23.8-24.4	24.2	12	23.9-24.4
В	DMW Perrier®(2A)	24.2	3	23.9-24.4	24.2	3	23.9-24.4
В	MHW (1)	24.2	3	23.8-24.4	24.2	3	23.9-24.4
E	Lab Water	25.0	12	25.0-25.0	25.0	12	25.0-25.0
E	DMW Perrier <sup>®</sup> (2A)	25.0	3	25.0-25.0	25.0	3	25.0-25.0
E	MHW (1)	25.0	3	25.0-25.0	25.0	3	25.0-25.0
F	Lab Water	25.4	12	25.1-25.9	25.5	12	25.0-26.0
F	DMW Perrier <sup>®</sup> (2A)	25.4	3	25.1-25.8	25.5	3	25.0-25.9
F	MHW (1)	25.4	3	25.1-25.8	25.5	3	25.0-25.9
G	Lab Water	25.6	11	25.3-26.0	25.3	11	24.8-25.9
G	DMW Perrier®(2A)	25.5	3	25.3-25.9	25.3	3	24.8-25.9
G	MHW (1)	25.7	3	25.3-25.9	25.3	3	24.8-25.9
1	Lab Water	26.7	12	26.1-27.0	26.7	12	26.4-26.9
I	DMW Perrier <sup>®</sup> (2A)	26.7	3	26.1-27.0	26.7	3	26.5-26.9
I	MHW (1)	26.5	3	25.9-27.0	26.7	3	26.6-26.9
М	Lab Water	24.9	8	24.2-26.1	25.5	8	24.9-26.0
Μ	DMW Perrier <sup>®</sup> (2A)	24.6	2	24.2-25.0	25.6	2	25.4-25.8
М	MHW (1)	24.7	2	24.4-25.0	25.6	2	25.4-25.8
N	Lab Water	25.2	12	25.0-25.7	25.4	12	24.7-25.8
N	DMW Perrier®(2A)	25.2	3	25.0-25.6	25.4	3	25.1-25.7
Ν	MHW (1)	25.2	3	25.0-25.6	25.5	3	25.1-25.7
0	Lab Water	25.2	12	25.0-25.3	25.2	12	25.0-25.3
0	DMW Perrier <sup>®</sup> (2A)	25.2	3	25.2-25.3	25.2	3	25.2-25.3
0	MHW (1)	25.2	3	25.4-25.5	25.2	3	25.0-25.3
Q	Lab Water	25.3	12	25.1-25.5	25.3	12	25.1-25.5
Q	DMW Perrier®(2A)	25.4	3	25.1-25.5	25.3	3	25.1-25.5
Q	MHW (1)	25.3	3	25.1-25.5	25.3	3	25.4-25.6

Table C34. Summary of hardness and alkalinity data collected in control chambers from the ten laboratories participating in the second *C. dubia* interlaboratory study. Water quality parameters are presented in two categories (before and after renewal of test solutions). N values refer to the number of tests conducted and included in the means.

		Hard	ness (mg	/L CaCO₃)	Alkaliı	nity (mg/	L CaCO₃)
Lab	Sample Type	Mean	N	Mean	Mean	N	Mean
				Range			Range
А	Lab Water	74	12	60-82	69.6	12	58-92
А	DMW Perrier®(2A)	82.3	3	69-91	90.3	3	88-93
А	MHW (1)	110	3	67-132	79.6	3	59-110
В	Lab Water	85.3	12	78-96	62	12	60-64
В	DMW Perrier®(2A)	85.3	3	82-88	79.3	3	78-80
В	MHW (1)	116.7	3	108-126	56	3	52-62
E	Lab Water	99.3	12	98-100	63.6	12	60-70
E	DMW Perrier <sup>®</sup> (2A)	103	3	100-107	87	3	85-90
E	MHW (1)	136.7	3	130-145	61.3	3	54-70
F	Lab Water	91.2	12	86-95	91.5	12	88-99
F	DMW Perrier <sup>®</sup> (2A)	87	3	84-89	83	3	80-89
F	MHW (1)	119	3	113-129	61	3	57-64
G	Lab Water	79.8	6	73-85	77.8	7	73-82
G	DMW Perrier <sup>®</sup> (2A)	91.3	6	81-104	77.7	4	72-82
G	MHW (1)	114.3	6	85-144	66.8	6	54-90
1	Lab Water	89.3	12	85-93	71.6	12	68-74
1	DMW Perrier <sup>®</sup> (2A)	93	3	84-98	66	3	56-79
1	MHW (1)	129.3	3	118-139	45	3	34-58
Μ	Lab Water	102	4	98-108	70.5	4	66-78
Μ	DMW Perrier®(2A)	90.5	4	86-94	82	3	80-84
Μ	MHW (1)	131	2	130-132	60	3	58-62
N	Lab Water	85.0	12	81-88.5	59.5	12	57-63
N	DMW Perrier®(2A)	86.3	3	82-91	75.3	3	73-78
N	MHW (1)	121	3	109-124	53.6	3	52-55
0	Lab Water	91.6	12	84-97	63.3	12	61-67
0	DMW Perrier®(2A)	91.6	3	88-96	85.3	3	80-94
0	MHW (1)	127.6	3	112-144	64.3	3	56-69
Q	Lab Water	92	10	91-93	89.1	10	86-91
Q	DMW Perrier®(2A)	87.7	3	87-88	92	1	92
Q	MHW (1)	124	3	111-133	63	3	61-65

Table C35. Summary of water quality data collected from the brood boards used to initiate the tests during the second *C. dubia* interlaboratory study. The data is divided into two categories: 'before' defined as water in test chambers for 24 hours, and 'after' defined as water quality measurements recorded after renewal in the test chambers. N values refer to the number of water quality measurements of the brood boards reported by the laboratories.

		Hardne	ess (m	ng/L CaCO₃)	A	Alkalinity		Conductivity (µS/cm)			рН		
					(mį	(mg/L CaCO₃)							
Lab	Sample Type	Mean	Ν	Mean	Mean	Ν	Mean	Mean	Ν	Mean	Mean	Ν	Mean
				Range			Range			Range			Range
А	Before	NM	-	-	NM	-	-	307	4	304-309	7.6	4	7.6-7.7
А	After	75	3	64-81	69	3	60-84	298	3	295-300	7.7	3	7.6-7.9
В	Before	NM	-	-	NM	-	-	366	3	360-378	7.2	3	7.2-7.4
В	After	86	6	78-96	63	6	56-70	361	3	353-368	7.0	З	6.9-7.1
Е	Before	100	2	100-100	68	2	66-70	366	3	364-368	8.0	3	8.0-8.1
Е	After	99	3	98-100	62	3	60-66	364	3	362-365	8.0	3	7.9-8.1
F	Before	101	7	96-112	96	7	83-111	210	7	207-214	8.2	7	8.1-8.3
F	After	90	9	86-94	91	9	87-93	195	7	194-197	8.2	7	8.1-8.2
G	Before	81	1	81	73	1	73	199	6	196-210	8.2	6	8.1-8.2
G	After	81	3	81-82	77	4	68-82	186	4	183-194	8.2	4	8.1-8.2
1	Before	NM	-	-	NM	-	-	367	6	361-375	7.9	6	7.8-8.0
1	After	93	3	90-97	72	3	55-87	342	6	340-343	7.9	6	7.8-8.0
Μ	Before	NM	-	-	NM	-	-	363	5	311-411	8.3	5	8.2-8.4
Μ	After	101	4	98-105	68	4	65-70	322	5	310-326	8.2	5	8.1-8.2
Ν	Before	NM	-	-	NM	-	-	277	8	264-297	8.0	8	8.0-8.4
Ν	After	85	7	83-87	59	7	58-60	269	5	255-286	8.1	5	7.9-8.3
0	Before	101	4	99-103	66	4	62-68	356	5	353-362	7.7	5	7.6-7.8
0	After	96	5	93-99	63	5	61-63	340	5	334-348	7.8	5	7.8-7.9
Q	Before	97	4	82-100	93	3	86-97	211	4	203-225	8.3	4	8.1-8.4
Q	After	91	7	79-98	87	7	83-92	187	8	182-193	8.3	8	8.1-8.4

Table C35. d	continued.
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		Dissolv	ed Ox	ygen (mg/L)	Water	Water Temperature (°C)			Air Temperature (°C)		
Lab	Sample Type	Mean	Ν	Mean	Mean	Ν	Mean	Mean	Ν	Mean	
				Range			Range			Range	
А	Before	7.0	4	6.8-7.2	24.3	4	24.0-25.0	26.6	4	26.5-26.6	
А	After	7.4	4	7.0-7.7	24.2	4	24.0-24.4	26.6	4	26.5-26.7	
В	Before	8.8	3	8.8-8.9	25.4	3	25.2-25.5	25.5	3	25.4-25.5	
В	After	8.8	3	8.6-9.1	25.4	3	25.2-25.5	25.5	3	25.4-25.5	
Е	Before	7.9	3	7.8-7.9	24.8	3	24.7-24.9	24.9	3	24.7-25.0	
Е	After	7.9	3	7.8-8.0	24.8	3	24.6-24.8	24.9	3	24.9-25.0	
F	Before	8.2	7	8.2-8.3	24.4	7	24.2-24.6	25.3	7	25.2-25.4	
F	After	8.0	7	7.9-8.0	25.0	7	24.8-25.3	25.3	7	25.2-25.3	
G	Before	7.8	6	7.5-8.1	24.8	6	24.6-25.1	25.8	6	25.1-26.3	
G	After	8.3	6	8.2-8.5	24.6	6	24.2-24.9	25.7	6	25.1-26.1	
1	Before	7.0	6	6.4-7.4	24.9	6	24.8-25.1	25.5	6	25.2-25.7	
I	After	7.8	6	7.6-7.8	24.9	6	24.8-25.0	25.3	6	25.0-25.7	
М	Before	6.8	5	6.4-6.9	25.6	5	24.4-27.2	25.3	5	24.6-25.9	
М	After	6.8	5	6.7-7.1	24.8	5	24.6-25.0	24.4	5	23.7-25.0	
Ν	Before	8.2	8	8.2-8.3	24.5	8	24.3-24.9	25.0	8	24.6-25.4	
Ν	After	8.2	8	8.1-8.4	24.7	8	24.4-25.0	25.0	8	24.7-25.6	
0	Before	7.7	5	7.5-8.0	24.9	5	24.7-25.2	25.3	5	25.2-25.4	
0	After	8.5	5	8.1-8.8	25.0	5	24.6-25.4	25.2	-5	25.1-25.3	
Q	Before	8.4	4	8.3-8.4	24.0	4	23.9-24.3	25.0	4	24.9-25.3	
Q	After	7.9	4	7.9-8.0	25.3	4	25.0-25.7	25.0	4	24.9-25.2	

### CONCENTRATION-RESPONSE DATA

Figure C12. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab A.





Figure C13. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab B.



Figure C14. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab E.



Figure C15. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab F.



Figure C16. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab G.

Figure C17: Second ILS NaCl Concentration Responses for Lab I. Reproduction in all the Perrier based concentrations was very low, including the unspiked sample. This lab did not participate in the Baseline ILS.





Figure C18. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab M.



Figure C19. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab N.



Figure C20. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab O.



Figure C21. Baseline (left) and second (right) ILS NaCl Concentration Responses for Lab Q.

# Appendix D – Supporing materials provided to the laboratories

#### D1 Guidance for Documenting Brood Board Health

This is intended to help with the determination of abnormal occurrences in the brood board for the second interlaboratory study (ILS). If any of the notable observations illustrated below occur in the brood boards used in the second ILS, they should be noted with appropriate brood board health code (**Table D1**) on the daily observation sheets and included in the electronic data submission. Notes are given about how some of these appear to the naked eye. If any of these are suspected greater magnification should be used for verification.

**Table D1**. Health codes and descriptions that must be used for documenting brood board health for the second ILS.

Health	Parameter	Description of Parameter
Code		
A	Unhealthy adult	Lack of normal movement, not normal shape, smaller than normal, or atypical coloration. Presence of ephippia in brood pouch. Empty brood pouch, lack of clear gutline, and undersized brood based on age (i.e. only 3-6 eggs present 5 or more day post initiation).
D	Dead adult	Self-explanatory.
К	All OK	Nothing in any of the other categories to report.
М	Male	An animal that produces no young and is microscopically examined to determine sex. Triangular abdomen and fast/irregular swimming.
N	Dead neonates	Self-explanatory. Can be some or all of brood.
U	Unhealthy neonates	Lack of normal movement, not normal shape, ephippia or atypical coloration.
0	Other occurrences	Growth on adults or neonates, biofilm in brood cups, foreign species, aborted broods, flocculent material, etc. Describe in comments section.
Y	Neonates used to initiate a test	Neonates from this brood board chamber were used to initiate a test.



Abnormal, non-reproductive female. These will sometimes appear as having an empty and translucent brood pouch or contain a small number of eggs (A) that are aborted or do not become viable neonates. This would fall under health code A, as an unhealthy adult.

Photo courtesy of Alison Briden Pacific EcoRisk



Adult female with ephippia (A) (resting or diapause stage embryo). Ephippia are not normally observed in healthy cultures This would receive health code A.

Photo courtesy of Alison Briden Pacific EcoRisk



Normal adult females will have visible eggs in the brood pouch (A) that are clearly differentiated, especially early in the brood cycle. As the organism gets closer to releasing the brood, unborn neonates should have visible eye spots. The adult should have a visible gut line and usually has a visible green coloration from feeding on the algal suspension. This would receive health code K.

Photo courtesy of Alison Briden Pacific EcoRisk



Male. Note the flatter back with absence of brood pouch (A) and longer antennules (B) than the female. Males are noticeably smaller than reproductive age females. Males also tend to have more erratic swimming behavior. This would be health code M.

Photo courtesy of Alison Briden Pacific EcoRisk



Male left and female right. Note the difference in size and shape (A) and difference in antennules (A) from the female Health code K for the female, M for the male.



Partially aborted brood with eggs in molted carapace. The aborted brood was visible to the naked eye as a small black dot inside the molted carapace. Unhealthy females will sometimes partially or completely abort underdeveloped eggs without molting the carapace; if a female is observed with an empty brood pouch, the replicate should be examined for aborted eggs which appear as small black dots at the bottom of the chamber under a microscope. This would receive health code O.



Female with stalked ciliate infection. This infection affected reproduction but was not fatal. The ciliates were shed with the carapace but quickly returned This would receive health code O.
Photo courtesy of Alison Briden Padific EcoRisk



Adult female withPeritrichs (stalked ciliates) on carapace. These appeared as reddish spots on the animal and bottom of cup to the naked eye. This infection was usually fatal. This would receive health code O.



Dead adult covered with biofilm. This receive health code D,O. Photo courtesy of Alison Briden Padific EcoRisk

#### D2 – Guidance for Randomization of Test Chambers

Randomization of the test chambers is a requirement in the test method. The manner of randomization is left to the individual laboratories. If a laboratory is currently randomizing their tests, they are encouraged to continue with their method. For laboratories not currently randomizing, below are two possible methods that can be used. Other acceptable methods may also be used. Either multiple sets of random numbers should be assigned for use in testing or new numbers can be chosen for each test performed. A single set of random numbers must not be used for each test.



Test board with chambers in each row assigned random numbers. Each row represents a random block from a single female. Colors and columns represent separate samples or concentrations. The small numbers identify each chamber.



Test board adjusted using the random numbers assigned in the left graphic to randomize within each row. This method represents the minimum level of randomization required.



In this form of randomization, each chamber is assigned a random number. The chambers are arranged in treatment blocks for addition of the neonates.

After addition of the neonates, the chambers are arranged in numerical order which randomizes the entire test. The use of colored labels will help to keep the treatments straight during subsequent water changes.

# D3 - Ceriodaphnia Food and Feeding Data Sheet for Second ILS

Test Start Date
Test Batch Numbers
Sheet of for this set of tests
Feeding Method: Direct or In Test Solution (Circle)
Each food individually Or Foods are Mixed and Fed (Circle)
ҮСТ
Source of Prepared YCT: inhouse or purchased (Circle; give supplier if purchased)
Is YCT filtered before use or does the YCT have solids strained out
Production Date YCT Batch ID
Laboratory Measured Solids mg/ml
Concentration fed to tests (x ml/x ml of mg/L TSS YCT)
Concentration in each test cup is calculated to be:
Algae
Source of Prepared Algae: inhouse or purchased (Circle; give supplier if purchased)
Production Date Algae Batch ID
Supplier Cells/ml
Laboratory Measured Cells/ml Measurement Method
Procedure used to determine cells/ml
Concentration fed to tests (x ml/x ml) of algae concentration, cells/ml)
Concentration in each test cup is calculated to be:

### D4 – Guidance for Identifying and Documenting Split Broods

Initial identification of possible split broods must be conducted at the time of each daily check. Notations of possible split broods must be made on the datasheets daily. Notes for indicators of split broods should be made on bench sheets or an observation recording sheet. Final determination of split broods may be made by a more senior employee, but again must be done on a daily basis and the data sheet initialed. Identification of split broods by bench analysts can be reviewed as part of the laboratory's data QA/QC process and any changes to a determination for split brood made by the reviewer should be done in conjunction with the analyst who performed the test and the notes that they took during testing. Determination of split broods cannot be conducted after the test has ended unless detailed bench notes are available from the daily observations.

The process for identifying split broods should involve multiple steps.

- When a female produces a small brood relative to other brood sizes on that test day (within the same test concentration), she should be examined to determine if there are remaining eyed neonates in her brood pouch which, in combination with observing the neonates present in the replicate look newly released, is an indication that the organism is actively releasing the brood during the changeover time. If so, this should be noted on the bench sheet. The presence of additional neonates can be seen with the naked eye and is easy to observe under a dissecting scope.
- On the day following a small brood, the size of the individual neonates should be compared to those released by other females. If the neonates are larger than those from other females, this would indicate that they are from the part of the previous day's brood.
- If a split brood is detected, it should be indicated on the bench sheet and the neonate counts from the two days must be circled to denote that they should be treated as a single brood.