TABLE OF CONTENTS

S1. Table of ion concentrations (mg/L) in the three control and dilution waters used for toxicity testing with *Ceriodaphnia dubia*, *Hyalella azteca*, and *Chironomus dilutus*

Water name	Na	K	Ca	Mg	Cl	SO$_4$	HCO$_3$	Br
Lake Superior water (LSW)	1.5	0.5	13.1	2.5	1.5	3.2	52	0.015
Amended LSW (ALSW)	6.5	1.5	14.6	4.1	7.7	15	52	0.015[a]
Amended LSW + Cl + Br (ALSW+)	11.3	1.5	14.6	4.1	15	15	52	0.055

[a] No Br addition specified in formula

S2. Table of chemicals and internal standards used

Acronym	Full Name	CASRN	Vendor Name	Internal Standard	m/z
PFBA	Perfluorobutanoic acid	375-22-4	Synquest	$^{13}C_3$-PFBA	213
PFHxA	Perfluorohexanoic acid	307-24-4	Synquest	$^{13}C_5$-PFHxA	313
PFOA	Perfluorooctanoic acid	335-67-1		$^{13}C_8$-PFOA	413
PFNA	Perfluorononanoic acid	375-95-1	Synquest	$^{13}C_9$-PFNA	463
PFDA	Perfluorodecanoic acid	335-76-2	Synquest	$^{13}C_6$-PFDA	513
K-PFBS	Potassium Perfluorobutanesulfonate	29420-49-3	Synquest	$^{13}C_3$-PFBS	299
K-PFHxS*	Potassium Perfluorohexanesulfonate	3871-99-6	Alfa Aesar	$^{13}C_3$-PFHxS	399
Na-PFHxS	Sodium Perfluorohexanesulfonate	82382-12-5	Toronto Research Chemical	$^{13}C_3$-PFHxS	399
Na-PFHpS	Sodium Perfluoroheptanesulfonate	21934-50-9	Toronto Research Chemical	$^{13}C_8$-PFOS	449
K-PFOS	Potassium Perfluorooctanesulfonate	2795-39-3		$^{13}C_8$-PFOS	499
Na-PFNS	Sodium Perfluorononanesulfonate	98789-57-2	Toronto Research Chemical	$^{13}C_8$-PFOS	549
Na-PFDS	Sodium Perfluorodecanesulfonate	2806-15-7	Toronto Research Chemical	$^{13}C_8$-PFOS	599
FBSA	Perfluorobutanesulfonamide	30334-69-1	Synquest	$^{13}C_3$-PFHxS	298
FHxSA	Perfluorohexanesulfonamide	41997-13-1	Synquest	$^{13}C_8$-PFOS	398
FOSA	Perfluorooctanesulfonamide	754-91-6	Synquest	$^{13}C_8$-PFOSA	498
6:2 FtS	1H, 1H, 2H, 2H-perfluoroocatanesulfonate	27619-97-2	Synquest	$^{13}C_2$-6:2 FtS	427
8:2 FtS	1H, 1H, 2H, 2H-perfluorodecanesulfonate	39108-34-4	Synquest	$^{13}C_2$-8:2 FtS	577
10:2 FtS	1H, 1H, 2H, 2H-perfluordodecanesulfonate	120226-60-0	Synquest	$^{13}C_2$-8:2 FtS	627
OS	Octane-1-Sulfonate (Dilution Standard)			N/A	193

S3. Purity evaluation

Each PFAS used for exposures was examined by high-resolution mass spectrometer (HRMS) for impurities that may affect the outcome of the toxicity tests. Each PFAS was prepared as individual 1 mg/L solutions in methanol and analyzed on a Orbitrap ID-X MS coupled to a Vanquish ultra-high-performance liquid chromatograph (Thermo Fisher Scientific, Waltham, MA) by electrospray ionization. Reagent blanks were run at the beginning of each analysis as well as in between each sample. A positive control (PFAC30PAR, Wellington Laboratories) which contained 30 different PFAS—including all the PFAS used for exposures except 10:2 FtS—was also analyzed as a positive control.

The instrument was operated using XCalibur software (v. 4.3.73.11; Thermo Fisher Scientific). Sample volumes of 2.0 uL were injected onto a Acquity HSS-C18 column (2.1 x 150 mm x 1.8 µm; Waters, Milford, MA) and seperated by gradient elution. The mobile phase was comprised of 5mM aqueous ammonium acetate (A) and methanol (B); the gradient conditions were 10% B held for 0.5 minutes then ramped to 90% B over 14.5 minutes. This gradient was followed by a wash at 100% B for 5 minutes and a re-equilibration at 10% B for 6 minutes. The flow rate was 300 uL/min throughout the gradient. The column compartment and autosampler were temperature controlled at 40 and 10 °C, respectively. The electrospray source was operated at -3500 V capillary potential, 350 °C vaporizer temperature, 40 psi sheath gas, 6 psi auxiliary gas, and 2 psi sweep gas. The mass spectrometer scanned from m/z 100-1000 for precursor ions (MS1) at a resolution of 120,000. Product ions (MS2) were generated through higher-energy C-trap dissociation (HCD) at 20, 40, and 70 eV. An ion intensity of 10^6 counts/s triggered a data dependent MS2 analysis of the associated precursor ion at a resolution of 30,000. Dynamic exclusion was used to minimize multiple MS2 analyses of the same precursor within a six second time window.

Samples were analyzed for potential impurities by suspect screening and non-target analysis. Briefly, suspect screening was performed in FreeStyle (v. 1.5.93.34; Thermo Fisher Scientific) by screening the sample data for the exact monoisotopic mass of PFAS homologs. The PFAS homologs included perfluoroalkyl carboxylates, sulfonates, and sulfonamides from chain-length 3-14. In addition, the polyfluoroalkyl telomer sulfonates from chain length 4:2-14:2 were screened. If a screen resulted in a detection, retention time and MS2 data (if available) were used for additional identification confidence. Non-target analysis was performed in Compound Discoverer (v. 3.2.0.421) using a generic workflow. The software picked chromatographic peaks, aligned retention times, detected compounds, and grouped the data across samples. The software then predicted elemental composition, removed features detected in blanks, and attempted to identify unknowns using m/z Cloud (local and online spectral database) and Chemspider (online compound database).

Purity was determined based on raw area counts (i.e., the signal of the parent compound ratioed against the total signal of everything detected in a sample). The combined results of suspect screening and non-target analysis follow. All compounds tested ranged from 91 – 100% in purity based on the above analysis.

Perfluoroalkyl carboxylic acids

PFBA

Compound	Retention Time	m/z	Area
PFBA	6.32	212.9792	1.6E+07

		% Contamination	% Purity
		0	100

PFHxA

Compound	Retention Time	m/z	Area
PFHxA	11.30	312.9728	5.0E+07
Unknown	10.50	294.9818	2.7E+05
Unknown	10.14	230.9860	2.3E+05

		% Contamination	% Purity
		0.98	99.0

PFOA

Compound	Retention Time	m/z	Area
PFOA	13.40	412.9664	2.9E+08
PFHxA	11.25	312.9728	3.4E+05
PFHpA	12.28	362.9696	8.8E+04
PFNA	14.11	462.9632	1.4E+04
PFDA	14.71	512.9600	8.8E+05

		% Contamination	% Purity
		0.45	99.5

PFNA

Compound	Retention Time	m/z	Area
PFNA	14.20	462.9632	8.1E+07
PFHxA	11.30	312.9728	3.5E+04
PFHpA	12.30	362.9696	8.6E+05
PFOA	13.46	412.9664	7.6E+04
Unknown	7.716	218.9884	1.8E+05
Unknown	10.26	284.9799	5.7E+05
Unknown	12.65	380.9761	2.0E+05

		% Contamination	% Purity
		2.34	97.7

PFDA

Compound	Retention Time	m/z	Area
PFDA	14.82	512.9600	9.7E+07
PFOA	13.47	412.9664	2.2E+04
Unknown	14.53	494.9694	1.5E+05

% Contamination	% Purity
0.18	99.8

Perfluoroalkyl sulfonic acids

PFBS

Compound	Retention Time	m/z	Area
PFBS	9.96	298.9430	1.1E+08

		% Contamination	% Purity
		0	100

PFHxS (TRC)

Compound	Retention Time	m/z	Area
PFHxS	12.68	398.9366	1.7E+08
PFBS	9.98	298.9430	6.9E+05
PFPeS	11.56	248.9462	5.8E+04

		% Contamination	% Purity
		0.43	99.6

PFHxS (Sigma)

Compound	Retention Time	m/z	Area
PFHxS	12.61	398.9362	1.35E+08
PFBS	9.96	298.9427	5.74E+04
PFPeS	11.51	348.9392	1.09E+06
PFHpS	13.49	448.9331	3.42E+05
PFOS	14.20	498.9301	3.29E+06
PFNS	14.81	548.9271	3.45E+05
PFDS	15.33	598.9243	3.23E+05
6:2 FTS	13.45	426.9678	2.77E+04
Unknown	15.343	295.2274	6.07E+05
Unknown	12.991	331.24866	2.67E+05
Unknown	12.095	360.93927	2.36E+05

		% Contamination	% Purity
		4.7	95.3

PFHxS (Alfa)

Compound	Retention Time	m/z	Area
PFHxS	12.51	398.9366	9.7E+08
PFPrS	8.05	248.9494	3.9E+05
PFBS	10.05	298.9426	7.7E+06
PFPeS	11.55	348.9398	1.2E+07
PFHpS	13.52	448.9334	2.1E+06
PFOS	13.96	498.9302	4.7E+07

PFNS	14.48	548.9270	1.7E+06
PFDS	14.94	598.9238	3.0E+05
Unknown	12.00	368.9844	5.6E+06
Unknown	11.60	360.9391	3.8E+06
Unknown	13.10	464.9285	2.7E+06
Unknown	11.90	386.9751	3.4E+06
Unknown	12.61	414.9312	8.2E+05

% Contamination	% Purity
8.3	91.7

PFHpS

Compound	Retention Time	m/z	Area
PFHpS	13.54	448.9329	2.3E+08
PFHxS	12.65	398.9366	1.4E+05

% Contamination	% Purity
0.061	99.9

PFOS

Compound	Retention Time	m/z	Area
PFOS	14.08	498.9302	1.2E+09
PFBS	10.02	298.9430	1.7E+04
PFPeS	11.51	348.9398	1.0E+05
PFHxS	12.57	398.9366	3.4E+06
PFHpS	13.41	448.9334	8.5E+06
PFNS	14.62	548.9270	2.5E+05
PFDS	15.11	598.9238	1.1E+03
Unknown	13.64	476.9285	1.5E+06
Unknown	13.99	514.9252	6.2E+05
Unknown	14.52	548.9276	1.1E+06
Unknown	13.15	442.9428	2.9E+06
Unknown	12.43	462.9492	2.6E+06
Unknown	13.27	480.9399	3.0E+06
Unknown	13.39	460.9335	6.3E+06

% Contamination	% Purity
2.5	97.5

PFNS

Compound	Retention Time	m/z	Area
PFNS	14.56	548.9270	9.1E+08
PFHxA	11.24	312.9723	9.4E+04
PFHpA	12.30	362.9696	8.6E+05

Compound	Retention Time	m/z	Area
PFOA	13.32	412.9664	1.9E+05
PFNA	13.94	462.9632	2.0E+06
PFHpS	13.57	448.9334	1.8E+04
PFOS	13.99	498.9302	4.5E+06
PFDS	14.97	598.9238	3.1E+05
Unknown	14.04	466.9089	2.3E+07
Unknown	11.56	360.9581	7.0E+05

	% Contamination	**% Purity**
	3.4	96.6

PFDS

Compound	Retention Time	m/z	Area
PFDS	14.78	598.9238	1.0E+09
PFHxA	11.24	312.9724	9.4E+04
PFHpA	12.30	362.9696	8.6E+05
PFOA	13.36	412.9664	1.9E+05
PFNA	13.93	462.9632	3.4E+04
PFDA	14.30	512.9600	5.3E+06
PFHpS	13.56	448.9334	1.61E+03
PFOS	14.00	498.9302	6.22E+04
PFNS	14.51	548.9270	2.91E+05
PFUdS	15.08	648.9206	2.29E+04
PFDoS	15.42	698.9174	6.78E+06

	% Contamination	**% Purity**
	1.3	98.7

Perfluoroalkane sulfonamides

PFBSA

Compound	Retention Time	m/z	Area
PFBSA	11.68	297.9590	5.5E+08
PFBS	10.11	298.9425	4.0E+05
PFPrSA	9.41	247.9622	4.7E+05
PFHxSA	14.01	397.9518	1.6E+04
Bis(perfluorobutanesulfonyl)imide	13.87	579.8995	1.4E+07
Unknown	9.05	297.9681	6.2E+06
Unknown	12.16	313.9536	5.4E+05

		% Contamination	% Purity
		3.7	96.3

PFHxSA

Compound	Retention Time	m/z	Area
PFHxSA	13.97	397.9518	6.8E+08
PFHxS	12.57	398.9366	2.5E+06
PFOSA	15.35	497.9462	2.3E+03
Bis(perfluorohexane-sulfonyl)imide	15.43	779.8874	1.5E+06
Unknown	14.01	479.9556	1.9E+06

		% Contamination	% Purity
		0.9	99.1

PFOSA

Compound	Retention Time	m/z	Area
PFOSA	15.39	497.9462	1.91E+08
PFOA	13.46	412.9664	4.01E+04
PFHxSA	14.11	397.9518	3.86E+05
PFHpSA	14.92	447.9494	2.11E+06
PFNSA	16.20	547.9436	6.53E+04
PFDSA	16.64	597.93981	2.02E+04
Unknown	14.71	479.9556	7.06E+05

		% Contamination	% Purity
		1.7	98.3

Fluorotelomer sulfonic acids

6:2 FtS

Compound	Retention Time	m/z	Area
6:2 FtS	13.44	426.9675	4.8E+07
4:2 FtS	11.18	326.9743	6.9E+04
8:2 FtS	14.88	526.9627	1.87E+05
10:2 FtS	15.85	626.9551	4.57E+04
12:2 FtS	16.42	726.9487	4.42E+04
Unknown	14.07	454.9989	3.32E+05
Unknown	13.93	460.9291	9.38E+04

% Contamination	% Purity
1.6	98.4

8:2 FtS

Compound	Retention Time	m/z	Area
8:2 FtS	14.82	526.9627	2.4E+08
PFOA	13.50	412.9664	1.4E+04
PFNA	14.21	462.9632	8.9E+05
4:2 FtS	11.33	326.9743	6.1E+04
6:2 FtS	13.48	426.9675	3.2E+04
7:2 FtS	14.22	476.9647	2.1E+06
10:2 FtS	15.76	626.9551	8.7E+03
Unknown	14.50	476.9789	3.8E+05
Unknown	15.04	510.9670	1.1E+06

% Contamination	% Purity
1.9	98.1

10:2 FtS

Compound	Retention Time	m/z	Area
10:2 FtS	15.66	626.9551	3.2E+08
PFNA	14.11	462.9632	1.5E+05
PFDA	14.70	512.9600	1.1E+05
PFUdA	15.19	562.9568	7.8E+06
8:2 FtS	14.72	526.9627	2.0E+06
9:2 FtS	15.23	576.9583	2.4E+04
12:2 FtS	16.31	726.9487	1.6E+05
Unknown	15.45	576.9728	1.0E+06
Unknown	15.83	610.9612	1.1E+06

% Contamination	% Purity
3.7	96.3

11

S4. Chemical solubility

Effective solubility limits were encountered for several chemicals over the course of testing. Stocks of PFDS-Na could not be made to exceed ~0.8 ppm. Undissolved chemical was visibly present in solution even after >8 days of stirring, and measured concentrations plateaued despite remaining well below nominal. Similar effective solubility limits were encountered for 8:2 FtS (~3 ppm), 10:2 FtS (~0.3 ppm), and PFOSA (~28 ppm). Limited data also indicates loss of PFOSA to volatilization at such high concentrations, based on negative deviation from nominal in 10 and 20 ppm treatments during rangefinding tests and air sampling of the headspace from stock bottles. Other chemicals appeared to encounter softer solubility caps, with particulates that may have been undissolved chemical present at high stock concentrations. This was true of 6:2 FTS (~500 ppm), PFBSA (113 ppm), and PFHxSA (58 ppm), although there was no functional need to make more concentrated stocks of these chemicals, so it remains to be seen if higher water concentrations could be obtained.

S5. Chemical stability

Results suggested that adsorptive loss was not a significant challenge in most chemicals tested, with average exposure solutions in *H. azteca* and *C. dilutus* tests measuring 80 - 128% (mean = 101%, SD = 7%) of that measured in the cubitainer on Day 0 or 1 of the test. Similarly, average measurements in 24-hour old solutions taken in *C. dubia* tests were within 75 - 120% (mean = 99%, SD = 8%) of the Day 0 new solution measurement.

Exposure concentrations were also generally stable in time, with CVs among replicate measurements across a test from 2 to 20% (mean = 8%, SD = 3%). Exceptions occurred in select chemicals with some combination of longer perfluorinated carbon chain length, more hydrophobic head group, and lower test concentrations more easily impacted by adsorptive losses. The four compounds in which issues were encountered were 8:2 FtS, 10:2 FtS, PFDS, and PFOSA. Both PFDS and PFOSA were less problematic, with measured exposure concentrations in *C. dilutus* and *H. azteca* tests on average 62 - 84% of Day 0 cubitainer measurement (mean = 77%, SD = 8%). PFOSA behaved similarly in *C. dubia* testing, with the average old solution measurements 62% - 94% of the Day 0 new solution measurement (mean = 78%, SD = 11). Variability across measurements was also higher for these chemicals, producing CVs from 12 - 25% (mean = 17%, SD = 4%), except for PFOSA in *C. dilutus*, for which CVs were closer to typical (8-13%, mean = 10%, SD = 2%). 8:2 FtS was stable at the single ~3 ppm concentration tested for *H. azteca* and *C. dilutus*, but unstable at the two additional lower concentrations tested for *C. dubia*, in which measured new solution concentrations dropped ~20% between Day 0 and Day 6 in both the 0.38 ppm and 0.07 ppm treatments. 10:2 FtS was highly unstable in all tests. Loss between new solution measurements on Days 0 and 6 was 76% in *C. dubia* testing. In *H. azteca* and *C. dilutus* testing, the average test solution measurements were 37% and 35% of the Day 0 cubitainer measurement, respectively. Quantifiable loss of chemical was also observed for PFDA in *C. dubia* testing and PFNS in *C. dilutus* testing, although not to a degree that bears further comment herein.

S6. PFAS exposure concentration data

Quality control checks for the analytical data include the following:

A secondary calibration verification (SCV) sample is run with every instrument calibration. A SCV is a calibration standard prepared from a secondary and unrelated source of analyte and is used to verify the primary calibration standards are prepared correctly and/or that there is no issue with the purity of the primary source. The acceptance of the SCV is ± 30% of the nominal concentration.

A report level verification (RLV) is a standard at the lowest calibration level run after a maximum of 20 samples and the end of the run. The acceptance of the RLV is ± 40%.

A continuing calibration verification (CCV) is a mid-level calibrator run after a maximum of 20 samples and at the end of the run. The acceptance of the CCV is ± 30%.

A method blank is reagent water prepared in the same manner as exposure samples and run with a every 20 samples at maximum. Any contamination or interference in the method blank must not be greater than 50% of the concentration in the lowest calibrator.

A laboratory control sample (LCS) is a method blank that is spiked with a known amount of analyte (typically within 10-fold of the lowest calibrator). The acceptance of the LCS is within ± 30% of the target concentration. LCSs are run with a maximum of every 20 samples.

A sampling duplicate is a second aliquot of sample taken from the same location and processed identically to the original sample. The relative percent difference between duplicates should not be more than 30%. A matrix spike (MS) is a sampling duplicate spiked with a known amount of analyte (typically with 10-fold of the lowest calibrator). The acceptance of the MS is within ± 30% of the target concentration after correcting for the analyte concentration measured in the associated sample. Each MS is run with a maximum of every 20 samples.

Internal standard response for each sample should be within 60-140% when compared to the average of the internal standard response in calibration standards.

Dilution standards were added as an additional quality control check midway through this study as a verification that sample dilutions were performed correctly. Octane-1-sulfonate was added to each sample aliquot at a concentration that when diluted would be equivalent to the concentration in calibration standards. The response of the dilution standard in samples were required to be within 60-140% of those in calibration standards.

Failure of any quality control was followed up by close examination of the data and corrective action if deemed necessary. Corrective actions included qualifying the data; reinjecting the suspect sample; repreparing and reanalyzing effected samples or sample batches; recalibrating the instrument; raising the report level; and applying correction factors.

S6. *C. dubia* PFAS exposure concentration data

***C. dubia* QC Summary**

	LCS	MS	SCV*	PFHxS SCV	MB
Mean	99	99	102	123	< MRL
Median	97	101	101	124	
Standard Deviation	7.0	8.7	9.9	7.8	
Minimum	83	70	85	114	
Maximum	121	118	122	138	
Confidence Level(95.0%)	1.1	1.4	2.8	6.0	

* excludes PFHxS from the summary statistics. The Alfa Asar source was used for those tests; several solution preperations of which were all about 20-25% different from the SCV. This issue was not observed with PFHxS from other vendors.

Notes about this summary:

- Values shown in **bold** indicate measured concentrations that are derived from one or more replicate samples.

- Day # is indicated starting at day 0 when organisms are first added to test vessels. "New" in parentheses indicates solutions were taken from fresh stock solutions. "Old" in parentheses indicates solutions were taken from 24-hour old test vessels.

- Concentrations are listed in **mg/L**, unless otherwise noted.

Test Species: C. dubia
Chemical Abbreviation: PFBA
Test Dates: 05/14/2021 - 05/21/2021

Date	5/14/2021	5/17/2021	Average	RSD
Control	<20 ppb	<20 ppb	<20 ppb	NA
Level 2	13	12	12	NA
Level 3	25	**24**	24	NA
Level 4	52	49	51	NA
Level 5	102	98	100	NA
Level 6	213	**199**	206	NA
Day	0 (New)	3 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFHxA
Test Dates: 05/14/2021 - 05/21/2021

Date	5/14/2021	5/17/2021	Average	RSD

15

Control	<20 ppb	<20 ppb		
Level 2	13	13	13	NA
Level 3	27	**28**	27	NA
Level 4	56	61	58	NA
Level 5	112	110	111	NA
Level 6	218	**213**	215	NA
Day	0 (New)	3 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFOA (test 1)
Test Dates: 03/12/2021 - 03/19/2021

Date	3/12/2021	3/13/2021	3/18/2021	3/19/2021	Average	RSD
Control	<10 ppb	<10 ppb	<10 ppb	<10 ppb		
Level 2	0.17	0.16	0.22	0.22	0.19	16
Level 3	0.29	0.28	0.35	**0.33**	0.31	11
Level 4	0.57	0.53	0.73	**0.66**	0.62	14
Level 5	1.3	1.2	**1.4**	1.5	1.3	11
Level 6	2.6	2.3	3.0	**2.8**	2.7	11
Level 7	5.1	5.1	5.7	6.0	5.5	8
Level 8	11	**10**	12	12	11	6
Level 9	**19**	20	26	23	22	13
Level 10	40	**43**	49	46	44	9
Level 11	79	**86**	97	100	91	10
Day	0 (New)	1 (Old)	6 (New)	7 (Old)		

Note: The sampling procedure employed in this exposure involved sampling an aliquot of test solution without methanol stabilization. The sample was then centrifuged and sub-sampled into an HPLC vial, wherein it was stabilized in methanol and spiked with internal standard. This differs from the sampling procedure used subsequently, in which samples were aliquotted directly into methanol and then centrifuged, to ensure adequate methanol stabilization throughout the sample preparation process.

A brief comparison of sampling directly into methanol vs. stabilizing later found that, for PFOS, there was approximately 20% and 5% loss at 50 ppm and 0.2 ppm, respectively, when samples were not stabilized immediately in methanol.

Test Species: C. dubia
Chemical Abbreviation: PFOA (test 2)
Test Dates: 04/09/2021 - 04/16/2021

Date	4/9/2021	4/10/2021	4/13/2021	4/15/2021	4/16/2021	Average	RSD
Control	<30 ppb	<30 ppb	<30 ppb	<30 ppb	<30 ppb		
Level 2	7.2	6.6	6.9	6.8	**5.9**	6.7	7
Level 3	**15**	14	**14**	**14**	12	14	8
Level 4	**30**	29	26	33	25	28	11
Level 5	53	54	**55**	66	46	55	13
Level 6	112	**109**	119	105	101	109	6
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFOA (test 3)
Test Dates: 06/18/2021 - 06/25/2021

Date	6/18/2021	6/19/2021	6/22/2021	6/24/2021	6/25/2021	Average	RSD
Control	<30 ppb	<30 ppb	<30 ppb	<30 ppb	<30 ppb		
Level 2	3.5	3.4	**3.7**	3.3	3.3	3.5	5
Level 3	6.8	**6.6**	6.7	6.8	**6.9**	6.8	2
Level 4	14	14	14	**15**	13	14	6
Level 5	29	30	30	32	**28**	30	6
Level 6	**57**	55	**59**	58	53	57	5
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFNA
Test Dates: 02/04/2022 - 02/11/2022

Date	2/4/2022	2/5/2022	2/9/2022	2/10/2022	2/11/2022	Average	RSD
Control	<1.7 ppb	<1.7 ppb	<1.7 ppb	<1.7 ppb	5 ppb		
Level 2	3.0	**2.9**	2.7	2.6	2.7	2.8	6
Level 3	6.1	6.0	5.4	**5.6**	5.7	5.7	5
Level 4	12	11	11	11	**12**	11	4
Level 5	25	24	**21**	21	**24**	23	9
Level 6	**49**	**47**	**43**	44	48	46	5
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFDA
Test Dates: 06/18/2021 - 06/25/2021

Date	6/18/2021	6/19/2021	6/22/2021	6/24/2021	6/25/2021	Average	RSD
Control	<30 ppb	<30 ppb	<30 ppb	<30 ppb	<30 ppb		
Level 2	1.1	0.85	**0.93**	0.85	0.80	0.90	11
Level 3	2.2	**1.9**	2.2	1.9	**1.8**	2.0	10
Level 4	4.6	3.8	4.1	**4.4**	3.9	4.2	8
Level 5	9.6	8.2	8.6	8.6	**7.8**	8.6	8
Level 6	**19**	15	**16**	16	14	16	12
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFBS
Test Dates: 05/14/2021 - 05/21/2021

Date	5/14/2021	5/17/2021	Average	RSD
Control	<20 ppb	<20 ppb		
Level 2	**13.51**	13.94	13.7	NA
Level 3	28.38	28.91	28.6	NA
Level 4	57.26	60.54	58.9	NA
Level 5	115.2	110.3	112.8	NA
Level 6	215.4	**231.4**	223.4	NA
Day	0 (New)	3 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFHxS (test 1)
Test Dates: 01/29/2021 - 02/05/2021

	1/29/2021	1/30/2021	2/1/2021	2/2/2021	2/4/2021	2/5/2021	Average	RSD
Control	<18 ppb	<18 ppb	<18 ppb	96 ppb	<18 ppb	<18 ppb		
Level 2	5.8	5.9	5.5	**5.1**	**5.1**	**4.9**	5.4	8
Level 3	12	11	11	**10**	10	9.8	11	6
Level 4	**23**	**24**	**23**	22	**21**	**21**	22	6
Level 5	**49**	**49**	**46**	43	43	42	45	7
Level 6	**99**	96	**94**	87	86	**86**	91	6
Day	0 (New)	1 (Old)	3 (New)	4 (Old)	6 (New)	7 (Old)		

Note: The sampling procedure employed in this exposure involved sampling an aliquot of test solution without methanol stabilization. The sample was then centrifuged and sub-sampled into an HPLC vial, wherein it was stabilized in methanol and spiked with internal standard. This differs from the sampling procedure used subsequently, in which samples were aliquotted directly into methanol and then centrifuged, to ensure adequate methanol stabilization throughout the sample preparation process.

A brief comparison of sampling directly into methanol vs. stabilizing later found that, for PFOS, there was approximately 20% and 5% loss at 50 ppm and 0.2 ppm, respectively, when samples were not stabilized immediately in methanol.

Due to poor agreement between neat material standards and an secondary standard, data was adjusted down by a factor of 0.77X based on the average of deviation amongst 11 replicate secondary sources run at different times against various calibrations. This neat material was switched for one that did not have such a bias in subsequent exposures.

Test Species: C. dubia
Chemical Abbreviation: PFHxS (test 2)
Test Dates: 02/12/2021 - 02/19/2021

Date	2/12/2021	2/13/2021	2/15/2021	2/16/2021	2/18/2021	2/19/2021	Average	RSD
Control	<18 ppb	<18 ppb	<18 ppb	124 ppb	<18 ppb	<18 ppb		NA
Level 2	4.3	**4.1**	-	**4.2**	**3.8**	**3.6**	4.0	7
Level 3	8.7	9.5	8.00	8.8	8.2	8.5	7.4	7
Level 4	**16**	18	-	**17**	**17**	**16**	17	6
Level 5	31	33	-	**29**	33	**32**	32	5
Level 6	65	**65**	-	62	**66**	64	64	3
Day	0 (New)	1 (Old)	3 (New)	4 (Old)	6 (New)	7 (Old)		

Note: The sampling procedure employed in this exposure involved sampling an aliquot of test solution without methanol stabilization. The sample was then centrifuged and sub-sampled into an HPLC vial, wherein it was stabilized in methanol and spiked with internal standard. This differs from the sampling procedure used subsequently, in which samples were aliquotted directly into methanol and then centrifuged, to ensure adequate methanol stabilization throughout the sample preparation process.

A brief comparison of sampling directly into methanol vs. stabilizing later found that, for PFOS, there was approximately 20% and 5% loss at 50 ppm and 0.2 ppm, respectively, when samples were not stabilized immediately in methanol.

Due to poor agreement between neat material standards and an secondary standard, data was adjusted down by a factor of 0.77X based on the average of deviation amongst 11 replicate secondary sources run at different times against various calibrations. This neat material was switched for one that did not have such a bias in subsequent exposures.

Test Species: C. dubia
Chemical Abbreviation: PFOS (test 1)
Test Dates: 02/12/2021 - 02/19/2021

Date	2/12/2021	2/13/2021	2/15/2021	2/16/2021	2/18/2021	2/19/2021	Average	RSD
Control	<19 ppb	<19 ppb	<19 ppb	<19 ppb	<19 ppb	<19 ppb		NA
Level 2	**2.8**	**2.4**	-	**2.0**	**2.1**	2.0	2.3	16
Level 3	4.6	4.8	-	**4.8**	4.0	3.9	4.4	10
Level 4	**8.6**	**9.2**	-	**9.2**	**8.6**	**8.5**	8.8	4

Level 5	17	18	18	19	19	**18**	18	2
Level 6	36	**38**	-	39	**39**	**38**	38	3
Day	0 (New)	1 (Old)	3 (New)	4 (Old)	6 (New)	7 (Old)		

Note: The sampling procedure employed in this exposure involved sampling an aliquot of test solution without methanol stabilization. The sample was then centrifuged and sub-sampled into an HPLC vial, wherein it was stabilized in methanol and spiked with internal standard. This differs from the sampling procedure used subsequently, in which samples were aliquotted directly into methanol and then centrifuged, to ensure adequate methanol stabilization throughout the sample preparation process.

A brief comparison of sampling directly into methanol vs. stabilizing later found that, for PFOS, there was approximately 20% and 5% loss at 50 ppm and 0.2 ppm, respectively, when samples were not stabilized immediately in methanol.

Test Species: C. dubia
Chemical Abbreviation: PFOS (test 2)
Test Dates: 03/12/2021 - 03/19/2021

Date	3/12/2021	3/13/2021	3/16/2021	3/18/2021	3/19/2021	Average	RSD
Control	<9.3 ppb	<9.3 ppb	<9.3 ppb	<9.3 ppb	<9.3 ppb		
Level 2	0.049	0.045	-	0.052	0.051	0.049	6
Level 3	0.095	0.076	-	0.094	0.093	0.090	10
Level 4	0.19	0.38	-	0.23	**0.20**	0.25	35
Level 5	0.40	0.37	-	0.47	0.46	0.42	12
Level 6	0.91	0.85	-	1.0	**0.90**	0.92	8
Level 7	1.9	1.7	-	2.1	**2.0**	1.9	9
Level 8	**3.8**	3.4	-	4.2	4.3	3.9	11
Level 9	6.8	**6.8**	8.5	8.7	9.3	8.0	14
Level 10	15	**15**	-	**18**	19	17	11
Level 11	33	**30**	-	38	39	35	12
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Note: The sampling procedure employed in this exposure involved sampling an aliquot of test solution without methanol stabilization. The sample was then centrifuged and sub-sampled into an HPLC vial, wherein it was stabilized in methanol and spiked with internal standard. This differs from the sampling procedure used subsequently, in which samples were aliquotted directly into methanol and then centrifuged, to ensure adequate methanol stabilization throughout the sample preparation process.

A brief comparison of sampling directly into methanol vs. stabilizing later found that, for PFOS, there was approximately 20% and 5% loss at 50 ppm and 0.2 ppm, respectively, when samples were not stabilized immediately in methanol.

Test Species: C. dubia
Chemical Abbreviation: PFOS (test 3)
Test Dates: 04/09/2021 - 04/16/2021

Date	4/9/2021	4/10/2021	4/13/2021	4/15/2021	4/16/2021	Average	RSD
Control	<28 ppb	<28 ppb	<28 ppb	<28 ppb	<28 ppb		
Level 2	2.8	2.7	2.7	2.5	**2.6**	2.7	4
Level 3	6.5	5.8	5.8	**5.6**	5.8	5.9	6
Level 4	**12**	11	11	12	11	11	5
Level 5	25	23	**22**	24	**20**	23	8
Level 6	50	**50**	46	45	40	46	9
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFNS (test 1)
Test Dates: 04/01/2022 - 04/08/2022

Date	4/1/2022	4/2/2022	4/5/2022	4/7/2022	4/8/2022	Average	RSD
Control	<160 ppb	<160 ppb	<160 ppb	<160 ppb	<160 ppb		
Level 2	**1.3**	1.2	1.1	1.2	1.2	1.2	5
Level 3	2.3	2.4	**2.2**	2.5	**2.4**	2.4	4
Level 4	4.8	**4.7**	4.6	5.7	4.8	4.9	9
Level 5	8.9	8.8	**8.7**	**12.0**	**9.0**	9.5	15
Level 6	18	**18**	17	19	N/A	18	4
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFNS (test 1)
Test Dates: 9/23/2022 - 9/30/2022

Date	9/23/2022	9/24/2022	9/27/2022	9/29/2022	9/30/2022	Average	RSD
Control	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb		
Level 2	**0.38**	0.42	**0.40**	0.41	0.41	0.40	4
Level 3	-	1.1	1.1	-	-	1.1	-
Level 4	2.4	2.5	2.6	2.0	**2.4**	2.4	10
Level 5	5.4	**6.5**	6.9	**5.5**	**4.9**	5.8	14
Level 6	14	**15**	**16**	**13**	12	14	12
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Note: Sampling errors on days 0, 6, and 7 led to unreliable data for level 3, as such, those values were censored. However, given the % RSD of < 15 for the other treatment levels indicate that the average of the two days are likely close

to the treatmetnt concentration over the course of the experiment.

Test Species: C. dubia
Chemical Abbreviation: PFDS
Test Dates: 9/23/2022 - 9/30/2022

Date	9/23/2022	9/24/2022	9/27/2022	9/29/2022	9/30/2022	Average	RSD
Control	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb		
Level 2	**0.75**	**0.76**	**1.2**	**0.95**	**0.58**	0.85	28

Test Species: C. dubia
Chemical Abbreviation: PFBSA
Test Dates: 04/01/2022 - 04/08/2022

Date	4/1/2022	4/2/2022	4/5/2022	4/7/2022	4/8/2022	Average	RSD
Control	<80 ppb	<80 ppb	<80 ppb	<80 ppb	<80 ppb		
Level 2	**0.94**	1.0	1.0	1.0	1.0	1.0	4
Level 3	2.0	2.1	**2.1**	2.0	**2.0**	2.0	2
Level 4	4.3	**4.3**	4.0	3.9	4.1	4.1	4
Level 5	8.4	9.2	**8.4**	**8.1**	8.1	8.4	5
Level 6	18	**19**	16	16	**17**	17	7
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: PFHxSA
Test Dates: 04/01/2022 - 04/08/2022

Date	4/1/2022	4/2/2022	4/5/2022	4/7/2022	4/8/2022	Average	RSD
Control	<80 ppb	<80 ppb	<80 ppb	<80 ppb	<80 ppb		
Level 2	**0.18**	0.19	0.18	0.17	0.20	0.18	5
Level 3	0.38	0.39	**0.37**	0.36	**0.40**	0.38	4
Level 4	0.71	**0.78**	0.75	0.75	0.81	0.76	5
Level 5	1.5	1.5	**1.6**	**1.6**	1.6	1.6	4
Level 6	3.0	**3.0**	3.3	3.1	3.5	3.2	7
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia

Chemical Abbreviation: PFOSA
Test Dates: 03/18/2022 - 03/25/2022

Date	3/18/2022	3/19/2022	3/22/2022	3/24/2022	3/25/2022	Average	RSD
Control	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb		
Level 2	**0.054**	**0.041**	0.050	0.056	0.039	0.048	16
Level 3	0.121	0.082	0.078	0.086	**0.066**	0.086	24
Level 4	0.23	0.18	**0.19**	0.16	0.14	0.18	18
Level 5	0.47	0.36	0.42	**0.36**	0.31	0.38	15
Level 6	0.97	**1.00**	0.98	0.75	**0.75**	0.89	14
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: 6:2 FTS
Test Dates: 04/01/2022 - 04/08/2022

Date	4/1/2022	4/2/2022	4/5/2022	4/7/2022	4/8/2022	Average	RSD
Control	<80 ppb	<80 ppb	<80 ppb	<80 ppb	<80 ppb		
Level 2	**13**	12	13	12	13	13	3
Level 3	25	27	**25**	24	**27**	26	5
Level 4	53	**56**	51	45	53	52	7
Level 5	92	104	**93**	**90**	105	97	7
Level 6	192	**216**	179	175	**191**	191	8
Day	0 (New)	1 (Old)	4 (Old)	6 (New)	7 (Old)		

Test Species: C. dubia
Chemical Abbreviation: 8:2 FTS
Test Dates: 03/18/2022 - 03/25/2022

Date	3/18/2022	3/19/2022	3/24/2022	Average	RSD
Control	<25 ppb	<25 ppb	<25 ppb		
Level 2	**0.068**	**0.086**	0.056	0.070	22
Level 3	0.37	0.46	**0.30**	0.377	22
Level 4	2.3	**2.7**	2.1	2.40	13
Day	0 (New)	1 (Old)	6 (New)		

Note: Exposure was externally calibrated due to intended nature as a rangefinding experiment.

Other Qualifications
- RLV fails for 8:2 FTS on Day 0 run.

Test Species: C. dubia
Chemical Abbreviation: 10:2 FTS
Test Dates: 03/18/2022 - 03/25/2022

Date	3/18/2022	3/19/2022	3/24/2022	Average	RSD
Control	<23 ppb	<23 ppb	<23 ppb		
Level 2	*	*	*	NA	NA
Level 3	**0.036**	**	**	NA	NA
Level 4	0.23	**0.12**	0.055	0.14	64
Day	0 (New)	1 (Old)	6 (New)		

*Targeted concentration was sufficiently low that analysis was not attempted for this rangefinding exposure.
**Due to adsorptive losses, planned quantitation of these treatments was below calibration and thus not accurate, useable data.

Note: Exposure was externally calibrated due to intended nature as a rangefinding experiment.

S6. *H. azteca* PFAS exposure concentration data

H. azteca QC Summary

	LCS	MS	SCV	MB
Mean	96	99	101	< MRL
Median	95	98	99	
Standard Deviation	8.9	9.3	9.9	
Minimum	80	69	86	
Maximum	126	125	126	
Confidence Level(95.0%)	1.5	1.6	3.0	

Notes about this summary:

- Values shown in bold indicate measured concentrations that are derived from one or more replicate samples.

- Day # is indicated starting at day 0 when organisms are first added to test vessels. "Pre" in parentheses indicates samples were taken pre-renewal on 24-hour old test solutions. "Post" in parentheses indicates samples were taken from test vessels immediately following renewal.

- Stock Samples: Are indicated by bold red font. These samples were taken only for early exposure verification of stock concentrations and thus are not included in final average treatment concentrations and RSD values.

- Concentrations are listed in **mg/L**, unless otherwise noted.

Test Species: H. azteca
Chemical Abbreviation: PFBA
Test Dates: 09/15/2021 - 09/22/2022

Date	9/15/2021	9/16/2021	9/16/2021	9/17/2021	9/19/2021	9/21/2022	9/21/2022	Average	RSD
Control	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb		
Level 2	**182**	**186**	**194**	**178**	**173**	**174**	**175**	180	5
Day	**0 (Stocks)**	1 (Pre)	1 (Post)	2 (Post)	4 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFHxA
Test Dates: 09/15/2021 - 09/22/2022

Date	9/15/2021	9/16/2021	9/16/2021	9/17/2021	9/19/2021	9/21/2022	9/21/2022	Average	RSD
Control	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb		
Level 2	**19**	18	18	**18**	**19**	18	**19**	18	2
Level 3	40	**36**	36	37	**35**	**39**	**39**	37	5
Level 4	74	75	**70**	89	71	**73**	71	75	10
Level 5	148	140	**142**	138	145	**154**	160	146	6

Level 6	**276**	**283**	283	**275**	278	323	**316**	293	7
Day	**0 (Stocks)**	1 (Pre)	1 (Post)	2 (Post)	4 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFOA
Test Dates: 09/15/2021 - 09/22/2022

Date	9/15/2021	9/16/2021	9/16/2021	9/17/2021	9/19/2021	9/21/2022	9/21/2022	Average	RSD
Control	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb	<200 ppb		
Level 2	**4.8**	5.1	5.2	**5.3**	**5.6**	5.3	5.1	5.3	3
Level 3	10	**10**	10	11	**10**	11	**12**	11	5
Level 4	22	20	**21**	19	20	**20**	**26**	21	13
Level 5	37	39	**42**	38	42	**41**	42	41	5
Level 6	**105**	**78**	85	**87**	82	90	**80**	83	6
Day	**0 (Stocks)**	1 (Pre)	1 (Post)	2 (Post)	4 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFNA
Test Dates: 12/01/2021 - 12/08/2021

Date	12/1/2021	12/3/2022	12/3/2022	12/5/2022	12/6/2022	12/7/2021	12/7/2021	Average	RSD
Control	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb		
Level 2	0.091	0.091	0.090	0.094	0.076	0.089	0.085	0.087	7
Level 3	0.26	0.26	**0.25**	0.27	0.22	**0.25**	0.24	0.25	7
Level 4	0.76	**0.77**	0.69	0.71	0.63	0.73	**0.68**	0.70	7
Level 5	2.2	2.4	2.1	2.3	1.9	2.3	2.1	2.2	9
Level 6	7.0	8.6	**6.8**	6.9	**6.2**	6.8	6.2	6.9	12
Level 7	**22**	23	20	**22**	19	22	20	21	6
Day	**0 (Stocks)**	2 (Pre)	2 (Post)	4 (Pre)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFDA
Test Dates: 12/01/2021 - 12/08/2021

Date	12/1/2021	12/3/2022	12/3/2022	12/5/2022	12/6/2022	12/7/2021	12/7/2021	Average	RSD
Control	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb		
Level 2	0.018	0.017	0.017	0.026	0.020	0.021	0.020	0.020	17
Level 3	0.054	0.061	**0.055**	0.059	0.049	0.056	0.051	0.055	8
Level 4	0.16	**0.17**	0.16	0.17	0.14	0.16	0.16	0.16	6
Level 5	0.52	0.53	0.51	0.52	0.48	**0.52**	0.43	0.50	8
Level 6	1.6	1.5	**1.5**	1.6	**1.5**	1.6	1.5	1.5	4

								Average	RSD
Level 7	**5.0**	4.9	4.5	**5.1**	4.4	4.5	**4.7**	4.7	6
Day	**0 (Stocks)**	2 (Pre)	2 (Post)	4 (Pre)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFBS
Test Dates: 07/07/2021 - 07/14/2021

Date	7/7/2021	7/8/2021	7/8/2021	7/9/2021	7/11/2021	7/13/2021	7/13/2021	Average	RSD
Control	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb		
Level 2	**11**	**11**	11	**10**	11	12	**11**	11	7
Level 3	24	21	**22**	24	**24**	**25**	23	23	6
Level 4	49	43	**49**	**42**	51	43	45	46	8
Level 5	90	106	84	**92**	**109**	85	**93**	95	11
Level 6	202	**224**	179	**175**	230	**173**	174	192	14
Day	**0 (Stocks)**	1 (Pre)	1 (Post)	2 (Post)	4 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFHxS
Test Dates: 07/07/2021 - 07/14/2021

Date	7/7/2021	7/8/2021	7/8/2021	7/9/2021	7/11/2021	7/13/2021	7/13/2021	Average	RSD
Control	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb		
Level 2	**12**	**12**	12	12	12	13	**12**	12	3
Level 3	26	24	**24**	22	**24**	**26**	23	24	6
Level 4	51	45	**46**	**44**	50	48	47	47	5
Level 5	100	79	95	**82**	**101**	**75**	**91**	87	12
Level 6	151	**170**	186	230	201	**159**	183	188	13
Day	**0 (Stocks)**	1 (Pre)	1 (Post)	2 (Post)	4 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFOS
Test Dates: 07/07/2021 - 07/14/2021

Date	7/7/2021	7/8/2021	7/8/2021	7/9/2021	7/11/2021	7/13/2021	7/13/2021	Average	RSD
Control	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb	<20 ppb		
Level 2	**0.97**	**0.85**	0.93	0.89	0.85	0.94	**0.89**	0.89	4
Level 3	1.9	1.5	**1.7**	1.7	**2.0**	**1.8**	**1.7**	1.7	10
Level 4	3.4	3.1	**3.3**	**3.2**	3.5	3.2	3.2	3.3	4
Level 5	6.6	7.9	6.2	**6.4**	**6.9**	5.8	**6.1**	6.5	11

Level 6	13	**12**	11	12	14	**12**	12	12	10
Level 7	23	33	**24**	26	27	24	24	26	14
Day	**0 (Stocks)**	1 (Pre)	1 (Post)	2 (Post)	4 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFNS
Test Dates: 09/21/2022 - 09/28/2022

Date	9/21/2022	9/23/2022	9/23/2022	9/24/2022	9/26/2022	9/27/2022	9/27/2022	Average	RSD
Control	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb		
Level 2	**0.45**	**0.42**	0.40	0.42	0.42	**0.48**	0.41	0.43	6
Level 3	1.1	**0.96**	0.98	1.0	**1.0**	**1.1**	**1.0**	1.0	4
Level 4	2.5	2.4	**2.2**	**2.4**	2.3	2.9	**2.3**	2.4	10
Level 5	6.0	5.4	**5.4**	5.1	**5.9**	6.1	6.4	5.7	8
Level 6	15	14	15	**14**	15	17	15	15	7
Day	**0 (Stocks)**	2 (Pre)	2 (Post)	3 (Post)	5 (Pre)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFDS
Test Dates: 09/21/2022 - 09/28/2022

Date	9/21/2022	9/23/2022	9/23/2022	9/24/2022	9/26/2022	9/27/2022	9/27/2022	Average	RSD
Control	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb	<240 ppb		
Level 2	**0.95**	**0.69**	**0.79**	**0.86**	**0.87**	**0.93**	**1.0**	0.86	13
Day	**0 (Stocks)**	1 (Pre)	3 (Pre)	3 (Post)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFBSA
Test Dates: 05/06/2022 - 05/13/2022

Date	4/6/2022	4/7/2022	4/9/2022	4/9/2022	4/11/2022	4/12/2022	4/12/2022	Average	RSD
Control	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb		
Level 2	0.059	**0.067**	0.074	0.066	**0.059**	0.069	0.060	0.066	9
Level 3	0.12	**0.14**	0.14	0.13	**0.12**	0.13	0.12	0.13	7
Level 4	0.25	0.28	**0.29**	0.27	0.24	**0.26**	0.25	0.27	7
Level 5	0.53	0.57	0.61	**0.56**	0.53	0.53	**0.52**	0.55	6
Level 6	1.1	1.2	**1.2**	1.1	1.0	1.1	**1.1**	1.1	7
Level 7	**2.1**	2.4	2.4	**2.3**	2.3	**2.1**	2.1	2.3	6
Day	**0 (Stocks)**	1 (Pre)	3 (Pre)	3 (Post)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFHxSA
Test Dates: 04/07/2022 - 04/14/2022   All concentrations for this test are in ug/L

Date	4/6/2022	4/7/2022	4/9/2022	4/9/2022	4/11/2022	4/12/2022	4/12/2022	Average	RSD
Control	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb		
Level 2	6.3	**6.1**	6.5	5.6	**6.9**	7.1	7	6.5	9
Level 3	12	**12**	14	11	**13**	14	14	13	10
Level 4	25	23	**28**	22	28	**29**	28	26	11
Level 5	50	50	61	**48**	62	64	**59**	57	12
Level 6	99	101	**116**	93	109	124	113	109	10
Level 7	**188**	188	159	**166**	212	**225**	244	199	17
Day	**0 (Stocks)**	1 (Pre)	3 (Pre)	3 (Post)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: PFOSA
Test Dates: 04/07/2022 - 04/14/2022   All concentrations for this test are in ug/L

Date	4/6/2022	4/7/2022	4/9/2022	4/9/2022	4/11/2022	4/12/2022	4/12/2022	Average	RSD
Control	<0.25 ppb	<0.25 ppb	<0.25 ppb	<0.25 ppb	<0.25 ppb	<0.25 ppb	<0.25 ppb		
Level 2	0.9	**0.78**	0.56	0.56	**0.67**	0.54	0.53	0.61	16
Level 3	1.8	**1.6**	1.3	1.1	**1.4**	1.1	1.3	1.3	15
Level 4	3.4	3.1	**2.6**	2.5	2.5	**2.2**	2.4	2.6	12
Level 5	6.1	6.1	4.7	**4.4**	5.1	4.3	**4.6**	4.9	14
Level 6	12	12	**9.3**	8.5	9.2	8.6	**9**	9.4	14
Level 7	**23**	22	17	**17**	17	**15**	17	18	13
Day	**0 (Stocks)**	1 (Pre)	3 (Pre)	3 (Post)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: 6:2 FTS
Test Dates: 12/15/2021 - 12/22/2021

Date	12/16/2021	12/17/2021	12/17/2021	12/18/2021	12/20/2021	12/21/2021	12/21/2021	Average	RSD
Control	<78 ppb	<78 ppb	<78 ppb	<78 ppb	<78 ppb	<78 ppb	<78 ppb		
Level 2	3.3	3.4	3.3	3.4	2.9	**3.4**	3.2	3.3	7
Level 3	7.1	**7.0**	6.3	6.8	**6.5**	6.9	7.0	6.7	4
Level 4	13	13	12	13	12	13	13	13	6
Level 5	26	27	24	**27**	26	23	27	26	7
Level 6	50	54	**48**	56	52	50	**53**	52	5
Level 7	**105**	108	90	101	106	102	101	101	6

| Level 8 | 239 | 217 | 180 | 196 | 199 | 194 | 212 | 200 | 7 |
| Day | 1 (Stocks) | 2 (Pre) | 2 (Post) | 3 (Pre) | 5 (Post) | 6 (Pre) | 6 (Post) | | |

Test Species: H. azteca
Chemical Abbreviation: 8:2 FTS
Test Dates: 04/07/2022 - 04/14/2022

Date	4/6/2022	4/7/2022	4/9/2022	4/9/2022	4/11/2022	4/12/2022	4/12/2022	Average	RSD
Control	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb		
Level 2	3.0	2.9	3.0	2.7	3.0	3.0	2.6	2.9	7
Day	0 (Stocks)	1 (Pre)	3 (Pre)	3 (Post)	5 (Post)	6 (Pre)	6 (Post)		

Test Species: H. azteca
Chemical Abbreviation: 10:2 FTS
Test Dates: 04/07/2022 - 04/14/2022

Date	4/6/2022	4/7/2022	4/9/2022	4/9/2022	4/11/2022	4/12/2022	4/12/2022	Average	RSD
Control	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb	<2.5 ppb		
Level 2	0.21	0.11	0.038	0.093	0.10	0.047	0.093	0.081	38
Day	0 (Stocks)	1 (Pre)	3 (Pre)	3 (Post)	5 (Post)	6 (Pre)	6 (Post)		

S6. *C. dilutus* **PFAS exposure concentration data**

C. dilutus **QC Summary**

	LCS	MS	SCV	MB
Mean	97	97	97	< MRL
Median	98	98	96	
Standard Deviation	8.0	8.1	9.3	
Minimum	75	75	81.6	
Maximum	118	128	117.8	
Confidence Level(95.0%)	1.3	1.6	2.3	

Notes about this summary:

- Values shown in bold indicate measured concentrations that are derived from one or more replicate samples.

- Day # is indicated starting at day 0 when organisms are first added to test vessels. "Pre" in parentheses indicates samples were taken pre-renewal on 24-hour old test solutions. "Post" in parentheses indicates samples were taken from test vessels immediately following renewal.

- Stock Samples: Are indicated by bold red font. These samples were taken only for early exposure verification of stock concentrations and thus are not included in final average treatment concentrations and RSD values.

Test Species: C. dilutus
Chemical Abbreviation: PFBA
Test Dates: 10/01/2021 - 10/08/2021

Date	10/1/2021	10/1/2021	10/2/2021	10/5/2021	10/5/2021	10/7/2021	10/7/2021	Average	RSD
Control	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm		
Level 2	**213**	**212**	**212**	**216**	**213**	**222**	**225**	217	2.6
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFHxA (test 1)
Test Dates: 10/01/2021 - 10/08/2021

Date	10/1/2021	10/1/2021	10/2/2021	10/5/2021	10/5/2021	10/7/2021	10/7/2021	Average	RSD
Control	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm		
Level 2	17	**16**	17	21	20	21	20	19	11
Level 3	36	37	36	**39**	39	**39**	38	38	3.7
Level 4	**74**	63	**72**	80	**78**	77	**77**	75	8.6
Level 5	152	**187**	144	145	**158**	**152**	155	157	10
Level 6	298	275	**291**	**319**	314	315	318	305	6.0

Day	0 (Stocks)	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)

Test Species: C. dilutus
Chemical Abbreviation: PFHxA (test 2)
Test Dates: 12/03/2021 - 12/10/2021

Date	12/3/2021	12/3/2021	12/5/2021	12/7/2021	12/7/2021	12/9/2021	12/9/2021	Average	RSD
Control	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb		
Level 2	20	20	21	**20**	19	20	**19**	20	3.8
Level 3	39	**40**	41	38	36	39	36	38	5.4
Level 4	81	81	79	79	**78**	75	77	78	2.6
Level 5	162	164	171	160	155	149	144	157	6.3
Level 6	213	**211**	202	204	206	**190**	190	201	4.3
Level 7	**325**	303	**343**	300	311	301	301	310	5.4
Day	**0 (Stocks)**	0 (Post)	2 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFOA (test 1)
Test Dates: 04/23/2021 - 04/30/2021

Date	4/23/2021	4/23/2021	4/24/2021	4/27/2021	4/27/2021	4/29/2021	4/29/2021	Average	RSD
Control	<3.0 ppm	<3.0 ppm	<3.0 ppm	<3.0 ppm	<3.0 ppm	<3.0 ppm	<3.0 ppm		
Level 2	**53**	**54**	**55**	52	48	55	**77**	57	18
Level 3	104	**159**	110	**107**	**108**	110	103	116	18
Level 4	211	229	136	219	208	186	201	197	17
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFOA (test 2)
Test Dates: 10/01/2021 - 10/08/2021

Date	10/1/2021	10/1/2021	10/2/2021	10/5/2021	10/5/2021	10/7/2021	10/7/2021	Average	RSD
Control	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm	<1.3 ppm		
Level 2	4.7	**4.7**	4.6	5.2	5.1	5.4	4.9	5.0	6.3
Level 3	9.9	**11**	11	**10**	11	**9.9**	11	11	5.1
Level 4	**18**	23	**24**	20	**22**	20	**20**	22	8.2
Level 5	38	**43**	55	41	**39**	**39**	**42**	43	14
Level 6	70	88	**75**	**78**	86	77	82	81	6.4
Level 7	152	175	169	158	166	160	158	164	4.2
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFOA (test 3)
Test Dates: 02/25/2022 - 03/04/2022

Date	2/25/2022	2/25/2022	2/27/2022	3/1/2022	3/1/2022	3/3/2022	3/3/2022	Average	RSD
Control	<35 ppb	<35 ppb	<35 ppb	<35 ppb	<35 ppb	<35 ppb	<35 ppb		
Level 2	**15**	15	15	**17**	17	17	**15**	16	5.8
Level 3	29	**30**	32	35	**32**	34	29	32	6.9
Level 4	60	60	**64**	67	61	**68**	59	63	5.7
Level 5	122	120	**125**	136	**125**	131	**114**	125	6.1
Level 6	236	**234**	246	**245**	254	**271**	254	250	4.9
Day	**0 (Stocks)**	0 (Post)	2 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFNA
Test Dates: 12/03/2021 - 12/10/2021

Date	12/3/2021	12/3/2021	12/5/2021	12/7/2021	12/7/2021	12/9/2021	12/9/2021	Average	RSD
Control	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb	<25 ppb		
Level 2	1.4	1.4	1.5	1.5	1.3	1.6	**1.5**	1.5	7.0
Level 3	2.8	**2.9**	3.3	3.2	3.0	3.4	3.1	3.2	5.9
Level 4	5.8	5.8	6.8	6.5	5.9	7.0	5.7	6.3	8.9
Level 5	11	12	12	13	11	14	13	13	8.4
Level 6	23	22	25	24	**23**	**26**	24	24	5.9
Level 7	**47**	45	**51**	**49**	45	52	46	48	6.5
Day	**0 (Stocks)**	0 (Post)	2 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFDA
Test Dates: 10/29/2021 - 11/05/2021

Date	10/29/2021	10/29/2021	10/30/2021	11/2/2021	11/2/2021	11/4/2021	11/4/2021	Average	RSD
Control	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb		
Level 2	0.078	**0.061**	0.071	0.077	0.073	0.086	**0.074**	0.074	11
Level 3	0.15	0.12	**0.15**	0.16	0.15	**0.17**	0.14	0.15	11
Level 4	0.30	0.25	**0.32**	0.31	**0.29**	0.32	0.26	0.29	11
Level 5	0.56	**0.62**	0.61	**0.62**	**0.57**	0.68	0.59	0.62	6.1
Level 6	**1.2**	1.1	1.3	**1.3**	1.1	1.3	1.3	1.2	7.9
Level 7	2.3	2.1	2.7	2.5	2.4	**2.9**	**2.6**	2.5	10
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFBS
Test Dates: 10/29/2021 - 11/05/2021

Date	10/29/2021	10/29/2021	10/30/2021	11/2/2021	11/2/2021	11/4/2021	11/4/2021	Average	RSD
Control	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb		
Level 2	17	**15**	17	17	17	18	**18**	17	5.9
Level 3	36	34	**34**	30	32	**35**	37	34	7.1
Level 4	70	63	**72**	68	**67**	75	73	70	6.4
Level 5	138	**121**	132	**128**	**143**	135	135	132	5.5
Level 6	**281**	257	283	**255**	307	**302**	**285**	282	7.8
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFHxS
Test Dates: 10/29/2021 - 11/05/2021

Date	10/29/2021	10/29/2021	10/30/2021	11/2/2021	11/2/2021	11/4/2021	11/4/2021	Average	RSD
Control	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb	<45 ppb		
Level 2	0.70	**0.63**	0.74	0.71	0.67	0.78	**0.72**	0.71	7.2
Level 3	1.3	1.3	**1.4**	1.3	1.3	**1.5**	1.4	1.4	6.6
Level 4	2.7	2.5	**2.9**	2.9	**2.8**	3.2	3.0	2.9	8.1
Level 5	5.4	**5.2**	5.3	**5.5**	**5.0**	5.8	5.6	5.4	5.2
Level 6	**11**	9.3	11	**9.8**	10	12	12	11	10.0
Level 7	21	19	24	21	23	**26**	**25**	23	12.1
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFHpS
Test Dates: 08/10/2021 - 08/17/2021

Date	8/10/2021	8/10/2021	8/11/2021	8/13/2021	8/13/2021	8/16/2021	8/16/2021	Average	RSD
Control	<4.0 ppb	<4.0 ppb	<4.0 ppb	<4.0 ppb	<4.0 ppb	<4.0 ppb	<4.0 ppb		
Level 2	0.062	**0.061**	0.061	0.058	**0.053**	0.059	**0.069**	0.060	8.9
Level 3	**0.13**	0.12	0.12	**0.12**	0.11	0.11	**0.13**	0.12	8.0
Level 4	0.26	0.27	**0.26**	**0.24**	0.23	**0.22**	0.30	0.25	11.4
Level 5	0.51	0.51	**0.51**	0.50	0.47	**0.44**	0.48	0.49	5.7
Level 6	1.0	**0.98**	1.0	0.98	**0.98**	0.86	**0.95**	0.96	5.2
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	3 (Pre)	3 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFOS (test 1)
Test Dates: 03/21/2021 - 03/28/2021

<p style="text-align:center; color:red">All concentrations for this test are in ug/L</p>

Date	3/21/2021	3/21/2021	3/22/2021	3/25/2021	3/25/2021	3/27/2021	3/27/2021	Average	RSD
Control	<0.37 ppb	<0.37 ppb	<0.37 ppb	<0.37 ppb	<0.37 ppb	<0.37 ppb	<0.37 ppb		
Level 2	**0.94**	**0.86**	0.88	0.88	1.0	0.92	1.1	0.94	10
Level 3	1.6	**1.5**	**1.6**	1.6	2.1	1.6	1.6	1.7	13
Level 4	3.2	3.3	2.9	**3.5**	3.3	3.1	3.9	3.3	10
Level 5	6.8	6.0	**6.8**	6.3	**6.2**	5.6	**6.0**	6.2	6.5
Level 6	13	11	17	12	11	**11**	11	12	20
Level 7	24	22	22	24	24	**24**	23	23	4.2
Level 8	50	45	**48**	48	50	50	**46**	48	4.3
Level 9	101	91	92	119	87	93	90	95	12
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFOS (test 2)
Test Dates: 02/25/2022 - 03/04/2022   All concentrations for this test are in ug/L

Date	2/25/2022	2/25/2022	2/27/2022	3/1/2022	3/1/2022	3/3/2022	3/3/2022	Average	RSD
Control	<1.0 ppb	<1.0 ppb	<1.0 ppb	<1.0 ppb	<1.0 ppb	<1.0 ppb	<1.0 ppb		
Level 2	**2.9**	2.8	2.8	**3.0**	2.8	3.1	**2.8**	2.9	4.3
Level 3	5.9	**5.6**	6.1	6.2	5.6	**6.1**	6.0	5.9	4.6
Level 4	12	11	13	12	11	**12**	12	12	4.7
Level 5	24	21	**24**	24	**23**	24	**23**	23	4.8
Level 6	49	**45**	46	**48**	50	**51**	46	48	5.1
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFNS (test 1)
Test Dates: 08/27/2021 - 09/03/2021

Date	8/27/2021	8/27/2021	8/28/2021	8/31/2021	8/31/2021	9/2/2021	9/2/2021	Average	RSD
Control	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb		
Level 2	0.013	**0.011**	0.010	0.014	**0.010**	0.013	**0.013**	0.012	13
Level 3	**0.028**	0.024	0.021	**0.024**	0.023	**0.026**	0.026	0.024	7.7
Level 4	0.050	0.045	**0.042**	0.052	**0.045**	**0.056**	0.054	0.049	11
Level 5	0.10	0.086	**0.090**	0.10	0.084	**0.11**	0.10	0.10	10
Level 6	**0.19**	**0.16**	0.17	0.17	0.16	0.19	0.17	0.17	6.4
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFNS (test 2)
Test Dates: 09/09/2022 - 09/16/2022

Date	9/9/2022	9/9/2022	9/10/2022	9/13/2022	9/13/2022	9/16/2022	9/16/2022	9/16/2022	Average	RSD
Control	<1.14 ppb	<1.14 ppb	<1.14 ppb	<1.14 ppb	<1.14 ppb	-	<1.14 ppb	<1.14 ppb		
Level 2	0.0017	0.0014	0.0012	**0.0016**	0.0015	0.0013	**0.0014**	0.0016	0.0014	8.9
Level 3	0.0032	0.0029	0.0025	**0.0032**	**0.0029**	-	0.0022	0.0027	0.0027	12.8
Level 4	0.0058	0.0053	**0.0051**	0.0063	**0.0061**	0.0061	0.0055	**0.0055**	0.0056	8.3
Level 5	0.013	**0.011**	**0.012**	0.012	0.012	-	**0.012**	0.012	0.012	5.5
Level 6	**0.028**	**0.023**	0.023	0.025	0.024	0.019	0.022	**0.026**	0.024	6.8
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	**6 (Stocks)**	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: PFDS
Test Dates: 08/27/2021 - 09/03/2021

Date	8/27/2021	8/27/2021	8/28/2021	8/31/2021	8/31/2021	9/2/2021	9/2/2021	Average	RSD
Control	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb		
Level 2	0.025	**0.018**	**0.017**	0.020	**0.017**	0.026	**0.028**	0.021	23
Level 3	**0.055**	**0.028**	0.023	**0.030**	0.035	**0.044**	0.044	0.034	25
Level 4	0.097	**0.065**	**0.053**	0.067	**0.073**	**0.084**	0.081	0.070	16
Level 5	0.20	**0.13**	**0.14**	**0.18**	0.13	0.17	**0.17**	0.15	14
Level 6	**0.34**	**0.26**	0.22	0.35	0.28	0.31	0.30	0.29	16
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: FBSA
Test Dates: 05/20/2022 - 05/27/2022

Date	5/20/2022	5/20/2022	5/21/2022	5/24/2022	5/24/2022	5/26/2022	5/26/2022	Average	RSD
Control	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb		
Level 2	0.24	0.26	**0.30**	0.26	0.26	0.29	0.30	0.28	7.1
Level 3	0.51	**0.41**	**0.52**	0.52	0.50	**0.55**	0.57	0.51	11
Level 4	1.0	**1.0**	1.1	**1.0**	1.0	**1.1**	1.0	1.1	2.8
Level 5	2.0	1.6	1.5	**2.0**	2.1	2.2	2.2	1.9	16
Level 6	4.2	4.5	4.1	4.0	**4.2**	4.2	**4.7**	4.3	5.4
Level 7	**8.8**	8.7	9.0	8.3	**8.2**	8.6	**9.0**	8.6	3.5
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: FHxSA
Test Dates: 05/20/2022 - 05/27/2022

Date	5/20/2022	5/20/2022	5/21/2022	5/24/2022	5/24/2022	5/26/2022	5/26/2022	Average	RSD
Control	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb		
Level 2	0.058	0.051	**0.061**	0.053	0.054	0.058	0.058	0.056	6.7
Level 3	0.11	**0.093**	**0.12**	0.11	0.11	**0.12**	0.11	0.11	8.6
Level 4	0.23	**0.21**	0.23	**0.21**	0.22	**0.23**	0.22	0.22	4.9
Level 5	0.47	0.46	0.47	**0.43**	0.43	0.48	0.45	0.45	4.6
Level 6	0.91	0.87	0.75	0.84	**0.85**	0.92	**0.88**	0.85	6.5
Level 7	**1.8**	1.4	1.7	1.7	**1.7**	1.7	**1.7**	1.7	7.6
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: FOSA (test 1)
Test Dates: 08/27/2021 - 09/03/2021

Date	8/27/2021	8/27/2021	8/28/2021	8/31/2021	8/31/2021	9/2/2021	9/2/2021	Average	RSD
Control	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb	<2.4 ppb		
Level 2	0.0044	**0.0033**	0.0032	0.0031	**0.0029**	0.0033	**0.0036**	0.0032	8.0
Level 3	**0.0071**	0.0056	0.0054	**0.0048**	0.0046	**0.0061**	0.0059	0.0054	11
Level 4	0.014	0.012	**0.012**	0.0099	**0.0095**	**0.011**	0.011	0.011	9.0
Level 5	0.027	**0.031**	0.029	**0.025**	0.024	0.024	**0.022**	0.026	13
Level 6	0.050	**0.043**	0.044	0.038	0.036	0.042	0.038	0.040	8.0
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: FOSA (test 2)
Test Dates: 09/09/2022 - 09/16/2022

Date	9/9/2022	9/9/2022	9/10/2022	9/13/2022	9/13/2022	9/16/2022	9/16/2022	9/16/2022	Average	RSD
Control	<1.14 ppb	<1.14 ppb	<1.14 ppb	<1.14 ppb	<1.14 ppb	-	<1.14 ppb	<1.14 ppb		
Level 2	0.0025	0.0022	0.0016	**0.0021**	0.0020	0.0021	**0.0018**	0.0019	0.0019	10.4
Level 3	0.0047	0.0039	0.0028	**0.0038**	**0.0038**	-	0.0036	0.0035	0.0035	11.5
Level 4	0.0097	0.0075	**0.0050**	0.0071	**0.0077**	0.0079	0.0074	**0.0072**	0.0070	14.5
Level 5	0.019	**0.017**	**0.011**	0.015	0.016	-	**0.014**	0.014	0.015	12.8
Level 6	**0.035**	0.028	0.017	0.028	0.030	0.027	0.028	**0.029**	0.027	18.2
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	**6 (Stocks)**	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: 6:2 FTS
Test Dates: 05/20/2022 - 05/27/2022

Date	5/20/2022	5/20/2022	5/21/2022	5/24/2022	5/24/2022	5/26/2022	5/26/2022	Average	RSD
Control	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb		
Level 2	9.7	12	**12**	10	11	10	11	11	6.5
Level 3	24	**25**	**25**	23	24	**24**	25	24	4.0
Level 4	50	**50**	48	**54**	53	**52**	52	52	4.2
Level 5	81	107	100	**101**	**104**	103	**102**	103	2.4
Level 6	188	194	209	187	**208**	215	**205**	203	5.1
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: 8:2 FTS
Test Dates: 05/20/2022 - 05/27/2022

Date	5/20/2022	5/20/2022	5/21/2022	5/24/2022	5/24/2022	5/26/2022	5/26/2022	Average	RSD
Control	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb		
Level 2	**2.7**	**2.6**	**2.4**	**2.6**	**2.9**	**3.1**	**3.1**	2.8	11
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

Test Species: C. dilutus
Chemical Abbreviation: 10:2 FTS
Test Dates: 05/20/2022 - 05/27/2022

Date	5/20/2022	5/20/2022	5/21/2022	5/24/2022	5/24/2022	5/26/2022	5/26/2022	Average	RSD
Control	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb	<10 ppb		
Level 2	**0.23**	**0.13**	**0.06**	**0.05**	**0.11**	**0.04**	**0.10**	0.081	45
Day	**0 (Stocks)**	0 (Post)	1 (Pre)	4 (Pre)	4 (Post)	6 (Pre)	6 (Post)		

S7. Analysis of standard deviation versus mean reproduction or growth among PFAS tests conducted with three species

Standard deviation is that of sublethal endpoints observed among replicate exposure chambers within an experimental treatment. Stars indicate average standard deviation among response categories equally divided across the range of observed responses

a. *C. dubia* reproduction: standard deviation had a constant relationship with treatment mean reproduction except when reproduction was very low, in which case a Poisson distribution was assumed (as below, between 0 and 10 young per female)
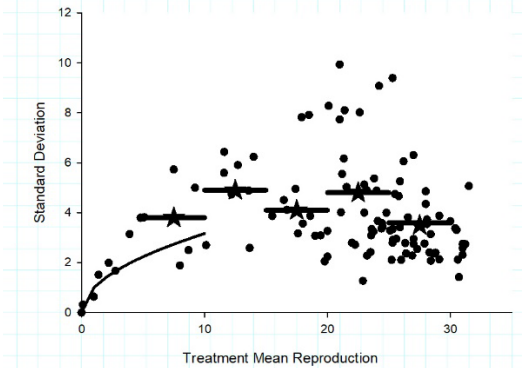
b. *H. azteca* biomass gain: standard deviation increased with treatment mean biomass gain
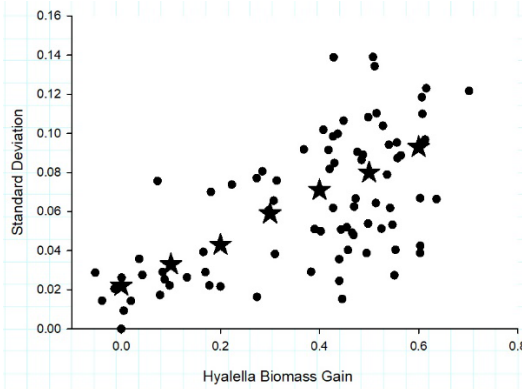
c. *C. dilutus* biomass gain: standard deviation was greatest among treatments with moderate effects on biomass gain, and decreased among treatments with either high effect (less biomass gain) or control-level biomass.
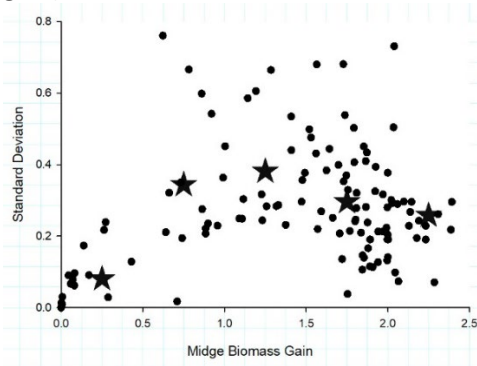
S8. Density dependent growth analysis for *H. azteca* and *C. dilutus*

7d *Hyalella* weight deviation as a function of survival (PFAS tests conducted 2021-2022)

7d midge weight deviation as a function of survival (PFAS tests conducted 2021-2022)
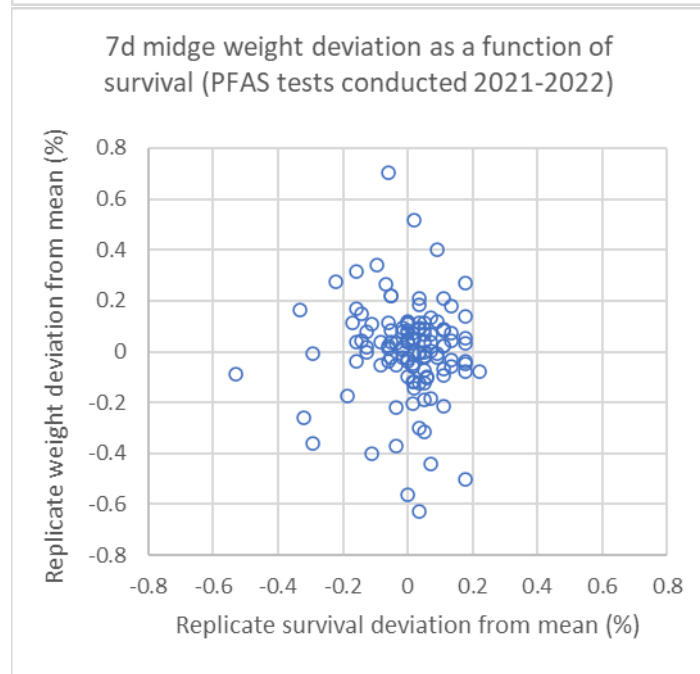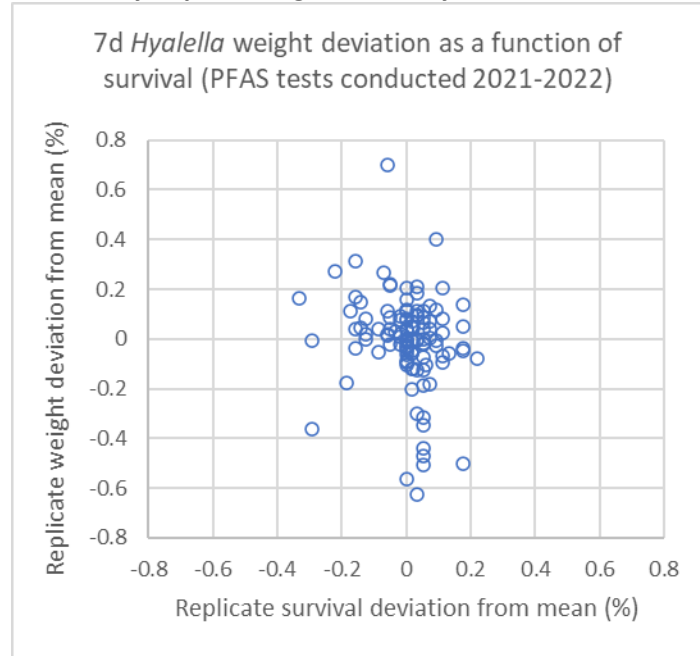
S9. Summaries of basic water chemistries in exposure chambers

C. dubia		Temp °C	pH	D.O. mg/L
PFBA	AVG	24.8	7.66	8.4
	SD	0.22	0.07	0.11
	MIN	24.4	7.56	8.1
	MAX	25.2	7.77	8.5

PFHxA	AVG	24.9	7.57	8.4
	SD	0.15	0.10	0.09
	MIN	24.6	7.4	8.3
	MAX	25.2	7.71	8.5

PFOA 1	AVG	24.7	7.72	8.5
	SD	0.31	0.06	0.26
	MIN	24.0	7.6	8.2
	MAX	25.2	7.78	8.8

PFOA 2	AVG	24.5	7.58	8.4
	SD	0.27	0.07	0.11
	MIN	24.0	7.47	8.2
	MAX	25.2	7.67	8.5

PFOA 3	AVG	24.9	7.64	8.3
	SD	0.51	0.03	0.07
	MIN	23.9	7.59	8.2
	MAX	25.8	7.69	8.4

PFNA	AVG	24.5	7.97	8.7
	SD	0.74	0.03	0.23
	MIN	23.2	7.93	8.3
	MAX	25.4	8.04	9.0

PFDA	AVG	24.7	7.58	8.4
	SD	0.42	0.02	0.11
	MIN	24.0	7.55	8.2
	MAX	25.4	7.62	8.5

PFBS	AVG	24.9	7.57	8.3
	SD	0.13	0.12	0.09
	MIN	24.6	7.33	8.1
	MAX	25.2	7.7	8.4

		Temp °C	pH	D.O. mg/L
PFHxS 1	AVG	24.6	7.68	8.5
	SD	0.33	0.07	0.15
	MIN	24.0	7.58	8.3
	MAX	25.2	7.77	8.7

PFHxS 2	AVG	24.5	7.74	8.8
	SD	0.33	0.03	0.17
	MIN	24.0	7.71	8.7
	MAX	25.0	7.79	9.1

PFOS 1	AVG	24.5	7.73	8.7
	SD	0.35	0.06	0.05
	MIN	23.8	7.64	8.7
	MAX	25.2	7.86	8.8

PFOS 2	AVG	24.4	7.76	8.4
	SD	0.19	0.03	0.13
	MIN	24.1	7.71	8.4
	MAX	24.8	7.81	8.8

PFOS 3	AVG	24.9	7.58	8.3
	SD	0.40	0.06	0.19
	MIN	24.3	7.49	8.1
	MAX	25.7	7.67	8.7

PFNS	AVG	24.7	7.97	8.8
	SD	0.41	0.07	0.19
	MIN	23.4	7.87	8.6
	MAX	25.3	8.07	9.1

PFOSA 1	AVG	24.6	7.95	8.6
	SD	0.45	0.04	0.16
	MIN	23.8	7.91	8.2
	MAX	25.2	8.01	8.6

PFOSA 2	AVG	24.6	7.94	8.6
	SD	0.22	0.04	0.16
	MIN	24.0	7.88	8.4
	MAX	24.9	8	8.9

		Temp °C	pH	D.O. mg/L
PFBSA	AVG	24.7	8.00	8.8
	SD	0.13	0.10	0.24
	MIN	24.4	7.9	8.5
	MAX	25.0	8.14	9.2

PFHxSA	AVG	24.6	8.00	8.9
	SD	0.40	0.07	0.25
	MIN	23.5	7.91	8.5
	MAX	25.2	8.1	9.2

6:2FTS	AVG	24.3	7.92	8.7
	SD	0.29	0.07	0.17
	MIN	23.6	7.81	8.5
	MAX	24.8	8.03	8.9

All tests	AVG	24.6	7.77	8.55
	MIN	23.2	7.33	8.10
	MAX	25.8	8.1	9.20

Average pH by class:

PFCAs	7.67
PFSAs	7.72
FASAs	7.97
FTSAs	7.92

H. azteca		Temp °C	pH	D.O. mg/L
PFBA	AVG	22.7	7.32	8.47
	SD	0.23	0.33	0.77
	MIN	22.2	6.88	5.50
	MAX	23.1	7.67	7.30
PFHxA	AVG	22.6	7.41	8.44
	SD	0.25	0.30	0.48
	MIN	22.2	6.96	5.50
	MAX	23.3	8.04	7.10
PFOA	AVG	22.6	7.31	8.44
	SD	0.26	0.30	0.57
	MIN	21.9	6.85	5.20
	MAX	23.2	7.76	7.00
PFNA	AVG	22.5	7.60	8.99
	SD	0.71	0.23	0.39
	MIN	21.2	7.18	6.20
	MAX	23.9	7.92	7.30
PFDA	AVG	22.5	7.52	9.04
	SD	0.74	0.22	0.45
	MIN	21.1	7.18	6.30
	MAX	23.9	7.91	7.70
PFBS	AVG	22.8	7.14	6.46
	SD	0.26	0.21	0.48
	MIN	22.2	6.89	5.70
	MAX	23.2	7.55	7.10
PFHxS	AVG	22.9	7.07	6.65
	SD	0.30	0.39	0.41
	MIN	22.2	5.52	5.90
	MAX	23.5	7.50	7.10
PFOS	AVG	22.8	7.11	6.60
	SD	0.31	0.22	0.69
	MIN	22.1	6.85	5.33
	MAX	23.3	7.46	7.40
PFNS	AVG	22.9	7.69	9.29
	SD	0.29	0.20	0.64
	MIN	22.3	7.34	6.60
	MAX	23.4	7.94	8.50

		Temp °C	pH	D.O. mg/L
PFDS	AVG	22.9	7.67	9.19
	SD	0.19	0.21	0.80
	MIN	22.6	7.32	6.90
	MAX	23.2	7.95	8.70
PFBSA	AVG	23.3	7.68	8.70
	SD	0.74	0.22	0.31
	MIN	22.2	7.43	5.80
	MAX	25.4	8.08	6.90
PFHxSA	AVG	22.8	7.67	8.69
	SD	0.33	0.21	0.25
	MIN	22.0	7.40	6.10
	MAX	23.6	8.01	7.00
PFOSA	AVG	23.1	7.67	8.73
	SD	0.45	0.18	0.50
	MIN	22.0	7.46	6.20
	MAX	24.3	7.95	8.60
6:2FTS	AVG	23.2	7.57	8.83
	SD	0.50	0.22	0.48
	MIN	22.2	7.25	6.70
	MAX	24.3	7.91	8.50
8:2FTS	AVG	22.6	7.66	
	SD	0.57	0.17	0.22
	MIN	21.5	7.48	6.60
	MAX	23.5	7.93	7.20
10:2FTS	AVG	22.8	7.67	
	SD	0.41	0.18	0.23
	MIN	22.1	7.48	6.60
	MAX	23.5	7.93	7.10
All tests	AVG	22.8	7.49	8.3
	MIN	21.1	5.52	5.20
	MAX	23.1	8.08	8.70

Average pH by class:

PFCAs	7.43
PFSAs	7.34
FASAs	7.68
FTSAs	7.63

C. dilutus		Temp C	pH	D.O. mg/L
PFBA	AVG	22.7	6.92	5.8
	SD	0.22	0.06	0.36
	MIN	22.3	6.83	5.3
	MAX	23.0	6.98	6.4
PFHxA	AVG	23.4	7.28	6.0
	SD	0.37	0.08	0.49
	MIN	22.7	7.12	5.2
	MAX	24.0	7.44	7.0
PFOA 1	AVG	22.7	6.96	6.2
	SD	0.21	0.13	1.0
	MIN	22.2	6.77	4.7
	MAX	23.0	7.19	7.9
PFOA2	AVG	23.3	6.96	5.8
	SD	0.20	0.07	0.51
	MIN	23.0	6.86	5.0
	MAX	23.8	7.11	6.8
PFOA 3	AVG	22.9	7.39	6.3
	SD	1.0	0.08	0.56
	MIN	20.3	7.26	5.3
	MAX	24.0	7.60	7.5
PFNA	AVG	22.8	7.36	6.2
	SD	0.55	0.08	0.53
	MIN	21.6	7.17	5.2
	MAX	23.5	7.44	7.4
PFDA	AVG	22.5	7.13	6.3
	SD	0.24	0.09	0.35
	MIN	21.9	6.97	5.8
	MAX	23.3	7.30	7.0
PFBS	AVG	22.7	7.14	5.8
	SD	0.18	0.12	0.36
	MIN	22.3	6.91	4.9
	MAX	23.1	7.31	6.3
PFHxS	AVG	22.8	7.16	6.0
	SD	0.48	0.10	0.44
	MIN	21.8	6.98	5.4
	MAX	24.0	7.34	6.8

		Temp C	pH	D.O. mg/L
PFHpS	AVG	23.0	6.96	5.4
	SD	0.24	0.10	0.72
	MIN	22.5	6.82	4.2
	MAX	23.7	7.08	6.5
PFOS 1	AVG	22.6	7.01	6.0
	SD	0.44	0.15	0.79
	MIN	21.7	6.54	2.3
	MAX	23.7	7.33	7.7
PFOS 2	AVG	22.9	7.38	6.1
	SD	1.0	0.04	0.37
	MIN	20.5	7.32	5.4
	MAX	23.9	7.47	6.8
PFNS 1	AVG	23.0	7.09	6.2
	SD	0.64	0.07	0.35
	MIN	21.6	6.92	5.6
	MAX	24.4	7.17	6.9
PFNS 2	AVG	22.5	7.43	5.7
	SD	0.30	0.07	0.33
	MIN	22.0	7.26	5.0
	MAX	23.2	7.55	6.3
PFDS	AVG	23.1	7.06	6.1
	SD	0.63	0.06	0.32
	MIN	22.1	6.95	5.7
	MAX	24.3	7.16	6.8
PFBSA	AVG	23.0	7.32	5.8
	SD	0.26	0.03	0.29
	MIN	22.3	7.28	5.1
	MAX	23.5	7.39	6.3
PFHxSA	AVG	23.2	7.34	6.0
	SD	0.23	0.05	0.27
	MIN	22.6	7.25	5.5
	MAX	23.7	7.46	6.5
PFOSA 1	AVG	23.1	7.00	5.8
	SD	0.34	0.06	0.53
	MIN	22.5	6.86	4.3
	MAX	23.6	7.16	7.0

		Temp C	pH	D.O. mg/L
PFOSA 2	AVG	22.6	7.43	5.7
	SD	0.33	0.07	0.27
	MIN	22.0	7.34	5.4
	MAX	23.2	7.60	6.4

		Temp C	pH	D.O. mg/L
6:2 FTS	AVG	23.0	7.29	5.8
	SD	0.31	0.08	0.39
	MIN	22.3	7.14	5.1
	MAX	23.5	7.47	6.6

		Temp C	pH	D.O. mg/L
8:2FTS	AVG	22.9	7.38	6.4
	SD	0.27	0.04	0.24
	MIN	22.2	7.33	6.0
	MAX	23.2	7.43	6.6

		Temp C	pH	D.O. mg/L
10:2FTS	AVG	22.9	7.38	6.4
	SD	0.26	0.06	0.53
	MIN	22.3	7.33	5.6
	MAX	23.2	7.49	7.2

		Temp C	pH	D.O. mg/L
All tests	AVG	22.9	7.20	6.0
	MIN	20.3	6.54	2.3
	MAX	24.4	7.60	7.9

Average pH by class:

PFCAs	7.14
PFSAs	7.15
FASAs	7.22
FTSAs	7.35

S10. *C. dubia* test designs, data, and analyses

S10.1 Overview

This section provides, for each PFAS toxicity test with *Ceriodaphnia dubia,* replicate-level test data and the results of concentration/response (CR) analyses of these data. For all tests, Section S10.2 tabulates the calculated effect concentrations (ECs) for both survival and reproduction, specifying for each test either (a) point EC estimates and confidence limits when test data were sufficient to conduct CR curve estimation or (b) ECs being greater than the highest test concentration, and the magnitude/ significance of effect at this concentration, when CR curves could not be estimated. For each test, Sections S10.3 to S10.24 provide: a table of test data, analysis software output for the survival CR curve estimation when conducted, and output for the reproduction CR curve estimation when conducted. Note, all control concentrations were assigned a value of 1/10th of the lowest treatment for CR analysis.

The output for the survival CR curve estimation has three components:

(1) Header text specifying the taxon ("Cerio"), the chemical, and the endpoint("Survival").

(2) A figure for fraction survival ("Effect Variable") versus the base-10 logarithm of the concentration (uM) that includes observed treatment-level survivals and the estimated CR curve. For tests with at least two partial effects, the solid-black plotted line is the maximum-likelihood solution. For tests with just one partial effect, parameter estimates are not based on a maximum likelihood solution, but rather on the average of their confidence limits, and the red-dashed line is based on those estimates and approximate/illustrative.

(3) A table regarding parameter estimation for the log-logistic CR model. The three model parameters are the base-10 logarithm of the LC50 ("logX50"), the standard deviation of the log-logistic distribution ("logStdDev"), and the control survival ("CtrlSurv"). For these three parameters, the table provides: initial guesses ("Guess") for the nonlinear optimization search, the allowed parameter range ("PMin", "PMax"), and a fraction of the allowed range over which the parameter search is initially restricted ("Delta"). The table then provides final estimates ("PEst") and 95% confidence limits ("95%LCL","95%UCL") for each parameter, as well as for the untransformed LC50, for the logarithm of the LC20 ("logX20"), and for the untransformed LC20. Parameter estimates and CLs in black denote maximum likelihood solution values, whereas parameter estimates in red denote alternative approximations (see text) and CLs in red denote those at their min/max.

The output for the reproduction CR curve estimation has three components:

(1) Header text specifying the taxon ("Cerio"), the endpoint ("Repro"), the chemical, and whether the number of partials effects was at least two ("2+P") to allow maximum likelihood curve fitting or just one ("1P") in which case ECs are approximated as the mean of their calculated confidence limits. The header also specifies whether any replicates were censored due to being outliers ("CensOut") or having parental mortality ("CensMort"), and whether high treatments were censored when not being needed for curve definition ("CensTrt").

(2) A figure that includes replicate-level data ("Number of Offspring in Replicate") versus "Log_{10} Exposure Concentration (uM)", and the estimated CR curve (black line). Data used for curve

estimation are indicated by black circles, with treatment means being a red "bowtie". Replicates censored due to parental mortality are indicated by small red "x"s. Replicates censored due to technical errors (e.g., solution lost or physical damage to parent during transfers) are indicated by large red "X"s. Red circles designate either censored outliers or complete censoring of high concentration treatments. For tests with at least two partial effects, the plotted line is the maximum-likelihood solution. For tests with just one partial effect, the line is approximate, being constrained by a maximum slope of 5.0, in which case the EC estimates were based on the mean of the confidence lines and not the equation for the line.

(3) A table regarding parameter estimation for the log-logistic CR model. The four model parameters are: the base-10 logarithm of the EC50 ("logEC50"), the slope of the line at the EC50 ("Slope"), the control reproduction ("Y0"), and the standard deviation when reproduction is >5 ("Y0SD"; see text for how standard deviation is specified at lower reproduction values). The table provides: initial guesses ("Guess") for these four parameters to use by the nonlinear optimization routine; final parameter estimates ("FinalEst") for the four parameters, for the base-10 logarithm of the EC20 ("logEC20"), and for the untransformed EC50 and EC20; and the lower and upper 95% confidence limits for the ECs ("95%LCL","95%UCL").

S10.2. PFAS Survival LC50s and LC20s, reproduction EC50s and EC20s, and 95% confidence limits (mg/L) for 7-d *C. dubia* tests

Chemical class	Molecular weight g/mol	Abbrev.	LC50	LC20	EC50	EC20
Perfluoroalkyl carboxylic acids	214.04	PFBA	>206	>206	>206	>206
	314.05	PFHxA	>215	>215	321 (235-750)	147 (90.9-217)
	414.07	PFOA	>91	>91	37.3 (33.8-40.8)	26.2 (21.7-32.6)
			>109	>109	35.9 (31.7-40.3)	25.1 (20.1-30.2)
			>57	>57	43.0 (41.0-45.9)	33.8 (31.1-37.5)
	464.08	PFNA			26.2 (23.7-28.8)	23.1 (21.0-25.4)
	514.08	PFDA	>16	>16	14.0 (12.6-15.7)	8.2 (6.4-10.0)
Perfluoroalkyl sulfonic acids	299.09	PFBS	>223	>223	>223	>223
	400.12	PFHxS	>91	>91	26.4 (23.0-30.0)	15.8 (11.8-19.4)
			>85	>85	28.4 (25.6-31.8)	17.3 (13.5-20.9)
	500.13	PFOS	>39	>39	16.0 (14.0-18.3)	10.0 (7.0-13.4)
			>36	>36	17.0 (16.0-18.0)	14.5 (13.1-15.8)
			32 (23-48)	17 (8.1-45)	17.2 (14.4-20.8)	9.8 (6.0-13.2)
	550.14	PFNS	11 (8.6-14.)	6.9 (4.0-9.4)	7.7 (6.4-9.6)	4.8 (3.5-7.1)
			6.1 (3.8-9.0)	2.9 (1.0-4.9)	8.3 (6.2-12.9)	5.4 (3.2-11.9)
	600.15	PFDS	>0.85[*]	>0.85[*]	>0.85[*]	>0.85[*]
Perfluoroalkane sulfonamides	299.12	FBSA	>17	>17	6.9 (5.1-9.4)	2.0 (0.8-3.5)
	399.13	FHxSA	>3.2	>3.2	1.3 (1.1-1.4)	0.95 (0.78-1.2)
	499.15	FOSA	>2.5	>2.5	0.54 (0.50-0.60)	0.41 (0.36-0.47)
Fluorotelomer sulfonic acids	428.17	6:2 FTS	>190	>190	212 (184-274)	124 (96.2-155)
	528.18	8:2 FTS	>2.4[*]	>2.4[*]	>2.4[*]	>2.4[*]
	628.2	10:2 FTS	>0.14[*]	>0.14[*]	>0.14[*]	>0.14[*]

*ECx greater than solubility limit

S10.3 C. dubia PFBA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
5.8* Control	1.2	1	100%	29	28.8	
		1		30		
		1		30		
		1		27		
		1		28		
58	12	1	100%	32	28.2	
		1		24		
		1		26		
		1		29		
		1		30		
114	24	1	80%	31	27.8	
		1		27		
		1		27		
		0		14		Y - mortality
		1		26		
237	51	1	100%	29	26.6	
		1		29		
		1		20		
		1		27		
		1		28		
467	100	1	100%	30	28.4	
		1		29		
		1		24		
		1		30		
		1		29		
962	206	1	100%	27	28.2	
		1		31		
		1		22		
		1		28		
		1		33		

Endpoint	Analysis method
Survival	No significant effects
Reproduction	No significant effects

S10.4 C. dubia PFHxA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
4.1 Control	1.3	1	100%	32	26.2	
		1		24		
		1		23		
		1		19		
		1		33		
41	13	1	100%	33	31.5	
		1		6		Y - outlier
		1		24		
		1		34		
		1		35		
87	27	1	100%	30	31.0	
		1		34		
		1		28		
		1		32		
		1		6		Y - outlier
185	58	1	100%	31	28.4	
		1		26		
		1		30		
		1		27		
		1		28		
353	111	1	100%	26	24.4	
		1		19		
		1		25		
		1		28		
		1		24		
686	215	1	100%	21	20.0	
		1		17		
		1		23		
		1		20		
		1		19		

Endpoint   Analysis method
Survival   No significant effects
Reproduction   Regression

Cerio   Repro   PFHxA   2+P,CensOut   CensMortAll   CensTrtNone
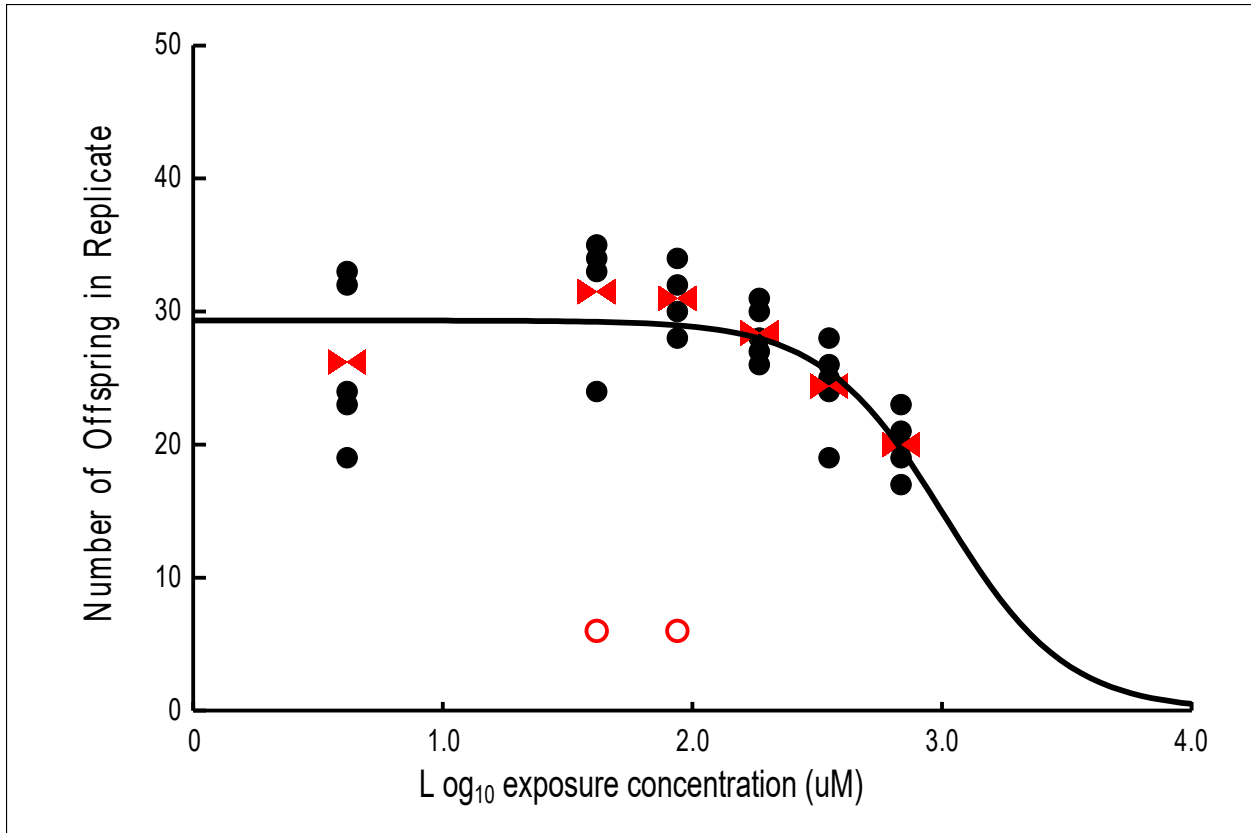
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	2.9000	3.0092	2.8736	3.3778
Slope	2.0000	1.0230		
Y0	26.200	29.327		
Y0SD	5.000	3.860		
logEC20		2.6704	2.4614	2.8405
EC50		1021.38	747.55	2386.98
EC20		468.18	289.36	692.57

S10.5 C. dubia PFOA test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.046 Control 1	0.019	1	100%	34	27.0	
		1		25		
		1		28		
		1		26		
		1		29		
		1		24		
		1		13		
		1		36		
		1		25		
		1		30		
0.046 Control 2	0.019	1	100%	31	25.9	
		1		29		
		1		26		
		1		28		
		1		12		
		1		27		
		1		26		
		1		24		
		1		29		
		1		27		
0.46	0.19	1	100%	31	28.3	
		1		31		
		1		27		
		1		29		
		1		25		
		1		25		
		1		26		
		1		29		
		1		31		
		1		29		
0.75	0.31	1	100%	29	26.0	
		1		30		
		1		26		
		1		23		
		1		23		
		1		28		
		1		28		
		1		28		
		1		19		
		1		25		

1.5	0.62	1	100%	29	26.9	
		1		28		
		1		27		
		1		26		
		1		25		
		1		29		
		1		25		
		1		24		
		1		25		
		1		31		
3.2	1.3	1	90%	25	26.3	
		1		24		
		0		0		Y - mortality
		1		27		
		1		23		
		1		25		
		1		24		
		1		29		
		1		29		
		1		31		
6.5	2.7	1	100%	27	25.3	
		1		28		
		1		23		
		1		27		
		1		28		
		1		26		
		1		23		
		1		30		
		1		21		
		1		20		
13	5.5	1	100%	26	25.6	
		1		26		
		1		25		
		1		24		
		1		26		
		1		26		
		1		19		
		1		31		
		1		27		
		1		26		
27	11	1	80%	25	23.1	
		1		27		
		1		23		
		0		24		Y - mortality
		1		25		
		1		18		
		1		21		

		1		23		
		0		12		Y - mortality
		1		28		
54	22	1	100%	23	23.5	
		1		22		
		1		24		
		1		22		
		1		19		
		1		24		
		1		22		
		1		27		
		1		26		
		1		26		
107	44	1	100%	9	8.7	
		1		8		
		1		8		
		1		10		
		1		9		
		1		13		
		1		6		
		1		5		
		1		12		
		1		7		
219	91	1	100%	0	0.0	Y*
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint   Analysis method
Survival   No significant effects
Reproduction   Regression

Cerio   Repro   PFOA 1   2+P   CensMortAll   CensTrtHigh1
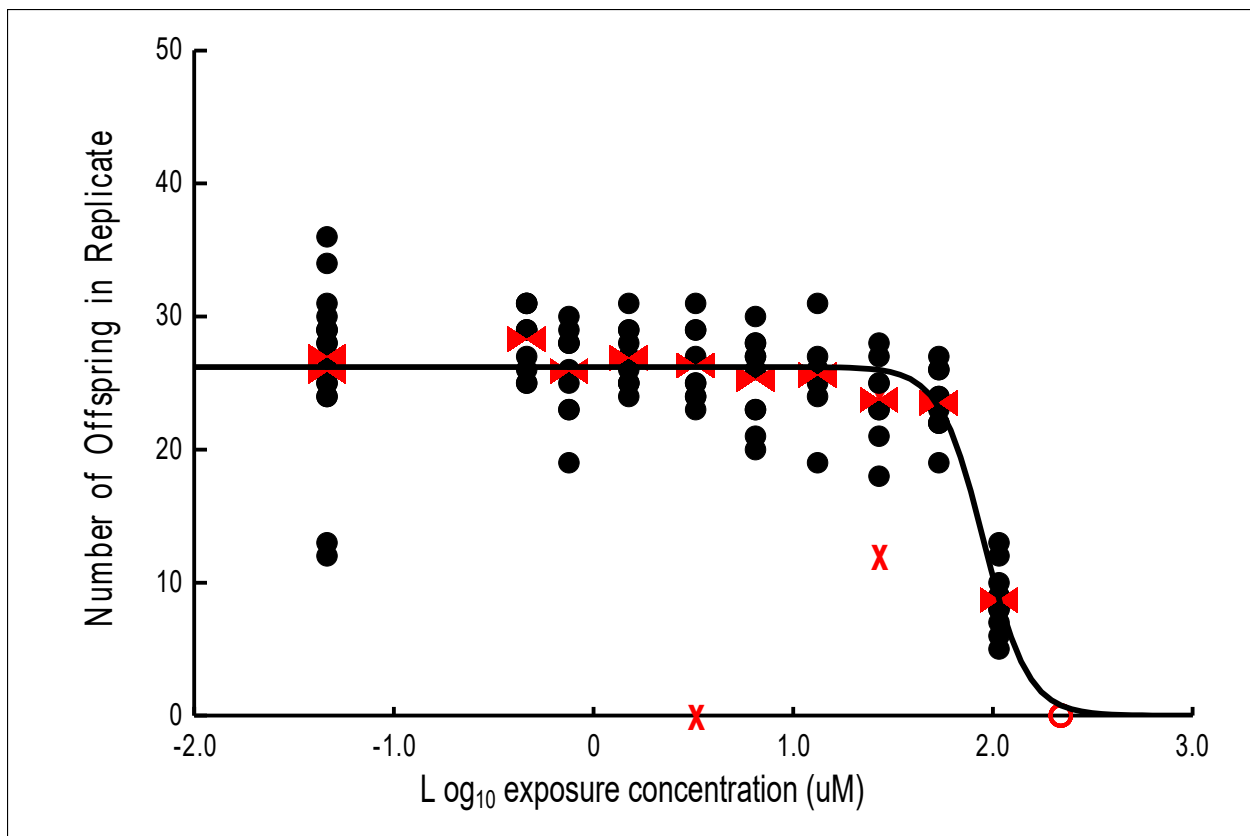
Parameter Summary

Parameter	Guess	Final Est	95% LCL	95% UCL
logEC50	1.9100	1.9542	1.9124	1.9932
Slope	2.0000	2.2634		
Y0	27.000	26.213		
Y0SD	5.000	3.531		
logEC20		1.8011	1.7183	1.8958
EC50		90.00	81.73	98.44
EC20		63.26	52.27	78.66

GLTED_PFAS_CerioReproAnalysi

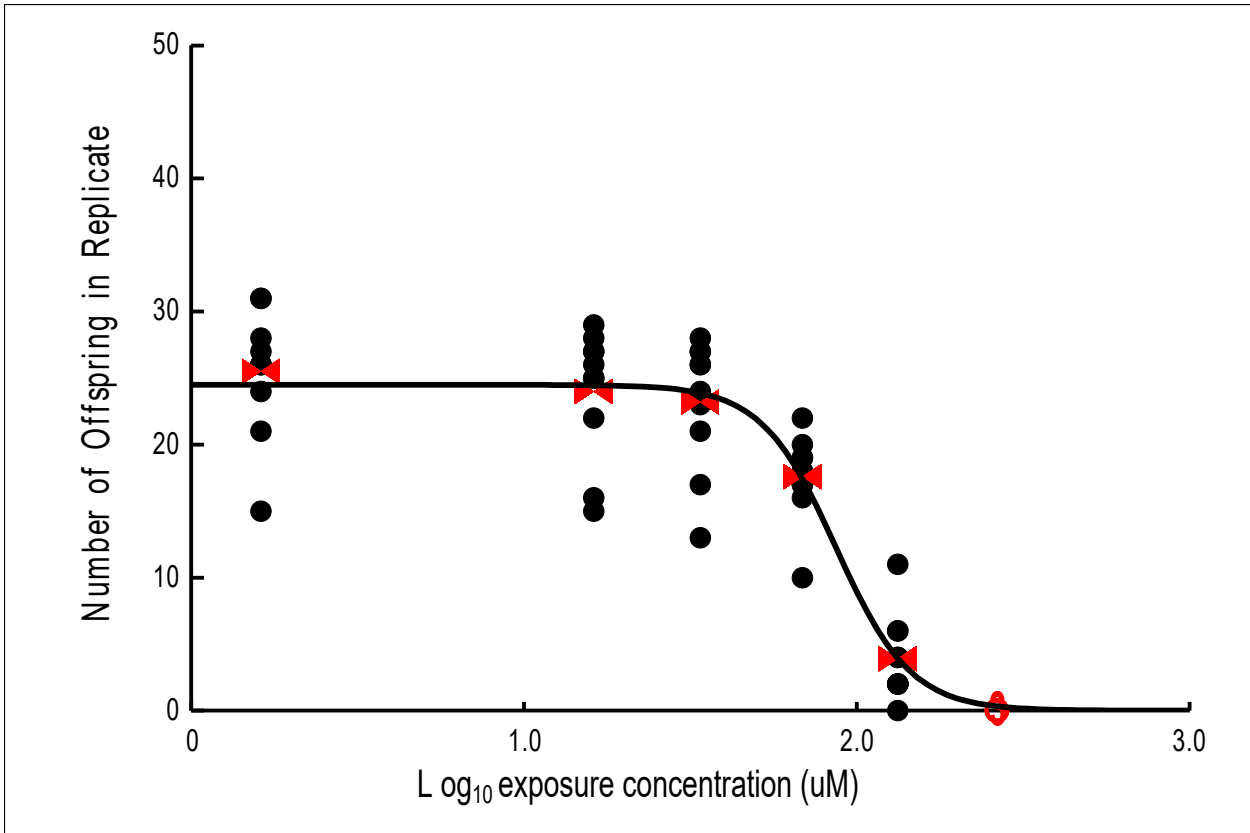## S10.6 C. dubia PFOA test 2 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
1.6 Control	0.67	1	100%	26	25.5	
		1		15		
		1		26		
		1		28		
		1		21		
		1		27		
		1		31		
		1		24		
		1		31		
		1		26		
16	6.7	1	100%	27	24.0	
		1		15		
		1		25		
		1		29		
		1		28		
		1		16		
		1		22		
		1		27		
		1		25		
		1		26		
34	14	1	100%	13	23.2	
		1		24		
		1		27		
		1		23		
		1		26		
		1		17		
		1		27		
		1		21		
		1		28		
		1		26		
69	28	1	100%	18	17.6	
		1		19		
		1		17		
		1		20		
		1		19		
		1		10		
		1		18		
		1		16		
		1		17		
		1		22		

133	55	1	100%	0	3.9	
		1		4		
		1		11		
		1		2		
		1		2		
		1		2		
		1		6		
		1		6		
		1		4		
		1		2		
264	109	1	80%	0	0.0	Y*
		1		0		Y
		0		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		0		0		Y

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method	
Survival	Means comparison	$P = 0.036$
Reproduction	Regression	

Cerio   Repro   PFOA 2   2+P   CensMortAll   CensTrtHigh1

Parameter Summary

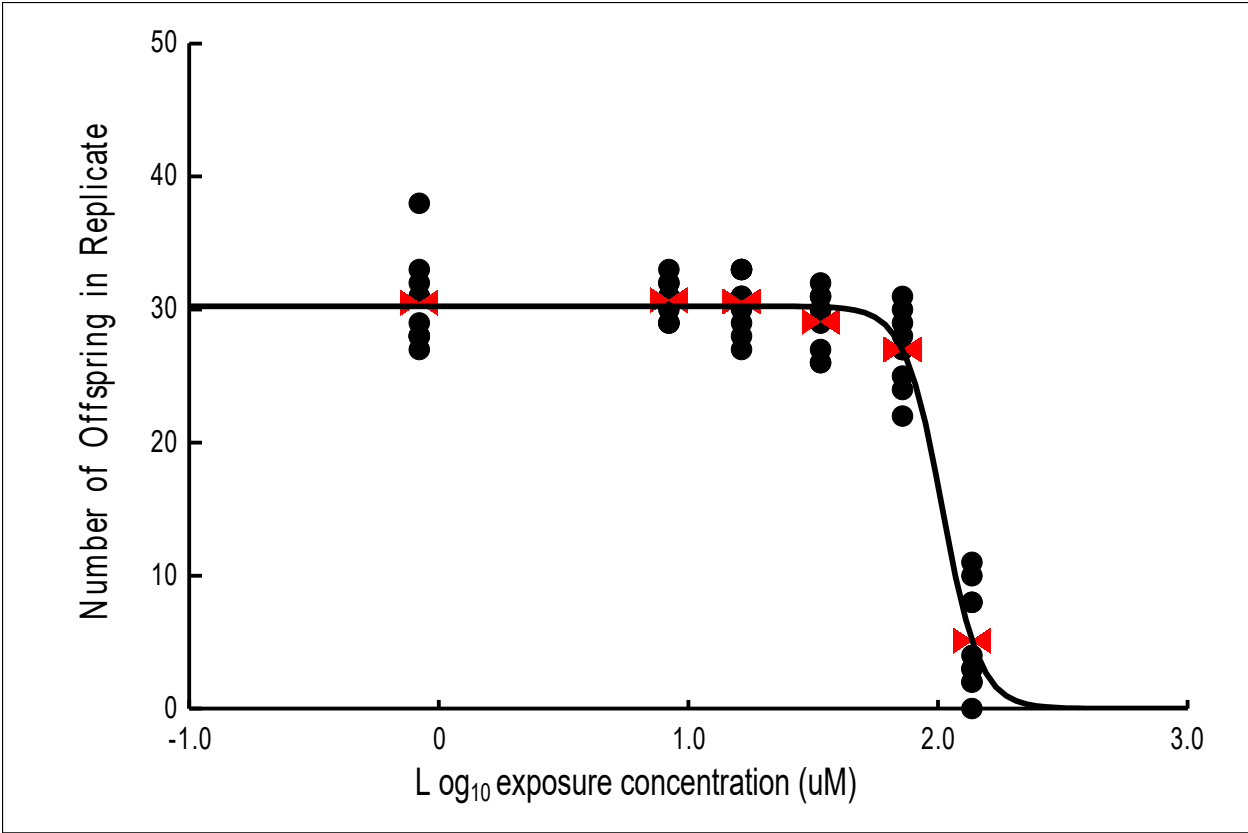| Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	2.0000	1.9379	1.8844	1.9882
Slope	2.0000	2.2342		
Y0	25.500	24.493		
Y0SD	5.000	4.290		
logEC20		1.7828	1.6862	1.8628
EC50		86.68	76.63	97.32
EC20		60.64	48.55	72.92

S10.7 C. dubia PFOA test 3 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.83 Control	0.35	1	100%	28	30.5	
		1		33		
		1		28		
		1		38		
		1		28		
		1		31		
		1		31		
		1		27		
		1		29		
		1		32		
8.3	3.5	1	100%	31	30.7	
		1		33		
		1		31		
		1		31		
		1		32		
		1		29		
		1		30		
		1		29		
		1		29		
		1		32		
16	6.8	1	100%	33	30.6	
		1		31		
		1		29		
		1		33		
		1		33		
		1		30		
		1		27		
		1		28		
		1		31		
		1		31		
34	14	1	100%	29	29.1	
		1		32		
		1		26		
		1		31		
		1		26		
		1		30		
		1		30		
		1		27		
		1		29		
		1		31		

72	30	1	100%	29	27.0	
		1		31		
		1		24		
		1		27		
		1		28		
		1		30		
		1		27		
		1		27		
		1		25		
		1		22		
137	57	1	100%	3	5.1	
		1		8		
		1		8		
		1		11		
		1		2		
		1		2		
		1		10		
		1		3		
		1		0		
		1		4		

Endpoint   Analysis method
Survival   No significant effects
Reproduction   Regression

Cerio   Repro   PFOA 3   2+P   CensMortAll   CensTrtNone

Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	2.0200	2.0165	1.9958	2.0444
Slope	2.0000	3.3275		
Y0	30.500	30.245		
Y0SD	5.000	2.607		
logEC20		1.9124	1.8762	1.9570
EC50		103.88	99.04	110.76
EC20		81.73	75.20	90.58

## S10.8 C. dubia PFNA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.60 Control	0.28	1	100%	15	19.9	
		1		28		
		1		14		
		1		20		
		1		17		
		1		22		
		1		18		
		TE		0		Y - TE
		1		18		
		1		27		
6.0	2.8	1	100%	26	21.1	
		1		21		
		1		25		
		1		21		
		1		20		
		1		19		
		1		25		
		1		16		
		1		14		
		1		24		
12	5.7	1	100%	20	17.4	
		1		19		
		1		20		
		1		19		
		1		21		
		1		18		
		1		17		
		1		16		
		1		20		
		1		4		Y - outlier
25	11	1	80%	17	19.0	
		1		19		
		1		23		
		0		21		Y - mortality
		1		20		
		1		19		
		1		23		
		1		17		
		1		14		
		0		0		Y - mortality

49	23	1	100%	20	15.5	
		1		11		
		1		13		
		1		16		
		1		19		
		1		21		
		1		17		
		1		16		
		1		12		
		1		10		
99	46	0	60%	0	0.0	Y - mortality
		1		0		
		1		0		
		0		0		Y - mortality
		1		0		
		0		0		Y - mortality
		1		0		
		1		0		
		0		0		Y - mortality
		1		0		

TE = Technical Error, replicate censored

Endpoint   Analysis method
Survival   Means comparison $P = 0.006$
Reproduction   Regression

Cerio   Repro   PFNA   1P,CensOut   CensMortAll   CensTrtNone
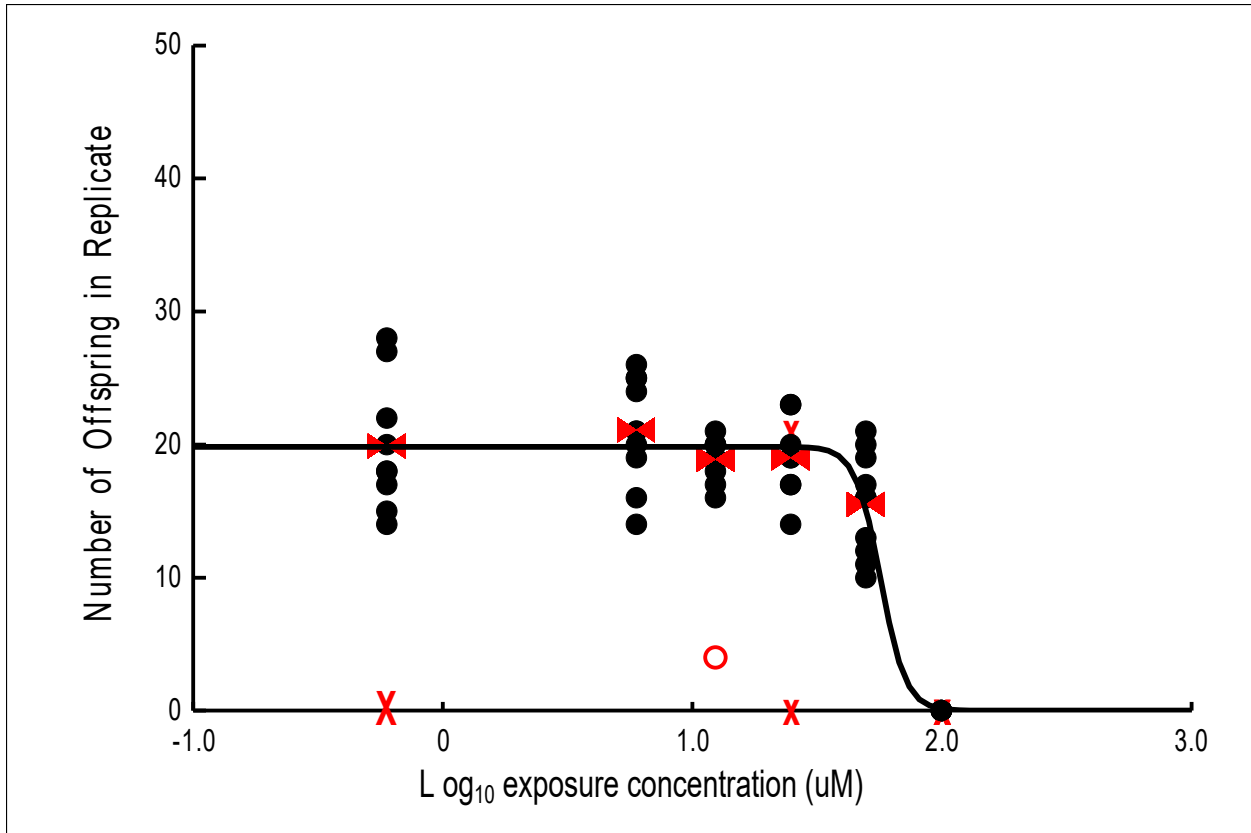
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.7000	1.751	1.7082	1.7934
Slope	4.0000	5.0000		
Y0	20.000	19.834		
Y0SD	4.000	3.575		
logEC20		1.697	1.6550	1.7380
EC50		56.36	51.08	62.15
EC20		48.77	45.19	54.70

S10.9 C. dubia PFDA data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.17 Control	0.09	1	100%	33	31.2	
		1		30		
		1		32		
		1		35		
		1		34		
		1		29		
		1		33		
		1		29		
		1		31		
		1		26		
1.7	0.90	1	100%	34	31.0	
		1		31		
		1		34		
		1		28		
		1		33		
		1		33		
		1		27		
		1		27		
		1		31		
		1		32		
3.9	2.0	1	100%	31	30.0	
		1		31		
		1		33		
		1		27		
		1		35		
		1		33		
		1		29		
		1		25		
		1		24		
		1		32		
8.1	4.2	1	100%	27	28.8	
		1		30		
		1		30		
		1		31		
		1		32		
		1		31		
		1		27		
		1		26		
		1		25		
		1		29		

17	8.6	1	100%	29	24.4	
		TE		0		Y - TE
		1		26		
		1		23		
		1		20		
		1		24		
		1		27		
		1		27		
		1		18		
		1		26		
31	16	1	100%	16	12.7	
		1		16		
		1		10		
		1		19		
		1		13		
		1		4		
		1		20		
		1		4		
		1		8		
		1		17		

TE = Technical Error, replicate censored

Endpoint	Analysis method
Survival	No significant effects
Reproduction	Regression

Cerio   Repro   PFDA   2+P   CensMortAll   CensTrtNone
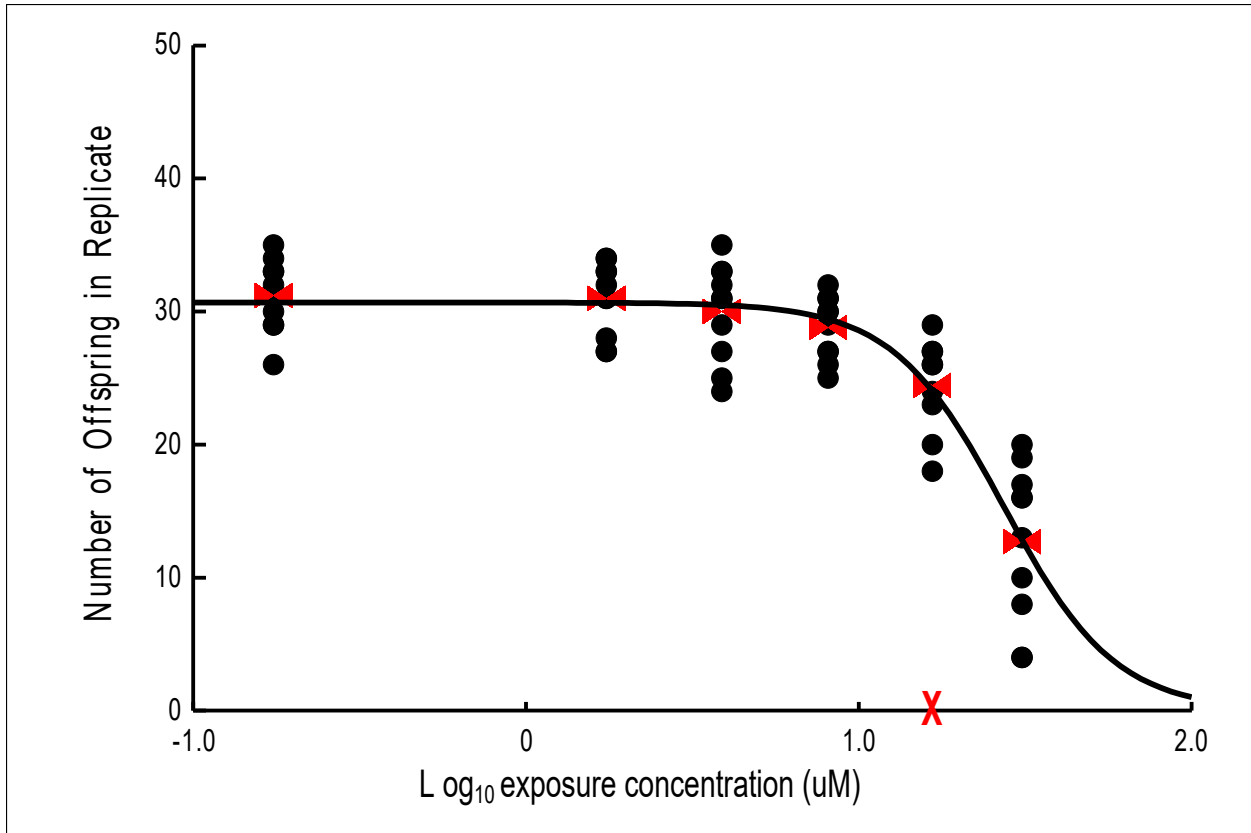
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.3000	1.4358	1.3907	1.4842
Slope	2.0000	1.4970		
Y0	31.200	30.679		
Y0SD	5.000	3.519		
logEC20		1.2043	1.0973	1.2885
EC50		27.28	24.59	30.49
EC20		16.01	12.51	19.43

S10.10 C. dubia PFBS test data and CR analysis output
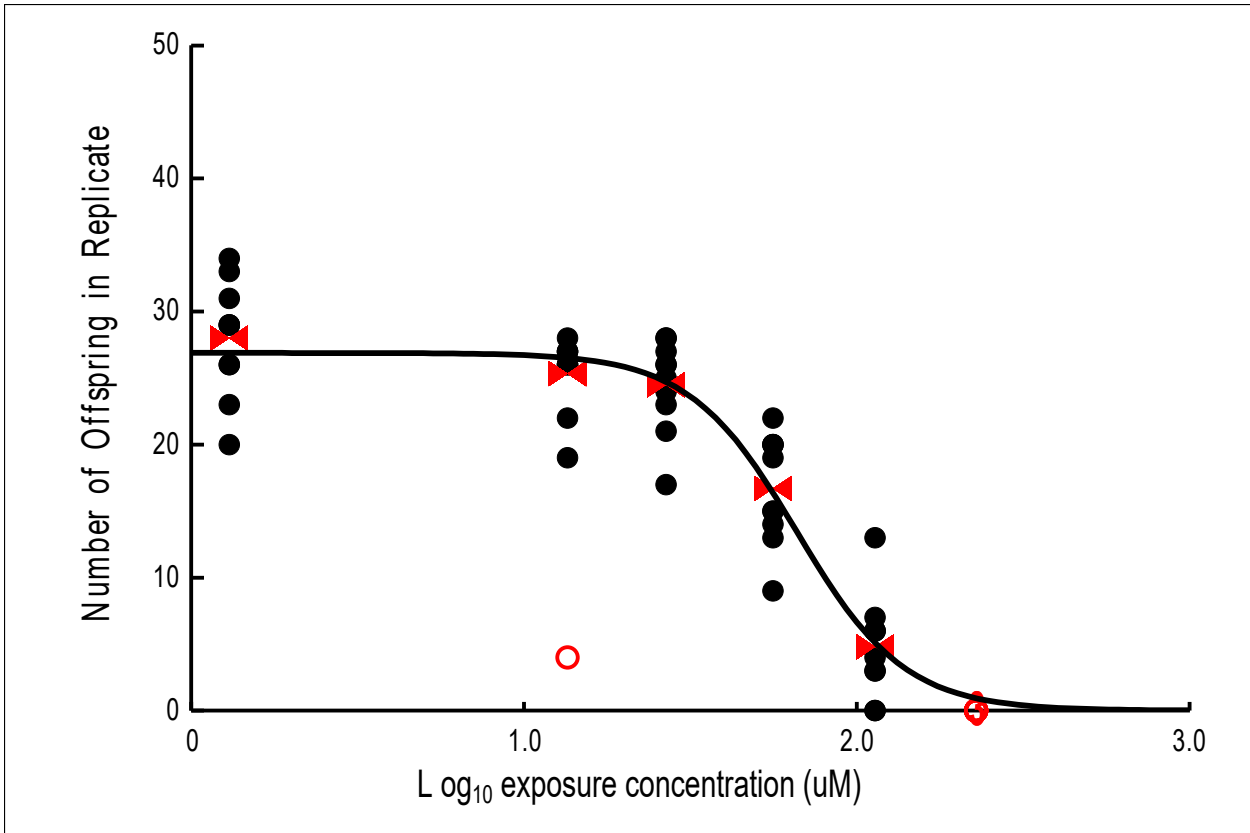
Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
4.6 Control	1.4	1	100%	1	20.8	
		1		28		
		1		28		
		1		23		
		1		24		
46	14	1	100%	22	27.8	
		1		27		
		1		28		
		1		32		
		1		30		
96	29	1	100%	33	31.0	
		1		31		
		1		29		
		1		31		
		1		31		
197	59	1	100%	17	26.6	
		1		29		
		1		27		
		1		32		
		1		28		
377	113	1	100%	30	30.0	
		1		24		
		1		34		
		1		25		
		1		37		
747	224	1	100%	14	25.2	
		1		25		
		1		32		
		1		24		
		1		31		

Endpoint	Analysis method
Survival	No significant effects
Reproduction	No significant effects

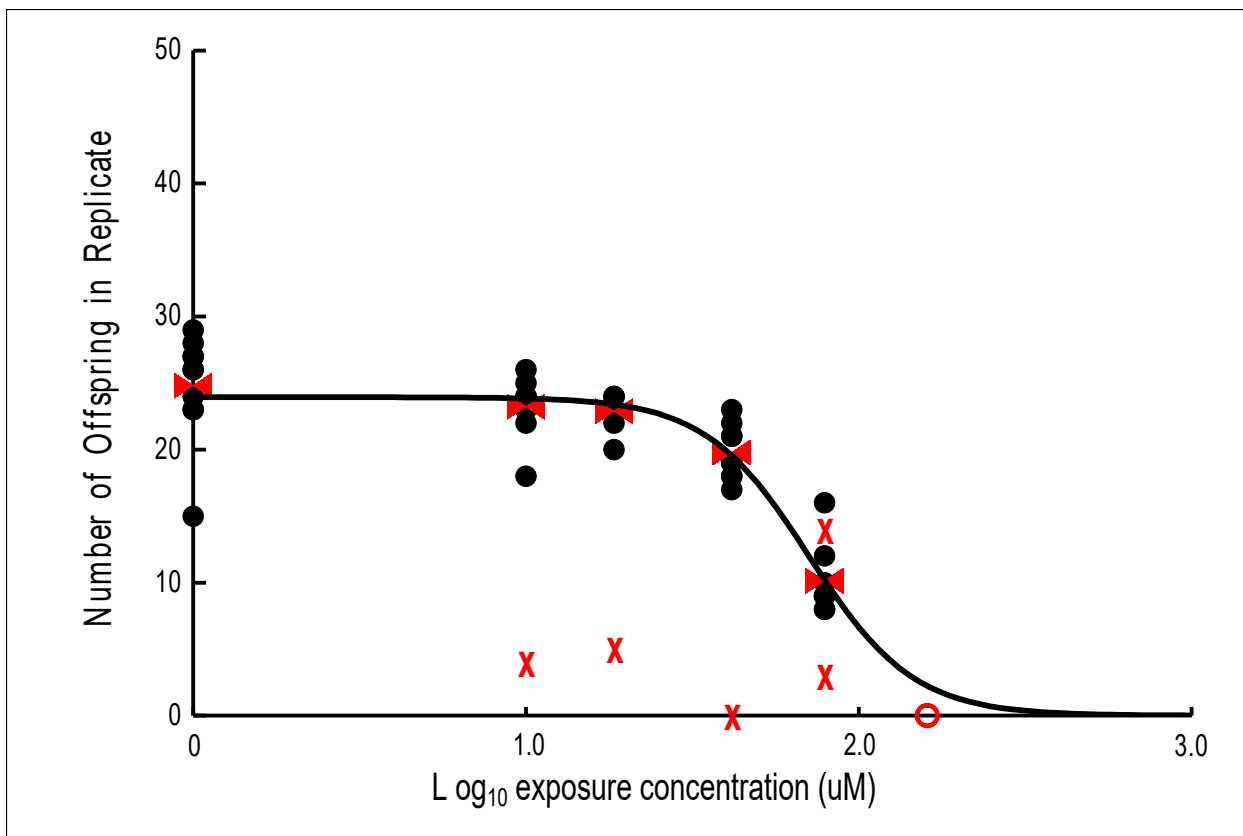## S10.11 C. dubia PFHxS test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
1.3 Control	0.54	1	100%	29	28.0	
		1		34		
		1		29		
		1		31		
		1		29		
		1		33		
		1		26		
		1		20		
		1		23		
		1		26		
13	5.4	1	100%	27	23.2	
		1		28		
		1		4		Y - outlier
		1		26		
		1		27		
		1		26		
		1		26		
		1		22		
		1		19		
		1		27		
27	11	1	100%	24	24.5	
		1		17		
		1		26		
		1		28		
		1		23		
		1		27		
		1		21		
		1		25		
		1		26		
		1		28		
56	22	1	100%	20	16.7	
		1		19		
		1		20		
		1		22		
		1		20		
		1		9		
		1		14		
		1		15		
		1		13		
		1		15		

113	45	1	100%	6	4.8	
		1		0		
		1		0		
		1		6		
		1		3		
		1		13		
		1		7		
		1		3		
		1		4		
		1		6		
228	91	1	80%	0	0.0	Y*
		0		0		Y
		0		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method	
Survival	Means comparison	$P = 0.037$
Reproduction	Regression	

Cerio	Repro	PFHxS1	2+P,CensOut	CensMortAll	CensTrtHigh1

Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	2.0000	1.8199	1.7601	1.8756
Slope	2.0000	1.5445		
Y0	25.920	26.891		
Y0SD	5.000	3.641		
logEC20		1.5955	1.4693	1.6858
EC50		66.06	57.56	75.09
EC20		39.40	29.47	48.50

S10.12 C. dubia PFHxS test 2 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
1.0 Control	0.40	1	100%	23	24.8	
		1		26		
		1		26		
		1		23		
		1		15		
		1		27		
		1		27		
		1		24		
		1		28		
		1		29		
10	4.0	1	90%	24	23.2	
		1		25		
		1		26		
		1		24		
		0		4		Y - mortality
		1		24		
		1		22		
		1		18		
		1		23		
		1		23		
18	7.4	1	90%	24	22.9	
		1		23		
		1		20		
		1		24		
		1		22		
		0		5		Y - mortality
		1		23		
		1		23		
		1		24		
		1		23		
42	17	1	90%	17	19.8	
		1		19		
		1		18		
		1		21		
		1		23		
		1		18		
		0		0		Y - mortality
		1		22		
		1		19		
		1		21		

79	32	0	80%	3	10.1	Y - mortality
		1		12		
		0		14		Y - mortality
		1		9		
		1		9		
		1		16		
		1		10		
		1		8		
		1		9		
		1		8		
161	64	1	100%	0	0.0	Y*
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	No significant effects
Reproduction	Regression

Cerio   Repro   PFHxS 2   2+P   CensMortAll   CensTrtHigh1

Parameter Summary

Parameter	Guess	Final Est	95%LCL	95%UCL
logEC50	1.9000	1.8511	1.8065	1.9001
Slope	2.0000	1.6033		
Y0	20.000	23.939		
Y0SD	5.000	2.549		
logEC20		1.6349	1.5265	1.7171
EC50		70.97	64.05	79.44
EC20		43.14	33.61	52.13

S10.13 C. dubia PFOS test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.45 Control	0.23	1	90%	31	27.9	
		1		23		
		1		29		
		1		26		
		0		9		Y - mortality
		1		30		
		1		25		
		1		31		
		1		28		
		1		28		
4.5	2.3	1	90%	25	23.6	
		1		22		
		1		29		
		1		24		
		1		23		
		1		25		
		0		0		Y - mortality
		1		18		
		1		21		
		1		25		
8.8	4.4	0	67%	13	24.5	Y - mortality
		0		4		Y - mortality
		1		29		
		1		25		
		1		26		
		1		18		
		0		11		Y - mortality
		1		27		
		1		22		
		TE		0		Y - TE
18	8.8	0	80%	5	22.3	Y - mortality
		1		18		
		1		26		
		1		23		
		1		22		
		1		26		
		1		21		
		1		21		
		1		21		
		0		3		Y - mortality

36	18	1	89%	9	10.4	
		1		12		
		1		9		
		1		15		
		1		3		
		1		15		
		1		10		
		1		10		
		0		0		Y - mortality
		TE		0		Y
76	38	1	50%	0	0.0	Y*
		1		0		Y
		1		0		Y
		1		0		Y
		0		0		Y
		0		0		Y
		0		0		Y
		0		0		Y
		0		0		Y
		1		0		Y

TE = Technical Error, replicate censored

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	No significant effects
Reproduction	Regression

Cerio   Repro   PFOS 1   2+P   CensMortAll   CensTrtHigh1
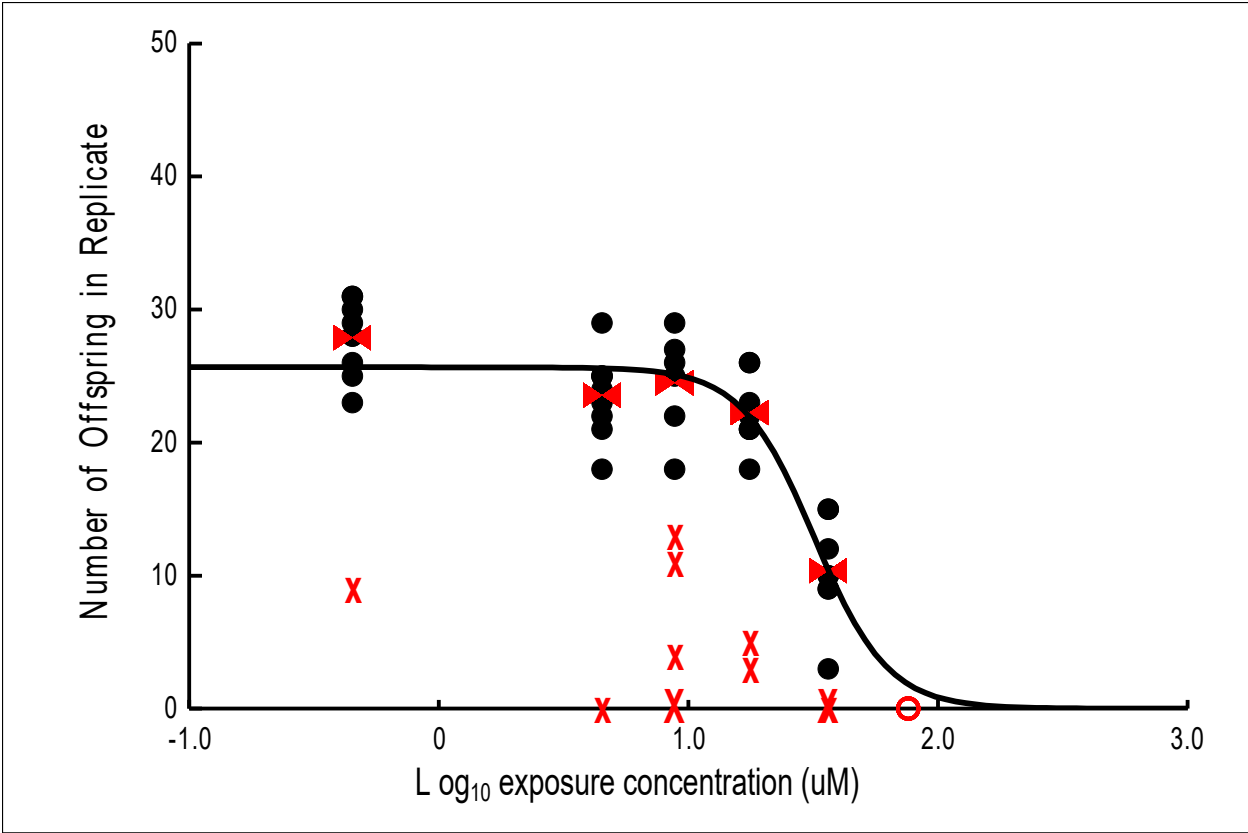
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.5000	1.5053	1.4474	1.5629
Slope	2.0000	1.7076		
Y0	27.889	25.663		
Y0SD	5.000	3.360		
logEC20		1.3023	1.1470	1.4269
EC50		32.01	28.02	36.55
EC20		20.06	14.03	26.73

## S10.14 C. dubia PFOS test 2 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.0098 Control 1	0.0049	1	100%	23	28.1	
		1		31		
		1		29		
		1		33		
		1		24		
		1		25		
		1		26		
		1		26		
		1		32		
		1		32		
0.0098 Control 2	0.0049	1	100%	25	25.8	
		1		36		
		1		24		
		1		32		
		1		21		
		1		22		
		1		23		
		1		24		
		1		25		
		1		26		
0.098	0.049	1	100%	19	23.0	
		1		20		
		1		21		
		1		19		
		1		18		
		1		25		
		1		27		
		1		29		
		1		26		
		1		26		
0.18	0.09	1	90%	24	26.6	
		1		29		
		1		30		
		1		27		
		1		26		
		1		18		
		0		8		Y - mortality
		1		26		
		1		29		
		1		30		

0.41	0.25	1	80%	19	22.6	
		1		28		
		1		22		
		1		25		
		1		5		Y - outlier
		0		0		Y - mortality
		0		11		Y - mortality
		1		24		
		1		31		
		1		27		
0.85	0.42	1	100%	29	26.0	
		1		25		
		1		24		
		1		26		
		1		25		
		1		24		
		1		28		
		1		28		
		1		28		
		1		23		
1.8	0.92	1	90%	24	21.6	
		1		23		
		1		23		
		1		25		
		1		14		
		1		12		
		1		22		
		1		25		
		1		26		
		0		0		Y - mortality
3.8	1.9	1	90%	21	21.3	
		1		22		
		1		27		
		1		19		
		1		14		
		1		19		
		0		11		Y - mortality
		1		12		
		1		30		
		1		28		
7.8	3.9	1	100%	22	22.3	
		1		27		
		1		23		
		1		21		
		TE		0		Y - TE
		1		26		
		1		12		

		1		21		
		1		27		
		1		22		
16	8.0	1	100%	25	20.6	
		1		15		
		1		22		
		1		26		
		1		25		
		TE		0		Y - TE
		1		18		
		1		24		
		1		14		
		1		16		
33	17	1	100%	0	13.6	Y - outlier
		1		17		
		1		13		
		1		13		
		1		16		
		1		15		
		1		11		
		1		12		
		1		11		
		1		14		
70	35	1	100%	0	0.0	Y*
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y
		1		0		Y

TE = Technical Error, replicate censored

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	No significant effects
Reproduction	Regression

Cerio Repro PFOS 2 1P CensOut CensMortAll CensTrtNone
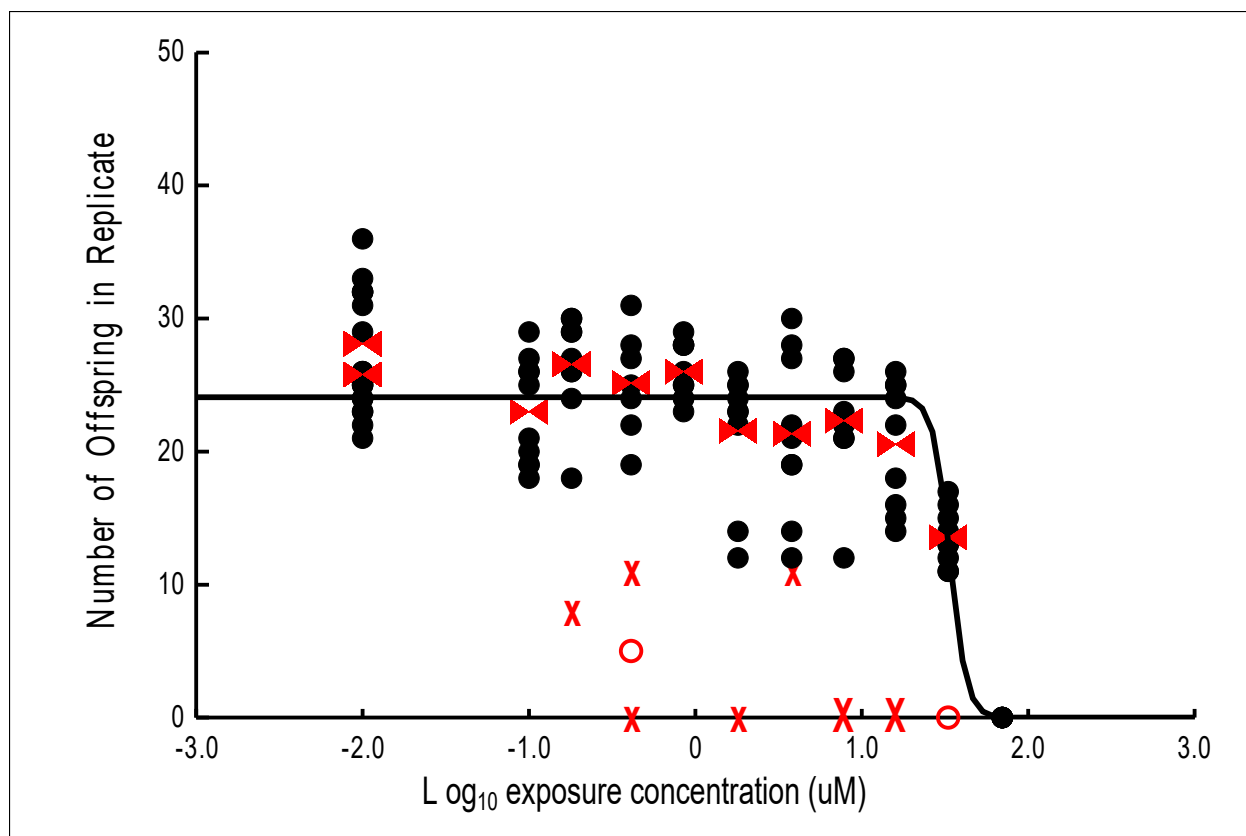
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.6000	1.5301	1.5036	1.5565
Slope	2.0000	5.0000		
Y0	25.000	24.097		
Y0SD	4.000	4.632		
logEC20		1.4613	1.4169	1.5057
EC50		33.89	31.88	36.01
EC20		28.93	26.12	32.04

S10.15 C. dubia PFOS test 3 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.53 Control	0.27	1	90%	28	28.1	
		1		28		
		1		32		
		1		31		
		1		26		
		1		30		
		1		29		
		0		0		Y - mortality
		1		28		
		1		21		
5.3	2.7	1	100%	20	23.8	
		1		21		
		1		29		
		1		32		
		1		28		
		1		22		
		1		27		
		1		16		
		1		17		
		1		26		
12	5.9	1	100%	18	24.1	
		1		19		
		1		28		
		1		22		
		1		25		
		1		29		
		1		24		
		1		27		
		1		23		
		1		26		
23	11	0	90%	4	19.4	Y - mortality
		1		17		
		1		15		
		1		22		
		1		25		
		1		21		
		1		21		
		1		17		
		1		19		
		1		18		

45	23	1	80%	0	7.5	
		1		8		
		1		6		
		1		16		
		0		7		Y - mortality
		1		0		
		1		14		
		1		8		
		0		0		Y - mortality
		1		8		
92	46	0	20%	0	0.0	Y
		0		0		Y
		0		0		Y
		0		0		Y
		0		0		Y
		1		0		Y
		1		0		Y
		0		0		Y
		0		0		Y
		0		0		Y

*High exposures with no reproduction were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	Regression
Reproduction	Regression

Cerio PFOS 3 Survival
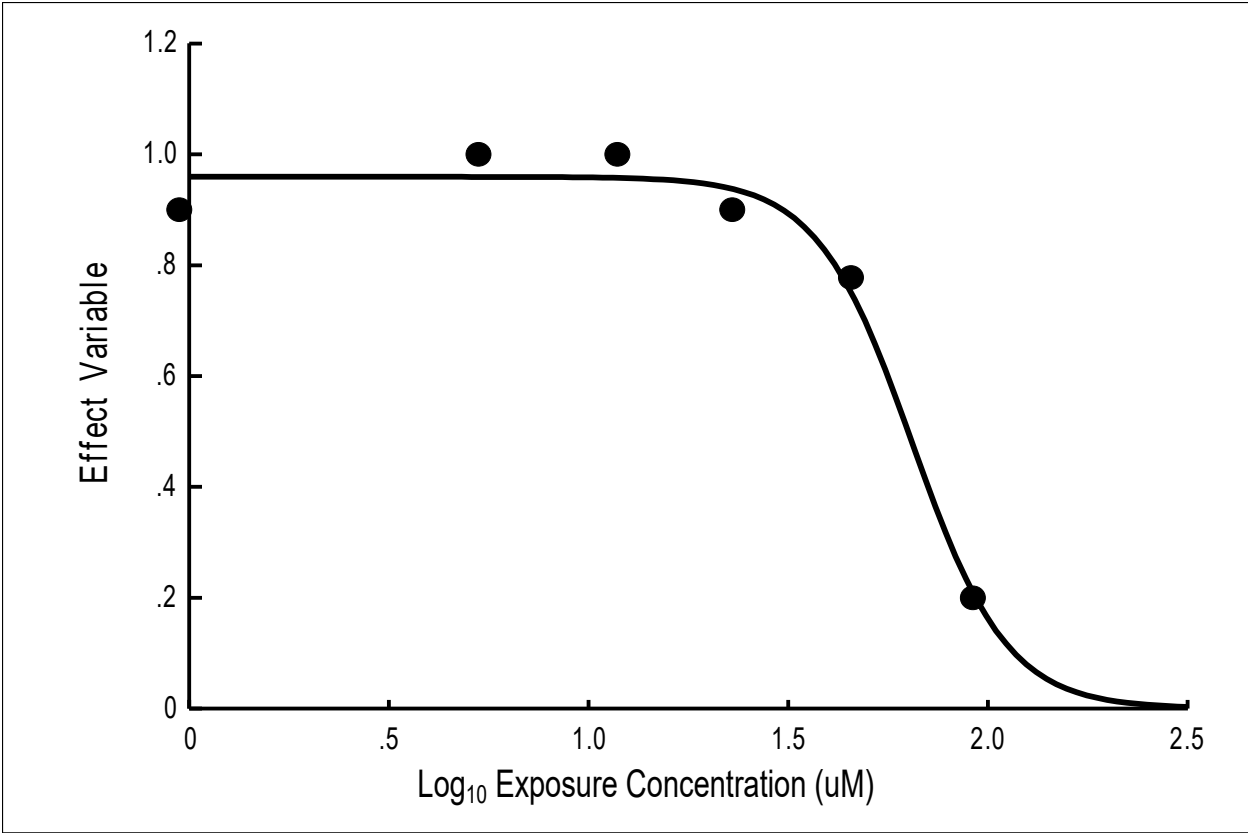
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	1.7041	0.5000	1.4041	2.0041	1.8101	1.6636	1.9787
					64.58	46.09	95.20
logStdDev	0.2869	0.5000	0.0100	0.5000	0.2162	0.0100	0.5000
CtrlSurv	0.9500	0.5000	0.8000	1.0000	0.9595	0.8584	0.9975
logX20					1.6448	1.4038	1.9524
					44.14	25.34	89.62

Cerio   Repro   PFOS 3   2+P   CensMortAll   CensTrtHigh1
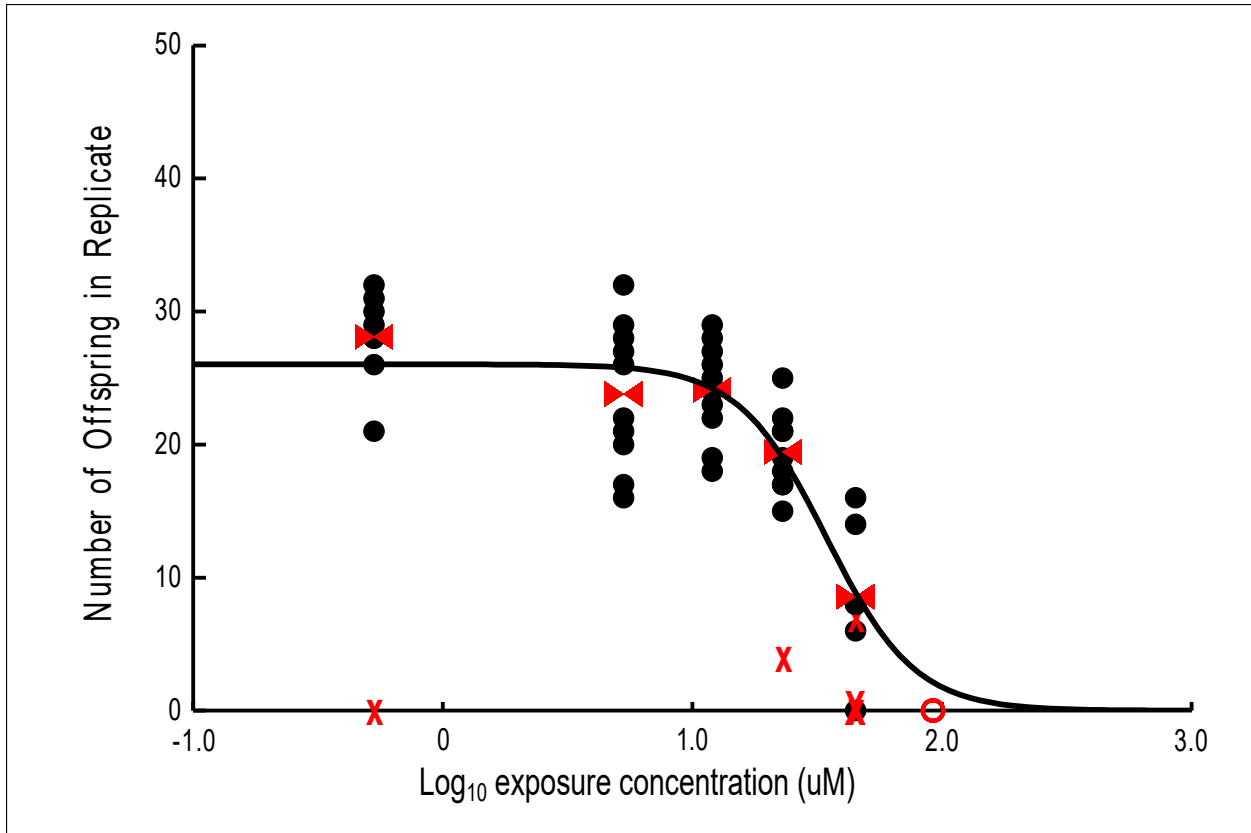
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.5000	1.5373	1.4601	1.6188
Slope	2.0000	1.4164		
Y0	25.864	26.037		
Y0SD	5.000	4.169		
logEC20		1.2926	1.0817	1.4216
EC50		34.46	28.85	41.57
EC20		19.61	12.07	26.40

S10.16 C. dubia PFNS test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.22 Control	0.12	1	100%	23	30.4	
		1		28		
		1		32		
		1		31		
		1		34		
		1		28		
		1		34		
		1		31		
		1		33		
		1		30		
2.2	1.2	1	90%	23	25.2	
		1		21		
		0		15		Y - mortality
		1		28		
		1		25		
		1		27		
		1		26		
		1		26		
		1		25		
		1		26		
4.3	2.4	1	100%	2	21.0	Y - outlier
		1		20		
		1		27		
		1		27		
		1		24		
		1		16		
		1		18		
		1		25		
		1		24		
		1		27		
8.9	4.9	1	100%	21	20.0	
		1		20		
		1		11		
		1		21		
		1		22		
		1		22		
		1		20		
		1		20		
		1		21		
		1		22		

17	9.5	1	60%	17	13.7	
		0		9		Y - mortality
		1		12		
		0		8		Y - mortality
		1		14		
		1		10		
		0		10		Y - mortality
		1		13		
		1		16		
		0		10		Y - mortality
33	18	1	10%	0	0.0	
		0		1		
		0		1		
		0		0		Y*
		0		1		
		0		0		Y
		0		2		
		0		2		Y
		0		1		
		0		0		Y

*In the high treatment, only mortalities that occurred early in the test were censored. Reproduction from females that died on day 6 or 7 was included because it provides information that anchors this treatment as a near-total effect concentration.

Endpoint	Analysis method
Survival	Regression
Reproduction	Regression

Cerio PFNS 1 Survival
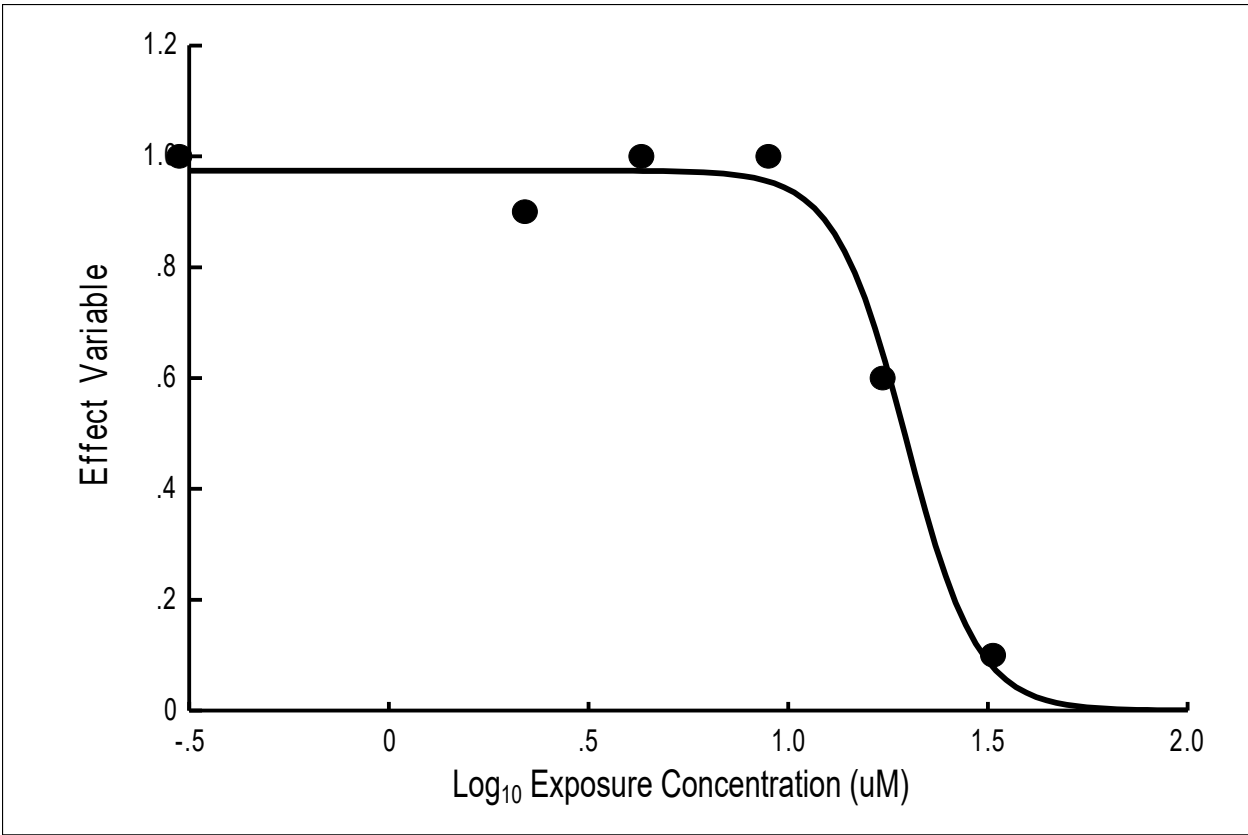
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	1.2627	0.5000	0.9627	1.5627	1.2959	1.1917	1.4113
					19.77	15.55	25.78
logStdDev	0.2084	0.5000	0.0100	0.5000	0.1612	0.0823	0.3418
CtrlSurv	0.9750	0.5000	0.8000	1.0000	0.9744	0.8921	1.0000
logX20					1.1727	1.0013	1.2887
					14.88	10.03	19.44

Cerio   Repro   PFNS1   2+P,CensOut   CensMortAll   CensTrtNone
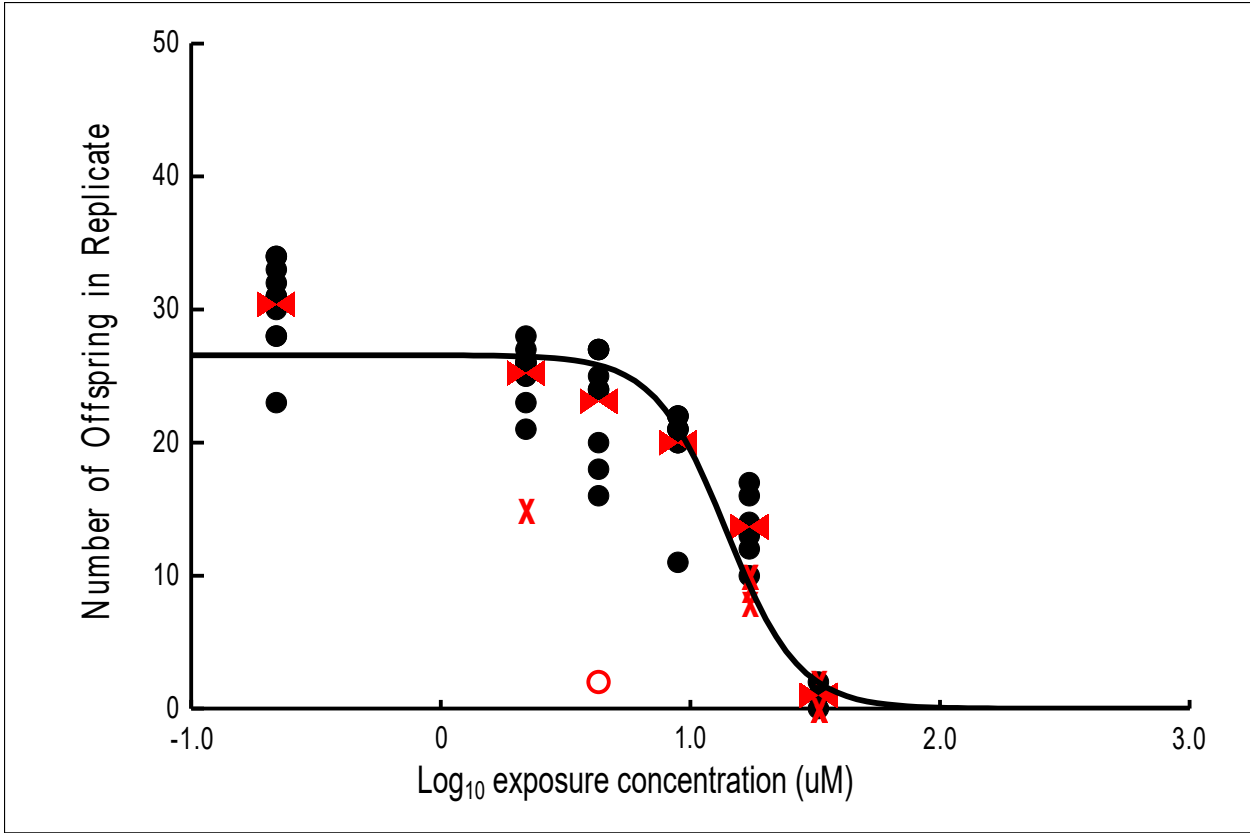
Parameter Summary

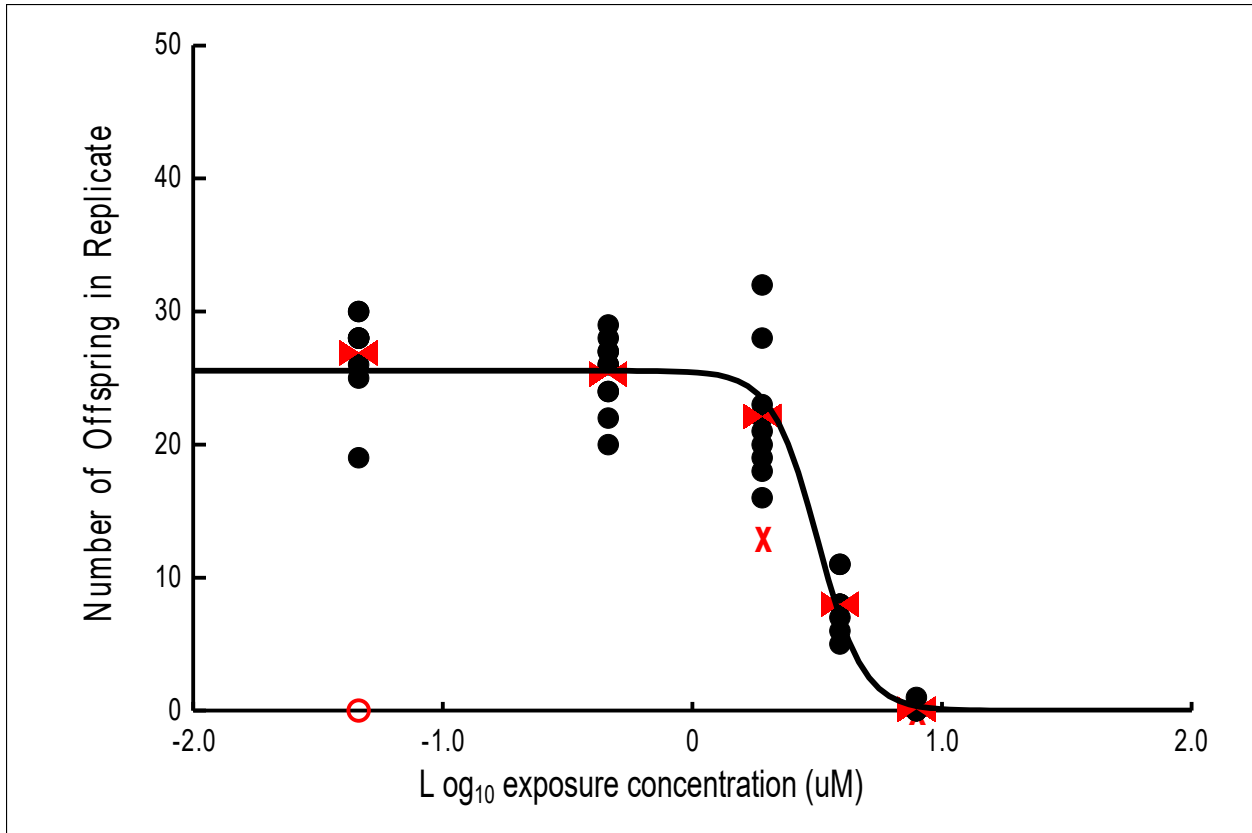| Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.2000	1.1460	1.0625	1.2426
Slope	1.5000	1.7233		
Y0	25.000	26.574		
Y0SD	4.000	4.143		
logEC20		0.9449	0.7985	1.1132
EC50		14.00	11.55	17.48
EC20		8.81	6.29	12.98

S10.17 C. dubia PFNS test 2 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.073 Control	0.040	1	100%	19	23.0	
		1		12		
		1		18		
		1		26		
		1		25		
		1		27		
		1		27		
		1		28		
		1		23		
		1		25		
0.73	0.40	1	100%	12	13.6	
		1		6		
		1		11		
		1		16		
		1		10		
		1		11		
		1		18		
		1		17		
		1		23		
		1		12		
2.0	1.1	1	90%	16	16.4	
		1		17		
		1		18		
		1		7		
		1		16		
		1		23		
		1		18		
		1		13		
		1		20		
		0		0		Y - mortality
4.3	2.4	1	100%	14	18.6	
		1		17		
		1		15		
		1		23		
		1		13		
		1		24		
		1		18		
		1		21		
		1		22		
		1		19		

11	5.8	0	67%	15	11.6	Y - mortality
		1		10		
		TE		0		Y
		0		11		Y - mortality
		1		12		
		1		12		
		0		5		Y - mortality
		1		16		
		1		15		
		1		16		
26	14	0	10%	3	4.0	
		0		2		
		0		4		
		0		3		
		0		5		Y*
		0		1		
		0		0		
		0		5		
		0		0		Y
		1		4		

TE = Technical Error, replicate censored

*In the high treatment, only mortalities that occurred early in the test were censored. Reproduction from females that died on day 6 or 7 was included because it provides information that anchors this treatment as a near-total effect concentration.

Endpoint	Analysis method
Survival	Regression
Reproduction	Regression

Cerio PFNS 2 Survival
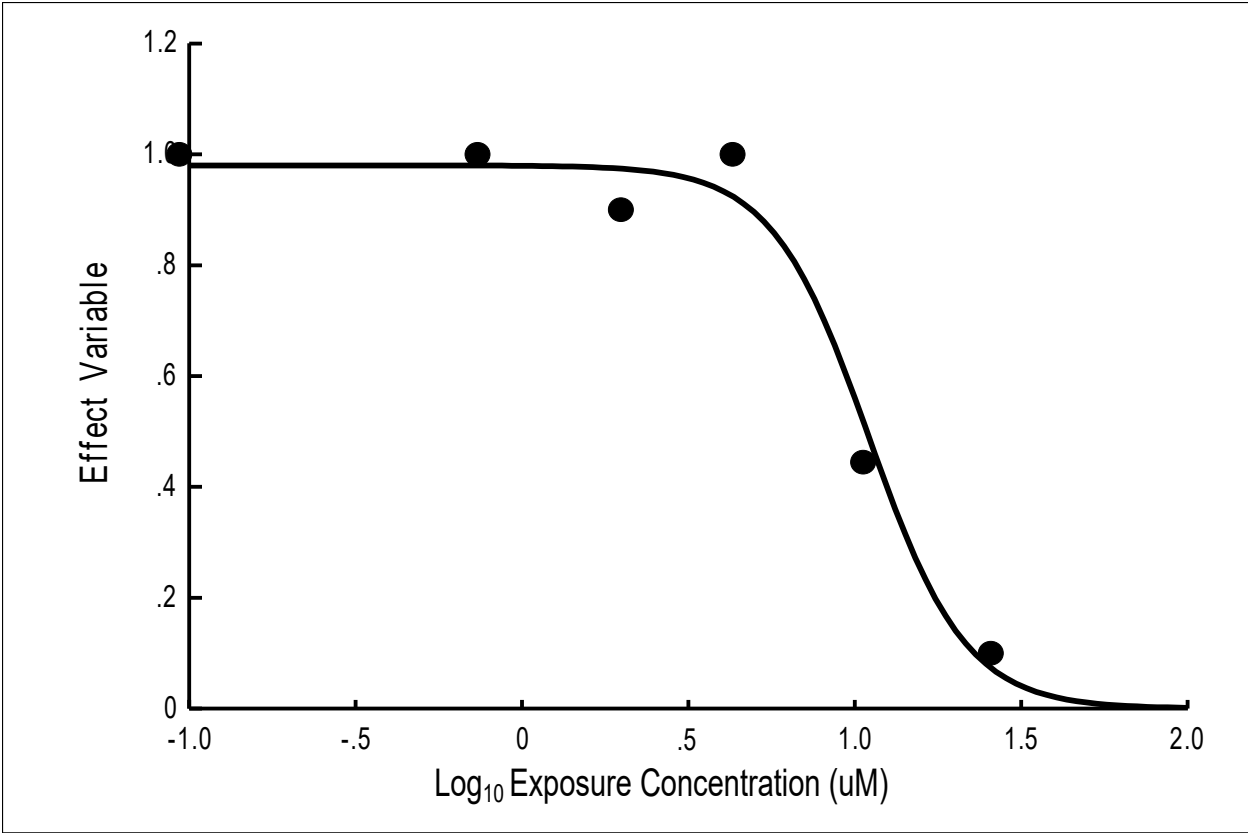
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	1.0637	0.5000	0.7637	1.3637	1.0422	0.8393	1.2150
					11.02	6.91	16.41
logStdDev	0.2872	0.5000	0.0100	0.5000	0.2646	0.1217	0.5000
CtrlSurv	0.9750	0.5000	0.8000	1.0000	0.9802	0.8948	1.0000
logX20					0.8400	0.4917	1.0293
					6.92	3.10	10.70

Cerio   Repro   PFNS 2   2+P   CensMortAll   CensTrtNone
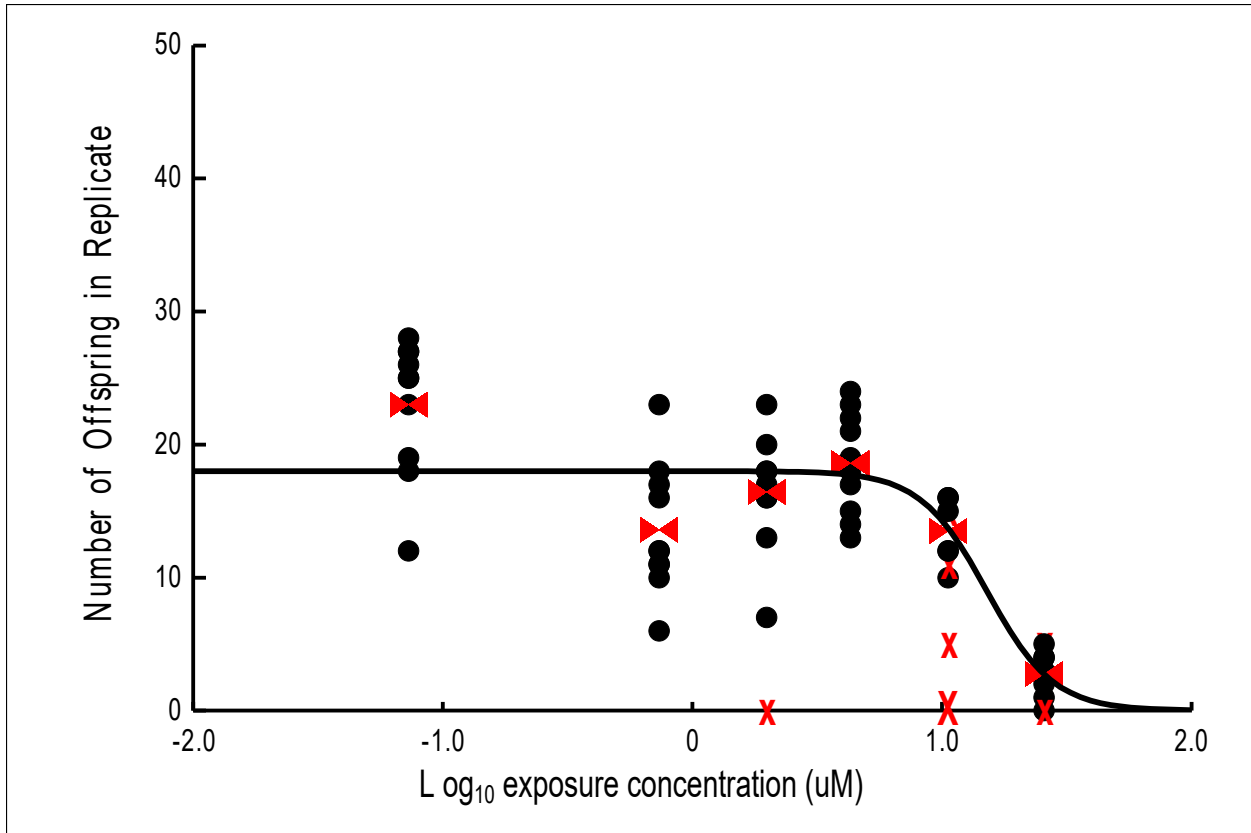
Parameter Summary

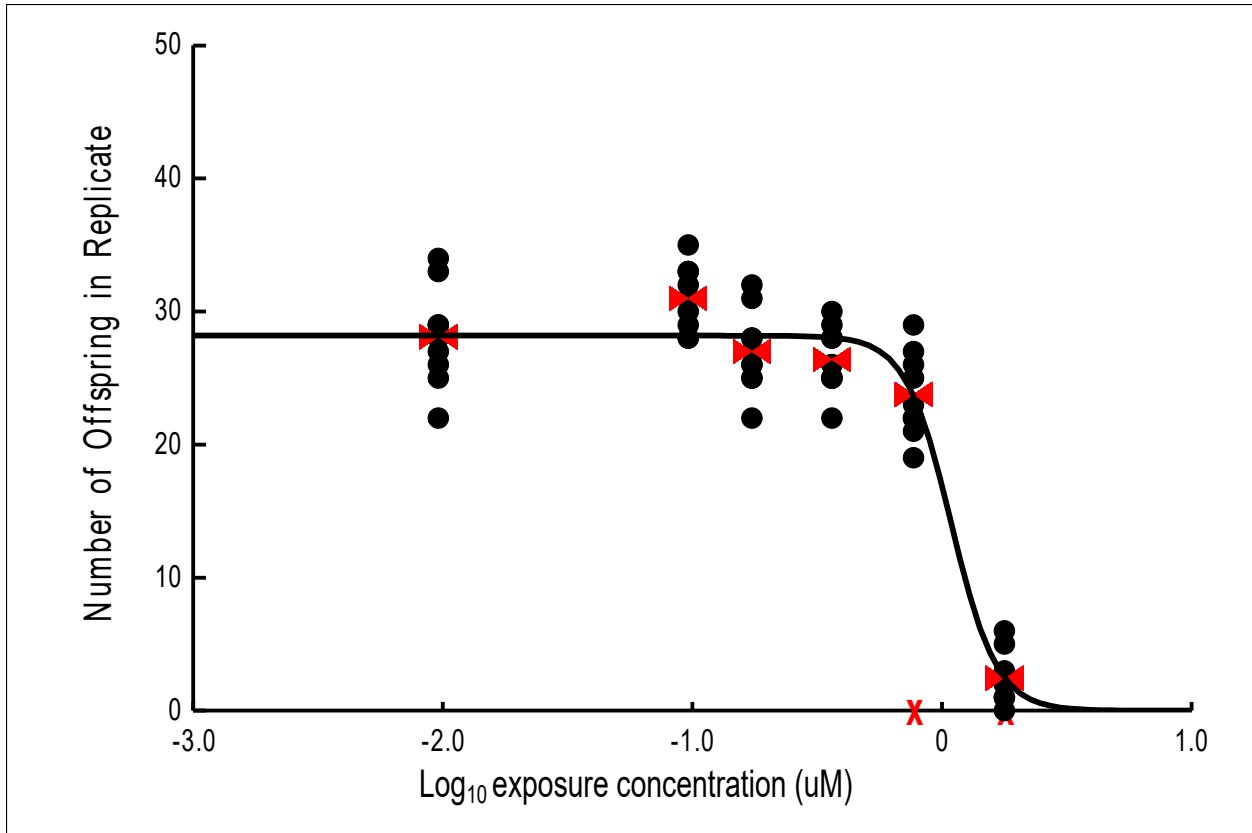| Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.0000	1.1794	1.0499	1.3708
Slope	1.5000	1.8749		
Y0	20.000	18.006		
Y0SD	4.000	5.265		
logEC20		0.9945	0.7655	1.3361
EC50		15.11	11.22	23.48
EC20		9.87	5.83	21.68

S10.18 C. dubia PFDS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.14 Control	0.085	1	90%	25	22.7	
		1		16		
		1		12		
		1		28		
		0		11		Y - mortality
		1		30		
		1		19		
		1		30		
		1		29		
		1		15		
1.4	0.85	1	100%	19	17.8	
		1		15		
		1		16		
		1		16		
		TE		0		Y
		1		14		
		1		20		
		1		19		
		1		23		
		1		18		

TE = Technical Error, replicate censored

Endpoint   Analysis method
Survival   No significant effects
Reproduction   Means comparison $P = 0.0426$

S10.19 C. dubia FBSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.34 Control	0.10	1	100%	27	27.3	
		1		26		
		1		24		
		1		32		
		1		28		
		1		26		
		1		26		
		1		28		
		1		25		
		1		31		
3.4	1.0	1	100%	20	23.6	
		1		25		
		1		22		
		1		21		
		1		27		
		1		27		
		1		21		
		1		26		
		1		19		
		1		28		
6.8	2.0	1	100%	23	22.0	
		1		22		
		1		28		
		1		22		
		1		18		
		1		22		
		1		22		
		1		24		
		1		20		
		1		19		
14	4.1	1	100%	21	18.0	
		1		19		
		1		15		
		1		14		
		1		14		
		1		21		
		1		22		
		1		23		
		1		16		
		1		15		

28	8.4	1	100%	15	11.6	
		1		11		
		1		5		
		1		13		
		1		0		
		1		18		
		1		17		
		1		4		
		1		18		
		1		15		
57	17	0	80%	0	1.4	Y - mortality
		1		4		
		1		3		
		1		0		
		1		0		
		1		2		
		1		0		
		1		1		
		0		0		Y - mortality
		1		1		

Endpoint   Analysis method
Survival   Means comparison $P = 0.036$
Reproduction   Regression

| Cerio | Repro | FBSA | 2+P,CensHi | CensMortAll | CensTrtNone |
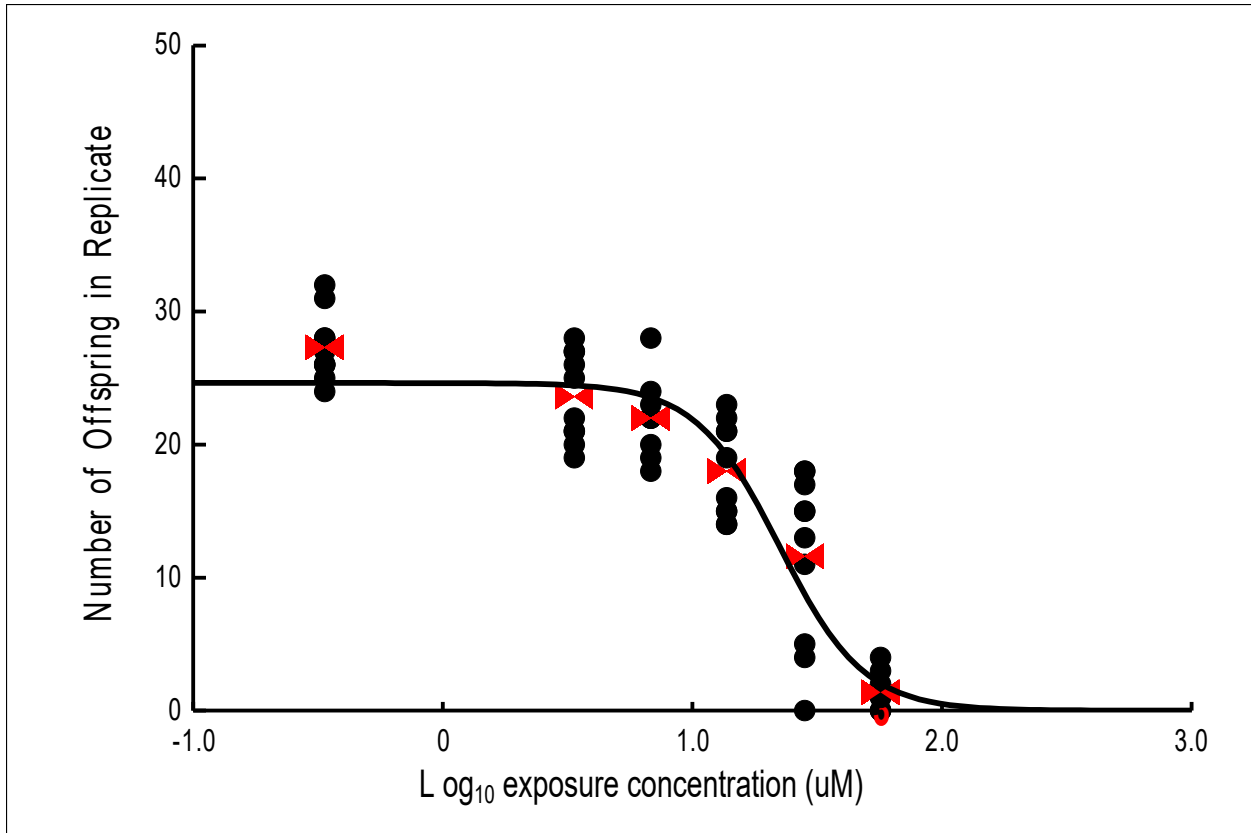
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	1.4000	1.3502	1.2536	1.4436
Slope	2.0000	1.4868		
Y0	27.300	24.630		
Y0SD	5.000	4.264		
logEC20		1.1171	0.9534	1.2764
EC50		22.40	17.93	27.77
EC20		13.10	8.98	18.90

GLTED_PFAS_CerioReproAnalysi

S10.20 C. dubia FHxSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.046 Control	0.018	1	100%	25	24.2	
		1		28		
		1		0		Y - outlier
		1		30		
		1		28		
		1		26		
		1		28		
		1		30		
		1		28		
		1		19		
0.46	0.18	1	100%	26	25.3	
		1		28		
		1		24		
		1		20		
		1		22		
		1		24		
		1		29		
		1		27		
		1		26		
		1		27		
1.9	0.76	1	90%	18	22.1	
		0		13		Y - mortality
		1		21		
		1		22		
		1		23		
		1		28		
		1		16		
		1		32		
		1		20		
		1		19		
3.9	1.6	1	100%	8	8.0	
		1		11		
		1		8		
		1		8		
		1		6		
		1		11		
		1		8		
		1		7		
		1		8		
		1		5		

7.9	3.2	1	90%	0	0.1	
		1		0		
		1		0		
		0		0		Y - mortality
		1		0		
		1		0		
		1		0		
		1		1		
		1		0		
		1		0		

Endpoint	Analysis method
Survival	No significant effects
Reproduction	Regression

| Cerio | Repro | FHxSA | 2+P,CensOut | CensMortAll | CensTrtNone |

Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	0.4600	0.5026	0.4454	0.5599
Slope	2.0000	2.7218		
Y0	24.200	25.548		
Y0SD	5.000	3.381		
logEC20		0.3753	0.2898	0.4829
EC50		3.18	2.79	3.63
EC20		2.37	1.95	3.04

GLTED_PFAS_CerioReproAnalysi

S10.21 C. dubia FOSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.0096 Control	0.0048	1	100%	29	28.1	
		1		28		
		1		25		
		1		26		
		1		28		
		1		34		
		1		33		
		1		29		
		1		22		
		1		27		
0.096	0.048	1	100%	31	31.0	
		1		35		
		1		29		
		1		30		
		1		31		
		1		33		
		1		33		
		1		28		
		1		28		
		1		32		
0.17	0.086	1	100%	28	27.0	
		1		31		
		1		32		
		1		26		
		1		28		
		1		26		
		1		25		
		1		27		
		1		22		
		1		25		
0.36	0.18	1	100%	26	26.4	
		1		25		
		1		26		
		1		28		
		1		28		
		1		25		
		1		30		
		1		22		
		1		25		
		1		29		

0.77	0.38	1	90%	29	23.8	
		1		21		
		0		0		Y - mortality
		1		23		
		1		25		
		1		22		
		1		27		
		1		26		
		1		19		
		1		22		
1.8	0.89	0	90%	0	2.4	Y - mortality
		1		1		
		1		2		
		1		1		
		1		2		
		1		6		
		1		5		
		1		0		
		1		2		
		1		3		

Endpoint	Analysis method
Survival	No significant effects
Reproduction	Regression

Cerio   Repro   FOSA   2+P   CensMortAll   CensTrtNone

Parameter Summary

Parameter	Guess	Final Est	95% LCL	95% UCL
logEC50	0.1100	0.0368	0.0014	0.0776
Slope	2.0000	2.7406		
Y0	28.000	28.189		
Y0SD	5.000	3.153		
logEC20		-0.0897	-0.1409	-0.0302
EC50		1.09	1.00	1.20
EC20		0.81	0.72	0.93

S10.22 C. dubia 6:2 FTS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
2.9 Control	1.3	1	100%	35	29.1	
		1		26		
		1		30		
		1		30		
		1		29		
		1		33		
		1		25		
		1		33		
		1		23		
		1		27		
29	13	1	100%	30	28.0	
		1		22		
		1		29		
		1		27		
		1		20		
		1		28		
		1		27		
		1		29		
		1		30		
		1		38		
60	26	1	100%	28	28.4	
		1		31		
		1		33		
		1		28		
		1		28		
		1		29		
		1		27		
		1		21		
		1		29		
		1		30		
120	52	1	100%	31	28.7	
		1		27		
		1		27		
		1		29		
		1		31		
		1		24		
		1		28		
		1		29		
		1		29		
		1		32		

226	97	1	100%	23	25.1	
		1		20		
		1		24		
		1		22		
		1		24		
		1		25		
		1		30		
		1		25		
		1		29		
		1		29		
445	191	0	70%	6	16.4	Y - mortality
		0		14		Y - mortality
		1		15		
		1		7		
		0		5		Y - mortality
		1		15		
		1		15		
		1		22		
		1		22		
		1		19		

Endpoint | Analysis method
Survival | Means comparison | $P = 0.004$
Reproduction | Regression

Cerio Repro 62FTS 2+P CensMortAll CensTrtNone
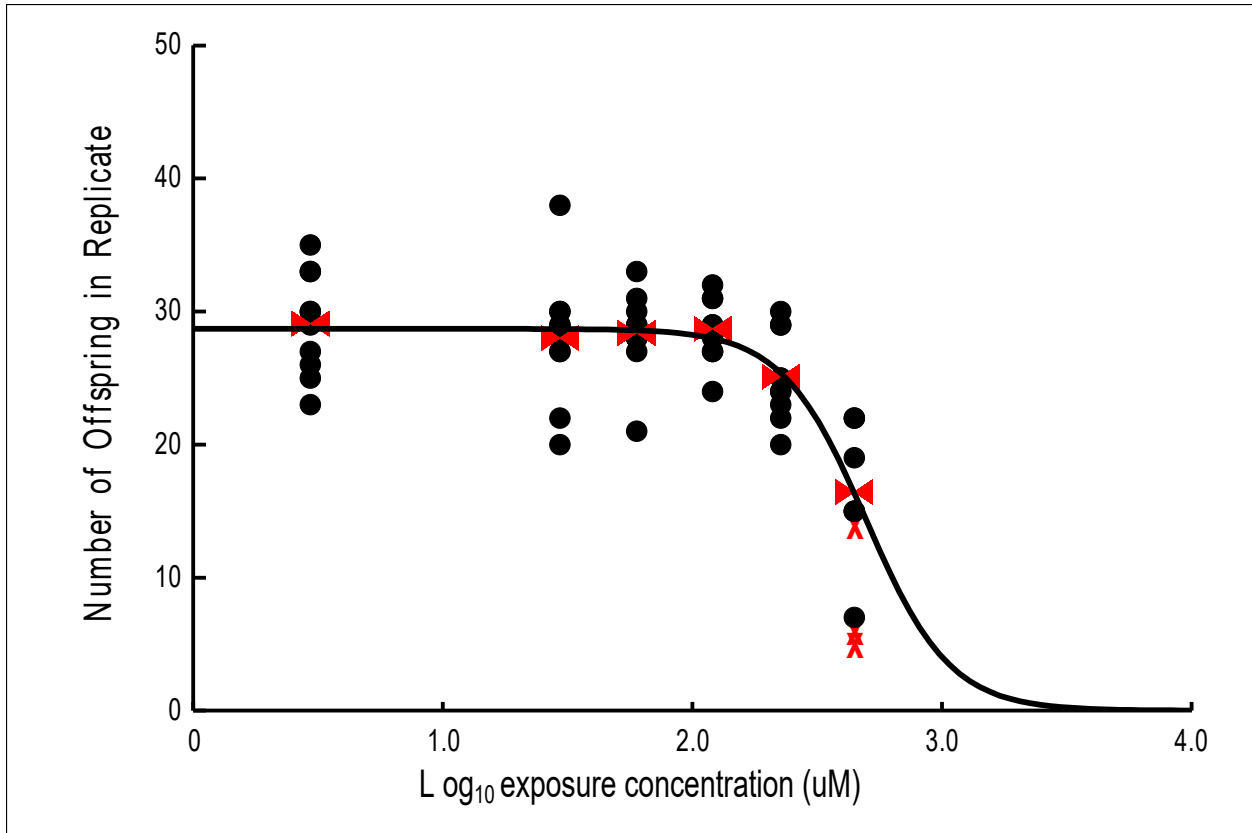
Parameter Summary

Parameter	Guess	FinalEst	95%LCL	95%UCL
logEC50	2.6000	2.6955	2.6323	2.8063
Slope	2.0000	1.4906		
Y0	29.100	28.704		
Y0SD	5.000	3.637		
logEC20		2.4630	2.3516	2.5579
EC50		496.07	428.89	640.15
EC20		290.43	224.71	361.36

S10.23 C. dubia 8:2 FTS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.013 Control	0.0070	1	100%	30	30.3	
		1		33		
		1		0		Y*
		1		25		
		1		33		
0.13	0.070	1	100%	28	27.3	
		1		29		
		1		0		Y
		1		27		
		1		25		
0.71	0.38	1	100%	31	29.3	
		1		27		
		1		28		Y
		1		29		
		1		30		
4.5	2.4	1	100%	25	28.0	
		1		27		
		1		31		Y
		1		29		
		1		28		

*Replicate C censored from each treatment of 8:2 FTS and 10:2 FTS tests because of poor performance in more than half of the replicates started with neonates from the same parent

Endpoint	Analysis method
Survival	No significant effects
Reproduction	No significant effects

S10.24 C. dubia 10:2 FTS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Survival (1 or 0)	Treatment average survival	Number of young produced	Treatment average per surviving female	Censored from reproduction analysis? Y
0.005 Control	0.00	1	100%	30	30.3	
		1		33		
		1		0		Y*
		1		25		
		1		33		
0.05	0.03	1	100%	28	30.5	
		1		30		
		1		31		Y
		1		34		
		1		30		
0.11	0.07	1	100%	30	28.3	
		1		26		
		1		0		Y
		1		27		
		1		30		
0.22	0.14	1	100%	22	19.0	
		1		27		
		1		0		Y
		1		21		
		1		25		

*Replicate C censored from each treatment of 8:2 FTS and 10:2 FTS tests because of poor performance in more than half of the replicates started with neonates from the same parent

Endpoint   Analysis method
Survival   No significant effects
Reproduction   Means comparison $P = 0.0013$

S11. *H. azteca* test designs, data, and analyses

S11.1 Overview

This section provides, for each PFAS toxicity test with *Hyalella azteca*, replicate-level test data and the results of concentration/response (CR) analyses of these data. For all tests, Section S11.2 tabulates the calculated effect concentrations (ECs) for both survival and growth, specifying for each test either (a) point EC estimates and confidence limits when test data were sufficient to conduct CR curve estimation or (b) ECs being greater than the highest test concentration and the magnitude/significance of effect at this concentration when CR curves could not be estimated. For each test, Sections S11.3 to S11.18 provide: a table of test data, analysis software output for the survival CR curve estimation when conducted, and output for the growth CR curve estimation when conducted. Note, all control concentrations were assigned a value of 1/10th of the lowest treatment for CR analysis.

The output for the survival CR curve estimation has three components:

(1) Header text specifying the taxon ("Hyalella"), the chemical, and the endpoint ("Survival").

(2) A figure for fraction survival ("Effect Variable") versus the base-10 logarithm of the concentration (uM) that includes observed treatment-level survivals and the estimated CR curve. For tests with at least two partial effects, the solid-black plotted line is the maximum-likelihood solution. For tests with just one partial effect, parameter estimates are not based on a maximum likelihood solution, but rather are approximated as described in the main text of this paper, and the red-dashed line is approximate/illustrative based on those parameter estimates.

(3) A table regarding parameter estimation for the log-logistic CR model. The three model parameters are the base-10 logarithm of the LC50 ("logX50"), the standard deviation of the log-logistic distribution ("logStdDev"), and the control survival ("CtrlSurv"). For these three parameters, the table provides: initial guesses ("Guess") for the nonlinear optimization search, the allowed parameter range ("PMin", "PMax"), and a fraction of the allowed range over which the parameter search is initially restricted ("Delta"). The table then provides final estimates ("PEst") and 95% confidence limits ("95%LCL","95%UCL") for each parameter, as well as for the untransformed LC50, the logarithm of the LC20 ("logX20"), and the untransformed LC20. Parameter estimates and CLs in black denote maximum likelihood solution values, whereas parameter estimates in red denote alternative approximations (see text) and CLs in red denote those at their min/max values.

The output for the growth CR curve estimation has four components:

(1) Header text specifying the taxon ("Hyalella"), the endpoint ("Biomass Gain"), the chemical abbreviation, and whether the number of partial effects was at least two ("2+Partial") to allow maximum likelihood curve fitting or just one ("1Partial") in which case alternative methods were used to approximate the maximum likelihood parameter values as described in the main text of this paper.

(2) A figure that includes replicate-level data ("Total Biomass Gain (mg AFDW)") versus "Log_{10} Exposure Concentration (uM)", and the estimated CR curve. Data used for curve estimation are

indicated by black circles, with treatment means being a red "bow tie". Red open circles denote replicates censored due to technical errors (e.g., solution lost), statistical outliers, or complete censoring of high concentration treatments not needed for curve definition. For tests with at least two partial effects, the plotted solid black line is the maximum-likelihood solution. For tests with just one partial effect, the plotted dashed red lines are illustrative, with the steeper line being the maximum likelihood solution at the maximum allowed slope and the shallower line being based on the alternative estimates for the parameter values (see text).

(3) A table regarding parameter estimation for the log-logistic CR model. The four model parameters are the base-10 logarithm of the EC50 ("logEC50"), the slope of the line at the EC50 ("Slope"), the control growth ("CtrlVal"), and a reference standard deviation ("StdDev"); see text for more details on weighting analysis regarding this standard deviation. For these four parameters, the table provides: initial guesses ("Guess") for the nonlinear optimization search, the allowed parameter range ("PMin", "PMax"), and a fraction of the allowed range over which the parameter search is initially restricted ("Delta"). The table then provides final maximum likelihood estimates ("PEst") and 95% confidence limits ("95%LCL","95%UCL") for each parameter, as well as for the untransformed EC50 (unlabeled line below the logEC50), the logarithm of the EC20 ("logEC20"), and the untransformed EC20. When there is just one partial effect, the maximum likelihood reached is constrained by the maximum allowed slope, so PEst deviates slightly from the unconstrained (and unrealistic) maximum likelihood solution. Parameter estimates then are alternatively estimated as described in the text and are entered in this table as "PAlt".

(4) The parameter optimization history, which provides values for the log likelihood and the four parameter values at different iterations (Iter) during the optimization.

S11.2. PFAS Survival LC50s and LC20s, growth EC50s and EC20s, and 95% confidence limits (mg/L) for 7-d *H. azteca* tests

Chemical class	Molecular weight g/mol	Abbrev.	LC50	LC20	EC50	EC20
Perfluoroalkyl carboxylic acids	214.04	PFBA	>180	>180	>180	>180
	314.05	PFHxA	>293	>293	213 (105-307)	55.6 (14.0-125)
	414.07	PFOA	>84	>84	9.6 (7.6-11.6)	5.0 (3.4-7.0)
	464.08	PFNA	>21	>21	2.4 (1.8-2.7)	1.2 (0.92-1.6)
	514.08	PFDA	>4.7	>4.7	0.85 (0.67-1.1)	0.49 (0.33-0.79)
Perfluoroalkyl sulfonic acids	299.09	PFBS	>192	>192	>192	>192
	400.12	PFHxS	85 (80-91)	69 (56-85)	47.0 (35.1-58.0)	27.3 (16.1-39.6)
	500.13	PFOS	>26	>26	20.1 (16.9-22.8)	7.2 (5.0-9.9)
	550.14	PFNS	>15	>15	11.1 (9.7-12.7)	6.5 (5.3-8.3)
	600.15	PFDS	>0.85[*]	>0.85[*]	>0.85[*]	>0.85[*]
Perfluoroalkane sulfonamides	299.12	FBSA	0.52 (0.48-0.57)	0.42 (0.32-0.54)	0.41 (0.34-0.49)	0.34 (0.27-0.42)
	399.13	FHxSA	0.15 (0.14-0.17)	0.08 (0.06-0.09)	0.10 (0.093-0.11)	0.08 (0.06-0.09)
	499.15	FOSA	0.023 (0.018-0.029)	0.013 (0.010-0.014)	0.017 (0.014-0.020)	0.010 (0.007-0.015)
Fluorotelomer sulfonic acids	428.17	6:2 FTS	>200	>200	14.3 (11.8-16.8)	5.9 (4.2-7.8)
	528.18	8:2 FTS	>2.9[*]	>2.9[*]	>2.9[*]	>2.9[*]
	628.2	10:2 FTS	>0.10[*]	>0.10[*]	>0.10[*]	>0.10[*]

*ECx greater than solubility limit

## S11.3. H. azteca PFBA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
84 control	18	10	10	100%	0.670	0.540	
		10	10		0.500		
		10	10		0.450		
		10	10		0.540		
841	180	10	10	98%	0.360	0.421	
		10	10		0.490		
		10	10		0.340		
		9	10		0.492		

Endpoint   Analysis method
Survival   No significant effects
Biomass gain   Means comparison $P = 0.0874$

S11.4. H. azteca PFHxA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
5.9 control	1.8	10	10	95%	0.490	0.368	
		8	10		0.274		
		10	10		0.380		
		10	10		0.330		
59	18	10	10	100%	0.440	0.390	
		10	10		0.320		
		10	10		0.390		
		10	10		0.410		
117	37	10	10	100%	TE	0.430	Y - TE
		10	10		0.370		
		10	10		TE		Y - TE
		10	10		0.490		
238	75	10	10	100%	0.230	0.308	
		10	10		0.300		
		10	10		0.310		
		10	10		0.390		
466	146	10	10	100%	0.212	0.181	
		10	10		0.240		
		10	10		0.080		
		10	10		0.190		
933	293	9	10	98%	0.190	0.178	
		10	10		0.200		
		10	10		0.170		
		10	10		0.150		

TE = Technical error; censored from analysis

Endpoint	Analysis method
Survival	No significant effects
Biomass gain	Regression

117

Hyalella   Biomass Gain   PFHxA   2+Partial
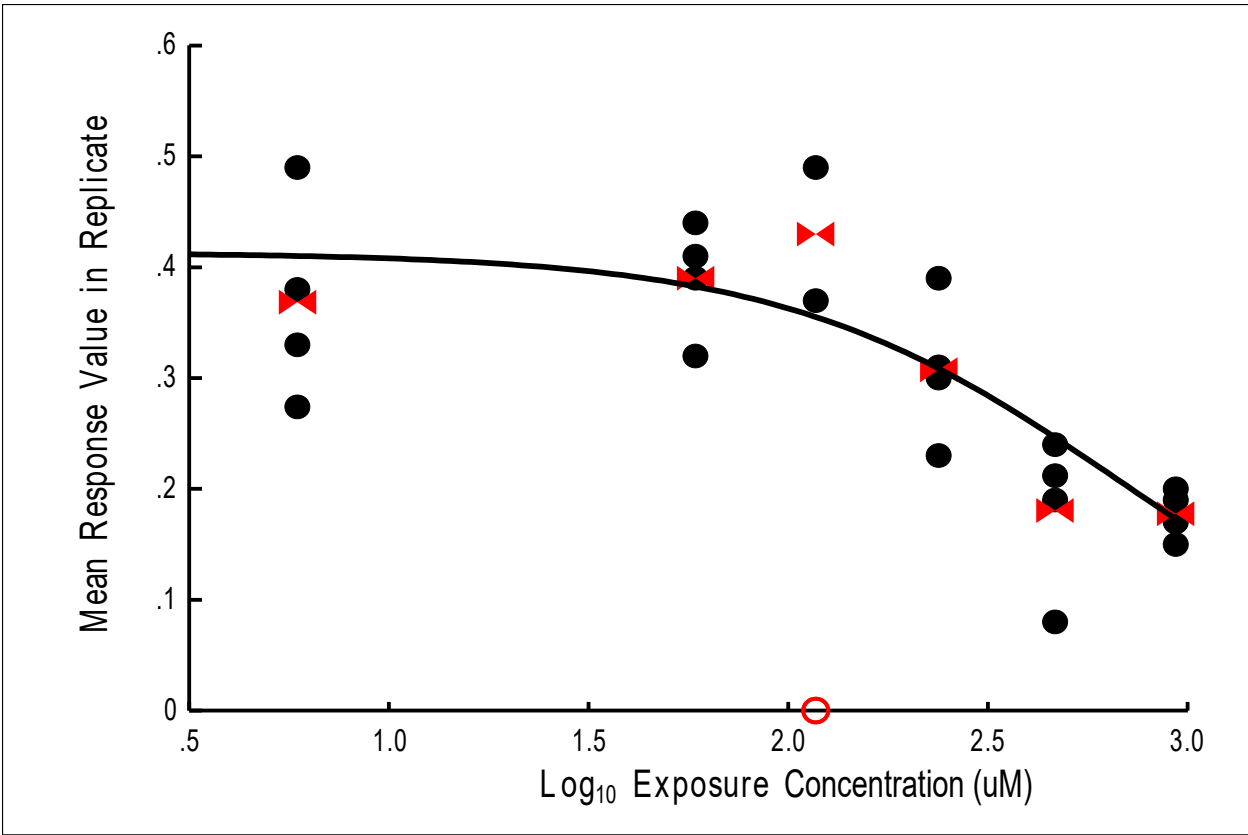
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	2.946	0.50	2.400	3.400	2.832	2.524	2.990	
					679.	334.	977.	
Slope	1.217	0.50	0.500	5.000	0.593	0.339	1.022	
CtrlVal	0.3894	0.50	0.2000	0.8000	0.4132	0.3536	0.5120	
StdDev	0.0673	0.50	0.0200	0.2000	0.0802	0.0610	0.1114	
logEC20					2.248	1.648	2.598	
					177.	44.	397.	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-25.1862	83.7675	2.782	3.120	0.500	2.275	0.3054	0.6017	0.0296	0.1421
11	-31.5067	-29.6823	2.763	2.877	0.531	0.728	0.3937	0.4420	0.0721	0.0859
51	-31.6549	-31.6458	2.826	2.839	0.581	0.601	0.4104	0.4158	0.0802	0.0818
111	-31.6578	-31.6578	2.832	2.832	0.593	0.593	0.4132	0.4132	0.0802	0.0803
224	-31.6578	-31.6578	2.832	2.832	0.593	0.593	0.4132	0.4132	0.0802	0.0802

Analysis Date: 01/22/2023 19:14   01/16/23 Continuous Variable Effects Analysis Program

## S11.5. H. azteca PFOA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
1.3 control	0.53	11	11	100%	0.553	0.498	
		10	10		0.440		
		10	10		0.380		
		10	10		0.620		
13	5.3	10	10	100%	0.400	0.458	
		10	10		0.460		
		10	10		0.490		
		10	10		0.480		
26	11	9	10	98%	0.322	0.297	
		10	10		0.320		
		10	10		0.340		
		11	11		0.207		
51	21	10	10	100%	0.170	0.133	
		10	10		0.120		
		10	10		0.130		
		10	10		0.110		
98	41	10	10	100%	0.010	0.043	
		10	10		0.060		
		10	10		0.070		
		10	10		0.030		
202	83	10	10	100%	0.010	0.000	Y**
		11	11		-0.038		Y
		10	10		0.020		Y
		10	10		0.010		Y

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint   Analysis method
Survival   No significant effects
Biomass gain   Regression

Hyalella   Biomass Gain   PFOA   2+Partial
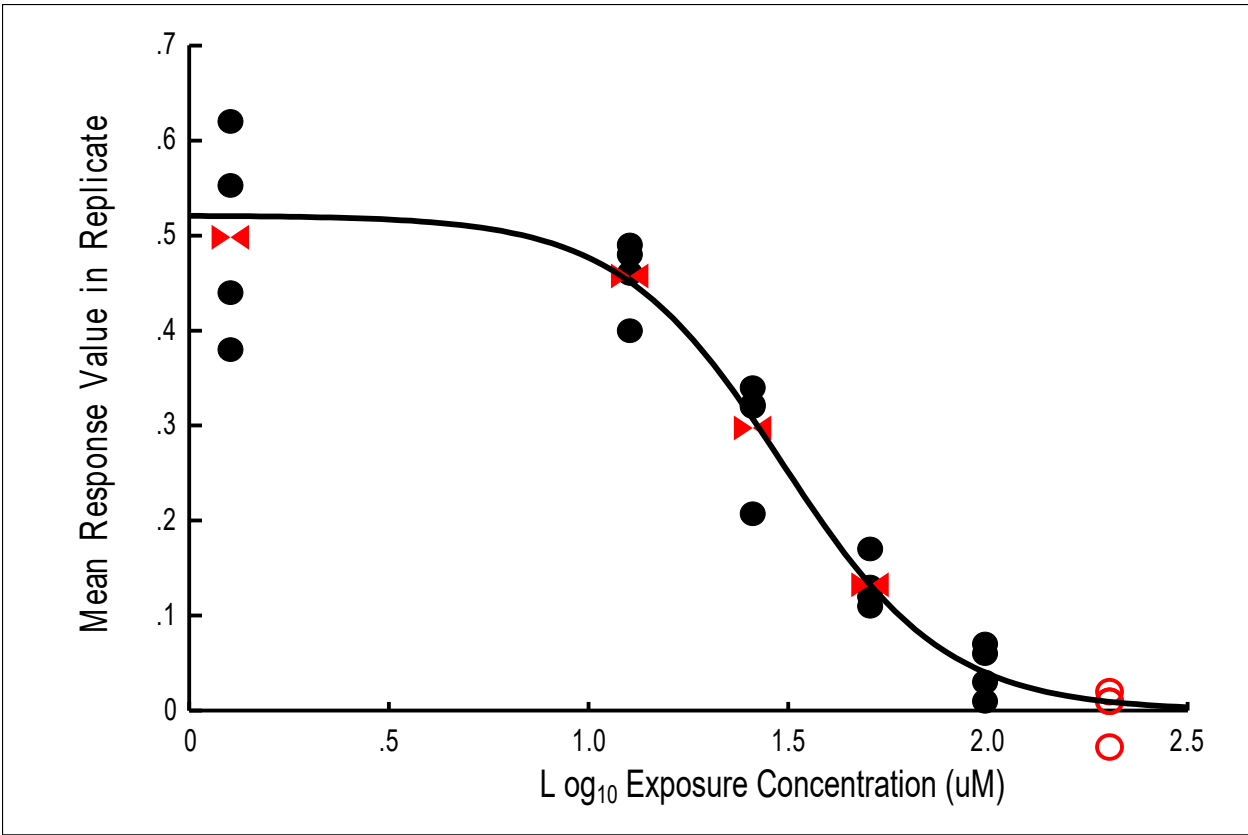
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.275	0.50	0.800	1.800	1.485	1.383	1.568	
					30.6	24.2	37.0	
Slope	4.871	0.50	0.500	5.000	1.223	0.967	1.613	
CtrlVal	0.4778	0.50	0.2000	1.0000	0.5210	0.4594	0.6014	
StdDev	0.0736	0.50	0.0200	0.2000	0.0746	0.0538	0.1054	
logEC20					1.202	1.034	1.345	
					15.9	10.8	22.1	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal	StdDev
1	3.3881	389.6639	1.079	1.459	4.105	4.882	0.3403 0.6094	0.0399 0.1047
51	-25.1945	-25.0600	1.660	1.669	4.776	4.846	0.4149 0.4279	0.1365 0.1431
161	-25.3618	-25.3334	1.664	1.665	4.694	4.788	0.4152 0.4176	0.1272 0.1293
331	-29.4650	-29.3396	1.632	1.635	1.704	1.828	0.4379 0.4460	0.1058 0.1084
673	-36.1011	-36.1011	1.485	1.485	1.223	1.223	0.5210 0.5210	0.0746 0.0746

Analysis Date: 01/22/2023 19:29   01/16/23 Continuous Variable Effects Analysis Program

S11.6. H. azteca PFNA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.019 control	0.0087	10	10	98%	0.395	0.511	
		9	10		0.429		
		10	10		0.525		
		10	10		0.695		
0.19	0.087	10	10	100%	0.455	0.558	
		10	10		0.575		
		10	10		0.665		
		10	10		0.535		
0.54	0.25	10	10	98%	0.515	0.469	
		9	10		0.429		
		10	10		0.505		
		10	10		0.425		
1.5	0.70	10	10	100%	0.495	0.470	
		10	10		0.405		
		10	10		0.545		
		10	10		0.435		
4.7	2.2	10	10	100%	0.255	0.274	
		10	10		0.265		
		10	10		0.285		
		10	10		0.289		
15	6.9	9	10	98%	0.020	0.036	
		10	10		0.075		
		10	10		0.055		
		10	10		-0.005		
45	21	10	10	100%	-0.015	-0.052	Y*
		10	10		-0.045		Y
		10	10		-0.075		Y
		10	10		-0.075		Y

*High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	No significant effects
Biomass gain	Regression

Hyalella   Biomass Gain   PFNA   2+Partial
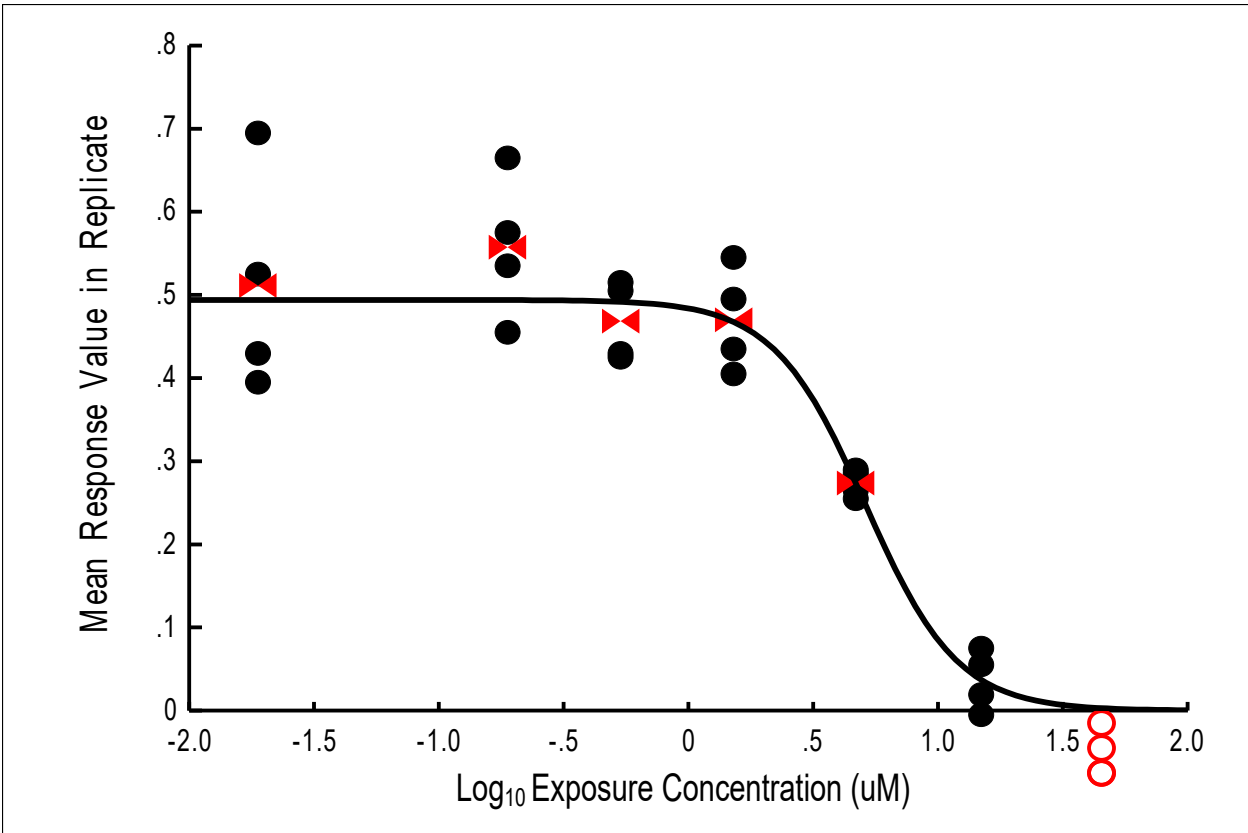
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	0.396	0.50	-0.100	0.900	0.710	1.383	1.568	
					5.13	24.16	36.95	
Slope	3.904	0.50	0.500	5.000	1.356	0.967	1.613	
CtrlVal	0.5018	0.50	0.3000	1.0000	0.4941	0.4594	0.6014	
StdDev	0.0855	0.50	0.0300	0.3000	0.0939	0.0538	0.1054	
logEC20					0.455	1.034	1.345	
					2.85	10.82	22.11	

Parameter Optimization History

Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	136.6204 428.7060	0.228 0.591	2.889 4.917	0.3343 0.6174	0.0384 0.1785
31	-34.5876 -33.7094	0.683 0.698	2.820 2.932	0.4630 0.4970	0.1282 0.1424
101	-35.9130 -35.9102	0.693 0.694	2.804 2.810	0.4849 0.4855	0.1032 0.1040
221	-37.8234 -37.5273	0.710 0.717	1.285 1.606	0.4820 0.4884	0.0897 0.0975
442	-37.9241 -37.9241	0.710 0.710	1.356 1.356	0.4941 0.4941	0.0939 0.0939

Analysis Date: 01/22/2023 19:27   01/16/23 Continuous Variable Effects Analysis Program

## S11.7. H. azteca PFDA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.0039 control	0.0020	10	10	100%	0.595	0.607	
		10	10		0.515		
		10	10		0.765		
		10	10		0.555		
0.039	0.020	10	10	98%	0.505	0.536	
		10	10		0.645		
		10	10		0.535		
		9	10		0.460		
0.11	0.055	10	10	98%	0.555	0.514	
		9	10		0.419		
		10	10		0.525		
		10	10		0.555		
0.31	0.16	11	11	100%	0.519	0.551	
		10	10		0.545		
		10	10		0.585		
		10	10		0.555		
0.97	0.50	10	10	100%	0.455	0.428	
		10	10		0.345		
		10	10		0.355		
		10	10		0.555		
3.0	1.5	10	10	100%	0.115	0.098	
		10	10		0.105		
		10	10		0.105		
		10	10		0.065		
9.1	4.7	10	10	100%	-0.050	-0.039	Y**
		10	10		-0.044		Y
		10	10		-0.017		Y
		10	10		-0.044		Y

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	No significant effects
Biomass gain	Regression

Hyalella   Biomass Gain   PFDA   2+Partial
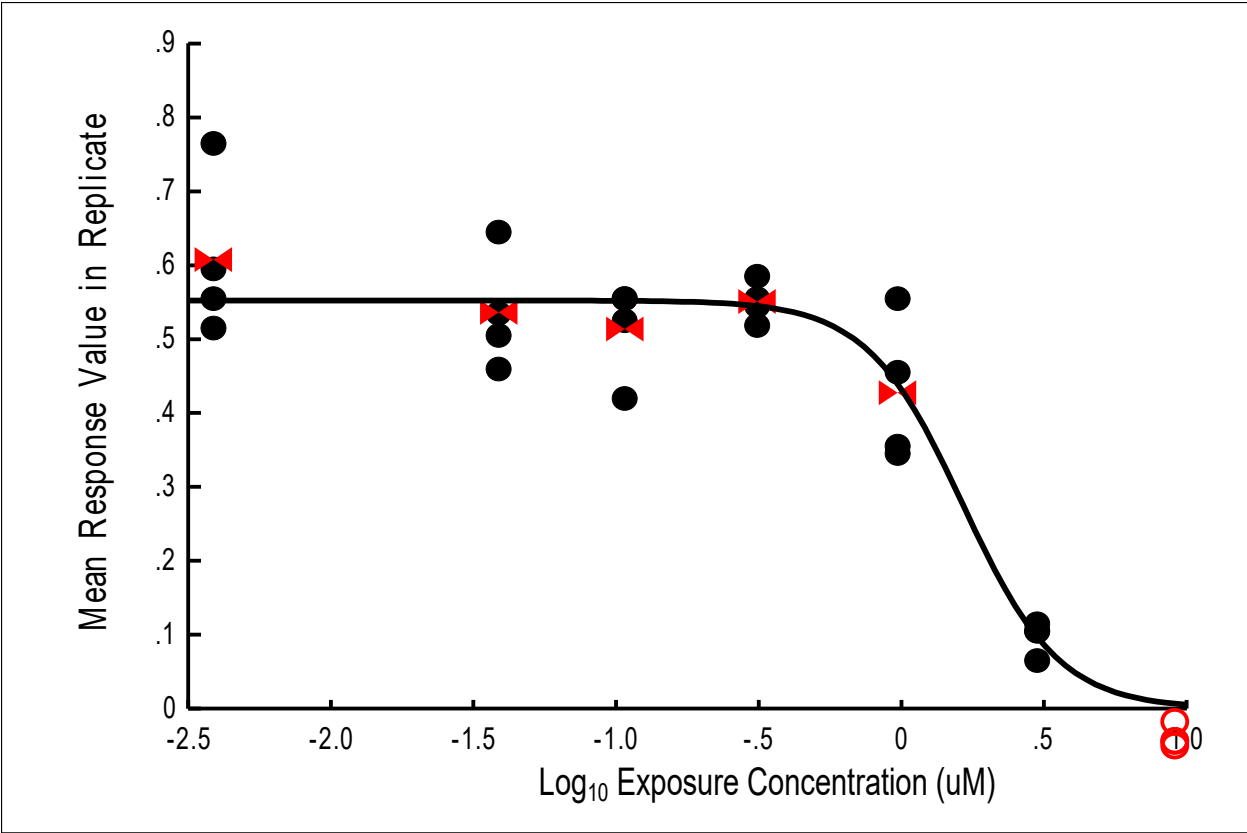
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	0.200	0.50	-0.300	0.500	0.216	0.114	0.324	
					1.64	1.30	2.11	
Slope	3.486	0.50	0.500	5.000	1.483	1.087	2.489	
CtrlVal	0.5520	0.50	0.3000	1.1000	0.5523	0.5271	0.5827	
StdDev	0.0745	0.50	0.0200	0.2000	0.0802	0.0611	0.1096	
logEC20					-0.018	-0.197	0.184	
					0.96	0.63	1.53	

Parameter Optimization History

| Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	-24.0887 63.5122	0.048 0.340	2.490 4.609	0.38950.7137	0.05170.1187
31	-33.7376 -33.0465	0.356 0.379	3.460 3.860	0.53220.5486	0.08760.1003
111	-34.1857 -34.0267	0.343 0.356	2.776 3.122	0.54100.5452	0.08810.0914
231	-36.4371 -36.3986	0.220 0.232	1.484 1.590	0.54930.5520	0.07710.0792
466	-36.4940 -36.4940	0.216 0.216	1.483 1.483	0.55230.5523	0.08020.0802

S11.8. H. azteca PFBS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
3.7 control	1.1	8	10	95%	0.354	0.429	
		10	10		0.540		
		10	10		0.270		
		10	10		0.550		
37	11	10	10	100%	0.550	0.418	
		10	10		0.360		
		10	10		0.410		
		11	11		0.353		
78	23	10	10	100%	0.510	0.448	
		10	10		TE		Y - TE
		10	10		0.510		
		11	11		0.325		
152	46	10	10	100%	0.400	0.403	
		10	10		0.470		
		10	10		0.350		
		10	10		0.390		
317	95	10	10	95%	0.480	0.444	
		10	10		0.490		
		9	10		0.382		
		9	10		0.422		
643	193	11	11	100%	0.507	0.437	
		10	10		0.460		
		10	10		0.490		
		10	10		0.290		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

TE = technical error; censored from analysis

Endpoint	Analysis method
Survival	No significant effects
Biomass gain	No significant effects

S11.9. H. azteca PFHxS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
3.0 control	1.2	10	10	100%	0.560	0.564	
		10	10		0.690		
		11	11		0.516		
		10	10		0.490		
30	12	10	10	98%	0.480	0.466	
		10	10		0.520		
		9	10		0.402		
		11	11		0.462		
60	24	10	10	100%	0.400	0.428	
		10	10		0.390		
		10	10		0.400		
		10	10		0.520		
117	47	10	10	100%	0.340	0.273	
		10	10		0.300		
		10	10		0.290		
		10	10		0.162		
217	87	7	10	45%	0.096	0.084	
		4	10		0.082		
		4	10		0.112		
		3	10		0.044		
470	188	0	10	0%	0.000	0.000	Y**
		0	10		0.000		Y
		0	10		0.000		Y
		0	10		0.000		Y

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	Regression
Biomass gain	Regression

Hyalella PFHxS Survival
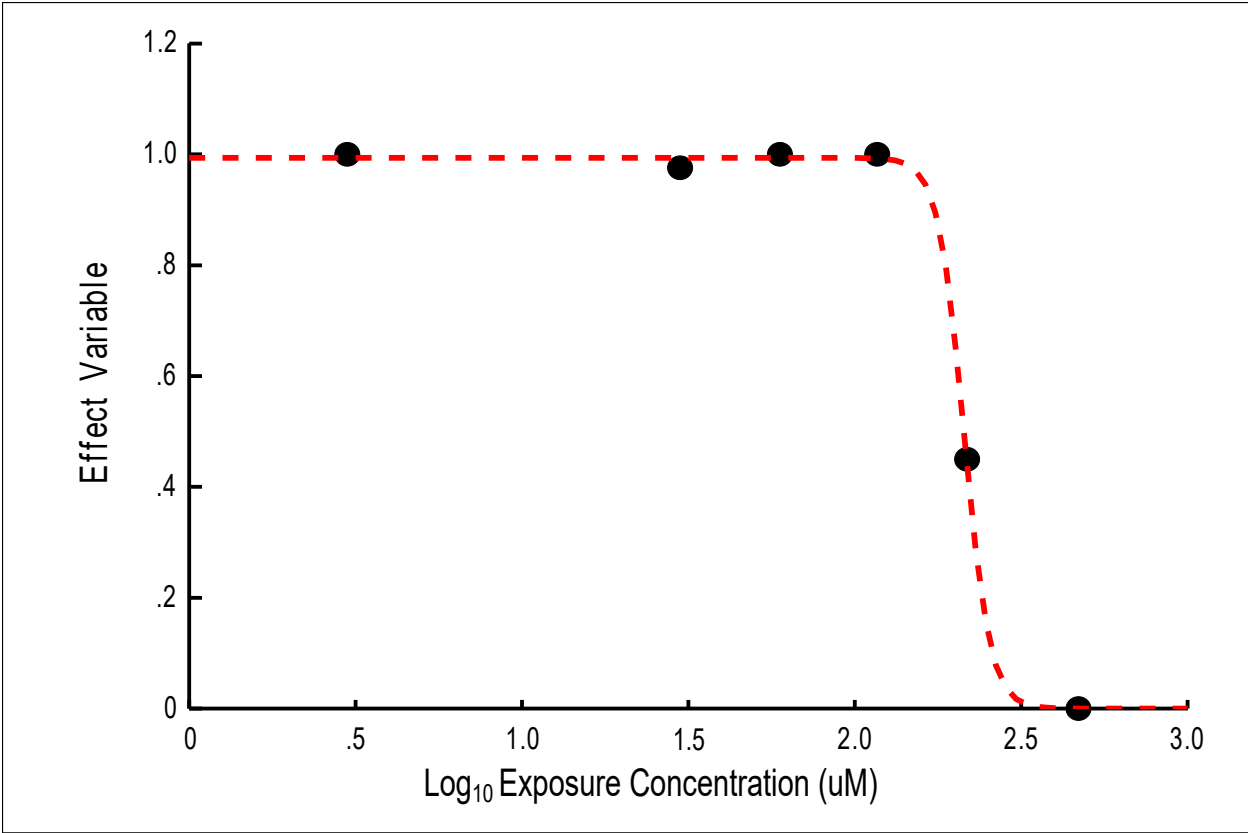
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	2.3696	0.5000	2.0696	2.6696	2.3281	2.2981	2.3581
					212.85	198.65	228.07
logStdDev	0.2018	0.5000	0.0100	0.5000	0.0708	0.0100	0.1416
CtrlSurv	0.9939	0.5000	0.8000	1.0000	0.9939	0.9731	1.0000
logX20					2.2696	2.2073	2.3319
					186.03	161.17	214.74

Hyalella   Biomass Gain   PFHxS   2+Partial
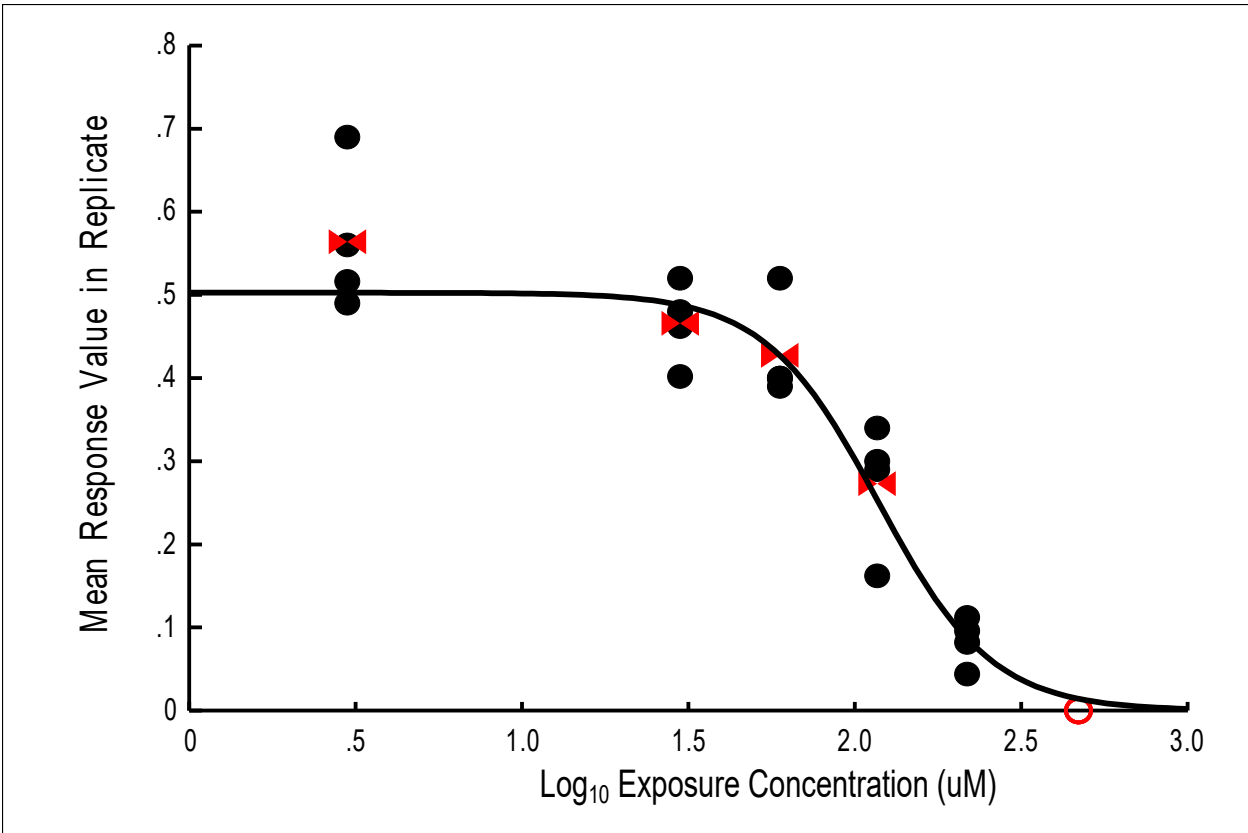
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	2.000	0.50	1.600	2.400	2.070	1.943	2.161	
					118.	88.	145.	
Slope	4.343	0.50	0.500	5.000	1.468	0.993	2.214	
CtrlVal	0.5641	0.50	0.3000	1.1000	0.5027	0.4528	0.5751	
StdDev	0.0769	0.50	0.0300	0.2000	0.0842	0.0636	0.1190	
logEC20					1.834	1.605	1.996	
					68.	40.	99.	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-15.3508	388.0726	1.807	2.164	3.076	4.663	0.4240	0.7129	0.0453	0.1132
21	-23.5846	-20.4252	2.114	2.256	2.536	3.441	0.4181	0.4922	0.1351	0.1801
71	-29.7852	-29.6314	2.092	2.108	1.566	1.695	0.4845	0.4920	0.0794	0.0850
151	-29.9519	-29.9518	2.070	2.071	1.465	1.472	0.5022	0.5029	0.0840	0.0843
317	-29.9520	-29.9520	2.070	2.070	1.468	1.468	0.5027	0.5027	0.0842	0.0842

Analysis Date: 01/22/2023 19:42   01/16/23 Continuous Variable Effects Analysis Program

S11.10. H. azteca PFOS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.18 control	0.089	10	10	100%	0.490	0.440	
		10	10		0.410		
		10	10		0.420		
		10	10		0.440		
1.8	0.89	10	10	100%	0.510	0.495	
		10	10		0.530		
		10	10		0.440		
		10	10		0.500		
3.4	1.7	10	10	100%	0.530	0.543	
		10	10		0.470		
		10	10		0.550		
		10	10		0.620		
6.5	3.3	10	10	100%	0.430	0.455	
		10	10		0.470		
		10	10		0.520		
		10	10		0.400		
13	6.5	10	10	100%	0.390	0.383	
		10	10		0.400		
		11	11		0.340		
		11	11		0.402		
24	12	10	10	100%	0.340	0.310	
		10	10		0.340		
		10	10		0.300		
		10	10		0.260		
52	26	10	10	100%	0.200	0.200	
		10	10		0.180		
		10	10		0.230		
		10	10		0.190		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	No significant effects
Biomass gain	Regression

Hyalella   Biomass Gain   PFOS   2+Partial
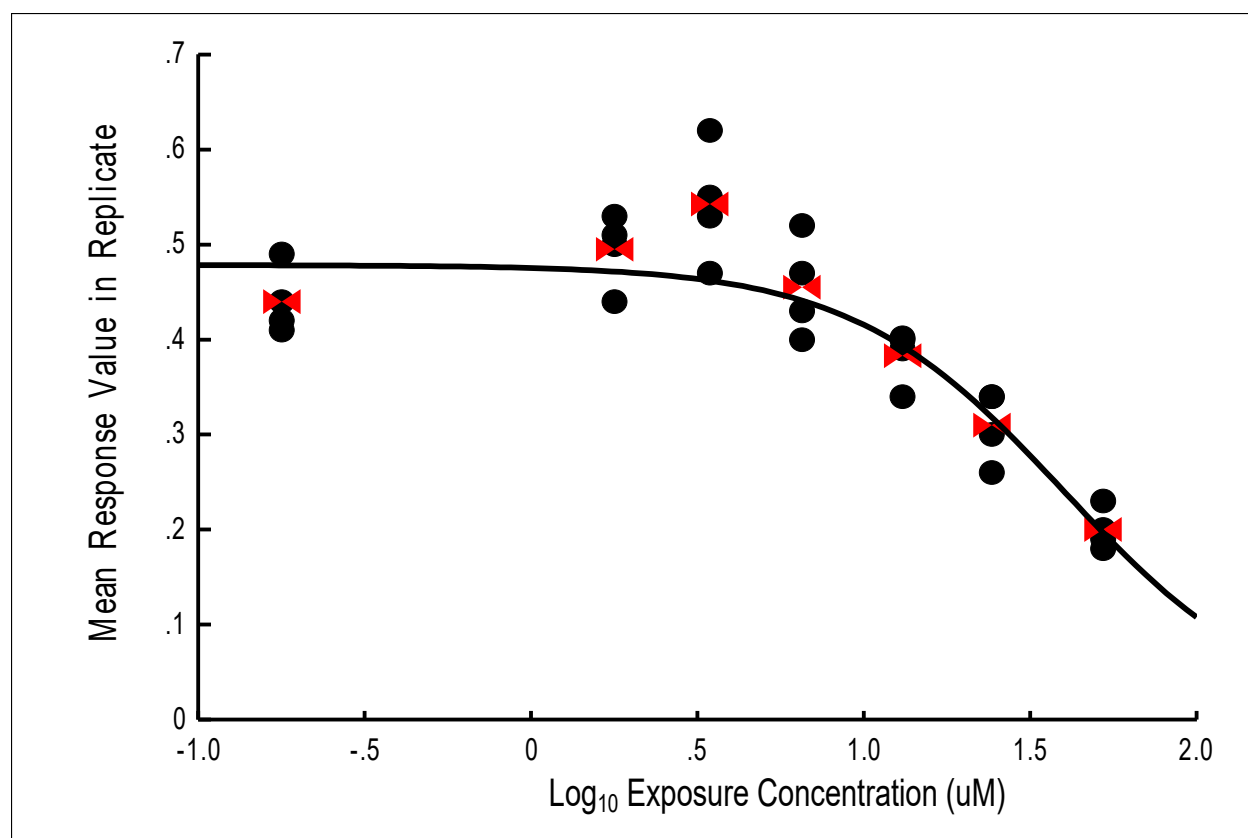
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.648	0.50	1.100	2.100	1.604	1.530	1.677	
					40.2	33.9	47.6	
Slope	1.024	0.50	0.500	5.000	0.781	0.607	1.010	
CtrlVal	0.4831	0.50	0.2000	1.0000	0.4785	0.4503	0.5084	
StdDev	0.0576	0.50	0.0200	0.2000	0.0742	0.0584	0.0989	
logEC20					1.161	1.001	1.297	
					14.5	10.0	19.8	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-35.7985	281.1404	1.404	1.888	0.720	2.289	0.2890	0.6777	0.0233	0.0980
11	-45.5294	-33.6741	1.577	1.696	0.635	1.127	0.4383	0.5001	0.0465	0.0710
51	-48.9846	-48.8779	1.613	1.624	0.749	0.798	0.4721	0.4776	0.0712	0.0756
121	-49.0444	-49.0444	1.604	1.605	0.781	0.781	0.4784	0.4785	0.0742	0.0743
260	-49.0445	-49.0445	1.604	1.604	0.781	0.781	0.4785	0.4785	0.0742	0.0742

Analysis Date: 01/22/2023 19:44   01/16/23 Continuous Variable Effects Analysis Program

S11.11. H. azteca PFNS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.077 control	0.043	9	10	98%	0.434	0.615	
		10	10		0.642		
		10	10		0.692		
		10	10		0.693		
0.77	0.43	10	10	98%	0.702	0.508	
		10	10		0.372		
		10	10		0.472		
		9	10		0.484		
1.9	1.0	10	10	100%	0.552	0.525	
		10	10		0.492		
		10	10		0.582		
		10	10		0.473		
4.4	2.4	10	10	98%	0.522	0.473	
		10	10		0.532		
		9	10		0.444		
		10	10		0.392		
10	5.7	10	10	100%	0.443	0.446	
		10	10		0.462		
		12	12		0.389		Y-overstocked
		10	10		0.432		
27	15	10	10	98%	0.152	0.165	
		10	10		0.222		
		10	10		0.132		
		9	10		0.154		

Endpoint	Analysis method
Survival	No significant effects
Biomass gain	Regression

Hyalella   Biomass Gain   PFNS   2+Partial
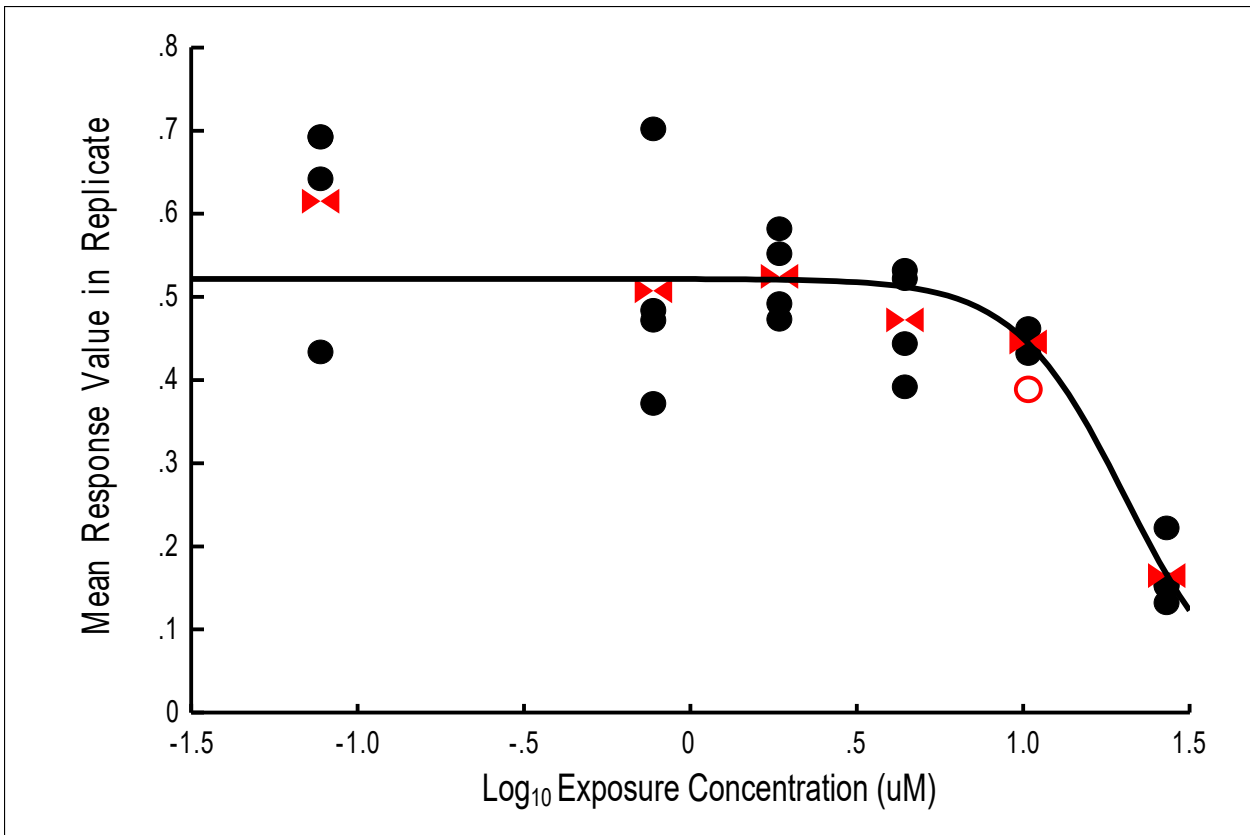
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.200	0.50	0.800	1.600	1.305	1.248	1.364	
					20.2	17.7	23.1	
Slope	0.541	0.50	0.500	5.000	1.509	1.161	2.060	
CtrlVal	0.6152	0.50	0.3000	1.2000	0.5217	0.4816	0.5639	
StdDev	0.1067	0.50	0.0400	0.3000	0.1194	0.0901	0.1687	
logEC20					1.075	0.982	1.180	
					11.9	9.6	15.1	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-3.5572	213.5596	1.032	1.383	0.503	1.443	0.3921	0.8063	0.0579	0.1695
11	-26.8282	-18.3844	1.208	1.285	1.012	1.305	0.5395	0.6051	0.0888	0.1101
51	-32.5362	-32.1383	1.280	1.314	1.371	1.560	0.5141	0.5397	0.1103	0.1195
121	-32.6062	-32.6062	1.305	1.305	1.509	1.509	0.5216	0.5217	0.1193	0.1194
250	-32.6062	-32.6062	1.305	1.305	1.509	1.509	0.5217	0.5217	0.1194	0.1194

Analysis Date: 01/22/2023 19:55   01/16/23 Continuous Variable Effects Analysis Program

S11.12. H. azteca PFDS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.14 control	0.086	10	10	98%	0.543	0.498	
		10	10		0.472		
		9	10		0.434		
		10	10		0.543		
1.4	0.86	10	10	98%	0.422	0.408	
		10	10		0.362		
		9	10		0.304		
		10	10		0.543		

Endpoint   Analysis method
Survival   No significant effects
Biomass gain   Means comparison $P = 0.0534$

S11.13. H. azteca FBSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.022 control	0.0066	10	10	100%	0.560	0.583	
		10	10		0.350		
		10	10		0.770		
		10	10		0.650		
0.22	0.066	10	10	100%	0.670	0.607	
		10	10		0.680		
		12	12		0.748		Y-overstocked
		10	10		0.470		
0.43	0.13	10	10	100%	0.490	0.533	
		10	10		0.610		
		10	10		0.320		
		10	10		0.710		
0.89	0.27	10	10	100%	0.590	0.553	
		10	10		0.560		
		10	10		0.510		
		13	13		0.632		Y-overstocked
1.9	0.55	5	10	40%	0.130	0.073	
		4	10		0.146		
		4	10		0.016		
		3	10		0.000		
3.7	1.1	0	10	0%	0.000	0.000	Y*
		0	10		0.000		Y
		0	10		0.000		Y
		0	10		0.000		Y
7.6	2.3	0	10	0%	0.000	0.000	Y
		0	10		0.000		Y
		0	10		0.000		Y
		0	10		0.000		Y

*High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	Regression
Biomass gain	Regression

Hyalella FBSA Survival
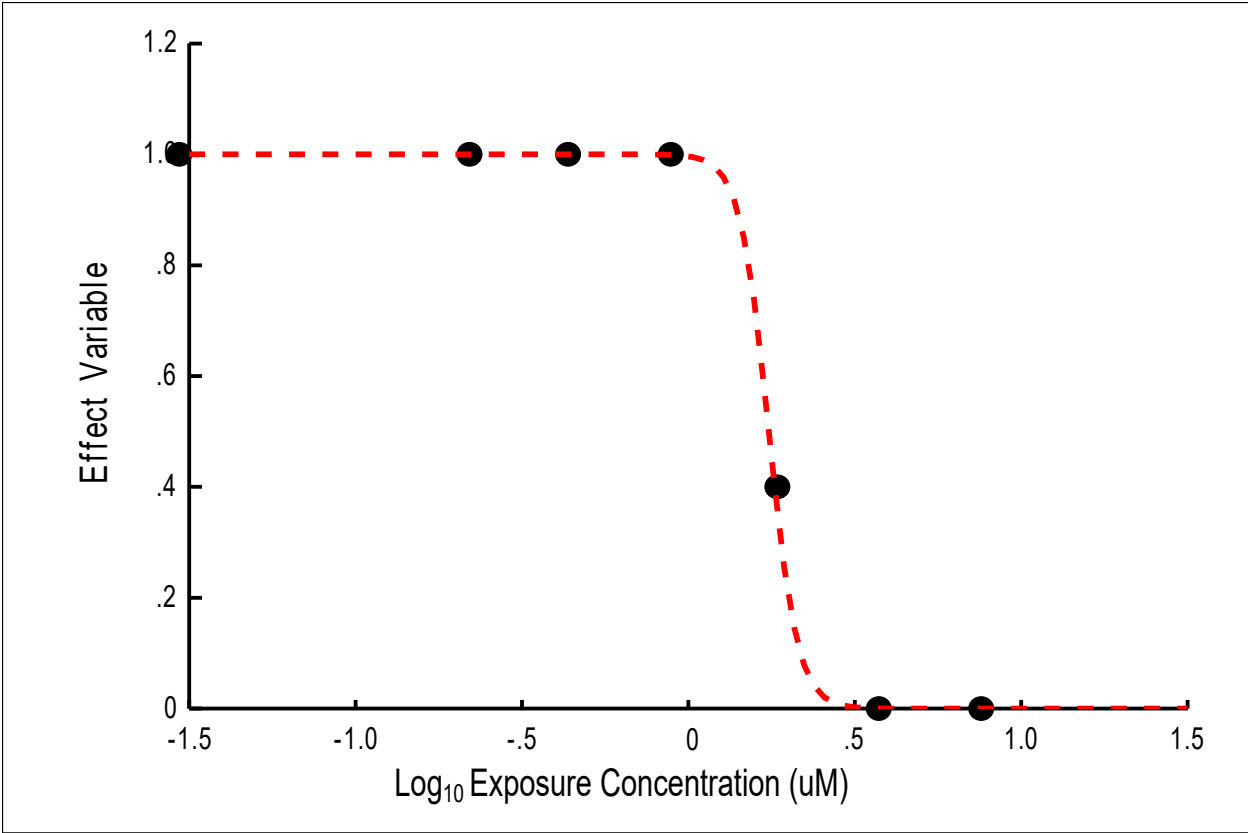
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.2597	0.5000	-0.0403	0.5597	0.2418	0.2068	0.2769
					1.75	1.61	1.89
logStdDev	0.2082	0.5000	0.0100	0.5000	0.0783	0.0100	0.1566
CtrlSurv	0.9990	0.5000	0.8000	1.0000	1.0000	0.9864	1.0000
logX20					0.1811	0.1015	0.2607
					1.52	1.26	1.82

Hyalella   Biomass Gain   FBSA   1Partial
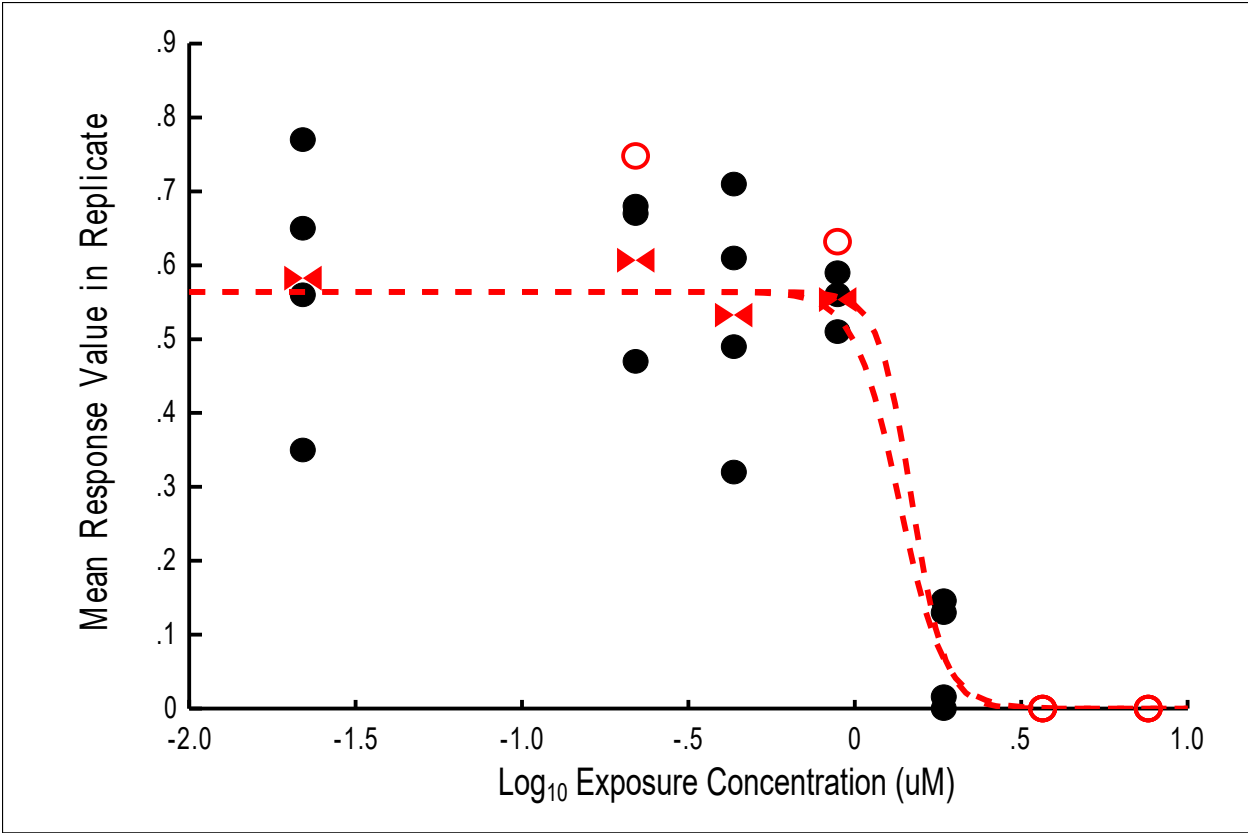
Parameter Summary   Optimization Errors: 0   0 99   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	0.052	0.50	-0.400	0.600	0.171	0.052	0.214	0.133
					1.48	1.13	1.64	1.36
Slope	8.647	0.50	0.500	5.000	5.000	2.464	5.000	3.732
CtrlVal	0.5671	0.50	0.3000	1.1000	0.5638	0.5267	0.6649	0.5638
StdDev	0.1257	0.50	0.0400	0.4000	0.1287	0.0946	0.1867	0.1406
logEC20					0.102	-0.044	0.144	0.050
					1.26	0.90	1.39	1.12

Parameter Optimization History

Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	-14.6660 275.8866	-0.029 0.468	5.000 5.000	0.3737 0.7379	0.0725 0.2149
11	-16.6895 -16.1406	0.098 0.154	5.000 5.000	0.5486 0.5909	0.1301 0.1514
41	-17.3746 -17.2981	0.163 0.172	5.000 5.000	0.5636 0.5688	0.1309 0.1355
101	-17.3851 -17.3851	0.171 0.171	5.000 5.000	0.5638 0.5639	0.1287 0.1287
203	-17.3851 -17.3851	0.171 0.171	5.000 5.000	0.5638 0.5638	0.1287 0.1287

Analysis Date: 01/22/2023 22:02   01/16/23 Continuous Variable Effects Analysis Program

S11.14. H. azteca FHxSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.0016 control	0.00065	9	10	95%	0.546	0.528	
		9	10		0.666		
		10	10		0.470		
		10	10		0.430		
0.016	0.0065	11	11	98%	0.658	0.636	
		10	10		0.720		
		10	10		0.580		
		9	10		0.586		
0.033	0.013	9	10	95%	0.556	0.603	
		10	10		0.650		
		9	10		0.596		
		10	10		0.610		
0.066	0.026	10	10	100%	0.570	0.515	
		10	10		0.560		
		10	10		0.580		
		10	10		0.350		
0.143	0.057	11	11	100%	0.595	0.556	
		10	10		0.640		
		10	10		0.420		
		10	10		0.570		
0.274	0.109	6	10	83%	0.124	0.223	
		9	10		0.286		
		8	10		0.272		
		10	10		0.210		
0.498	0.199	2	10	18%	0.018	0.020	
		2	10		0.028		
		3	10		0.032		
		0	10		0.000		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint   Analysis method
Survival   Regression
Biomass gain   Regression

Hyalella FHxSA Survival
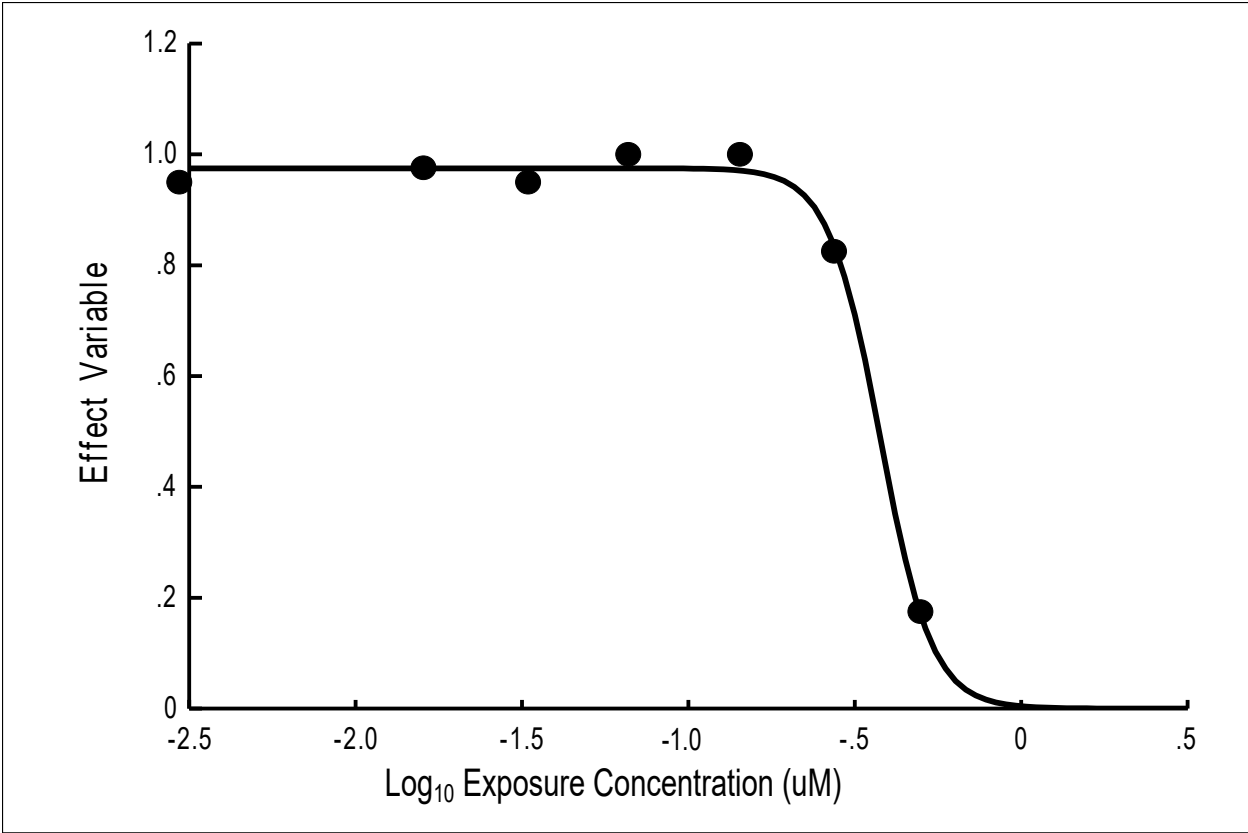
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.5162	0.5000	-0.8162	-0.2162	-0.4233	-0.4718	-0.3738
					0.38	0.34	0.42
logStdDev	0.2189	0.5000	0.0100	0.5000	0.1405	0.0999	0.2029
CtrlSurv	0.9751	0.5000	0.8000	1.0000	0.9749	0.9470	0.9909
logX20					-0.5307	-0.5981	-0.4684
					0.29	0.25	0.34

Survival Analysis Program for PFAS Project

Hyalella   Biomass Gain   FHxSA   2+Partial
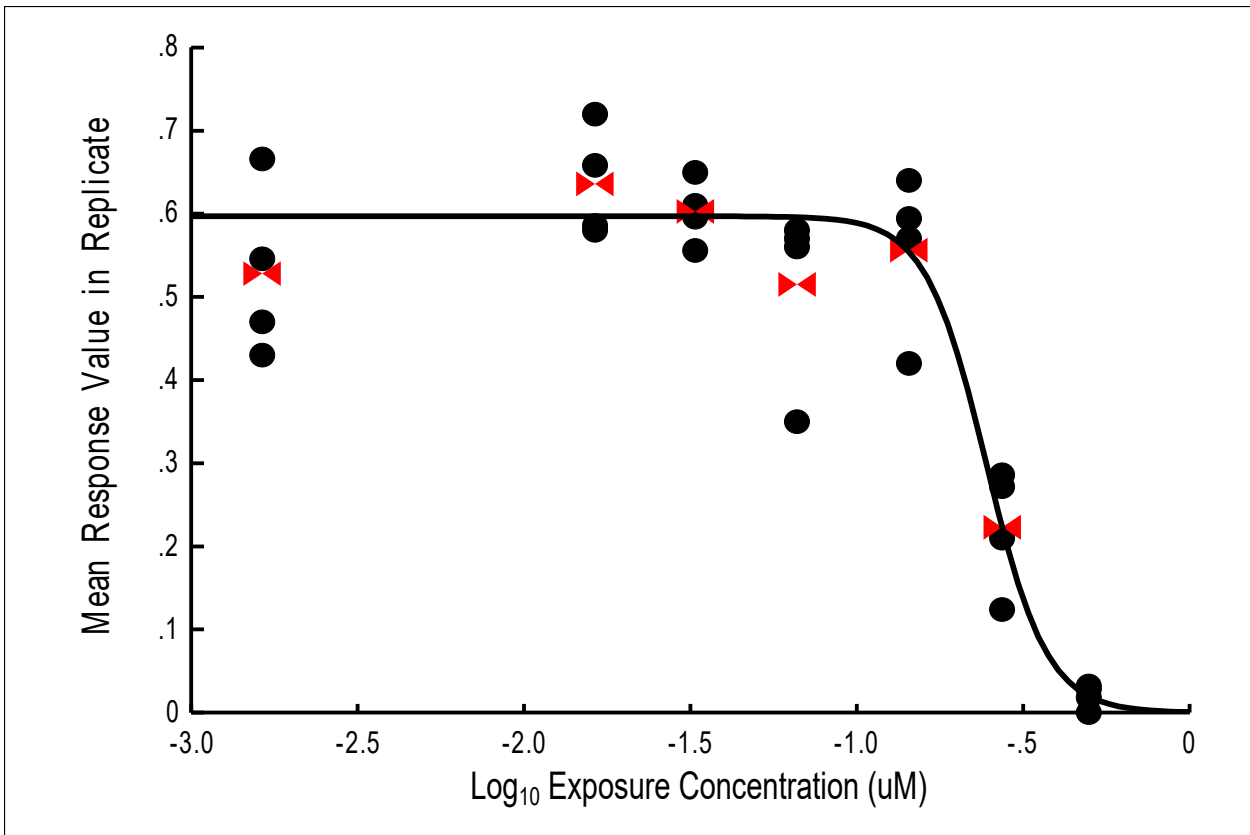
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-0.549	0.50	-1.000	0.000	-0.610	-0.673	-0.564	
					0.245	0.212	0.273	
Slope	3.007	0.50	0.500	5.000	2.746	2.018	5.000	
CtrlVal	0.5676	0.50	0.3000	1.1000	0.5971	0.5669	0.6274	
StdDev	0.0881	0.50	0.0300	0.3000	0.1005	0.0776	0.1373	
logEC20					-0.736	-0.836	-0.651	
					0.183	0.146	0.223	

Parameter Optimization History

Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	-32.2703 188.9216	-0.920 -0.330	1.983 3.569	0.4604 0.7278	0.0486 0.1574
11	-38.6728 -33.2991	-0.636 -0.573	2.459 2.786	0.5548 0.6085	0.0904 0.1115
51	-39.0109 -39.0046	-0.613 -0.611	2.695 2.721	0.5962 0.5977	0.0991 0.1005
111	-39.0164 -39.0164	-0.610 -0.610	2.746 2.747	0.5970 0.5971	0.1005 0.1005
230	-39.0164 -39.0164	-0.610 -0.610	2.746 2.746	0.5971 0.5971	0.1005 0.1005

Analysis Date: 01/22/2023 22:06   01/16/23 Continuous Variable Effects Analysis Program

## S11.15. H. azteca FOSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.00012 control	0.000061	10	10	100%	0.550	0.547	
		11	11		0.549		
		10	10		0.480		
		10	10		0.610		
0.0012	0.00061	10	10	100%	0.490	0.673	
		12	12		0.528		Y-overstocked
		10	10		0.850		
		10	10		0.680		
0.0026	0.0013	9	10	95%	0.716	0.613	
		10	10		0.560		
		9	10		0.506		
		10	10		0.670		
0.0051	0.0026	10	10	100%	0.630	0.608	
		10	10		0.590		
		10	10		0.720		
		10	10		0.490		
0.0097	0.0049	10	10	100%	0.760	0.702	
		10	10		0.840		
		11	11		0.567		
		10	10		0.640		
0.0189	0.0094	10	10	98%	0.350	0.477	
		9	10		0.516		
		10	10		0.560		
		10	10		0.480		
0.035	0.018	5	10	68%	0.210	0.285	
		9	10		0.376		
		6	10		0.224		
		7	10		0.328		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass gain	Regression

Hyalella FOSA Survival
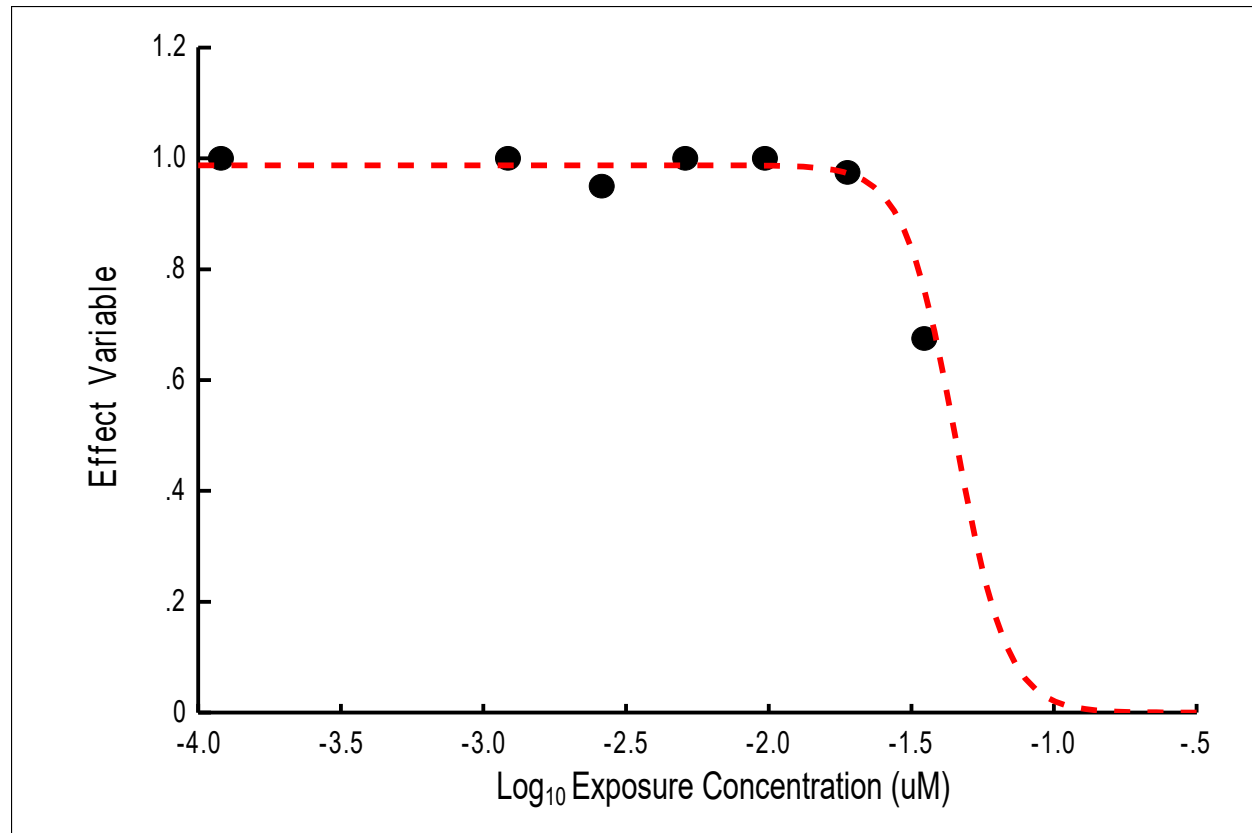
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.2979	0.5000	-1.5979	-0.9979	-1.3446	-1.4541	-1.2352
					0.05	0.04	0.06
logStdDev	0.2987	0.5000	0.0100	0.5000	0.1631	0.0100	0.3262
CtrlSurv	0.9875	0.5000	0.8000	1.0000	0.9875	0.9668	1.0000
logX20					-1.4600	-1.4793	-1.4406
					0.03	0.03	0.04

Hyalella   Biomass Gain   FOSA 2   2+Partial
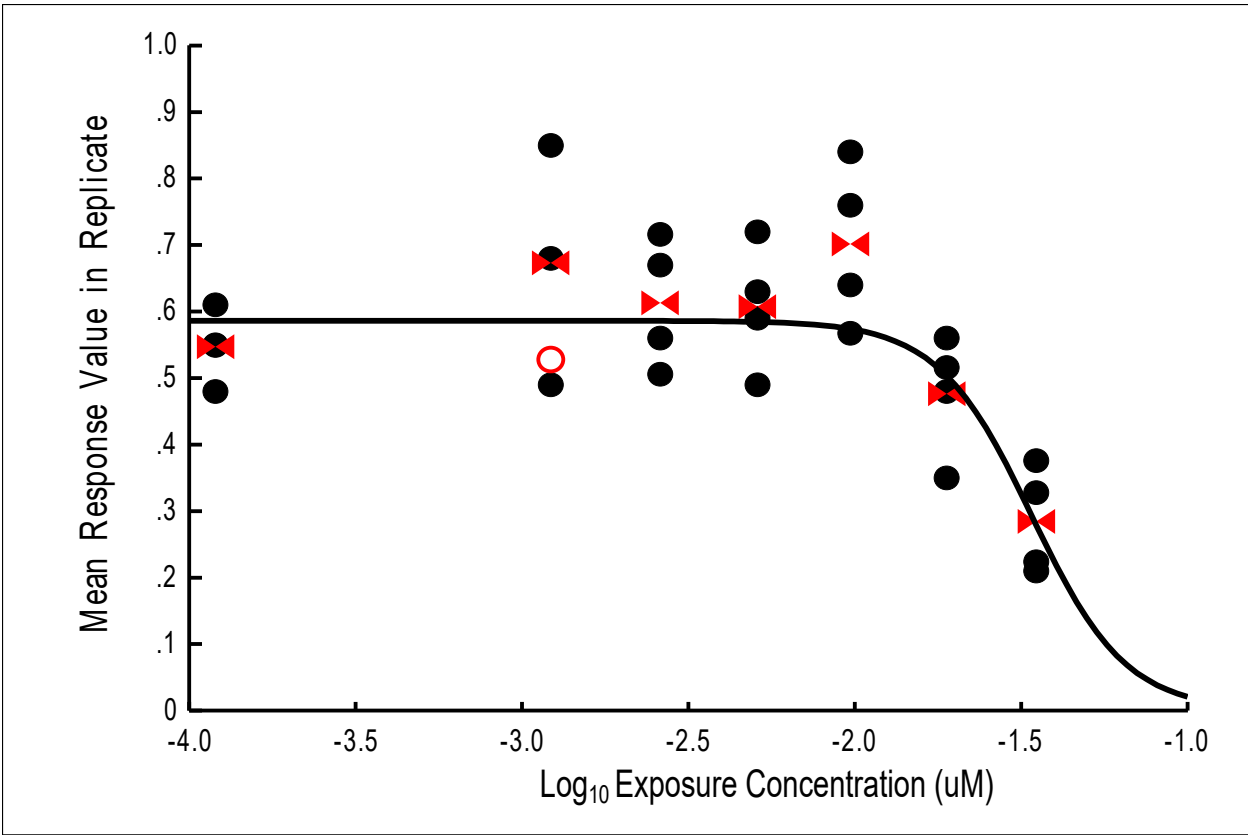
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.440	0.50	-1.900	-0.900	-1.468	-1.548	-1.355	
					0.0340	0.0283	0.0441	
Slope	2.184	0.50	0.500	5.000	1.765	0.959	5.000	
CtrlVal	0.6262	0.50	0.3000	1.3000	0.5861	0.5453	0.6276	
StdDev	0.1099	0.50	0.0400	0.3000	0.1305	0.1021	0.1748	
logEC20					-1.665	-1.815	-1.513	
					0.0216	0.0153	0.0307	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-17.8477	110.5301	-1.623	-1.207	1.201	2.762	0.3945	0.8464	0.0501	0.1878
21	-24.5211	-17.7324	-1.568	-1.420	1.290	2.316	0.5083	0.6467	0.1296	0.2047
61	-24.8556	-24.8499	-1.470	-1.466	1.819	1.852	0.5842	0.5871	0.1296	0.1313
141	-24.8625	-24.8625	-1.468	-1.468	1.765	1.766	0.5861	0.5862	0.1304	0.1305
289	-24.8625	-24.8625	-1.468	-1.468	1.765	1.765	0.5861	0.5861	0.1305	0.1305

Analysis Date: 01/22/2023 22:08   01/16/23 Continuous Variable Effects Analysis Program

S11.16. H. azteca 6:2 FTS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.76 control	0.33	10	10	100%	0.613	0.603	
		10	10		0.543		
		10	10		0.613		
		10	10		0.643		
7.6	3.3	10	10	100%	0.693	0.603	
		10	10		0.543		
		10	10		0.563		
		10	10		0.613		
15.7	6.7	9	10	98%	0.469	0.485	
		10	10		0.383		
		10	10		0.493		
		10	10		0.593		
30	13	10	10	100%	0.413	0.314	
		10	10		0.323		
		10	10		0.283		
		11	11		0.234		
60	26	10	10	98%	0.153	0.170	
		10	10		0.183		
		10	10		0.203		
		9	10		0.139		
122	52	10	10	100%	0.063	0.078	
		10	10		0.073		
		10	10		0.073		
		10	10		0.103		
236	101	10	10	100%	0.013	-0.014	Y**
		10	10		-0.017		Y
		10	10		-0.037		Y
		10	10		-0.015		Y
466	200	8	10	88%	0.005	0.005	Y
		8	10		-0.005		Y
		10	10		0.003		Y
		9	10		0.017		Y

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method	
Survival	Means comparison	$P = 0.001$
Biomass gain	Regression	
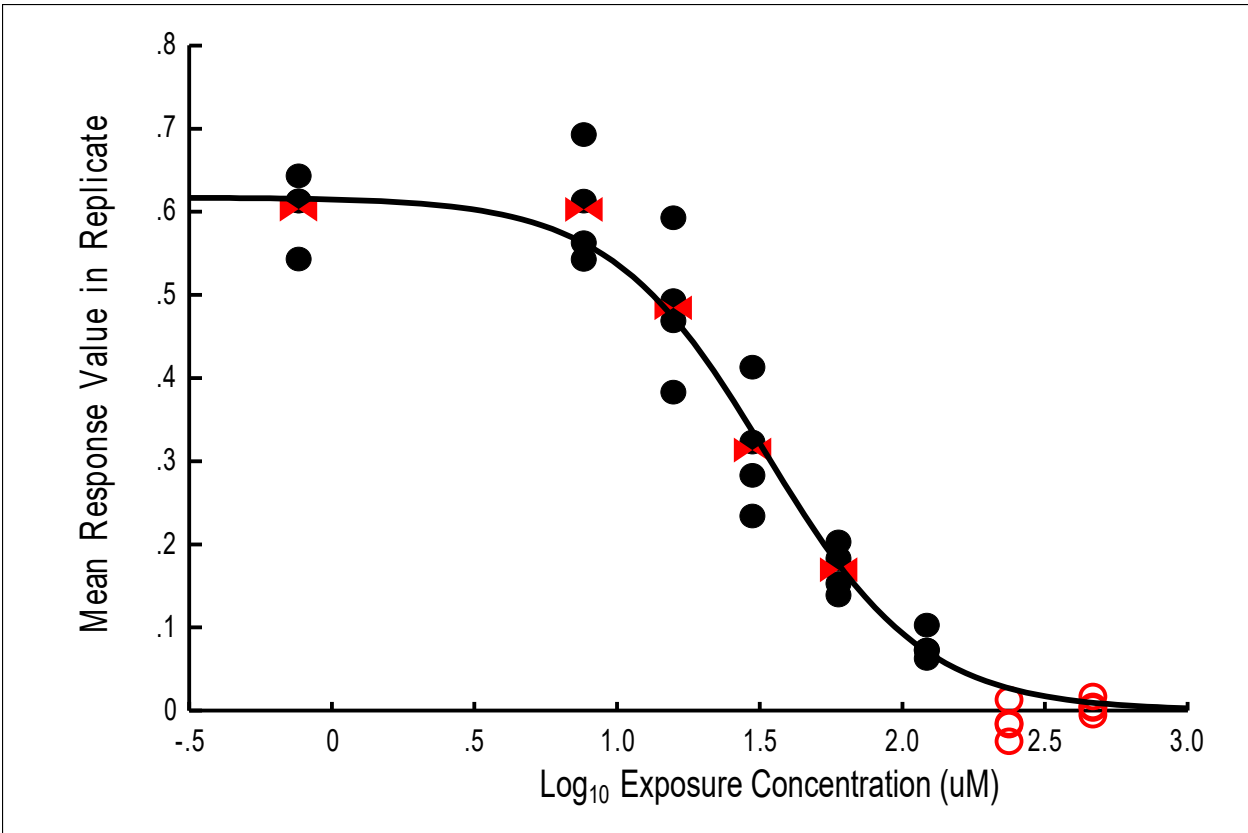
Hyalella   Biomass Gain   62FTS   2+Partial

Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.164	0.50	0.700	1.700	1.524	1.441	1.594	
					33.4	27.6	39.3	
Slope	3.566	0.50	0.500	5.000	0.908	0.756	1.085	
CtrlVal	0.6031	0.50	0.3000	1.2000	0.6173	0.5798	0.6566	
StdDev	0.0485	0.50	0.0200	0.1000	0.0523	0.0393	0.0727	
logEC20					1.142	0.993	1.263	
					13.9	9.8	18.3	

Parameter Optimization History

Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	98.6840 335.1854	0.915 1.366	2.621 4.278	0.3035 0.7913	0.0391 0.0736
31	-28.3586 -17.4151	1.569 1.700	1.075 2.201	0.5431 0.6879	0.0953 0.1000
101	-35.0740 -34.9743	1.500 1.541	0.835 0.922	0.6146 0.6349	0.0973 0.0978
221	-40.7965 -39.2085	1.505 1.582	0.826 1.013	0.6107 0.6364	0.0507 0.0658
450	-41.3167 -41.3167	1.524 1.524	0.908 0.908	0.6173 0.6173	0.0523 0.0523

Analysis Date: 01/22/2023 22:15   01/16/23 Continuous Variable Effects Analysis Program

Hyalella   Biomass Gain   62FTS   2+Partial

Data Summary

logC	#Meas	YVar	Mean	logC	#Meas	YVar	Mean	logC	#Meas	YVar	Mean
-0.116	1	0.6130	0.6033	1.475	1	0.4130	0.3133	2.374	-1	0.0130	-1.0000
	1	0.5433			1	0.3230			-1	-0.0170	
	1	0.6133			1	0.2830			-1	-0.0370	
	1	0.6433			1	0.2340			-1	-0.0150	
0.884	1	0.6930	0.6030	1.776	1	0.1530	0.1695	2.668	-1	0.0050	-1.0000
	1	0.5430			1	0.1830			-1	-0.0050	
	1	0.5630			1	0.2030			-1	0.0030	
	1	0.6130			1	0.1390			-1	0.0170	
1.197	1	0.4690	0.4845	2.086	1	0.0630	0.0780				
	1	0.3830			1	0.0730					
	1	0.4930			1	0.0730					
	1	0.5930			1	0.1030					

Confidence Limits Calculations

(LogLikelihoodTarget: -39.3967)   Reoptimized Other Parameters at CL

Parm	Type	logLike@CL	CLValue	logX50	Slope	CtrlVal	StdDev	ErrorFlags
logEC50	LCL	-39.3967	1.441		0.788	0.641	0.058	0 0 0 0 0
	UCL	-39.3967	1.594		1.019	0.595	0.058	0 0 0 0 0
Slope	LCL	-39.3967	0.756	1.462		0.632	0.058	0 0 0 0 0
	UCL	-39.3967	1.085	1.572		0.604	0.053	0 0 0 0 0
CtrlVal	LCL	-39.3967	0.580	1.566	0.958		0.057	0 0 0 0 0
	UCL	-39.3967	0.657	1.474	0.841		0.058	0 0 0 0 0
StdDev	LCL	-39.3968	0.039	1.525	0.930	0.616		0 0 0 0 0
	UCL	-39.3967	0.073	1.523	0.894	0.618		0 0 0 0 0
logEC20	LCL	-39.3967	0.993		0.765	0.638	0.058	0 0 0 0 0
	UCL	-39.3967	1.263		1.065	0.598	0.056	0 0 0 0 0

S11.17. H. azteca 8:2 FTS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.54	0.29	10	10	98%	0.380	0.474	
		9	10		0.496		
		10	10		0.570		
		10	10		0.450		
5.4	2.9	9	10	98%	0.486	0.519	
		10	10		0.600		
		10	10		0.570		
		10	10		0.420		

Endpoint	Analysis method
Survival	No significant effect
Biomass gain	No significant effect

S11.18. H. azteca 10:2 FTS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Total # of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)	Censored from growth analysis? Y
0.013	0.0081	10	10	98%	0.380	0.474	
		9	10		0.496		
		10	10		0.570		
		10	10		0.450		
0.13	0.081	10	10	100%	0.610	0.605	
		10	10		0.600		
		10	10		TE		Y - TE
		10	10		TE		Y - TE

TE = technical error; censored from analysis

Endpoint	Analysis method
Survival	No significant effect
Biomass gain	No significant effect

S12. *C. dilutus* test designs, data, and analyses

S12.1 Overview

This section provides, for each PFAS toxicity test with *Chironomus dilutus*, replicate-level test data and the results of concentration/response (CR) analyses of these data. For all tests, Section S12.2 tabulates the calculated effect concentrations (ECs) for both survival and growth, specifying for each test either (a) point EC estimates and confidence limits when test data were sufficient to conduct CR curve estimation or (b) ECs being greater than the highest test concentration and the magnitude/significance of effect at this concentration when CR curves could not be estimated. For each test, Sections S12.3 to S12.25 provide: a table of test data, analysis software output for the survival CR curve estimation when conducted, and output for the growth CR curve estimation when conducted. Note, all control concentrations were assigned a value of 1/10th of the lowest treatment for CR analysis.

The output for the survival CR curve estimation has three components:

(1) Header text specifying the taxon ("Midge"), the chemical, and the endpoint ("Survival").

(2) A figure for fraction survival ("Effect Variable") versus the base-10 logarithm of the concentration (uM) that includes observed treatment-level survivals and the estimated CR curve. For tests with at least two partial effects, the solid-black plotted line is the maximum-likelihood solution. For tests with just one partial effect, parameter estimates are not based on a maximum likelihood solution, but rather are approximated as described in the main text of this paper, and the red-dashed line is approximate/illustrative based on those parameter estimates.

(3) A table regarding parameter estimation for the log-logistic CR model. The three model parameters are the base-10 logarithm of the LC50 ("logX50"), the standard deviation of the log-logistic distribution ("logStdDev"), and the control survival ("CtrlSurv"). For these three parameters, the table provides: initial guesses ("Guess") for the nonlinear optimization search, the allowed parameter range ("PMin", "PMax"), and a fraction of the allowed range over which the parameter search is initially restricted ("Delta"). The table then provides final estimates ("PEst") and 95% confidence limits ("95%LCL","95%UCL") for each parameter, as well as for the untransformed LC50, the logarithm of the LC20 ("logX20"), and the untransformed LC20. Parameter estimates and CLs in black denote maximum likelihood solution values, whereas parameter estimates in red denote alternative approximations (see text) and CLs in red denote those at their min/max values.

The output for the growth CR curve estimation has four components:

(1) Header text specifying the taxon ("Midge"), the endpoint ("Biomass"), the chemical abbreviation, and whether the number of partial effects was at least two ("2+Partial") to allow maximum likelihood curve fitting or just one ("1Partial") in which case alternative methods were used to approximate the maximum likelihood parameter values as described in the main text of this paper.

(2) A figure that includes replicate-level data ("Total Biomass (mg AFDW)") versus "Log_{10} Exposure Concentration (uM)", and the estimated CR curve. Data used for curve estimation are

indicated by black circles, with treatment means being a red "bow tie". Red open circles denote replicates censored due to technical errors (e.g., solution lost), statistical outliers, or complete censoring of high concentration treatments not needed for curve definition. For tests with at least two partial effects, the plotted solid black line is the maximum-likelihood solution. For tests with just one partial effect, the plotted dashed red lines are illustrative, with the steeper line being the maximum likelihood solution at the maximum allowed slope and the shallower line being based on the alternative estimates for the parameter values (see text).

(3) A table regarding parameter estimation for the log-logistic CR model. The four model parameters are the base-10 logarithm of the EC50 ("logEC50"), the slope of the line at the EC50 ("Slope"), the control growth ("CtrlVal"), and a reference standard deviation ("StdDev"); see text for more details on weighting analysis regarding this standard deviation. For these four parameters, the table provides: initial guesses ("Guess") for the nonlinear optimization search, the allowed parameter range ("PMin", "PMax"), and a fraction of the allowed range over which the parameter search is initially restricted ("Delta"). The table then provides final maximum likelihood estimates ("PEst") and 95% confidence limits ("95%LCL","95%UCL") for each parameter, as well as for the untransformed EC50 (unlabeled line below the logEC50), the logarithm of the EC20 ("logEC20"), and the untransformed EC20. When there is just one partial effect, the maximum likelihood reached is constrained by the maximum allowed slope, so PEst deviates slightly from the unconstrained (and unrealistic) maximum likelihood solution. Parameter estimates then are alternatively estimated as described in the text and are entered in this table as "PAlt".

(4) The parameter optimization history, which provides values for the log likelihood and the four parameter values at different iterations (Iter) during the optimization.

S 12.2. PFAS Survival LC50s and LC20s, growth EC50s and EC20s, and 95% confidence limits (mg/L) for 7-d *C. dilutus* tests

| Chemical class	Molecular weight g/mol	Abbrev.	LC50	LC20	EC50	EC20
Perfluoroalkyl carboxylic acids	214.04	PFBA	>217	>217	>217	>217
	314.05	PFHxA	>310	>310	664 (234->628)	206 (168-497)
	414.07	PFOA	106 (88-118)	73 (48-94)	93.2 (69.7-109)	70.6 (44.1-93.1)
			134 (110-163)	98 (60-159)	116 (93-144)	94.0 (72.0-123)
			132 (122-142)	103 (87-123)	121 (112-131)	94.2 (80.6-110)
	464.08	PFNA	4.3 (3.7-5.0)	2.5 (1.8-3.3)	3.53 (3.0-4.1)	2.29 (1.82-2.84)
	514.08	PFDA	1.0 (0.88-1.1)	0.65 (0.51-0.82)	0.72 (0.64-0.81)	0.56 (0.48-0.66)
Perfluoroalkyl sulfonic acids	299.09	PFBS	>282	>282	>282	>282
	400.12	PFHxS	27.0 (17-44)	10.4 (4.8-23)	18.3 (13.3-24.1)	11.00 (6.46-19.6)
	450.12	PFHpS	0.25 (0.22-0.29)	0.16 (0.11-0.20)	0.24 (0.10-0.35)	0.18 (0.05-0.30)
	500.13	PFOS	0.044 (0.039-0.050)	0.025 (0.019-0.031)	0.025 (0.020-0.030)	0.018 (0.012-0.024)
			0.025 (0.023-0.029)	0.017 (0.014-0.020)	0.020 (0.018-0.023)	0.016 (0.013-0.020)
	550.14	PFNS	0.015 (0.012-0.017)	0.009 (0.006-0.011)	0.013 (0.012-0.014)	0.010 (0.009-0.012)
			0.013 (0.012-0.014)	0.010 (0.008-0.012)	0.011 (0.010-0.012)	0.009 (0.007-0.011)
	600.15	PFDS	0.13 (0.11-0.16)	0.063 (0.043-0.090)	0.11 (0.081-0.16)	0.073 (0.047-0.13)
Perfluoroalkane sulfonamides	299.12	FBSA	>8.6	>8.6	10.9 (9.4-13.7)	4.2 (2.3-5.8)
	399.13	FHxSA	1.8 (1.4-2.3)	1.0 (0.62-1.6)	0.72 (0.61-0.80)	0.47 (0.31-0.65)
	499.15	FOSA	0.037 (0.031-0.048)	0.017 (0.010-0.011)	0.011 (0.007-0.029)	0.005 (0.002-0.024)
			>0.027	>0.027	0.011 (0.008-0.015)	0.005 (0.003-0.010)
Fluorotelomer sulfonic acids	428.17	6:2 FTS	178 (148-229)	92 (44-197)	80.8 (65.7-100)	52.7 (38.2-73.9)
	528.18	8:2 FTS	>2.8[*]	>2.8[*]	>2.8[*]	>2.8[*]
	628.2	10:2 FTS	>0.16[*]	>0.16[*]	>0.16[*]	>0.16[*]

*ECx greater than solubility limit

S12.3. C. dilutus PFBA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
101 control	21.7	9	10	93%	1.81	2.035	
		8	10		1.61		
		10	10		2.76		
		10	10		1.96		
1012	217	17	17	100%		1.563	Y-overstocked
		11	11		1.29		
		11	11		1.53		
		10	10		1.87		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

| Endpoint	Analysis method	
Survival	No significant effect	
Biomass	Means comparison	$P = 0.0334$

151

S12.4. C. dilutus PFHxA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
6.32 control	1.98	9	10	95%	1.910	1.999	
		10	10		2.050		
		9	10		1.680		
		10	10		2.350		
		11	11		1.843		
		10	10		2.170		
		9	10		1.940		
		9	10		2.050		
63.2	20	10	10	100%	2.160	2.138	
		10	10		2.490		
		11	11		1.851		
		10	10		2.050		
122	38.3	10	10	98%	1.820	1.993	
		10	10		1.900		
		9	10		1.930		
		10	10		2.320		
249	78.2	9	10	93%	2.060	1.892	
		10	10		2.010		
		11	11		1.636		
		8	10		1.860		
500	157	9	10	97%	1.790	1.883	
		11	11		1.777		
		12	12		2.000		Y-overstocked
		11	11		1.967		
638	201	10	10	83%	0.820	0.920	
		8	10		0.750		
		7	10		0.420		
		8	10		1.690		
987	310	10	10	93%	1.950	1.285	
		9	10		0.770		
		8	10		0.660		
		11	11		1.760		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	No significant effect
Biomass	Regression

Midge   Biomass   PFHxA   2Partials,Weighted
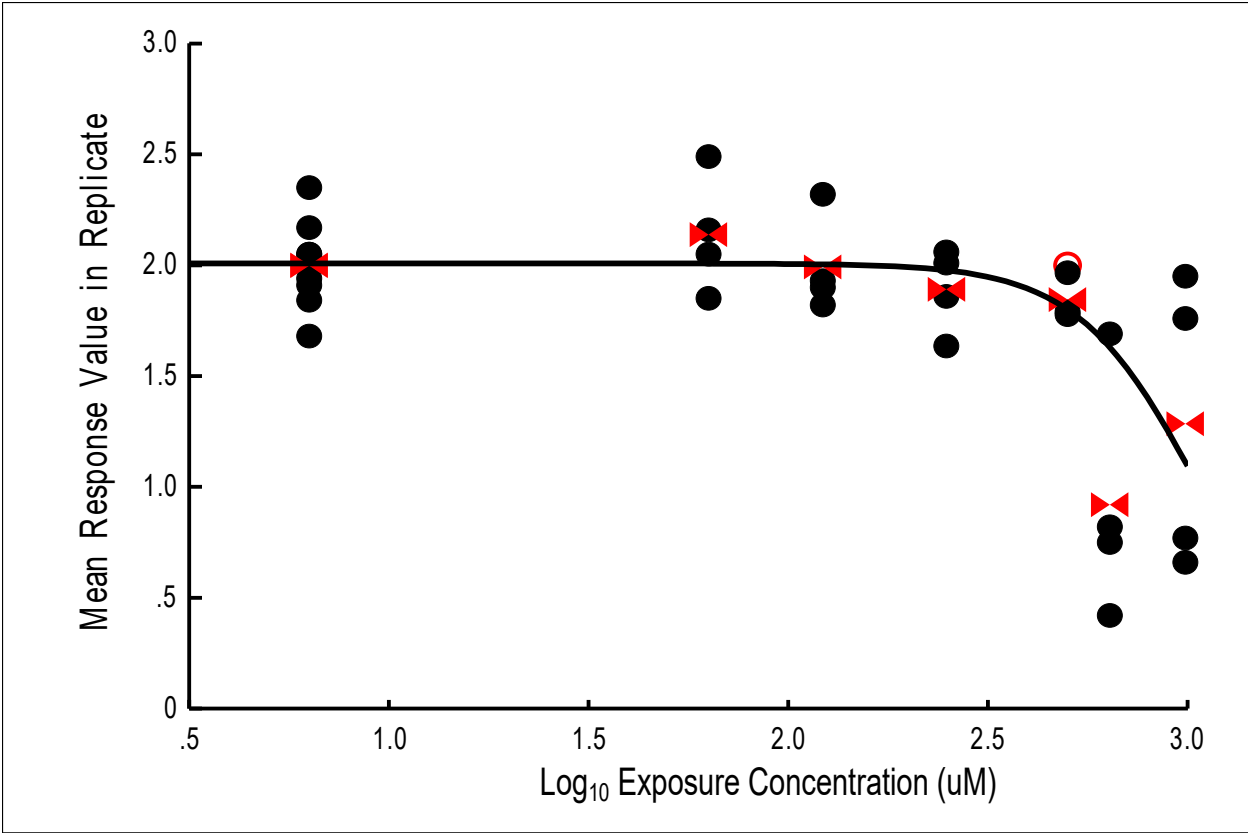
Parameter Summary   Optimization Errors: 0   0   0   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	3.135	0.50	2.600	5.000	3.027	2.872	4.113	
					1065.	745.	12969.	
Slope	1.626	0.50	0.200	5.000	1.645	0.347	3.279	
CtrlVal	1.984	0.50	1.000	3.000	2.008	1.911	2.118	
StdDev	0.202	0.50	0.100	0.800	0.240	0.189	0.315	
logEC20					2.817	2.729	3.199	
					656.	536.	1582.	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	6.0096	195.4158	2.602	3.626	0.200	2.747	1.587	2.561	0.144	0.368
21	3.6047	4.7865	3.060	3.185	1.079	1.269	1.980	2.059	0.202	0.227
71	3.2264	3.2426	3.132	3.140	1.167	1.197	2.013	2.021	0.236	0.241
161	3.0153	3.0154	3.027	3.028	1.643	1.647	2.008	2.008	0.240	0.240
325	3.0153	3.0153	3.027	3.027	1.645	1.645	2.008	2.008	0.240	0.240

S12.5. C. dilutus PFOA test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
13.7 control	5.7	10	10	85%	1.770	1.65	
		11	11		2.436		
		8	10		1.480		
		9	10		1.560		
		10	10		1.980		
		4	10		0.700		
137	57	6	10	78%	0.780	1.25	
		8	10		1.110		
		9	10		1.540		
		9	10		1.610		
		7	10		0.760		
		8	10		1.730		
280	116	3	10	32%	0.290	0.38	
		6	10		0.430		
		3	10		0.600		
		2	10		0.400		
		2	10		0.190		
		3	10		0.360		
475	197	0	10	2%	0.000	0.00	
		0	10		0.000		
		0	10		0.000		
		0	10		0.000		
		1	10		0.020		
		0	10		0.000		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFOA 1 Survival
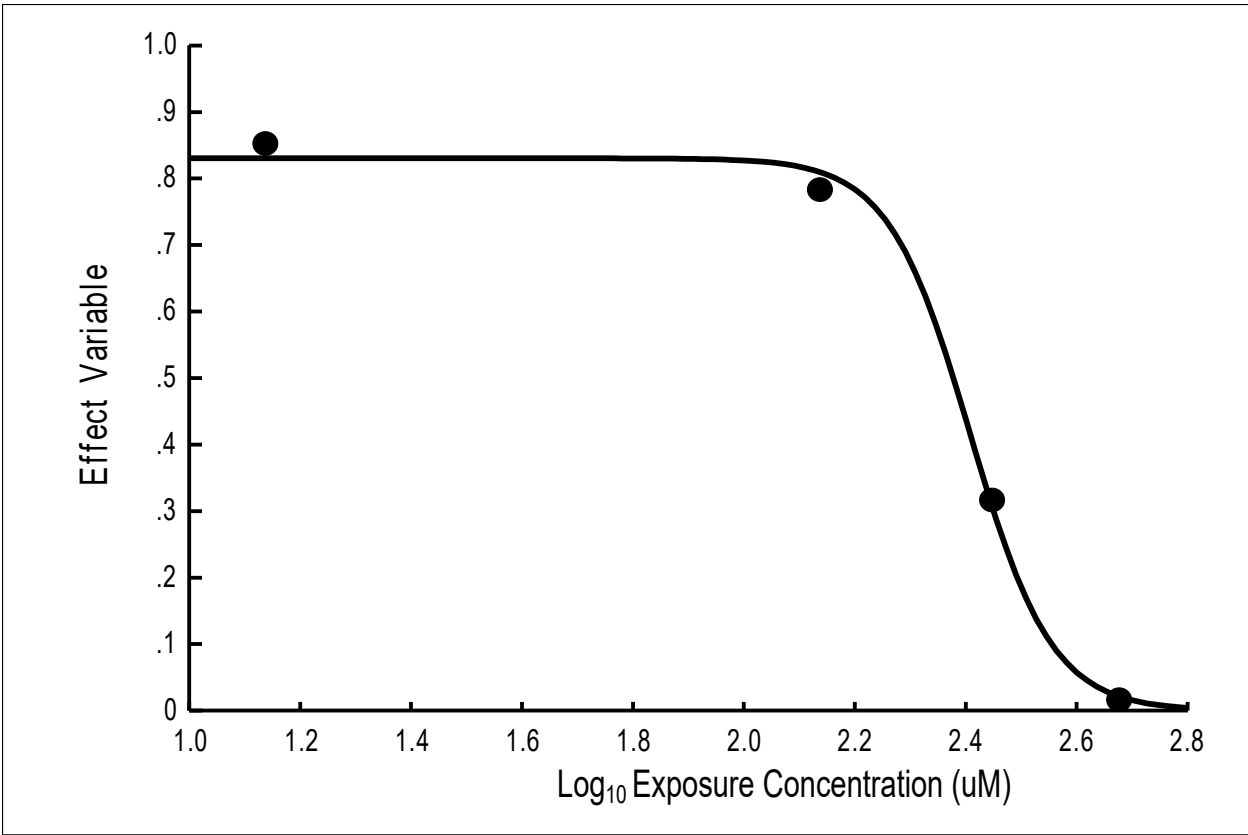
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	2.3364	0.5000	2.0364	2.6364	2.4082	2.3282	2.4561
					256.00	212.90	285.80
logStdDev	0.2345	0.5000	0.0100	0.5000	0.1337	0.0671	0.2320
CtrlSurv	0.8179	0.5000	0.8000	1.0000	0.8304	0.8000	0.9122
logX20					2.3060	2.1657	2.3884
					202.31	146.47	244.57

Midge   Biomass   PFOA 1   2Partial,Weighted
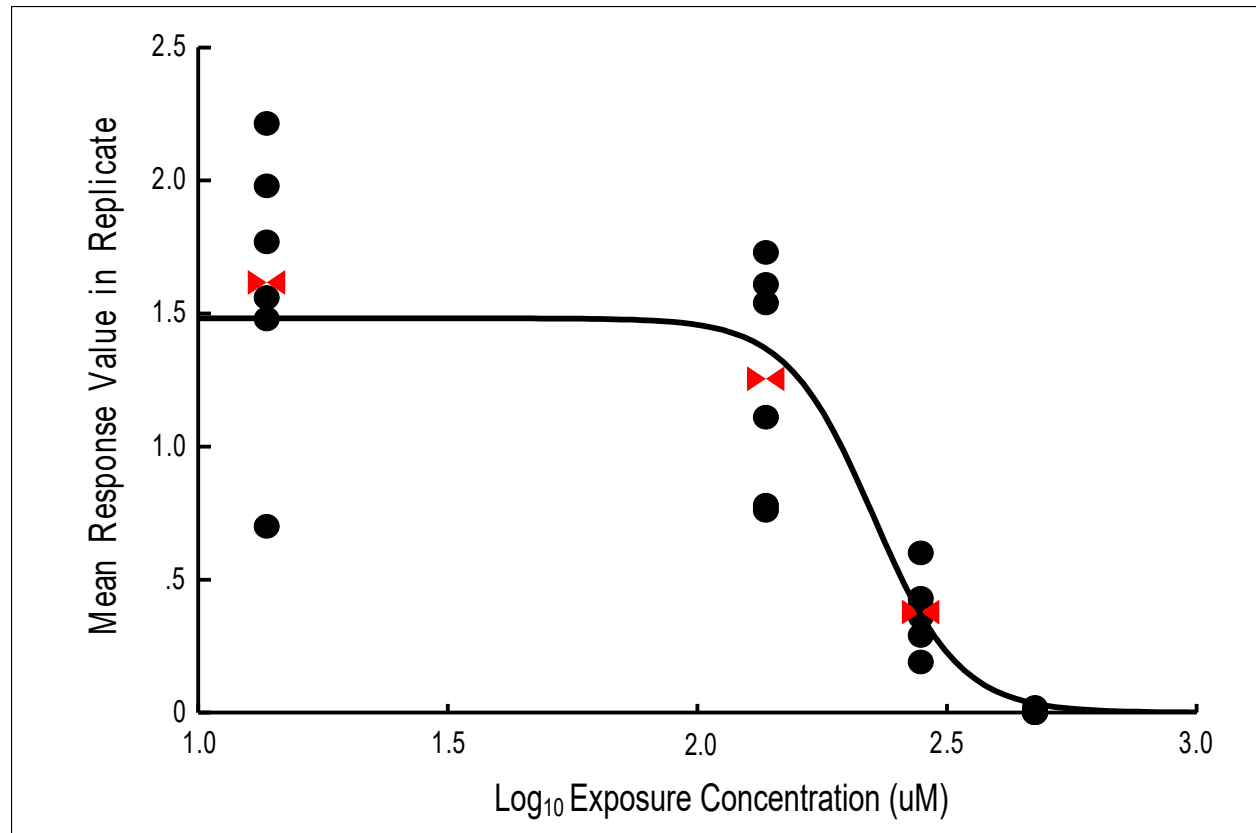
Parameter Summary   Optimization Errors: 0   0   0   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	2.329	0.50	1.800	2.800	2.352	2.226	2.421	
					225.	168.	264.	
Slope	2.870	0.50	0.500	5.000	2.899	1.677	5.000	
CtrlVal	1.617	0.50	1.000	3.000	1.482	1.148	1.916	
StdDev	0.479	0.50	0.200	0.800	0.492	0.357	0.716	
logEC20					2.232	2.027	2.352	
					171.	107.	225.	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-3.7782	24.7915	2.209	2.453	1.836	3.826	1.151	2.034	0.348	0.609
11	-4.2825	-3.6473	2.321	2.349	2.642	2.859	1.537	1.657	0.463	0.491
51	-4.4074	-4.4050	2.344	2.346	2.746	2.788	1.493	1.505	0.488	0.492
121	-4.4119	-4.4119	2.352	2.352	2.898	2.902	1.482	1.482	0.492	0.492
259	-4.4119	-4.4119	2.352	2.352	2.899	2.899	1.482	1.482	0.492	0.492

Analysis Date: 01/16/2023 19:50   01/16/23 Continuous Variable Effects Analysis Program

S12.6. C. dilutus PFOA test 2 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
1.2 control	0.50	10	10	100%	2.39	2.48	
		10	10		2.41		
		12	12		2.11		Y-overstocked
		10	10		2.64		
12	5.0	10	10	95%	2.33	2.03	
		10	10		2.22		
		11	11		1.75		
		8	10		1.80		
26	11	8	10	93%	1.57	1.93	
		9	10		1.75		
		10	10		2.48		
		11	11		1.90		
52	22	10	10	98%	2.34	2.23	
		10	10		2.45		
		10	10		2.22		
		9	10		1.92		
104	43	8	10	93%	1.34	1.81	
		9	10		1.95		
		11	11		2.06		
		10	10		1.89		
196	81	9	10	90%	2.10	2.00	
		9	10		2.02		
		8	10		1.73		
		10	10		2.16		
397	164	3	10	20%	0.32	0.26	
		3	10		0.21		
		0	10		0.00		
		2	10		0.52		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFOA 2 Survival
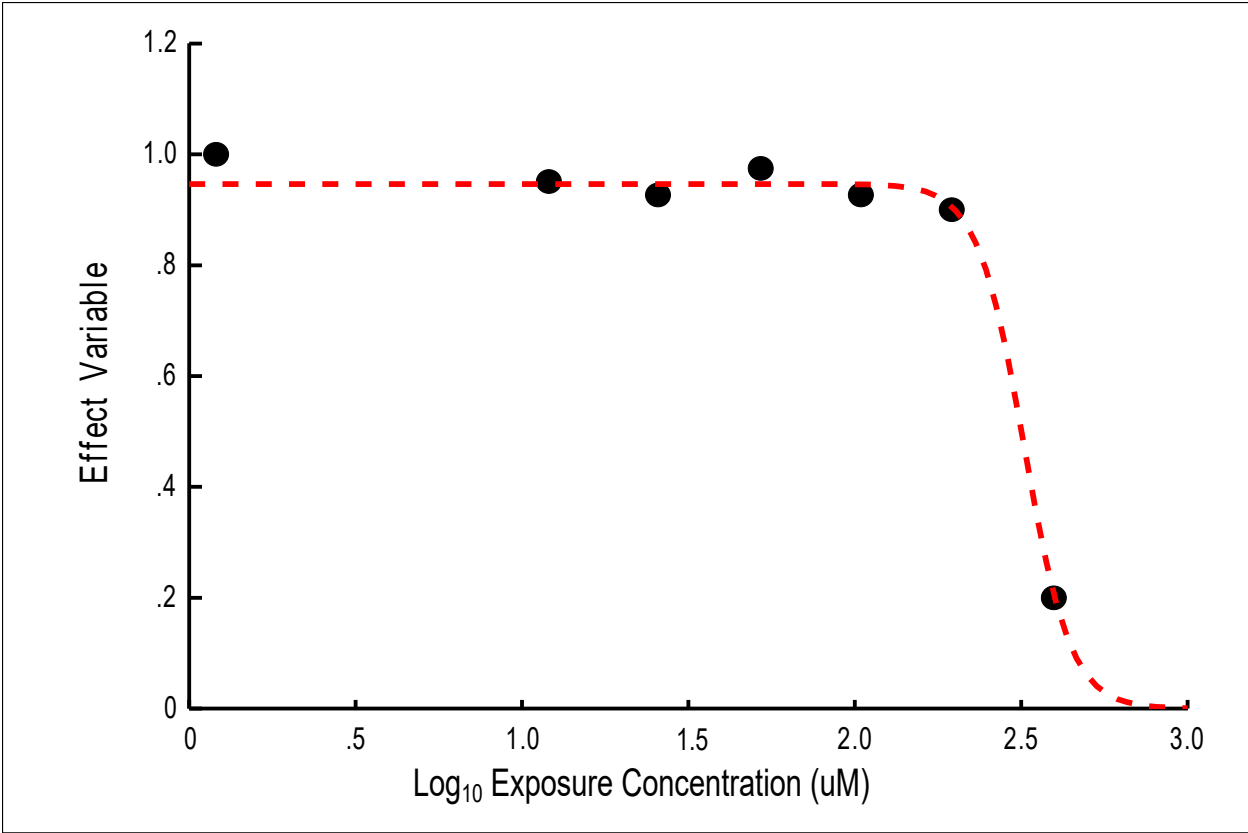
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	2.4670	0.5000	2.1670	2.7670	2.5088	2.4222	2.5954
					322.72	264.38	393.94
logStdDev	0.1462	0.5000	0.0100	0.5000	0.1274	0.0100	0.2547
CtrlSurv	0.9466	0.5000	0.8000	1.0000	0.9466	0.9140	0.9839
logX20					2.4274	2.2670	2.5878
					267.53	184.91	387.07

Midge	**Biomass**	**PFOA 2**	**1Partial,Weighted**
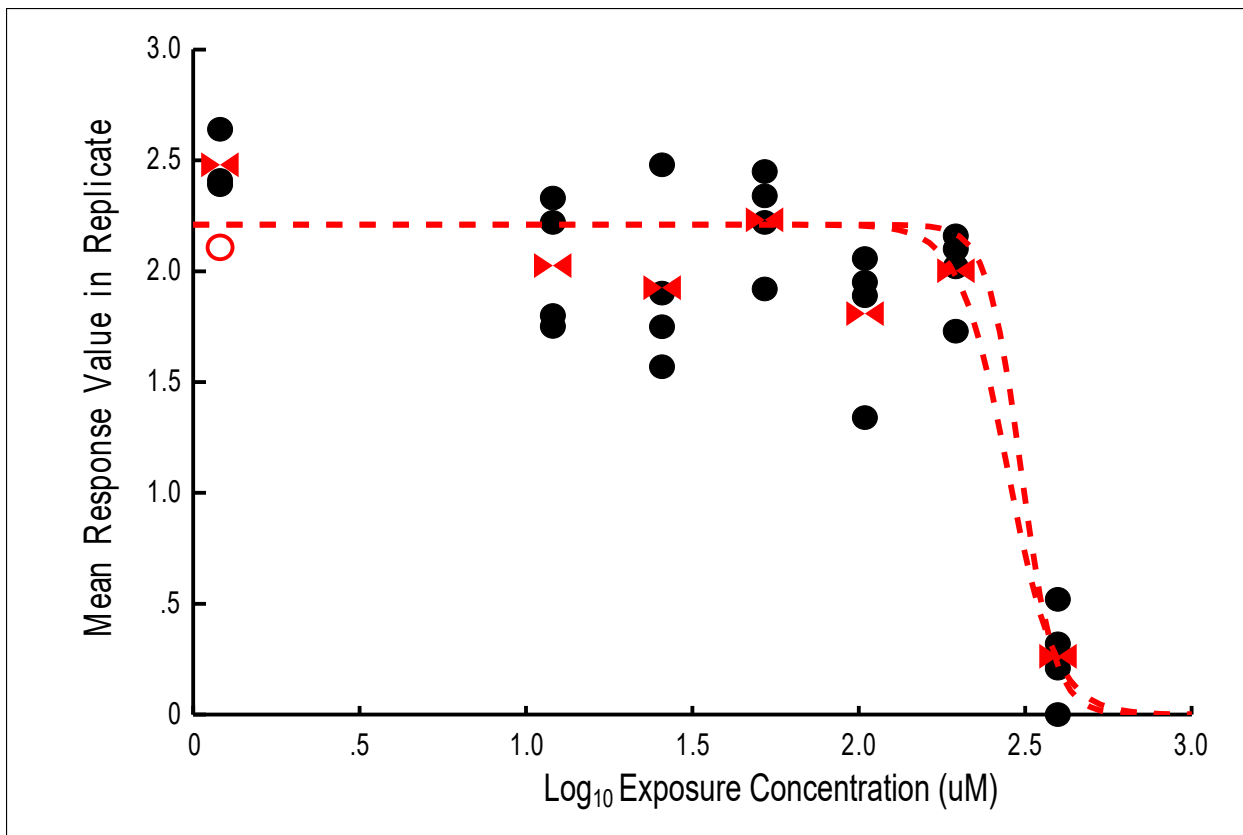
Parameter Summary   Optimization Errors: 0   0 99 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	2.161	0.50	1.700	2.700	2.488	2.353	2.542	2.447
					307.	225.	348.	280.
Slope	1.442	0.50	0.500	5.000	5.000	1.903	5.000	3.451
CtrlVal	2.145	0.50	1.000	3.000	2.210	2.083	2.441	2.210
StdDev	0.320	0.50	0.200	0.800	0.483	0.337	0.680	0.509
logEC20					2.418	2.240	2.472	2.356
					262.	174.	297.	227.

Parameter Optimization History

Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	41.9358 359.1656	2.051 2.406	5.000 5.000	1.786 2.400	0.301 0.543
11	8.3658 51.5790	2.267 2.626	5.000 5.000	1.786 2.462	0.435 0.768
41	7.6085 7.6677	2.468 2.489	5.000 5.000	2.191 2.223	0.470 0.486
101	7.5976 7.5976	2.487 2.488	5.000 5.000	2.210 2.210	0.482 0.483
205	7.5976 7.5976	2.488 2.488	5.000 5.000	2.210 2.210	0.483 0.483

Analysis Date: 01/16/2023 20:05   01/16/23 Continuous Variable Effects Analysis Program

S12.7. C. dilutus PFOA test 3 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
3.9 control	1.6	10	10	95%	1.44	1.87	
		10	10		2.06		
		8	10		1.62		
		10	10		2.34		
39	16	10	10	100%	1.74	1.89	
		10	10		1.97		
		10	10		1.99		
		10	10		1.86		
77	32	11	11	90%	1.88	1.97	
		9	10		2.27		
		11	11		1.96		
		7	10		1.78		
152	63	10	10	100%	2.10	1.94	
		10	10		2.09		
		11	11		1.64		
		10	10		1.93		
302	125	5	10	58%	1.11	0.89	
		7	10		0.92		
		4	10		0.58		
		7	10		0.93		
605	250	0	10	0%	0.00	0.00	
		0	10		0.00		
		0	10		0.00		
		0	10		0.00		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFOA 3 Survival
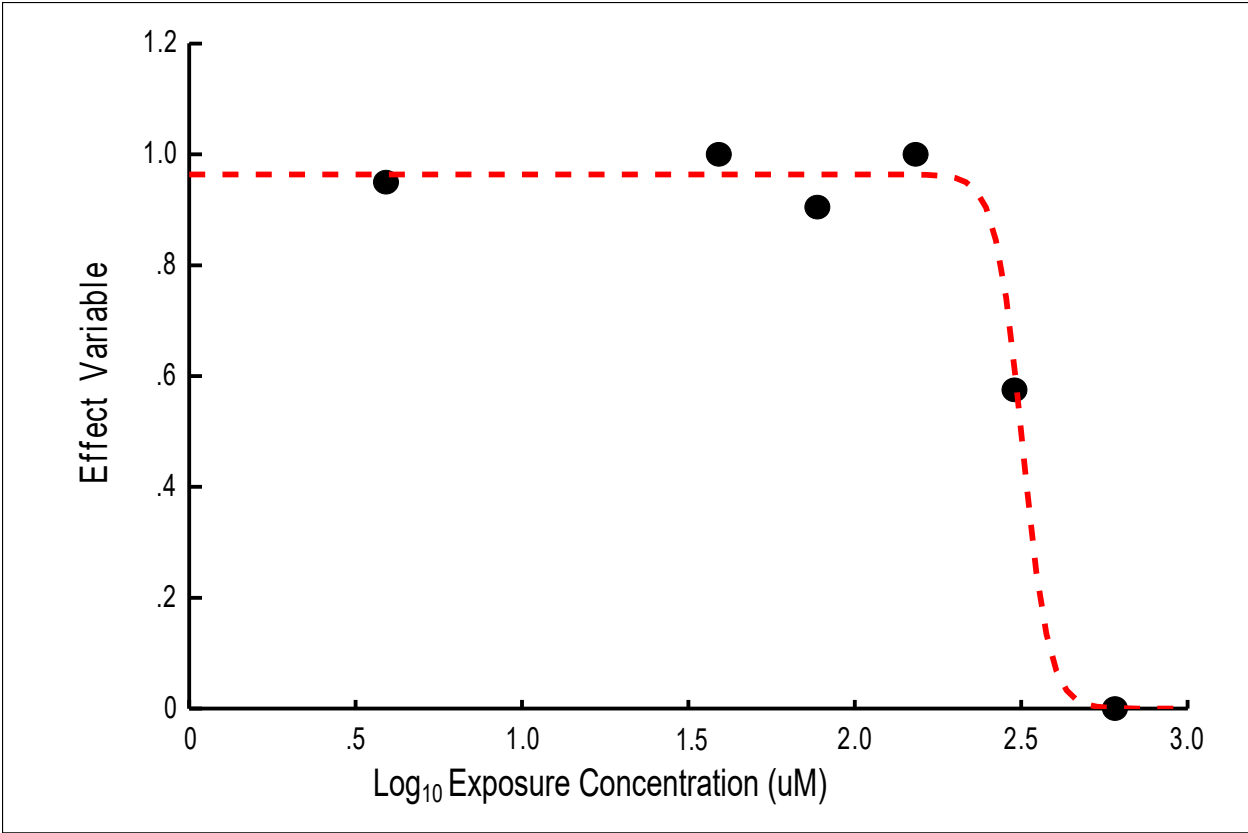
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	2.4820	0.5000	2.1820	2.7820	2.5028	2.4692	2.5364
					318.30	294.60	343.89
logStdDev	0.1997	0.5000	0.0100	0.5000	0.0726	0.0100	0.1453
CtrlSurv	0.9637	0.5000	0.8000	1.0000	0.9637	0.9268	0.9852
logX20					2.4294	2.3802	2.4787
					268.81	239.99	301.09

Midge   Biomass   PFOA 3   1Partial,Weighted
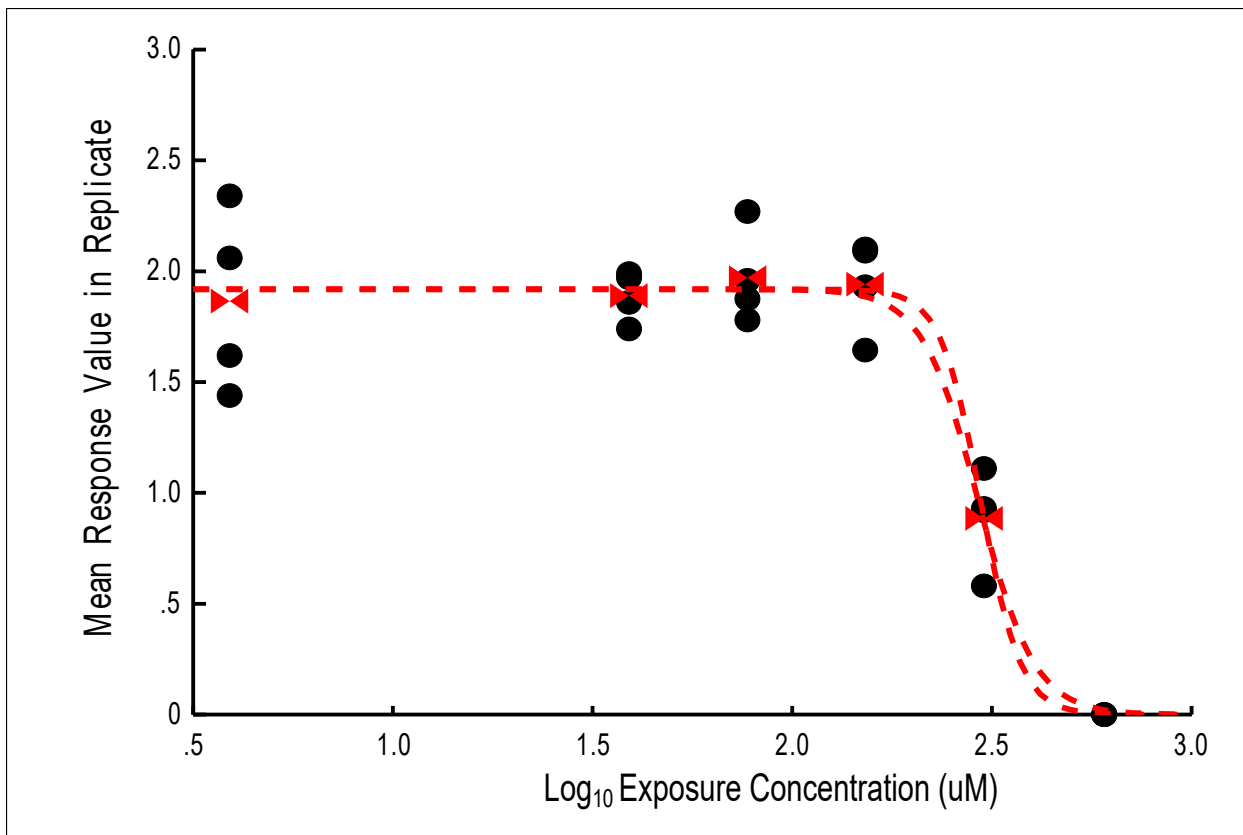
Parameter Summary   Optimization Errors: 0   0 99   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	2.494	0.50	2.000	3.000	2.473	2.432	2.501	2.467
					297.	271.	317.	293.
Slope	3.474	0.50	0.500	5.000	5.000	2.136	5.000	3.568
CtrlVal	1.917	0.50	1.000	3.000	1.918	1.814	2.028	1.918
StdDev	0.229	0.50	0.100	0.800	0.203	0.157	0.278	0.217
logEC20					2.403	2.289	2.426	2.357
					253.	194.	267.	228.

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-2.3231	65.4434	2.256	2.698	5.000	5.000	1.468	2.365	0.122	0.365
11	-3.0587	-0.9110	2.450	2.499	5.000	5.000	1.835	2.007	0.229	0.281
51	-4.1813	-4.0931	2.471	2.477	5.000	5.000	1.898	1.925	0.199	0.212
111	-4.1945	-4.1945	2.473	2.473	5.000	5.000	1.918	1.918	0.203	0.203
223	-4.1945	-4.1945	2.473	2.473	5.000	5.000	1.918	1.918	0.203	0.203

Analysis Date: 01/17/2023 10:40   01/16/23 Continuous Variable Effects Analysis Program

## S12.8. C. dilutus PFNA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.32 control	0.15	9	10	93%	1.64	1.85	
		9	10		1.95		
		10	10		1.95		
		9	10		1.84		
3.2	1.5	11	11	95%	1.65	1.72	
		8	10		1.72		
		10	10		1.91		
		10	10		1.60		
6.8	3.1	10	10	70%	1.25	1.09	
		5	10		0.72		
		6	10		1.17		
		7	10		1.22		
14	6.3	4	10	20%	0.55	0.27	
		1	10		0.17		
		0	10		0.00		
		3	10		0.37		
27	12	0	10	0%	0.00	0.00	
		0	10		0.00		
		0	10		0.00		
		0	10		0.00		
52	24	0	10	0%	0.00	0.00	Y*
		0	10		0.00		Y
		0	10		0.00		Y
		0	10		0.00		Y
103	48	0	10	0%	0.00	0.00	Y
		0	10		0.00		Y
		0	10		0.00		Y
		0	10		0.00		Y

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

| Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFNA Survival
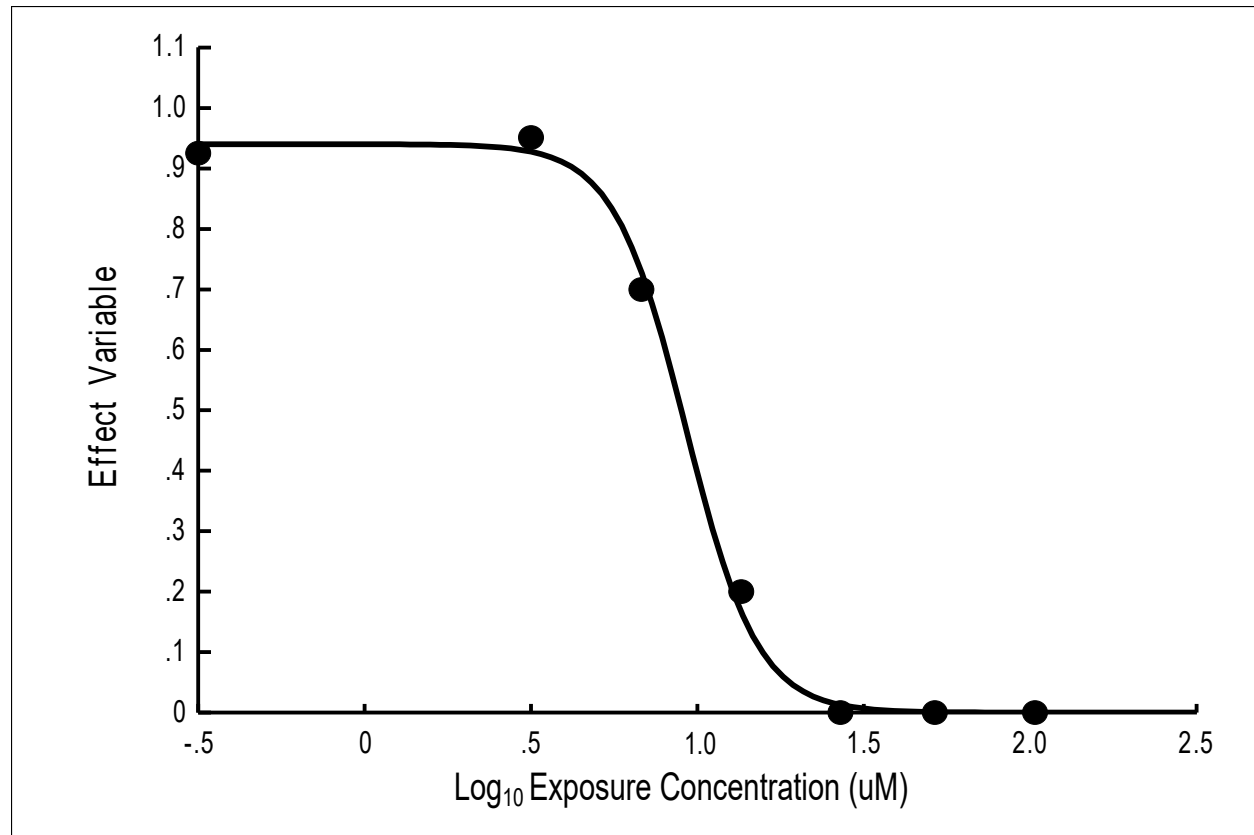
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.9412	0.5000	0.6412	1.2412	0.9655	0.8977	1.0359
					9.24	7.90	10.86
logStdDev	0.3261	0.5000	0.0100	0.5000	0.1961	0.1385	0.2749
CtrlSurv	0.9381	0.5000	0.8000	1.0000	0.9402	0.8692	0.9821
logX20					0.8156	0.7142	0.9125
					6.54	5.18	8.18

Midge   Biomass   PFNA   2Partial,Weighted
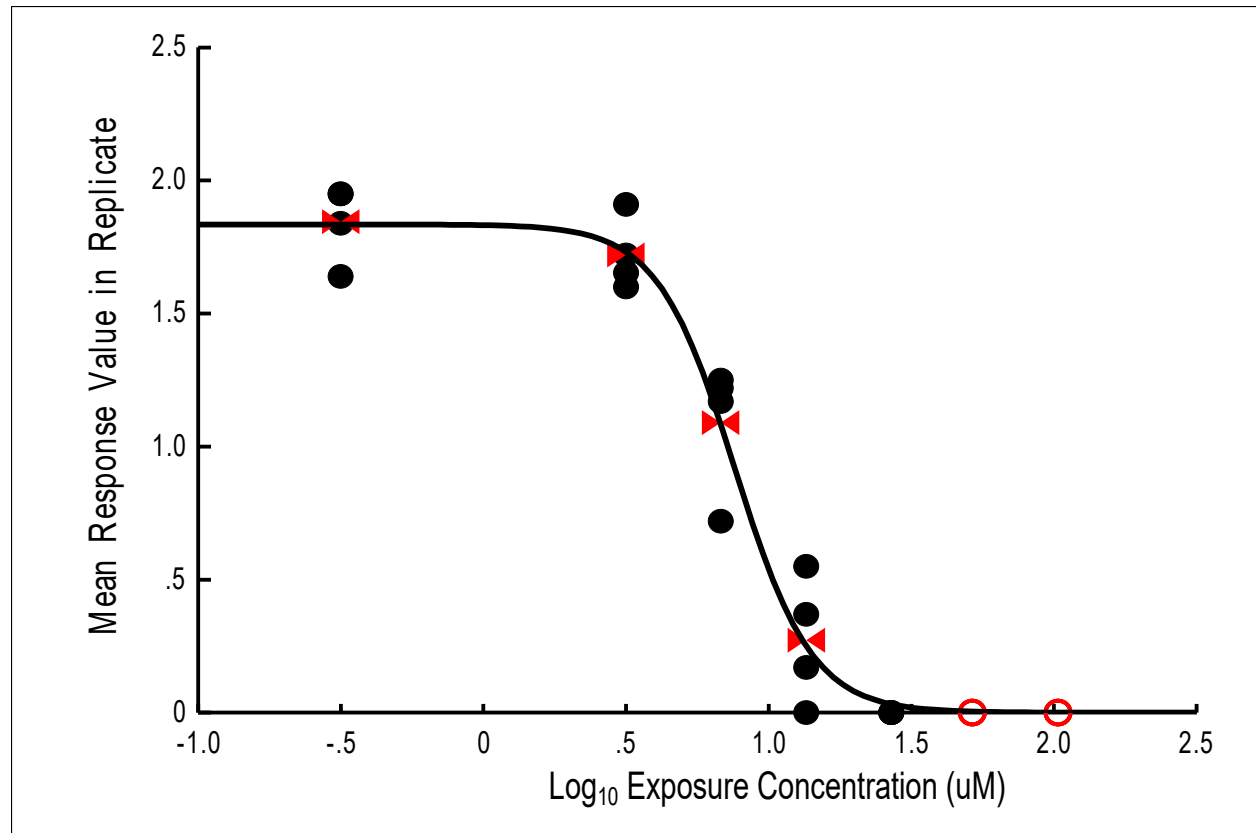
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	0.931	0.50	0.400	1.400	0.881	0.815	0.947	
					7.61	6.54	8.85	
Slope	2.004	0.50	0.500	5.000	1.844	1.391	2.980	
CtrlVal	1.783	0.50	1.000	3.000	1.835	1.723	1.955	
StdDev	0.137	0.50	0.050	0.800	0.137	0.094	0.204	
logEC20					0.693	0.594	0.788	
					4.94	3.92	6.13	

Parameter Optimization History

| Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	-10.5415 65.6013	0.744 1.116	0.994 3.067	1.192 2.280	0.098 0.281
11	-10.5415 -7.2864	0.889 0.977	1.841 2.270	1.680 1.827	0.127 0.167
51	-11.7292 -11.6053	0.882 0.893	1.976 2.092	1.800 1.820	0.136 0.142
111	-11.8602 -11.8601	0.881 0.881	1.841 1.845	1.835 1.835	0.137 0.137
225	-11.8602 -11.8602	0.881 0.881	1.844 1.844	1.835 1.835	0.137 0.137

S12.9. C. dilutus PFDA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.014 control	0.007	10	10	100%	1.89	1.94	
		10	10		1.87		
		10	10		2.13		
		10	10		1.87		
0.14	0.07	11	11	98%	1.67	1.77	
		11	11		1.51		
		10	10		1.95		
		9	10		1.94		
0.29	0.15	10	10	93%	1.98	1.64	
		8	10		1.14		
		10	10		1.81		
		12	12		1.51		Y-overstocked
0.57	0.29	10	10	98%	1.65	1.88	
		10	10		2.02		
		10	10		1.87		
		9	10		1.98		
1.2	0.6	9	10	93%	1.21	1.33	
		9	10		1.29		
		9	10		1.08		
		10	10		1.74		
2.4	1.2	2	10	23%	0.15	0.08	
		0	10		0.00		
		4	10		0.09		
		3	10		0.08		
4.9	2.5	0	10	3%	0.00	0.00	
		0	10		0.00		
		1	10		-0.01		
		0	10		0.00		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFDA Survival
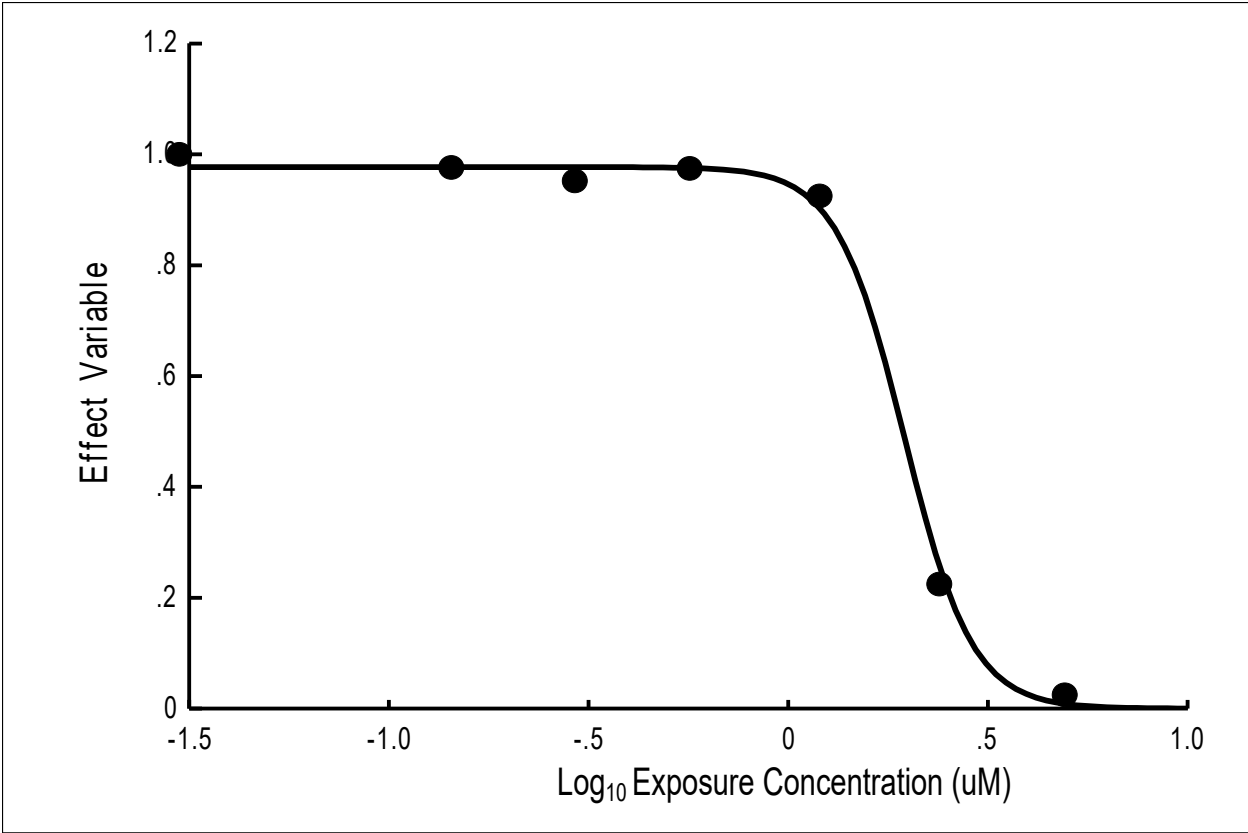
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.3685	0.5000	0.0685	0.6685	0.2912	0.2351	0.3436
					1.96	1.72	2.21
logStdDev	0.2273	0.5000	0.0100	0.5000	0.1541	0.1013	0.2243
CtrlSurv	0.9657	0.5000	0.8000	1.0000	0.9772	0.9475	0.9933
logX20					0.1734	0.0889	0.2489
					1.49	1.23	1.77

Midge   Biomass   PFDA   2Partial,Weighted
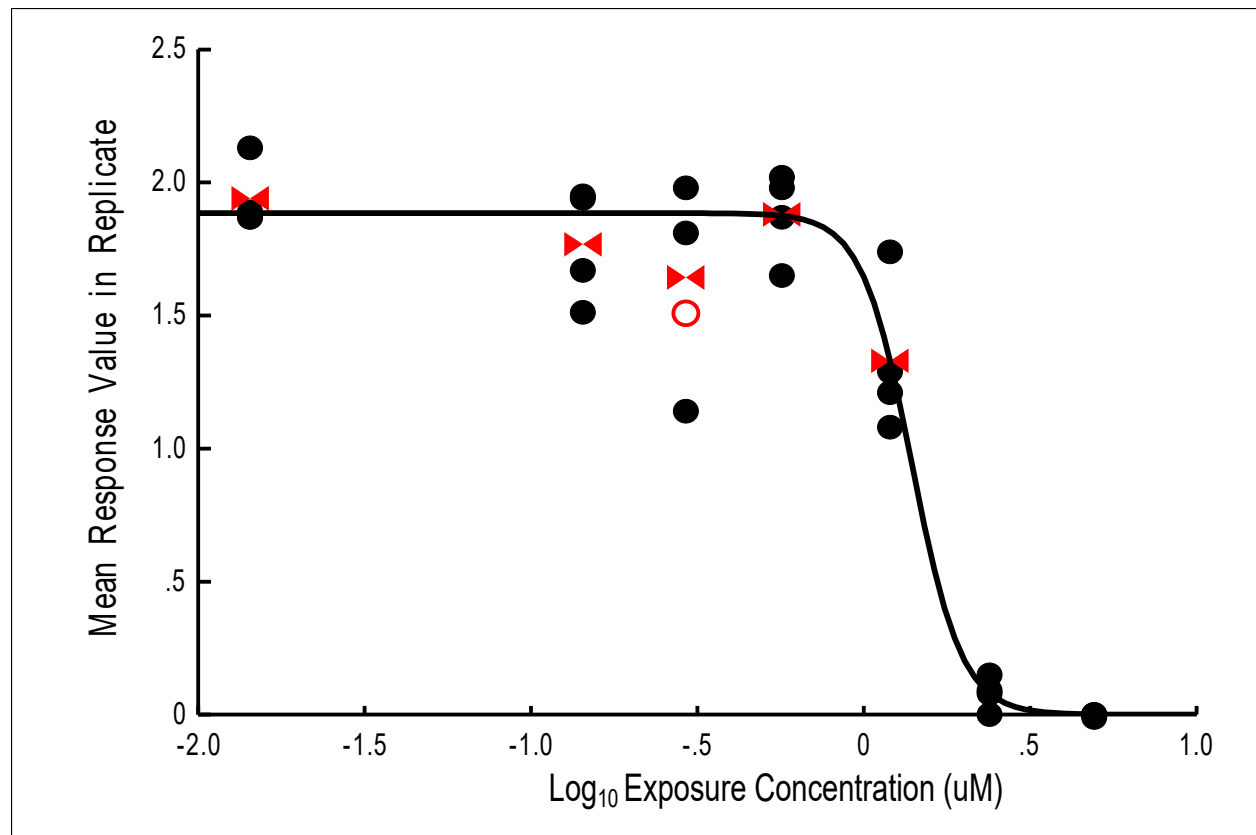
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	0.207	0.50	-0.300	0.700	0.144	0.097	0.195	
					1.39	1.25	1.57	
Slope	2.067	0.50	0.500	5.000	3.338	2.412	5.000	
CtrlVal	1.819	0.50	1.000	3.000	1.885	1.796	1.974	
StdDev	0.237	0.50	0.100	0.800	0.260	0.190	0.371	
logEC20					0.040	-0.032	0.106	
					1.10	0.93	1.28	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-12.0307	13.0037	0.000	0.167	2.026	3.042	1.694	2.062	0.235	0.370
11	-12.0307	-5.8681	0.044	0.202	2.554	3.606	1.778	2.073	0.258	0.370
61	-13.6589	-13.6206	0.137	0.149	3.181	3.324	1.882	1.896	0.246	0.260
131	-13.6883	-13.6883	0.144	0.144	3.337	3.338	1.885	1.885	0.260	0.260
269	-13.6883	-13.6883	0.144	0.144	3.338	3.338	1.885	1.885	0.260	0.260

Analysis Date: 01/16/2023 20:19   01/16/23 Continuous Variable Effects Analysis Program

S12.10. C. dilutus PFBS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
5.7 control	1.7	11	11	100%	1.91	2.00	
		11	11		1.92		
		10	10		2.19		
		10	10		1.97		
57	17	8	10	95%	1.74	1.84	
		11	11		1.74		
		10	10		2.15		
		11	11		1.72		
113	34	10	10	98%	2.08	1.87	
		11	11		1.50		
		10	10		1.80		
		9	10		2.09		
233	70	10	10	100%	2.31	2.13	
		10	10		1.89		
		10	10		1.98		
		10	10		2.34		
443	133	10	10	100%	1.23	1.87	
		10	10		2.00		
		10	10		2.09		
		10	10		2.17		
941	282	9	10	93%	1.19	1.59	
		10	10		1.71		
		9	10		1.75		
		9	10		1.72		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method	
Survival	No significant effects	
Biomass	Means comparison	$P = 0.0139$

S12.11. C. dilutus PFHxS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.18 control	0.075	10	10	93%	1.77	1.88	
		11	11		1.83		
		9	10		2.22		
		8	10		1.68		
1.8	0.71	10	10	98%	2.26	2.23	
		10	10		2.48		
		10	10		2.16		
		9	10		2.03		
3.4	1.4	9	10	93%	1.93	2.05	
		9	10		2.08		
		10	10		2.01		
		9	10		2.16		
7.2	2.9	9	10	98%	2.24	2.31	
		10	10		2.57		
		10	10		2.45		
		11	11		1.98		
13	5.4	10	10	95%	2.35	2.00	
		10	10		1.71		
		10	10		2.30		
		8	10		1.64		
27	11	10	10	83%	2.13	1.57	
		8	10		1.56		
		10	10		1.96		
		5	10		0.61		
58	24	5	10	48%	0.58	0.73	
		3	10		0.32		
		6	10		1.10		
		5	10		0.93		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFHxS Survival
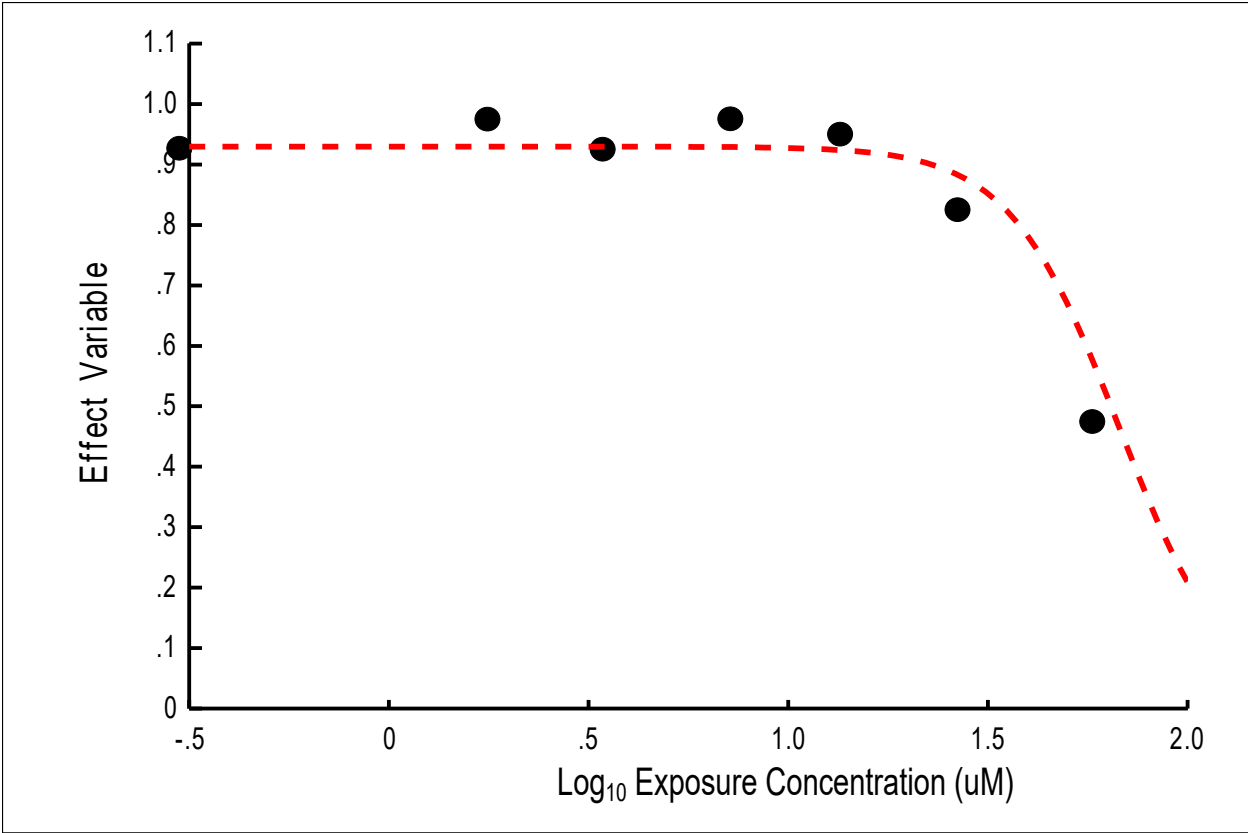
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	1.7371	0.5000	1.4371	2.0371	1.8293	1.6151	2.0435
					67.50	41.22	110.54
logStdDev	0.3212	0.5000	0.0100	0.5000	0.2500	0.0100	0.5000
CtrlSurv	0.9296	0.5000	0.8000	1.0000	0.9296	0.8877	0.9894
logX20					1.5299	1.3022	1.7576
					33.88	20.05	57.23

Midge   Biomass   PFHxS   2Partial,Weighted
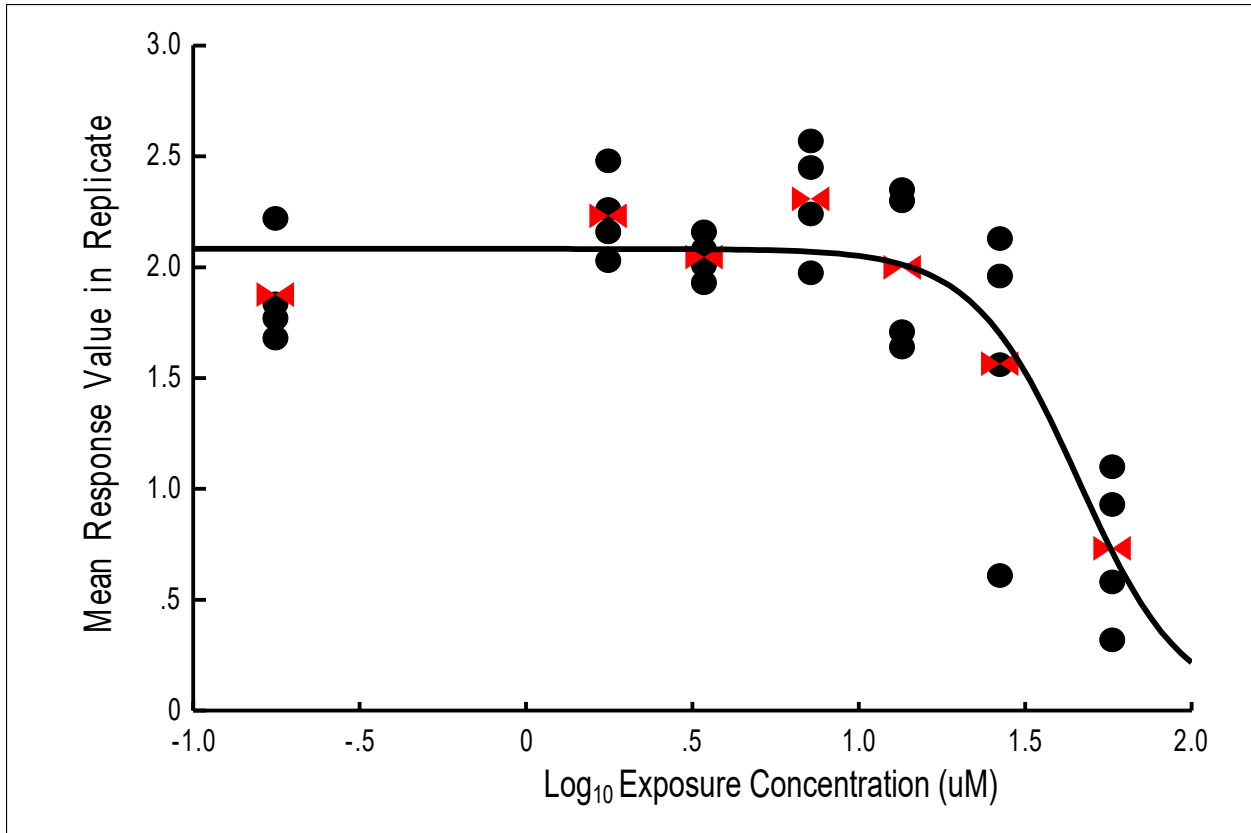
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.659	0.50	1.200	2.200	1.660	1.521	1.779	
					45.7	33.2	60.1	
Slope	1.712	0.50	0.500	5.000	1.582	0.844	5.000	
CtrlVal	2.092	0.50	1.000	3.000	2.083	1.999	2.170	
StdDev	0.268	0.50	0.200	0.800	0.323	0.252	0.430	
logEC20					1.440	1.208	1.690	
					27.6	16.1	48.9	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	4.8517	47.5439	1.538	1.829	0.500	2.408	1.713	2.623	0.230	0.369
11	4.3616	5.0291	1.646	1.719	1.544	1.895	2.049	2.119	0.305	0.339
51	4.2701	4.2728	1.657	1.660	1.591	1.617	2.080	2.084	0.320	0.322
111	4.2687	4.2687	1.659	1.660	1.581	1.582	2.083	2.083	0.323	0.323
234	4.2687	4.2687	1.660	1.660	1.582	1.582	2.083	2.083	0.323	0.323

Analysis Date: 01/16/2023 20:24   01/16/23 Continuous Variable Effects Analysis Program

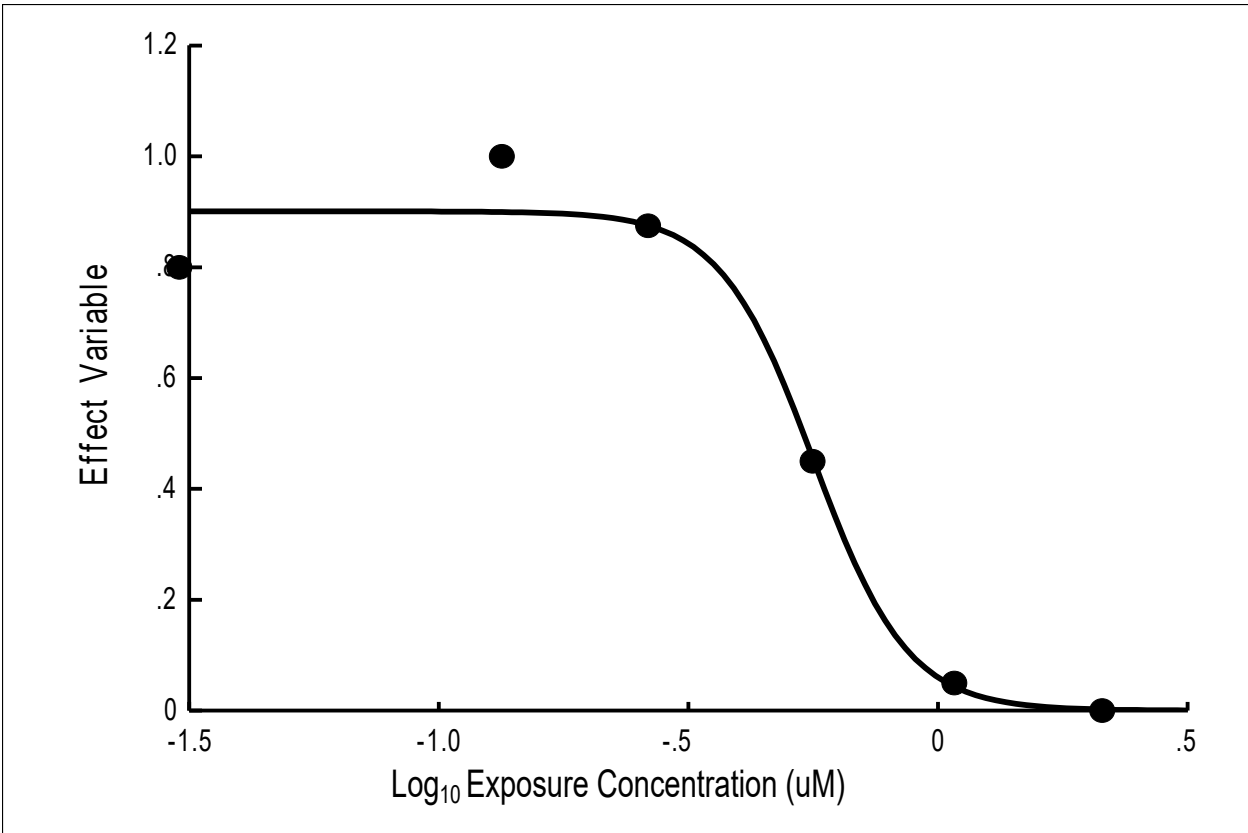## S12.12. C. dilutus PFHpS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.013 control	0.006	8	10	80%	1.79	1.73	
		6	10		0.84		
		8	10		1.78		
		10	10		2.50		
0.13	0.06	10	10	100%	1.59	1.56	
		10	10		2.16		
		11	11		1.22		
		10	10		1.27		
0.26	0.12	10	10	88%	1.85	1.19	
		9	10		1.35		
		6	10		0.39		
		10	10		1.18		
0.56	0.25	8	10	45%	1.44	0.78	
		6	10		1.27		
		2	10		0.26		
		2	10		0.16		
1.1	0.49	0	10	5%	0.00	0.04	
		0	10		0.00		
		0	10		0.00		
		2	10		0.18		
2.1	0.96	0	10	0%	0.00	0.00	Y**
		0	10		0.00		Y
		0	10		0.00		Y
		0	10		0.00		Y

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFHpS Survival

Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.1908	0.5000	-0.4908	0.1092	-0.2486	-0.3166	-0.1868
					0.56	0.48	0.65
logStdDev	0.3464	0.5000	0.0100	0.5000	0.1706	0.1069	0.2534
CtrlSurv	0.8917	0.5000	0.8000	1.0000	0.9008	0.8350	0.9503
logX20					-0.3791	-0.4860	-0.2976
					0.42	0.33	0.50

Survival Analysis Program for PFAS Project

Midge   Biomass   PFHpS   2+Partial,Weighted
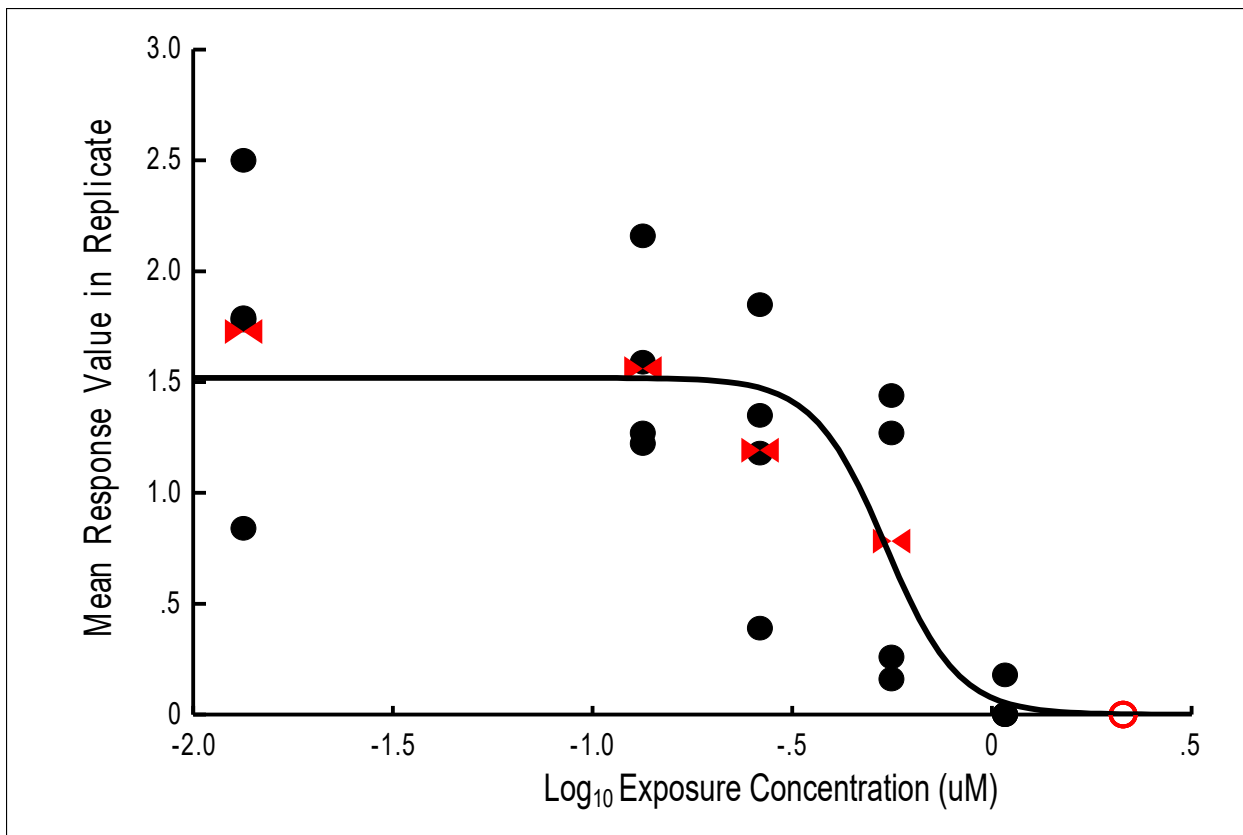
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-0.547	0.50	-1.000	0.000	-0.266	-0.654	-0.109	
					0.542	0.222	0.778	
Slope	2.465	0.50	0.500	5.000	2.765	1.084	5.000	
CtrlVal	1.644	0.50	1.000	3.000	1.519	1.209	2.152	
StdDev	0.501	0.50	0.200	0.800	0.528	0.386	0.753	
logEC20					-0.391	-0.951	-0.178	
					0.406	0.112	0.663	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	9.0189	21.2097	-0.729	-0.307	1.341	3.649	1.150	2.114	0.460	0.610
21	8.8099	9.0065	-0.316	-0.226	2.439	3.251	1.412	1.655	0.564	0.625
71	8.5742	8.5933	-0.247	-0.236	3.496	3.694	1.479	1.509	0.550	0.564
161	8.4152	8.4153	-0.266	-0.266	2.757	2.768	1.519	1.519	0.528	0.528
322	8.4152	8.4152	-0.266	-0.266	2.765	2.765	1.519	1.519	0.528	0.528

Analysis Date: 01/16/2023 20:30   01/16/23 Continuous Variable Effects Analysis Program

S12.13. C. dilutus PFOS test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.00019 control	0.000094	11	11	90%	1.21	1.05	
		11	11		1.30		
		8	10		0.63		
		8	10		1.16		
		10	10		1.27		
		8	10		1.27		
		9	10		0.91		
		12	12		1.14		Y-overstocked
		6	10		0.52		
		10	10		1.22		
		9	10		1.60		
		10	10		1.26		
		9	10		0.72		
		9	10		0.89		
		11	11		0.65		
		9	10		1.08		
0.0019	0.00094	11	11	90%	1.45	1.32	
		9	10		1.56		
		10	10		1.45		
		9	10		1.00		
		9	10		0.99		
		7	10		1.41		
		10	10		1.70		
		8	10		0.99		
0.0033	0.0017	11	11	84%	1.16	1.12	
		5	10		0.70		
		8	10		0.86		
		8	10		1.10		
		10	10		1.70		
		9	10		0.94		
		9	10		1.19		
		8	10		1.28		
0.0066	0.0033	10	10	88%	1.16	0.90	
		10	10		0.70		
		8	10		0.72		
		9	10		0.83		
		7	10		0.59		
		6	10		0.99		
		10	10		0.93		
		10	10		1.27		
0.012	0.0062	8	10	89%	1.56	1.23	
		10	10		1.44		
		9	10		1.19		
		9	10		1.25		
		8	10		1.20		
		9	10		0.83		
		8	10		0.97		
		11	11		1.41		
0.024	0.012	12	12	83%	1.37	0.95	Y-overstocked
		9	10		1.49		
		10	10		1.23		
		8	10		0.75		

		10	10		0.86		
		7	10		0.37		
		9	10		1.49		
		5	10		0.47		
0.046	0.023	8	10	86%	1.17	0.66	
		10	10		0.99		
		8	10		0.38		
		9	10		0.61		
		9	10		0.74		
		9	10		0.78		
		8	10		0.26		
		8	10		0.36		
0.096	0.048	3	10	34%	0.05	0.07	
		2	10		0.16		
		5	10		0.00		
		5	10		0.02		
		2	10		0.01		
		1	10		0.01		
		3	10		0.20		
		6	10		0.12		
0.19	0.095	1	10	6%	-0.02	0.00	
		1	10		0.00		
		0	10		0.00		
		0	10		0.00		
		0	10		0.00		
		2	10		0.03		
		1	10		0.00		
		0	10		0.00		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFOS 1 Survival
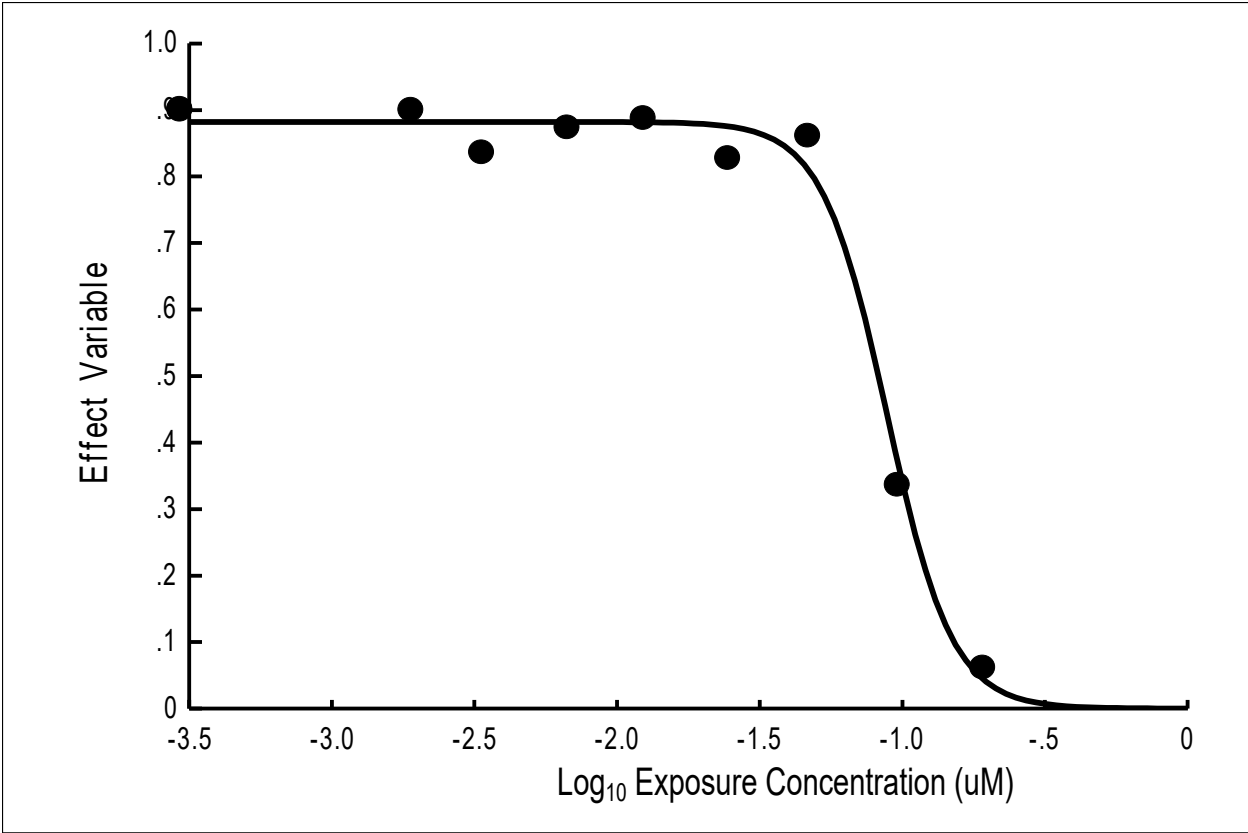
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.0558	0.5000	-1.3558	-0.7558	-1.0532	-1.1057	-1.0038
					0.09	0.08	0.10
logStdDev	0.2560	0.5000	0.0100	0.5000	0.2070	0.1517	0.2807
CtrlSurv	0.8747	0.5000	0.8000	1.0000	0.8821	0.8539	0.9073
logX20					-1.2114	-1.2983	-1.1402
					0.06	0.05	0.07

Midge   Biomass   PFOS 1   2+Partial,Weighted
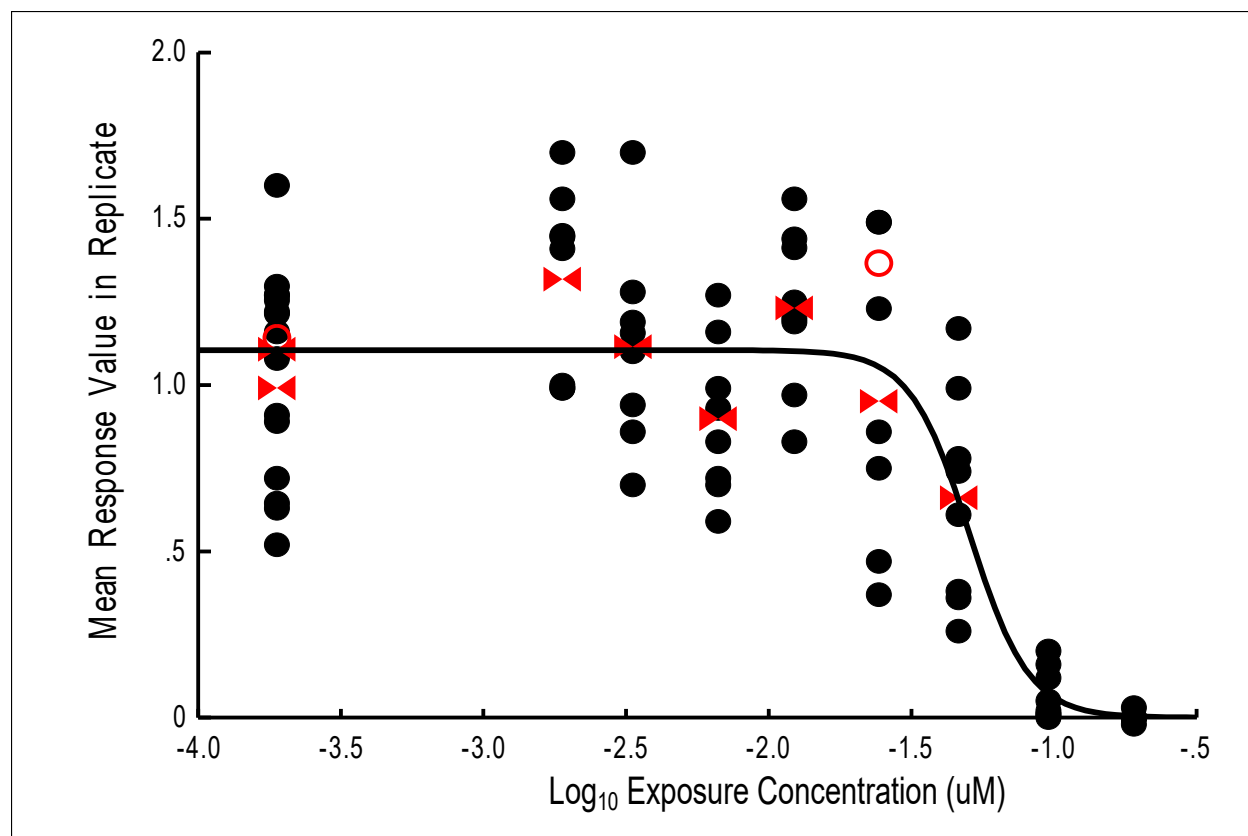
Parameter Summary   Optimization Errors: 0   0   0   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.254	0.50	-1.800	-0.800	-1.296	-1.399	-1.216	
					0.0506	0.0399	0.0608	
Slope	1.867	0.50	0.500	5.000	2.405	1.567	4.738	
CtrlVal	1.111	0.50	1.000	3.000	1.105	1.024	1.187	
StdDev	0.300	0.50	0.200	0.800	0.337	0.286	0.407	
logEC20					-1.440	-1.606	-1.326	
					0.0363	0.0247	0.0472	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-0.2715	64.8355	-1.374	-1.076	1.640	2.811	1.025	1.455	0.203	0.421
11	-0.2715	11.1523	-1.658	-1.250	1.398	2.675	1.000	1.257	0.295	0.517
51	-3.9193	-3.8568	-1.310	-1.298	2.218	2.319	1.097	1.113	0.331	0.341
121	-3.9346	-3.9346	-1.296	-1.296	2.405	2.407	1.105	1.105	0.337	0.337
257	-3.9346	-3.9346	-1.296	-1.296	2.405	2.405	1.105	1.105	0.337	0.337

Analysis Date: 01/17/2023 06:20   01/16/23 Continuous Variable Effects Analysis Program

S12.14. C. dilutus PFOS test 2 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.00058 control	0.00029	10	10	90%	2.08	1.81	
		6	10		1.42		
		10	10		1.89		
		10	10		1.84		
0.0058	0.0029	9	10	98%	1.76	1.79	
		10	10		2.41		
		10	10		1.18		
		10	10		1.82		
0.012	0.0059	9	10	93%	1.77	1.80	
		9	10		1.63		
		9	10		1.65		
		10	10		2.15		
0.024	0.012	10	10	95%	2.08	2.04	
		11	11		1.81		
		9	10		3.01		
		9	10		1.26		
0.046	0.023	6	10	58%	0.92	0.64	
		7	10		0.41		
		5	10		0.60		
		5	10		0.63		
0.095	0.048	0	10	3%	0.00	0.00	
		0	10		0.00		
		1	10		0.01		
		0	10		0.00		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFOS 2 Survival
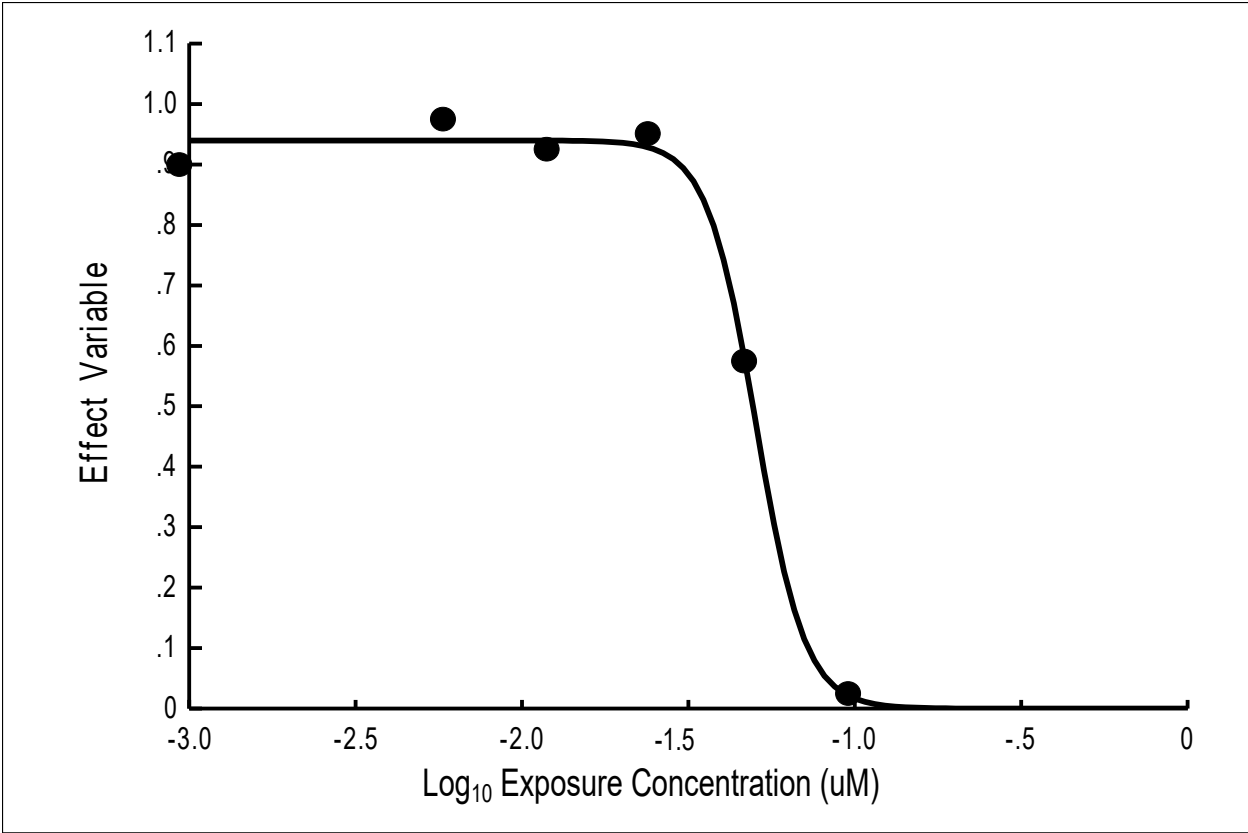
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.3287	0.5000	-1.6287	-1.0287	-1.2965	-1.3451	-1.2424
					0.05	0.05	0.06
logStdDev	0.2165	0.5000	0.0100	0.5000	0.1334	0.0786	0.2091
CtrlSurv	0.9378	0.5000	0.8000	1.0000	0.9396	0.8948	0.9707
logX20					-1.3985	-1.4775	-1.3421
					0.04	0.03	0.05

Midge   Biomass   PFOS 2   1Partial,Weighted
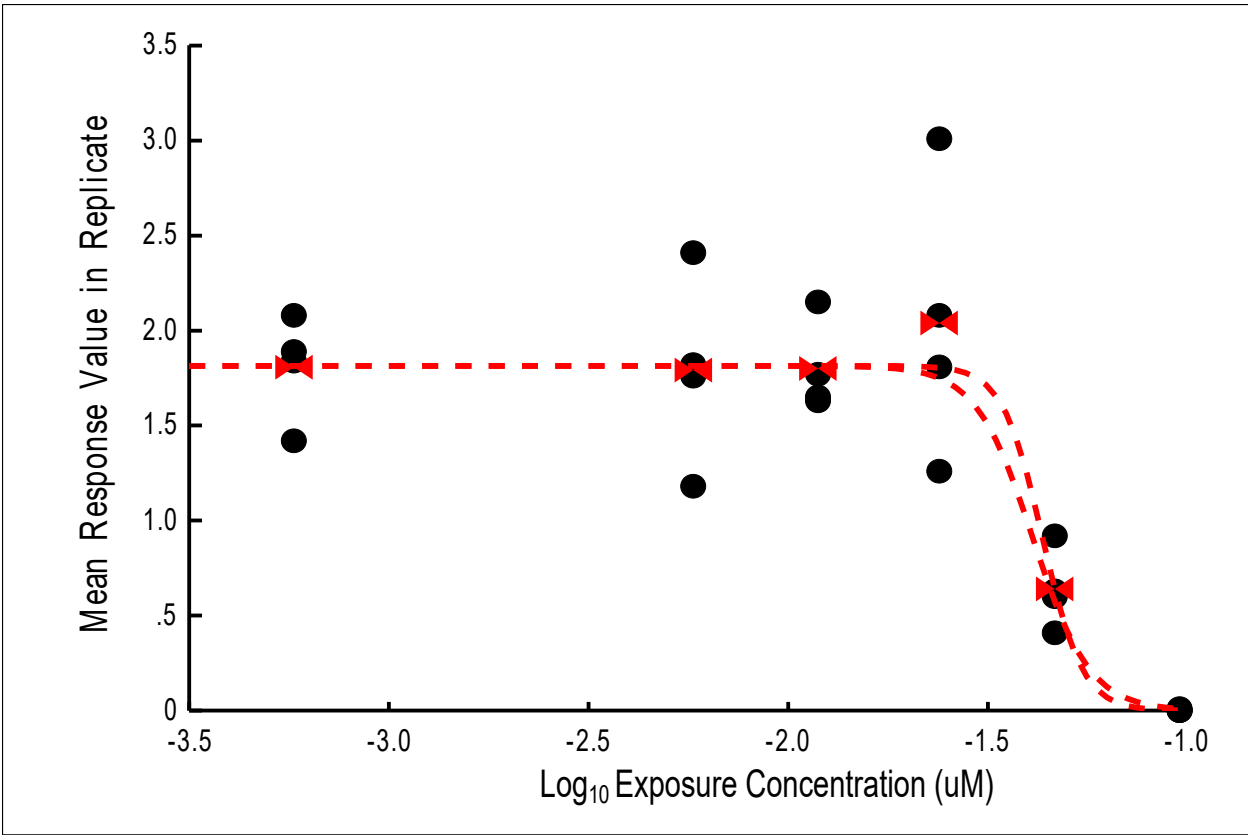
Parameter Summary   Optimization Errors: 0 0 99 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.286	0.50	-1.800	-0.800	-1.363	-1.435	-1.340	-1.388
					0.0434	0.0367	0.0457	0.0410
Slope	3.747	0.50	0.500	5.000	5.000	2.015	5.000	3.507
CtrlVal	1.860	0.50	1.000	3.000	1.815	1.664	1.966	1.815
StdDev	0.429	0.50	0.200	0.800	0.435	0.329	0.615	0.472
logEC20					-1.432	-1.598	-1.409	-1.503
					0.0370	0.0253	0.0390	0.0314

Parameter Optimization History

| Iter	-LogLike	logEC50	Slope	CtrlVal	StdDev
1	14.2513 178.0504	-1.506 -1.057	5.000 5.000	1.395 2.265	0.281 0.569
11	8.3655 20.1202	-1.766 -1.308	5.000 5.000	1.306 2.437	0.415 0.800
51	-0.4705 0.0759	-1.372 -1.355	5.000 5.000	1.780 1.882	0.485 0.538
111	-0.7771 -0.7770	-1.363 -1.363	5.000 5.000	1.814 1.815	0.435 0.436
224	-0.7771 -0.7771	-1.363 -1.363	5.000 5.000	1.815 1.815	0.435 0.435

S12.15. C. dilutus PFNS test 1 data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)	Treatment average biomass (mg)	Censored from growth analysis? Y
0.0022 control	0.0012	10	10	95%	2.22	2.15	
		10	10		1.81		
		8	10		2.04		
		10	10		2.51		
0.022	0.012	7	10	65%	1.49	1.26	
		9	10		1.48		
		5	10		0.90		
		5	10		1.16		
0.043	0.024	1	10	13%	0.05	0.01	
		0	10		0.00		
		1	10		-0.02		
		3	10		0.00		
0.089	0.049	0	10	0%	0.00	0.00	
		0	10		0.00		
		0	10		0.00		
		0	10		0.00		
0.17	0.10	0	10	0%	0.00	0.00	Y*
		0	10		0.00		Y
		0	10		0.00		Y
		0	10		0.00		Y
0.31	0.17	0	10	0%	0.00	0.00	Y
		0	10		0.00		Y
		0	10		0.00		Y
		0	10		0.00		Y

*High exposures with little/no growth were censored when not necessary for EC20 and EC50 estimation

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFNS 1 Survival
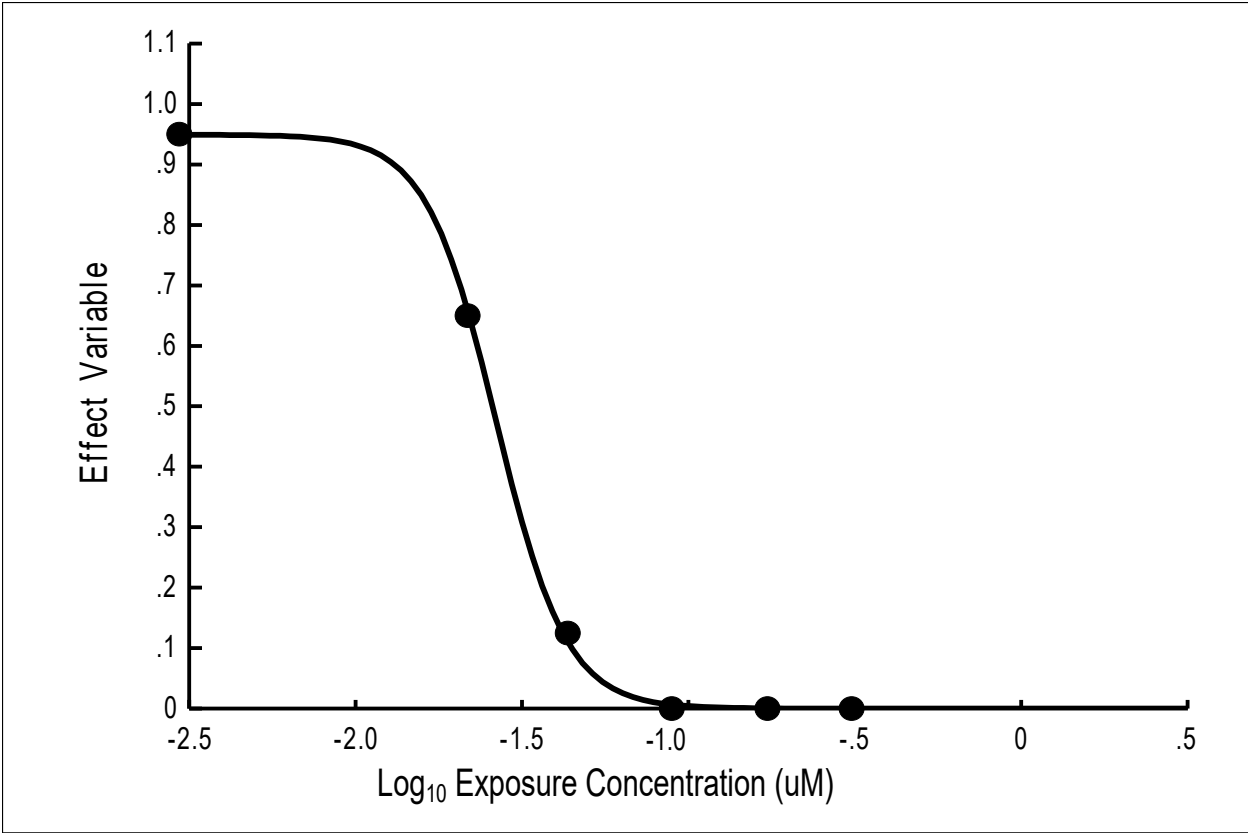
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.5219	0.5000	-1.8219	-1.2219	-1.5770	-1.6472	-1.5073
					0.03	0.02	0.03
logStdDev	0.3143	0.5000	0.0100	0.5000	0.1914	0.1335	0.2860
CtrlSurv	0.9500	0.5000	0.8000	1.0000	0.9492	0.8511	0.9914
logX20					-1.7233	-1.8434	-1.6315
					0.02	0.01	0.02

Midge   Biomass   PFNS 1 1Partial,Weighted
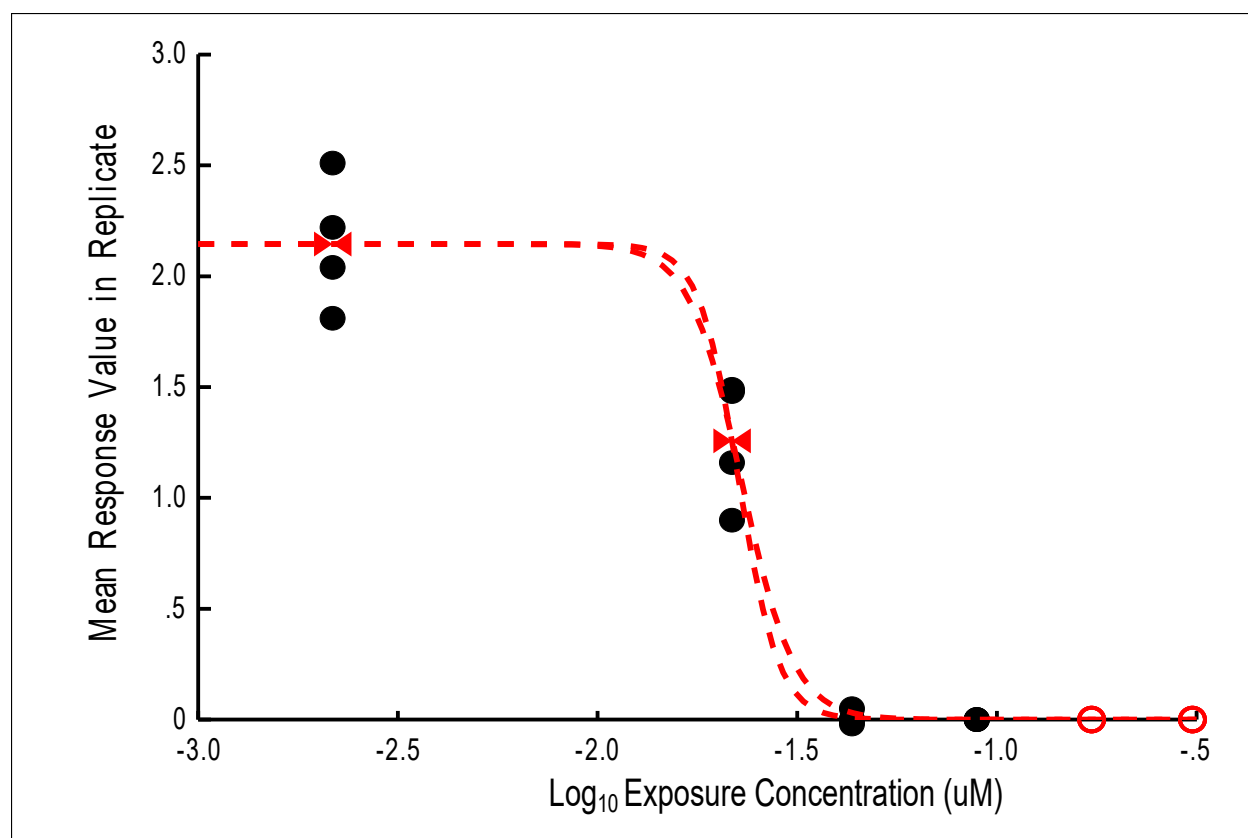
Parameter Summary   Optimization Errors: 0   0 99   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.619	0.50	-2.100	-1.100	-1.646	-1.678	-1.600	-1.639
					0.0226	0.0210	0.0251	0.0230
Slope	3.879	0.50	0.500	5.000	5.000	2.664	5.000	3.832
CtrlVal	2.145	0.50	1.000	3.000	2.145	1.861	2.429	2.145
StdDev	0.256	0.50	0.100	0.800	0.256	0.168	0.459	0.314
logEC20					-1.715	-1.794	-1.679	-1.736
					0.0193	0.0161	0.0210	0.0184

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-8.3552	171.0591	-1.838	-1.398	5.000	5.000	1.560	2.219	0.104	0.381
11	-9.9816	-5.7258	-1.672	-1.596	5.000	5.000	1.916	2.070	0.222	0.309
51	-10.5439	-10.4546	-1.646	-1.640	5.000	5.000	2.075	2.119	0.229	0.245
111	-10.6541	-10.6540	-1.646	-1.646	5.000	5.000	2.143	2.146	0.255	0.257
238	-10.6541	-10.6541	-1.646	-1.646	5.000	5.000	2.145	2.145	0.256	0.256

Analysis Date: 01/17/2023 06:30   01/16/23 Continuous Variable Effects Analysis Program

S12.16. C. dilutus PFNS test 2 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)	Treatment average biomass (mg)	Censored from growth analysis? Y
0.00026 control	0.00014	10	10	93%	2.23	1.85	
		10	10		2.15		
		7	10		1.24		
		10	10		1.79		
0.00264	0.0014	10	10	100%	1.70	1.80	
		10	10		1.78		
		10	10		2.34		
		10	10		1.36		
0.0051	0.0027	9	10	95%	1.41	1.52	
		10	10		1.94		
		10	10		1.87		
		9	10		0.86		
0.0104	0.0056	10	10	98%	1.69	1.63	
		9	10		1.51		
		10	10		2.11		
		10	10		1.19		
0.022	0.012	4	10	55%	0.58	0.74	
		4	10		0.57		
		7	10		0.95		
		7	10		0.86		
0.043	0.024	0	10	0%	0.00	0.00	
		0	10		0.00		
		0	10		0.00		
		0	10		0.00		

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFNS 2 Survival
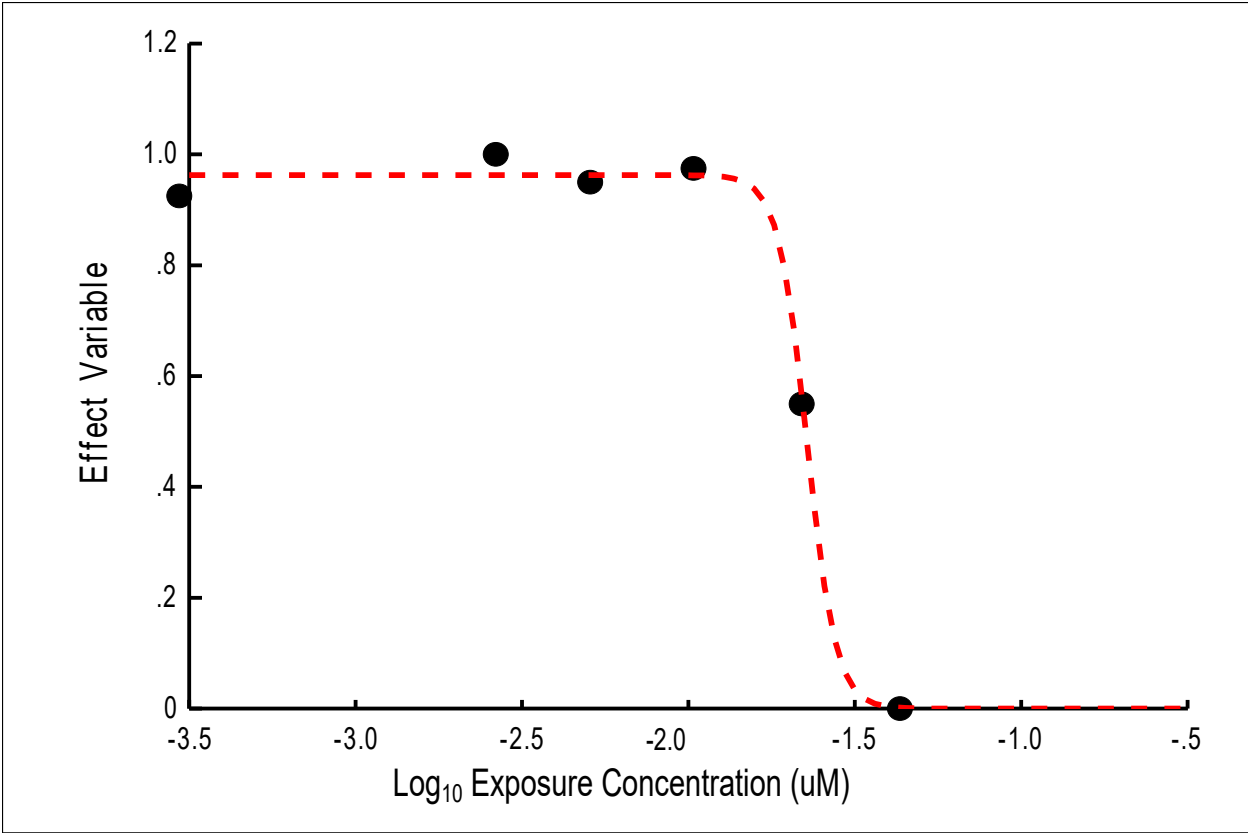
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.6822	0.5000	-1.9822	-1.3822	-1.6435	-1.6780	-1.6090
					0.02	0.02	0.02
logStdDev	0.2120	0.5000	0.0100	0.5000	0.0790	0.0100	0.1580
CtrlSurv	0.9625	0.5000	0.8000	1.0000	0.9625	0.9255	0.9849
logX20					-1.7208	-1.7799	-1.6617
					0.02	0.02	0.02

Analysis Date: 02/13/2023 11:44

Survival Analysis Program for PFAS Project

Midge   Biomass Gain   PFNS 2   1Partial,Weighted
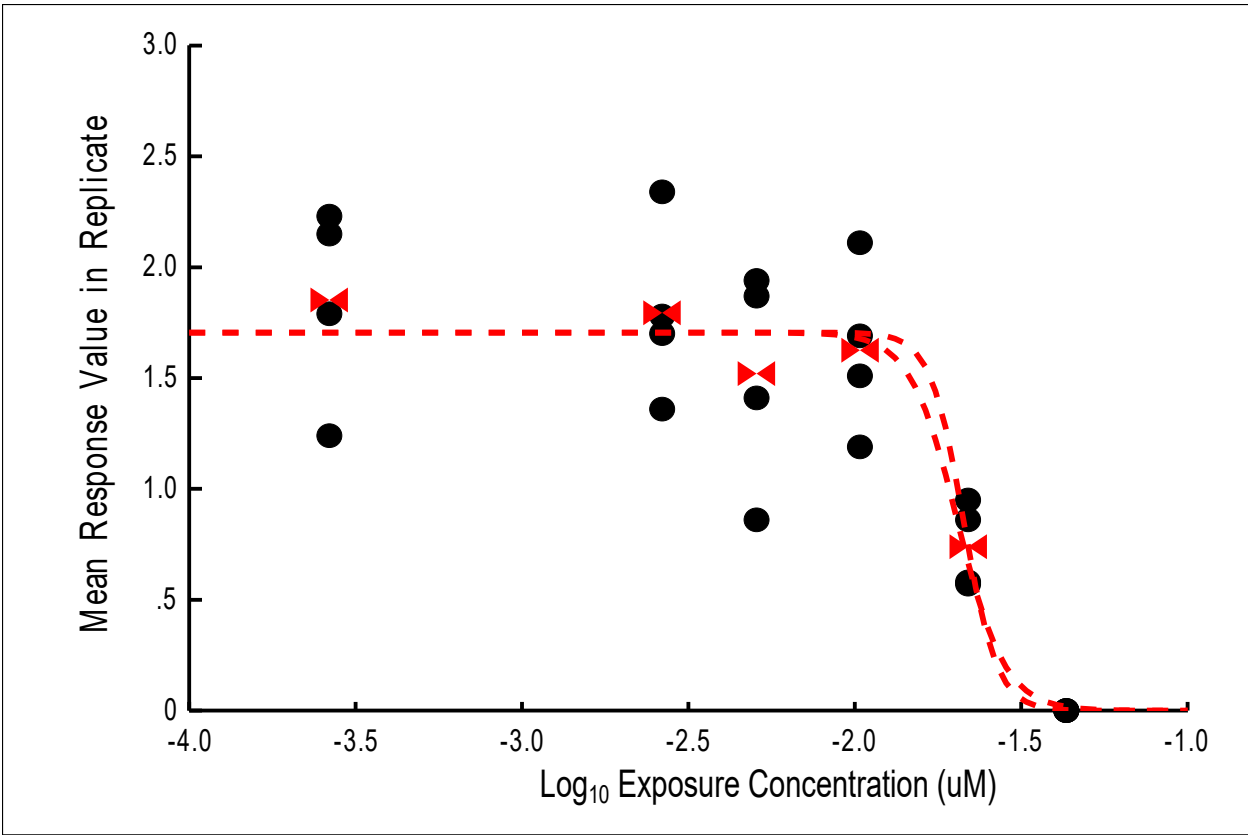
Parameter Summary   Optimization Errors: 0   0 99   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.991	0.50	-2.500	-1.500	-1.673	-1.738	-1.645	-1.691
					0.0212	0.0183	0.0226	0.0204
Slope	1.595	0.50	0.500	5.000	5.000	2.019	5.000	3.510
CtrlVal	1.824	0.50	1.000	3.000	1.705	1.506	1.915	1.705
StdDev	0.373	0.50	0.200	0.800	0.389	0.294	0.550	0.422
logEC20					-1.742	-1.900	-1.717	-1.809
					0.0181	0.0126	0.0192	0.0155

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	13.9342	130.6249	-2.134	-1.748	5.000	5.000	1.516	2.318	0.244	0.493
11	3.1365	17.3621	-1.784	-1.500	5.000	5.000	1.000	2.043	0.438	0.800
41	1.0692	1.2016	-1.678	-1.671	5.000	5.000	1.713	1.759	0.423	0.443
101	0.8643	0.8643	-1.673	-1.673	5.000	5.000	1.705	1.706	0.389	0.389
213	0.8643	0.8643	-1.673	-1.673	5.000	5.000	1.705	1.705	0.389	0.389

Analysis Date: 01/17/2023 06:34   01/16/23 Continuous Variable Effects Analysis Program

S12.17. C. dilutus PFDS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.0035 control	0.0021	10	10	85%	1.94	1.73	
		8	10		1.81		
		6	10		1.21		
		10	10		1.96		
0.035	0.021	10	10	98%	2.18	2.06	
		11	11		2.12		
		10	10		2.30		
		9	10		1.64		
0.057	0.034	10	10	95%	1.75	1.97	
		9	10		1.73		
		10	10		2.41		
		9	10		1.99		
0.12	0.07	7	10	78%	1.36	1.53	
		10	10		2.06		
		6	10		0.96		
		8	10		1.74		
0.26	0.15	5	10	38%	0.77	0.62	
		6	10		1.64		
		2	10		0.02		
		2	10		0.06		
0.48	0.29	2	10	10%	0.07	0.08	
		0	10		0.00		
		1	10		0.04		
		1	10		0.22		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge PFDS Survival
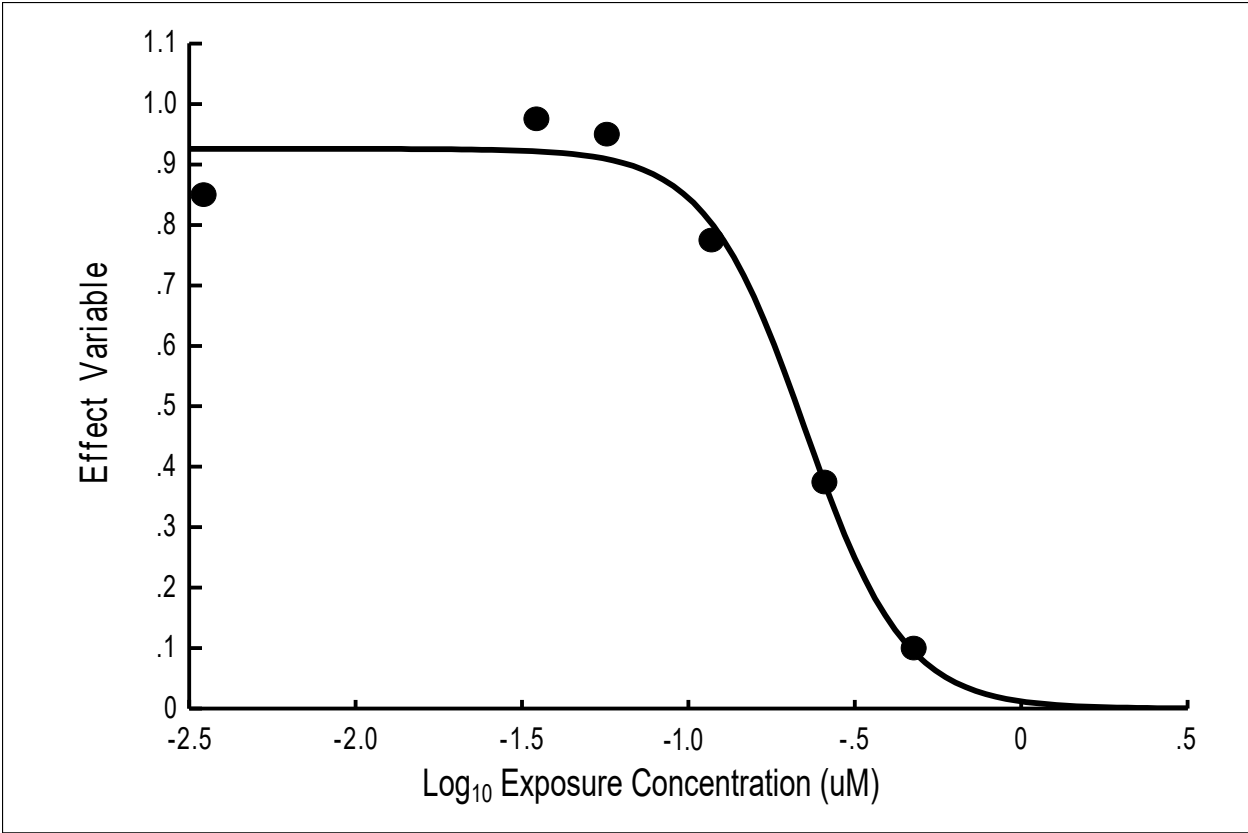
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.6833	0.5000	-0.9833	-0.3833	-0.6497	-0.7317	-0.5675
					0.22	0.19	0.27
logStdDev	0.3004	0.5000	0.0100	0.5000	0.2713	0.1864	0.3794
CtrlSurv	0.8877	0.5000	0.8000	1.0000	0.9258	0.8677	0.9665
logX20					-0.8570	-0.9811	-0.7371
					0.14	0.10	0.18

Midge   Biomass   PFDS   2+Partial,Weighted
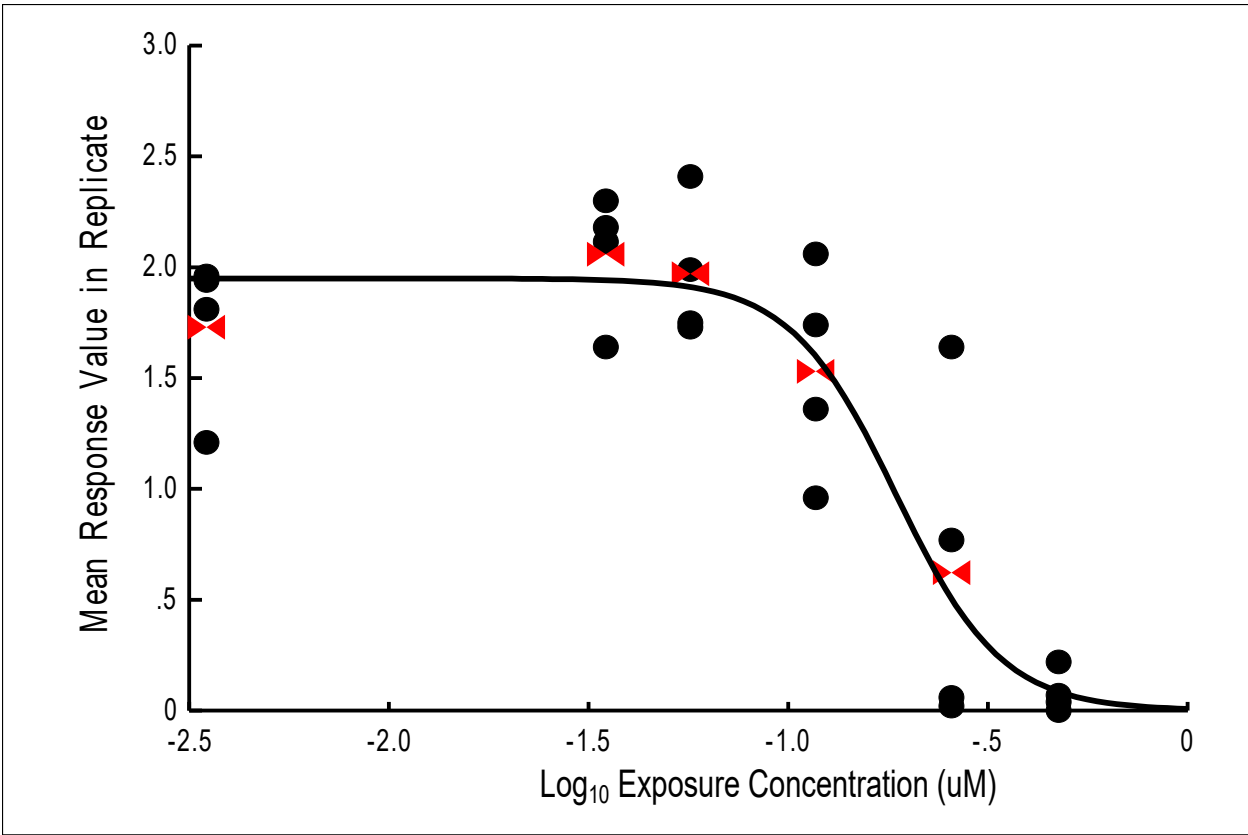
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-0.705	0.50	-1.200	-0.200	-0.730	-0.868	-0.561	
					0.186	0.136	0.275	
Slope	1.893	0.50	0.500	5.000	1.892	1.325	3.835	
CtrlVal	1.920	0.50	1.000	3.000	1.950	1.764	2.149	
StdDev	0.310	0.50	0.200	0.800	0.336	0.253	0.457	
logEC20					-0.913	-1.103	-0.668	
					0.122	0.079	0.215	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	5.3687	30.4873	-0.942	-0.470	0.828	2.981	1.500	1.920	0.225	0.390
11	5.3687	9.4653	-0.856	-0.509	1.893	3.980	1.797	2.057	0.263	0.421
51	5.1181	5.1239	-0.735	-0.725	1.869	1.921	1.943	1.954	0.330	0.335
121	5.1158	5.1158	-0.730	-0.730	1.892	1.892	1.950	1.950	0.336	0.336
251	5.1158	5.1158	-0.730	-0.730	1.892	1.892	1.950	1.950	0.336	0.336

Analysis Date: 01/17/2023 06:37   01/16/23 Continuous Variable Effects Analysis Program

S12.18. C. dilutus FBSA test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.093 control	0.028	10	10	93%	2.42	2.19	
		9	10		2.10		
		10	10		2.35		
		8	10		1.89		
0.93	0.28	10	10	100%	2.36	2.29	
		10	10		2.30		
		10	10		2.19		
		10	10		2.29		
1.7	0.51	8	10	93%	1.74	2.02	
		11	11		1.78		
		9	10		2.25		
		10	10		2.31		
3.5	1.1	11	11	98%	1.88	2.10	
		10	10		2.46		
		9	10		2.22		
		10	10		1.83		
6.4	1.9	8	10	93%	1.76	1.74	
		10	10		0.97		
		10	10		2.15		
		9	10		2.07		
14.4	4.3	10	10	100%	1.85	1.86	
		11	11		1.70		
		12	12		1.83		Y- overstocked
		10	10		2.04		
29	8.6	8	10	95%	1.19	1.30	
		10	10		1.34		
		10	10		1.30		
		10	10		1.38		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	No significant effect
Biomass	Regression

Midge   Biomass   FBSA   2+Partial,Weighted
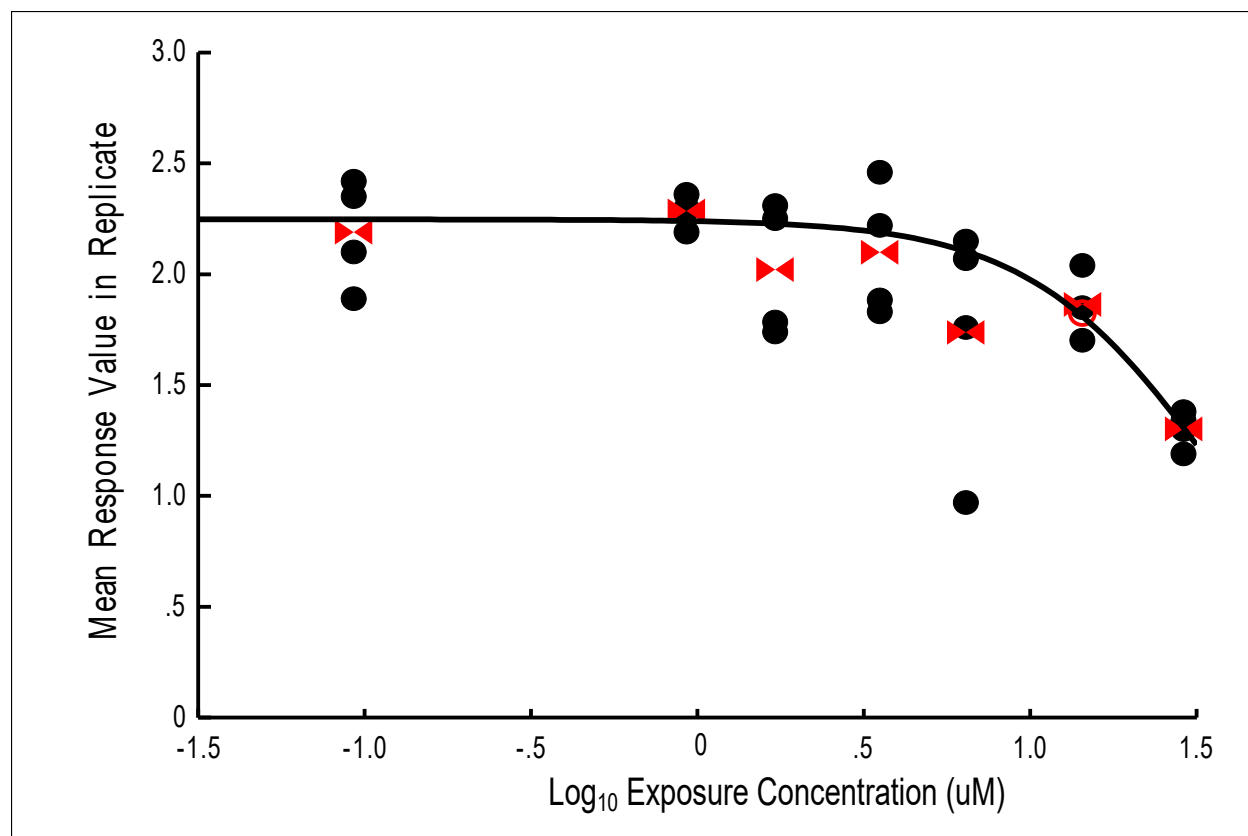
Parameter Summary   Optimization Errors: 0   0   0   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.699	0.50	1.200	2.200	1.553	1.494	1.661	
					35.8	31.2	45.8	
Slope	0.637	0.50	0.500	5.000	0.896	0.500	1.589	
CtrlVal	2.149	0.50	1.000	3.000	2.248	2.166	2.383	
StdDev	0.235	0.50	0.200	0.800	0.270	0.201	0.378	
logEC20					1.166	0.872	1.300	
					14.7	7.4	20.0	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-0.7820	111.3551	1.265	1.738	0.637	1.740	1.948	2.570	0.200	0.305
21	-4.8267	-4.6755	1.520	1.539	1.163	1.228	2.212	2.241	0.275	0.282
81	-5.3111	-5.3098	1.551	1.554	0.893	0.911	2.245	2.248	0.269	0.269
171	-5.3119	-5.3118	1.553	1.554	0.895	0.898	2.247	2.248	0.270	0.271
351	-5.3119	-5.3119	1.553	1.553	0.896	0.896	2.248	2.248	0.270	0.270

Analysis Date: 01/17/2023 06:43   01/16/23 Continuous Variable Effects Analysis Program

S12.19. C. dilutus HxSA test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.014 control	0.0056	11	11	95%	1.83	1.92	
		9	10		1.90		
		11	11		1.59		
		9	10		2.37		
0.14	0.056	10	10	95%	1.72	2.00	
		9	10		2.14		
		10	10		2.10		
		9	10		2.03		
0.28	0.11	9	10	93%	2.04	2.18	
		9	10		2.02		
		10	10		2.21		
		9	10		2.44		
0.55	0.22	10	10	97%	1.71	1.75	
		10	10		1.78		
		12	12		1.87		Y- overstocked
		9	10		1.77		
1.1	0.45	8	10	93%	1.42	1.71	
		10	10		1.89		
		10	10		1.82		
		9	10		1.69		
2.1	0.85	9	10	87%	0.72	0.71	
		13	13		0.68		Y- overstocked
		9	10		0.72		
		8	10		0.69		
4.2	1.7	3	10	48%	0.01	0.12	
		5	10		0.13		
		4	10		0.05		
		7	10		0.31		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge FHxSA Survival
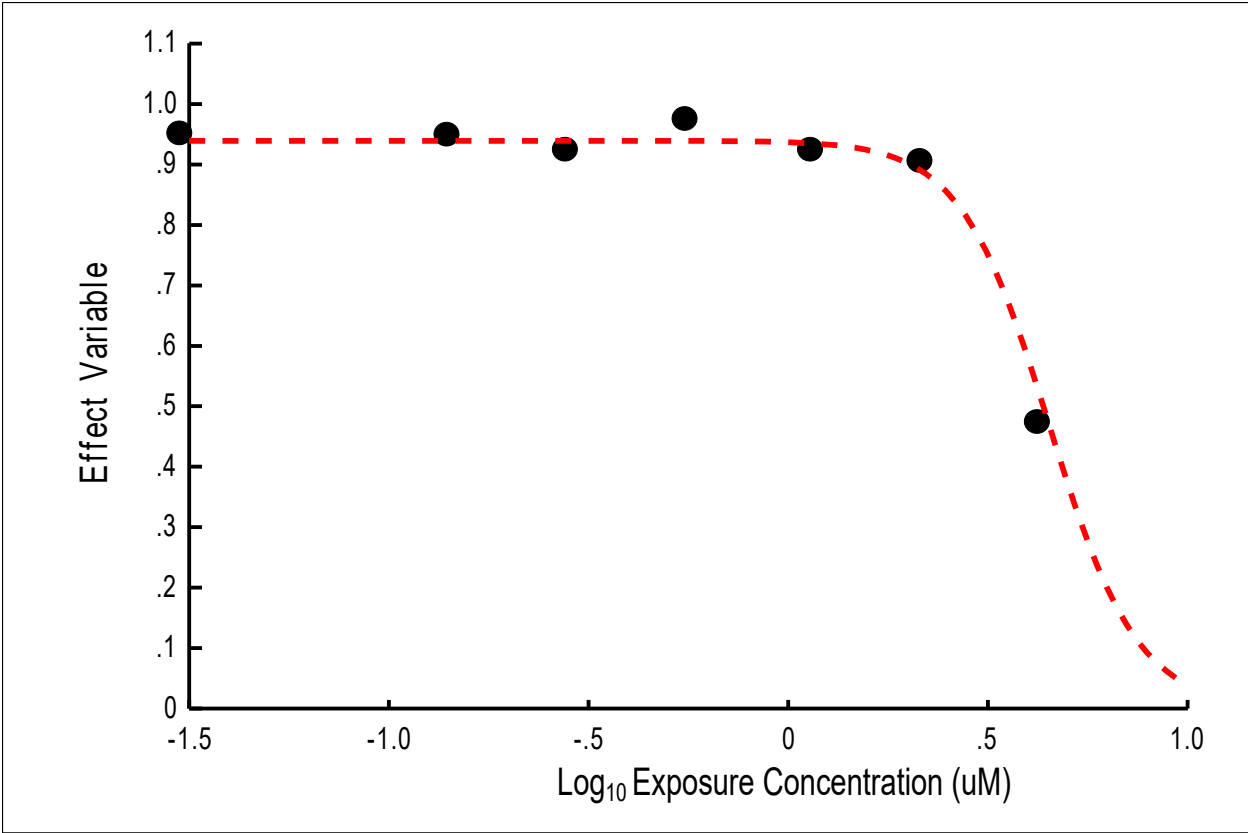
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.6059	0.5000	0.3059	0.9059	0.6531	0.5548	0.7514
					4.50	3.59	5.64
logStdDev	0.2272	0.5000	0.0100	0.5000	0.2012	0.0100	0.4023
CtrlSurv	0.9393	0.5000	0.8000	1.0000	0.9393	0.9049	0.9766
logX20					0.4854	0.3516	0.6192
					3.06	2.25	4.16

Analysis Date: 02/13/2023 11:47   Survival Analysis Program for PFAS Project

Midge   Biomass   FHxSA   2Partial,Weighted
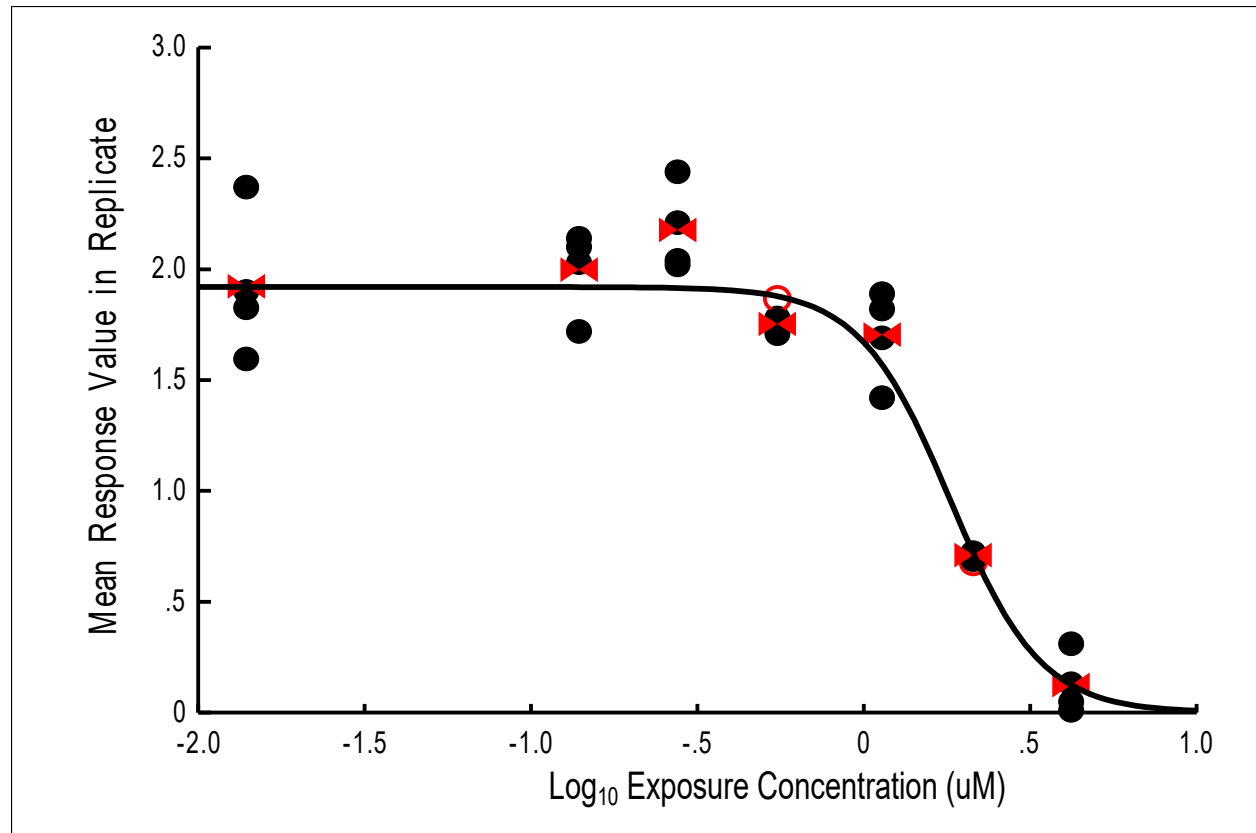
Parameter Summary   Optimization Errors: 0   0   0   0   0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	0.170	0.50	-0.300	0.700	0.259	0.186	0.302	
					1.82	1.54	2.00	
Slope	1.535	0.50	0.500	5.000	1.833	1.157	3.936	
CtrlVal	1.977	0.50	1.000	3.000	1.920	1.794	2.066	
StdDev	0.240	0.50	0.200	0.800	0.308	0.222	0.457	
logEC20					0.070	-0.105	0.211	
					1.18	0.78	1.63	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	-5.6634	77.8797	0.031	0.409	0.730	2.616	1.586	2.405	0.211	0.382
11	-5.6634	-3.4596	0.186	0.248	1.226	1.688	1.916	2.064	0.271	0.303
51	-7.2200	-7.0919	0.242	0.272	1.655	1.962	1.903	1.945	0.290	0.302
121	-7.3389	-7.3387	0.259	0.260	1.825	1.839	1.919	1.921	0.308	0.309
259	-7.3390	-7.3390	0.259	0.259	1.833	1.833	1.920	1.920	0.308	0.308

Analysis Date: 01/17/2023 06:56   01/16/23 Continuous Variable Effects Analysis Program

S12.20. C. dilutus FOSA test 1 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.00065 control	0.00032	9	10	98%	2.33	2.39	
		11	11		2.00		
		10	10		2.62		
		10	10		2.62		
0.0065	0.0032	10	10	96%	2.12	2.00	
		9	10		2.16		
		10	10		1.71		
		10	10		2.01		
0.011	0.0054	9	10	95%	1.12	1.47	
		9	10		1.36		
		10	10		1.80		
		10	10		1.61		
0.022	0.011	9	10	93%	1.94	1.75	
		9	10		1.32		
		12	12		2.02		Y- overstocked
		10	10		1.98		
0.052	0.026	5	10	70%	0.13	0.86	
		5	10		0.70		
		9	10		1.06		
		9	10		1.55		
0.081	0.040	3	10	43%	0.05	0.17	
		5	10		0.25		
		6	10		0.23		
		3	10		0.15		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge FOSA 1 Survival
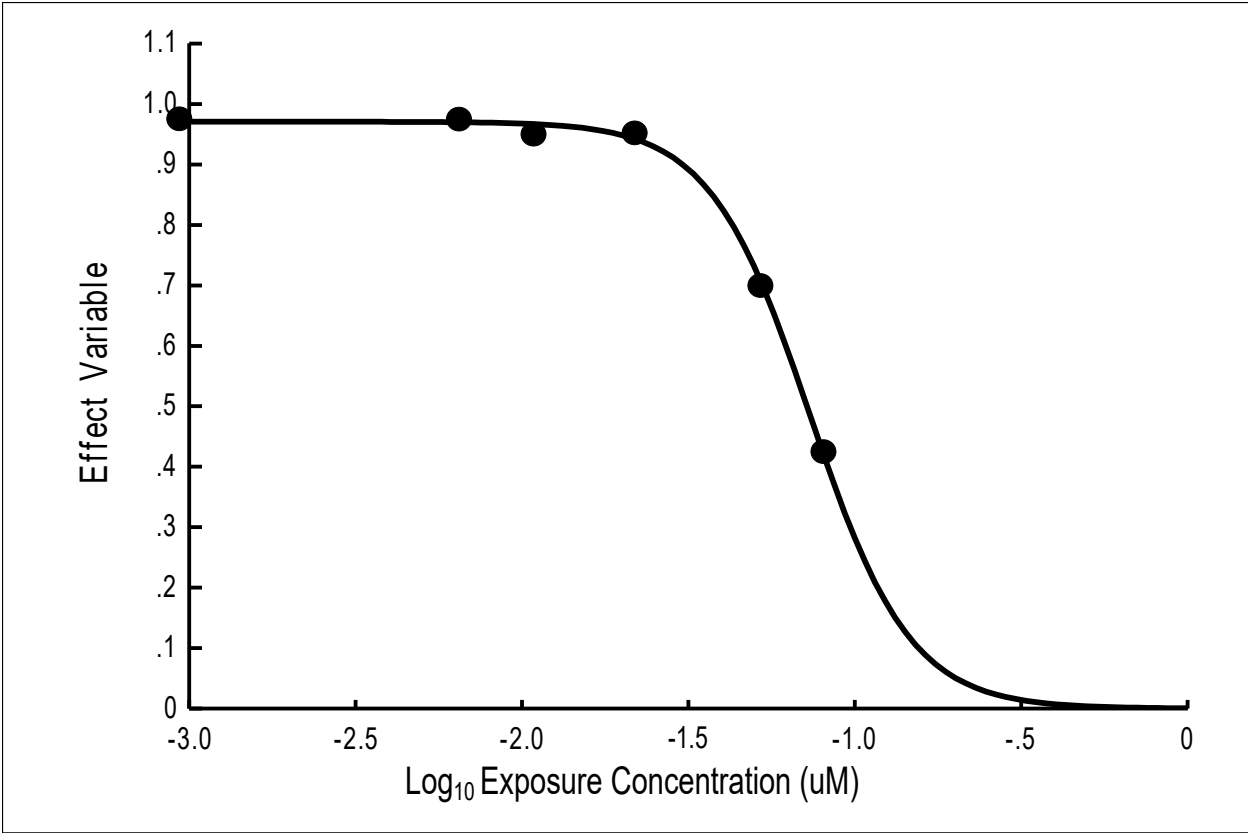
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.1746	0.5000	-1.4746	-0.8746	-1.1344	-1.2064	-1.0202
					0.07	0.06	0.10
logStdDev	0.3585	0.5000	0.0100	0.5000	0.2732	0.1546	0.5000
CtrlSurv	0.9632	0.5000	0.8000	1.0000	0.9707	0.9318	0.9959
logX20					-1.3432	-1.4744	-1.4244
					0.05	0.03	0.04

Midge   Biomass   FOSA 1   2+Partial,Weighted
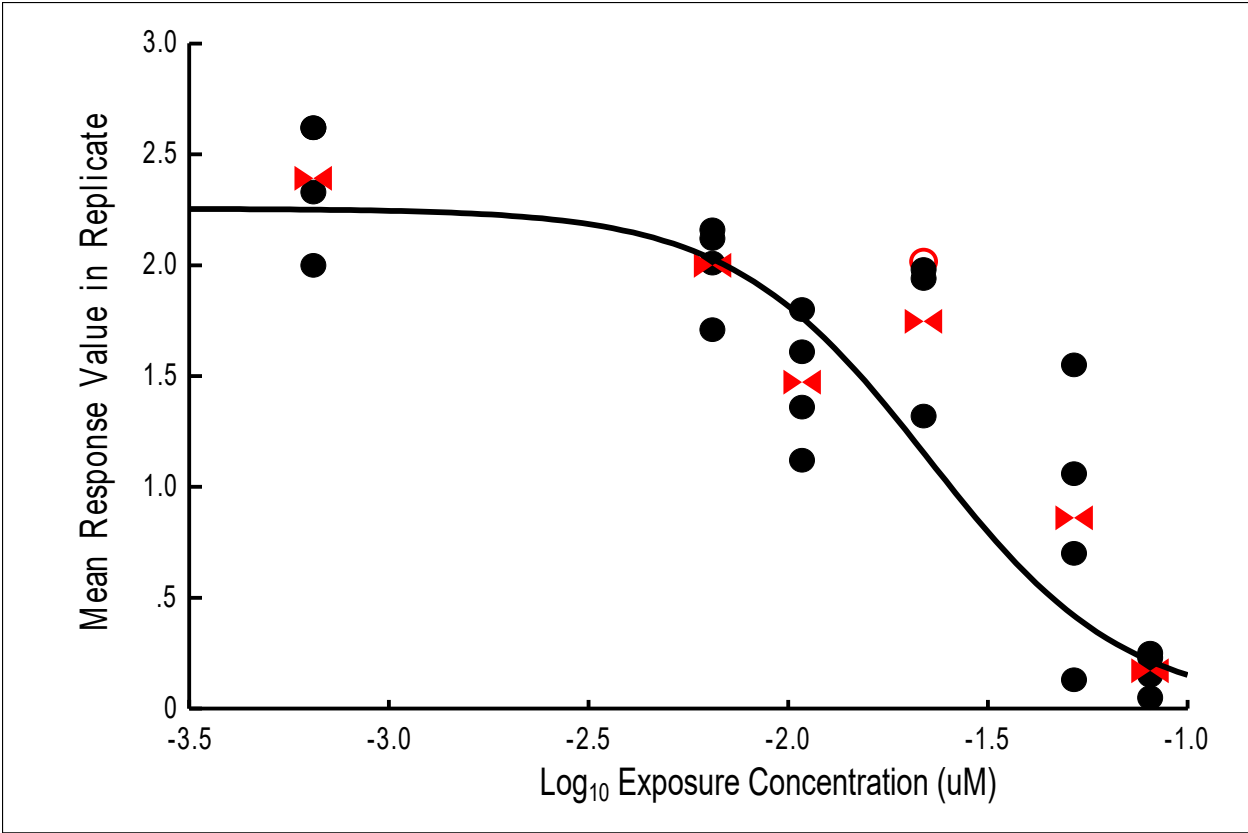
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.708	0.50	-2.200	-1.200	-1.649	-1.858	-1.238	
					0.0224	0.0139	0.0578	
Slope	1.047	0.50	0.500	5.000	1.012	0.733	4.180	
CtrlVal	2.392	0.50	1.000	3.000	2.255	1.836	2.688	
StdDev	0.256	0.50	0.200	0.800	0.359	0.275	0.494	
logEC20					-1.992	-2.308	-1.324	
					0.0102	0.0049	0.0474	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	11.6603	58.8165	-1.919	-1.487	0.530	2.155	1.918	2.794	0.213	0.370
21	7.0625	7.7008	-1.637	-1.539	1.025	1.288	2.105	2.244	0.292	0.326
61	6.5248	6.5573	-1.554	-1.538	1.217	1.256	2.088	2.109	0.338	0.344
141	6.1898	6.1905	-1.652	-1.647	1.005	1.017	2.254	2.261	0.357	0.358
296	6.1892	6.1892	-1.649	-1.649	1.012	1.012	2.255	2.255	0.359	0.359

Analysis Date: 01/17/2023 07:00   01/16/23 Continuous Variable Effects Analysis Program

S12.21. C. dilutus FOSA test 2 data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.00039 control	0.00019	10	10	96%	1.94	1.91	
		10	10		1.74		
		10	10		1.98		
		10	10		1.97		
0.0039	0.0019	10	10	94%	1.39	1.49	
		10	10		1.63		
		8	10		1.03		
		9	10		1.92		
0.0071	0.0035	10	10	95%	1.18	1.23	
		11	11		0.80		
		10	10		1.43		
		8	10		1.50		
0.014	0.0070	8	10	95%	1.55	1.38	
		10	10		1.37		
		10	10		1.53		
		10	10		1.05		
0.029	0.015	9	10	98%	0.64	0.96	
		10	10		1.12		
		10	10		1.13		
		10	10		0.94		
0.054	0.027	5	10	60%	0.29	0.29	
		8	10		0.32		
		5	10		0.25		
		6	10		0.29		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge FOSA 2 Survival
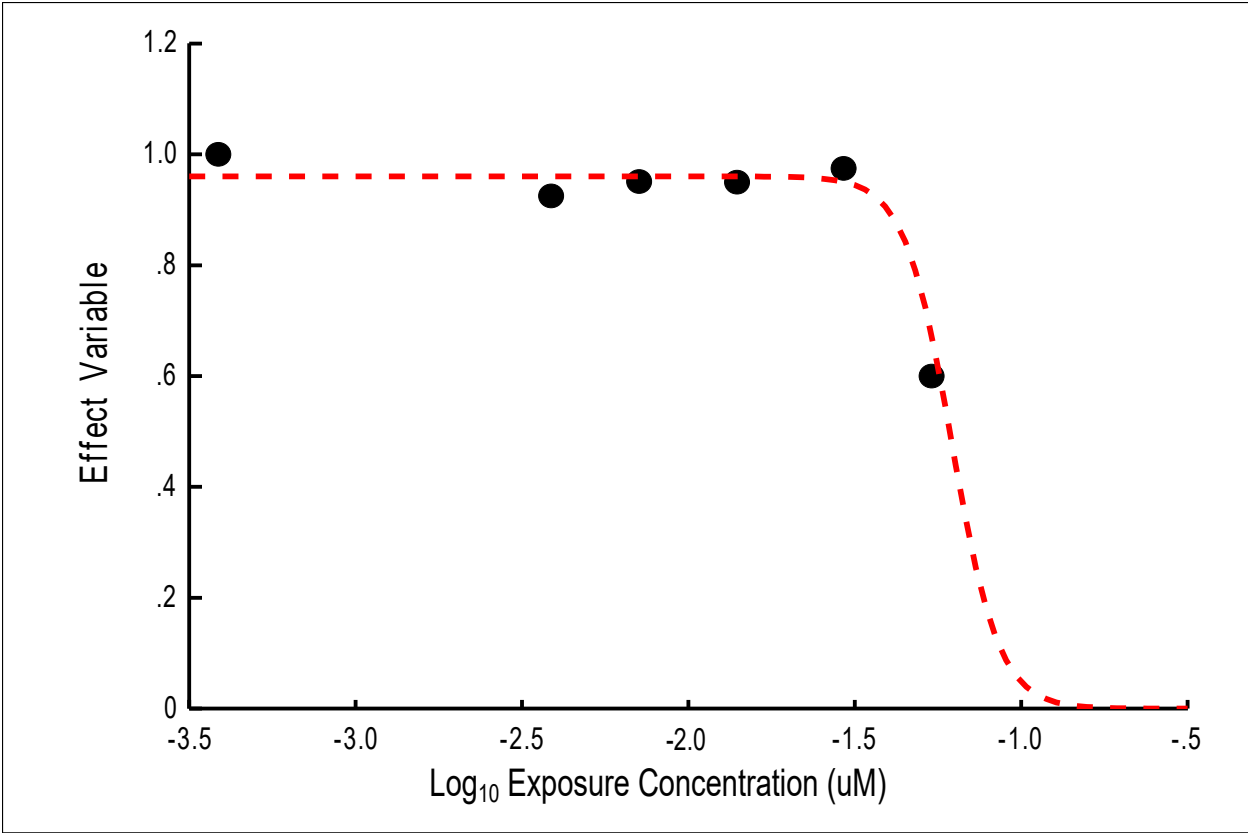
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-1.1982	0.5000	-1.4982	-0.8982	-1.2088	-1.2754	-1.1423
					0.06	0.05	0.07
logStdDev	0.2351	0.5000	0.0100	0.5000	0.1288	0.0100	0.2576
CtrlSurv	0.9602	0.5000	0.8000	1.0000	0.9602	0.9272	0.9816
logX20					-1.3320	-1.3946	-1.2695
					0.05	0.04	0.05

Survival Analysis Program for PFAS Project

Midge   Biomass   FOSA 2   2+Partial,Weighted
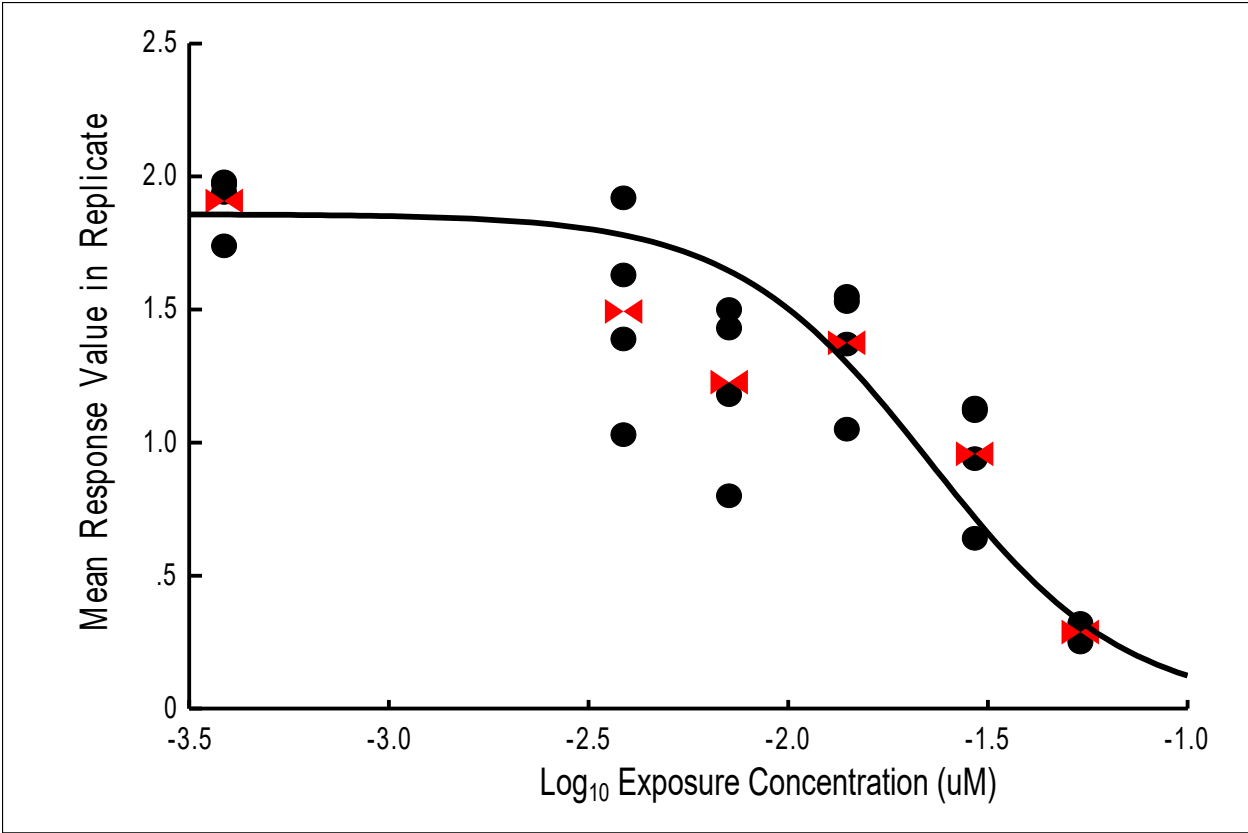
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	-1.901	0.50	-2.400	-1.400	-1.647	-1.813	-1.511	
					0.0226	0.0154	0.0309	
Slope	0.828	0.50	0.500	5.000	1.019	0.688	1.664	
CtrlVal	1.908	0.50	1.000	3.000	1.858	1.708	2.010	
StdDev	0.098	0.50	0.100	0.800	0.138	0.103	0.197	
logEC20					-1.987	-2.301	-1.725	
					0.0103	0.0050	0.0188	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	12.8192	33.6571	-2.134	-1.626	0.500	1.772	1.450	2.361	0.098	0.315
11	-0.8042	3.7204	-1.748	-1.594	0.847	1.318	1.671	2.073	0.141	0.205
51	-1.6643	-1.6604	-1.654	-1.647	0.997	1.025	1.854	1.866	0.137	0.140
121	-1.6688	-1.6688	-1.647	-1.647	1.019	1.019	1.858	1.858	0.138	0.138
245	-1.6688	-1.6688	-1.647	-1.647	1.019	1.019	1.858	1.858	0.138	0.138

Analysis Date: 01/17/2023 07:02   01/16/23 Continuous Variable Effects Analysis Program

S12.22. C. dilutus 6:2 FTS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
2.6 control	1.1	10	10	98%	2.16	2.07	
		9	10		2.08		
		10	10		1.99		
		10	10		2.03		
26	11	7	10	80%	1.52	1.80	
		10	10		1.93		
		12	12		1.96		Y- overstocked
		7	10		1.96		
56	24	7	10	80%	1.10	1.70	
		8	10		1.90		
		8	10		1.87		
		9	10		1.92		
120	52	9	10	90%	1.43	1.57	
		9	10		1.65		
		8	10		1.36		
		11	11		1.84		
240	103	5	10	76%	0.64	0.86	
		11	11		0.65		
		8	10		1.22		
		7	10		0.94		
474	203	2	10	35%	0.12	0.06	
		2	10		0.00		
		6	10		0.11		
		4	10		0.00		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method
Survival	Regression
Biomass	Regression

Midge 62FTS Survival
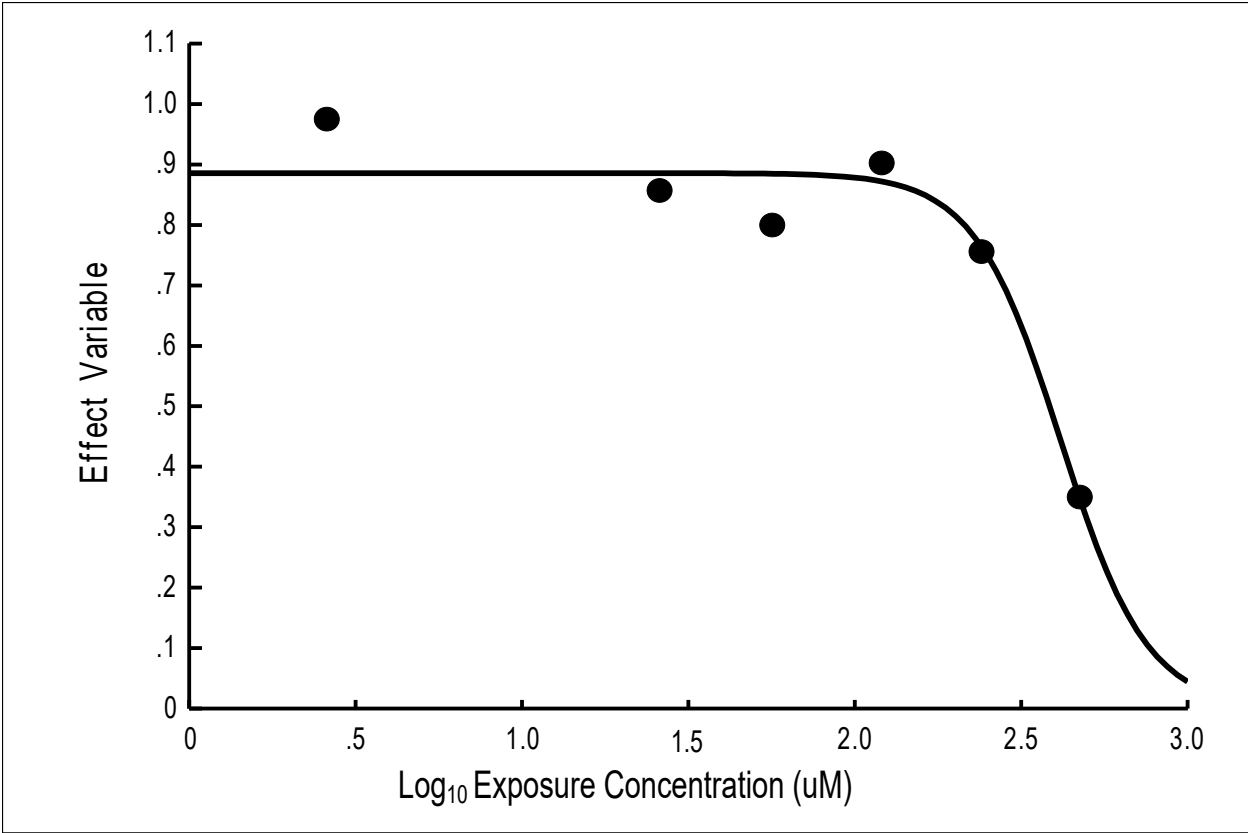
Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	2.5141	0.5000	2.2141	2.8141	2.6192	2.5376	2.7287
					416.11	344.83	535.41
logStdDev	0.3591	0.5000	0.0100	0.5000	0.2353	0.0100	0.5000
CtrlSurv	0.8836	0.5000	0.8000	1.0000	0.8857	0.8282	0.9347
logX20					2.4394	2.2374	2.6621
					275.01	172.74	459.27

Midge   Biomass   62FTS   2+Partial,Weighted
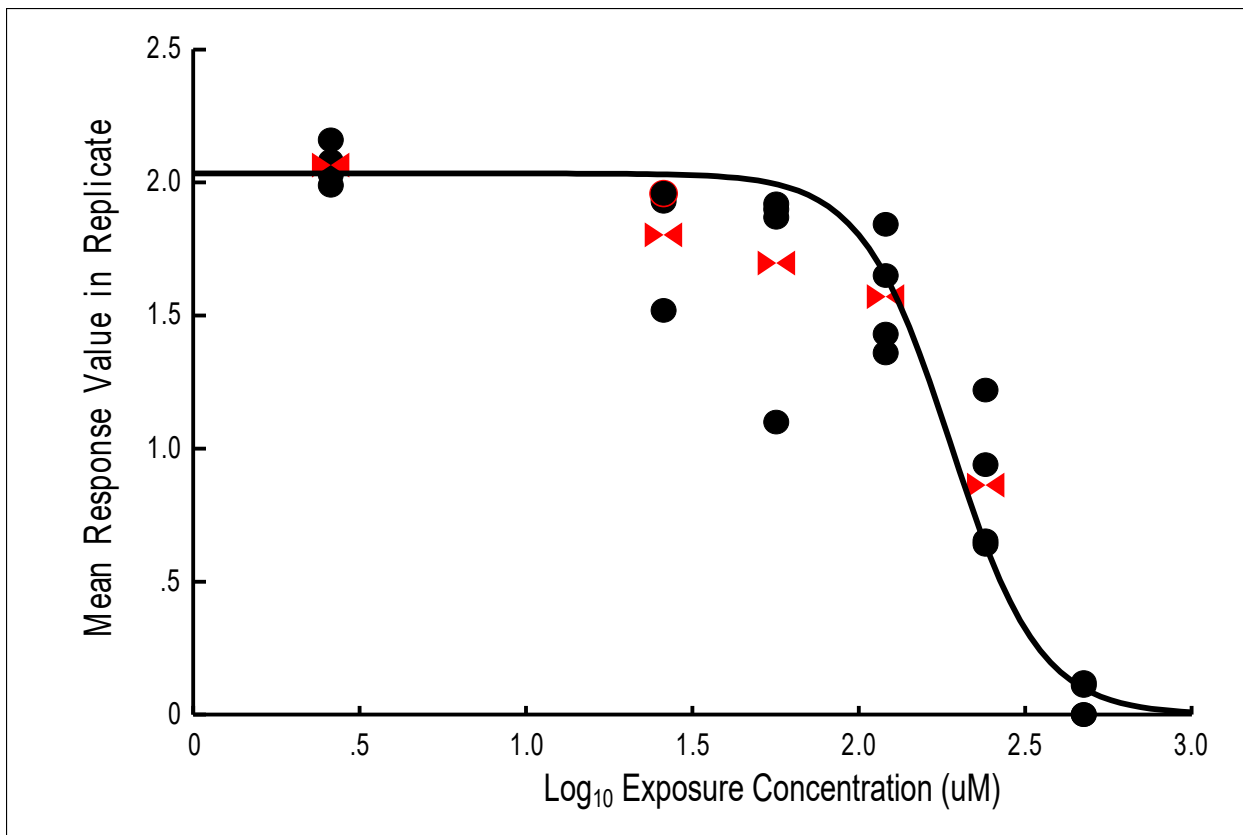
Parameter Summary   Optimization Errors: 0 0 0 0 0

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL	PAlt
logEC50	1.971	0.50	1.500	2.500	2.276	2.186	2.370	
					189.	154.	235.	
Slope	1.004	0.50	0.500	5.000	1.863	1.367	2.874	
CtrlVal	2.065	0.50	1.000	3.000	2.034	1.940	2.129	
StdDev	0.063	0.50	0.100	0.800	0.100	0.100	0.122	
logEC20					2.090	1.951	2.237	
					123.	89.	172.	

Parameter Optimization History

Iter	-LogLike		logEC50		Slope		CtrlVal		StdDev	
1	8.8440	34.0837	1.748	2.331	0.744	1.979	1.843	2.464	0.063	0.274
21	4.2923	6.4068	2.010	2.224	1.080	1.673	1.904	2.155	0.143	0.186
81	-4.5701	-4.4373	2.276	2.300	1.939	2.078	2.016	2.046	0.100	0.102
171	-4.6677	-4.6677	2.276	2.276	1.863	1.864	2.034	2.034	0.100	0.100
350	-4.6677	-4.6677	2.276	2.276	1.863	1.863	2.034	2.034	0.100	0.100

Analysis Date: 01/17/2023 07:08   01/16/23 Continuous Variable Effects Analysis Program

S12.23. C. dilutus 8:2 FTS test data and CR analysis output

Exposure concentration μM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.52 control	0.28	10	10	90%	1.76	1.41	
		9	10		0.64		
		7	10		1.45		
		11	11		1.78		
5.2	2.8	9	10	80%	1.83	1.66	
		8	10		1.59		
		9	10		1.89		
		6	10		1.34		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method	
Survival	Means comparison	$P = 0.226$
Biomass	Means comparison	$P = 0.212$

S12.24. C. dilutus 10:2 FTS test data and CR analysis output

Exposure concentration µM	Exposure concentration mg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass (mg)*	Treatment average biomass (mg)	Censored from growth analysis? Y
0.013 control	0.0081	10	10	90%	1.76	1.41	
		9	10		0.64		
		7	10		1.45		
		11	11		1.78		
0.13	0.081	10	10	83%	1.16	0.89	
		7	10		0.66		
		9	10		0.88		
		7	10		0.84		

*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

Endpoint	Analysis method	
Survival	Means comparison	$P = 0.346$
Biomass	Means comparison	$P = 0.059$