Supplemental information for Mount et al. Toxicity of DDT congeners to *Hyalella*

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S1. Analytes and conditions for GCMS analyses

Gas Chromatograph: Thermo Trace 1310

Oven Parameters

Temp Program	90 °C for 2 min; 30 °C/min to 180 °C; hold 0.00 min; 2.0 °C/min to 200 °C; hold 10.0 min; 30.0 °C/min to 300 °C; hold 5.00 min

Column

Agilent J&W DB-EUPAH 20m × 0.18mm × 0.14 µm (PN: 121-9627)

SSL Parameters

Liner:	Restek Topaz Uniliner, bottom hole NO glass wool
SSL Mode	Splitless
Flow Rate	0.8 mL/min
Split Flow	50.0 mL/min
Splitless time	1.80 min
Inlet Temp	200 °C
Transfer Line	270 °C

Mass Spectrometer: GC-QExactive Orbitrap

Ion Source Mode	EI Positive
Source Temp	270 °C
Electron Energy	43 eV
Emission Current	50 eV
Scan Mode	Full Scan (100-400 *m/z*)
Resolution Power	120,000

Analyte	Quant Ion	Qual Ion	RT	Surrogate Name
PCB-52-(13C12)	303.9591	301.9622	11.32	(ISTD)
44-DDMU	212.0380	281.9756	15.36	44-DDE-(Ring 13C12)
24-DDE	245.9991	247.9960	15.48	44-DDE-(Ring 13C12)
44-DDE	245.9991	247.9960	17.29	44-DDE-(Ring 13C12)
44-DDE-(Ring 13C12)	258.0393	260.0363	17.28	PCB-52-(13C12)
44-DDMS	235.0069	237.0038	17.54	44-DDE-(Ring 13C12)
24-DDD	235.0069	237.0038	19.03	44-DDE-(Ring 13C12)
24-DDT	235.0069	237.0038	21.48	24-DDT-(Ring 13C12)
24-DDT-(Ring 13C12)	247.0471	249.0441	21.45	PCB-52-(13C12)
44-DDD	235.0069	237.0038	22.47	44-DDE-(Ring 13C12)
44-DDT	235.0070	237.0039	25.49	44-DDT-(Ring 13C12)
44-DDT-(Ring 13C12)	247.0473	249.0443	25.46	PCB-52-(13C12)

S2. Summaries of basic water chemistries in exposure chambers

Temperature °C

Chemical	Mean	St. Dev	Min	Max
p,p'DDT	23.0	0.62	22.0	24.0
o,p'DDT	22.9	0.20	22.1	23.4
p,p'DDD	22.9	0.25	22.1	23.7
o,p'DDD	23.0	0.35	22.0	23.8
p,p'DDE	22.6	0.26	22.0	23.0
p,p'DDMU	22.9	0.50	21.5	23.9
			%CV	
All tests	**22.9**	**0.164**	**0.718**	

pH

Chemical	Mean	St. Dev	Min	Max
p,p'DDT	7.70	0.09	7.49	7.78
o,p'DDT	8.00	0.11	7.64	8.14
p,p'DDD	7.70	0.04	7.64	7.78
o,p'DDD	7.87	0.12	7.53	8.02
p,p'DDE	7.79	0.15	7.41	7.94
p,p'DDMU	8.16	0.08	7.99	8.32
			%CV	
All tests	**7.87**	**0.038**	**0.483**	

Dissolved oxygen mg/L

Chemical	Mean	St. Dev	Min	Max
p,p'DDT	8.50	0.35	8.10	9.30
o,p'DDT	8.80	0.34	7.50	9.40
p,p'DDD	8.50	0.16	8.30	8.90
o,p'DDD	8.60	0.28	7.80	9.10
p,p'DDE	8.60	0.28	8.30	9.20
p,p'DDMU	9.10	0.25	8.70	9.50
			%CV	
All tests	**8.68**	**0.069**	**0.799**	

S3. Test design, exposure concentrations, raw data, and effects analysis

Overview

This section provides, for each toxicity test, replicate-level test data and the results of concentration/response (CR) analyses of these data. CR analysis includes software output for both the survival CR curve estimation and the biomass CR curve estimation. Note, all control concentrations were assigned a value of 1/10th of the lowest treatment for CR analysis.

The outputs for survival and biomass CR curve estimation have three components:

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

(2) A figure for fraction survival or biomass ("Effect Variable") versus the base-10 logarithm of the concentration (µg/L) ("Exposure Variable") that includes observed treatment-level survival or replicate-level biomass data used for curve estimation (black solid circles), treatment mean biomass (red bow ties), and the estimated CR curve. For tests with at least two partial effect concentrations, the solid black curve is the maximum-likelihood solution. For tests with just one partial effect concentration, parameter estimates are not based on a maximum likelihood solution, but rather are approximated as an average of their confidence limits, and the red-dashed curve is approximate/illustrative based on those parameter estimates. Black open circles denote replicates censored due to technical errors (e.g., drying pan containing organisms lost) or statistical outliers.

(3) A table regarding parameter estimation for the log-logistic CR model. The three survival 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"). The four growth model parameters are the base-10 logarithm of the EC50 ("logX50"), the slope of the line at the EC50 ("Slope"), the control growth ("CtrlVal"), and the relative standard deviation of the log-logistic distribution ("RelStdv"). For these parameters, the tables provide: 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.

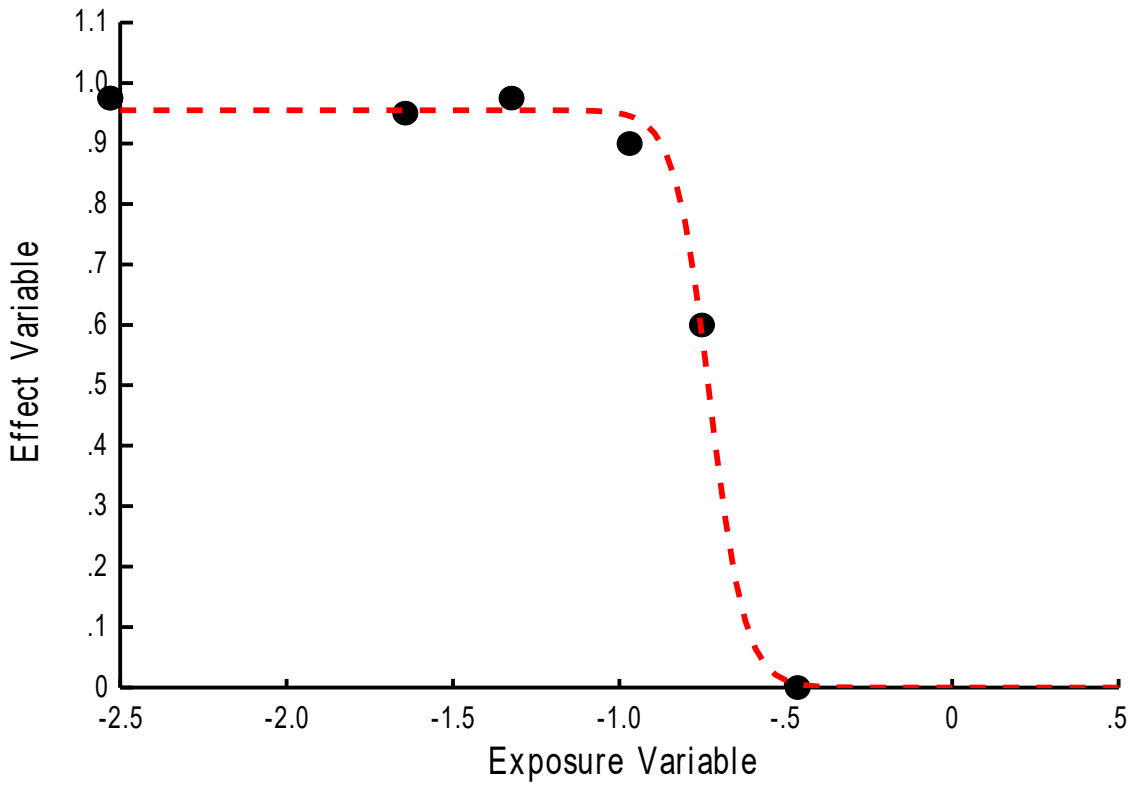
S3.1. p,p'DDT

Exposure concentration µg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)
Control	10	10	98%	0.576	0.502
	10	10		0.456	
	9	10		0.423	
	10	10		0.556	
MeOH blank	9	10	98%	0.403	0.510
	10	10		0.656	
	10	10		0.546	
	10	10		0.436	
0.023	10	10	95%	0.526	0.509
	9	10		0.533	
	9	10		0.493	
	10	10		0.486	
0.047	9	10	98%	0.373	0.472
	10	10		0.456	
	10	10		0.586	
	10	10		0.476	
0.107	9	10	90%	0.433	0.381
	9	10		0.303	
	10	10		0.456	
	8	10		0.330	
0.177	7	10	60%	0.178	0.125
	5	10		0.123	
	7	10		0.138	
	5	10		0.063	
0.343	0	10	0%	0.000	0.000
	0	10		0.000	
	0	10		0.000	
	0	10		0.000	

t-test control vs MeOH (current test only):

p = 0.916

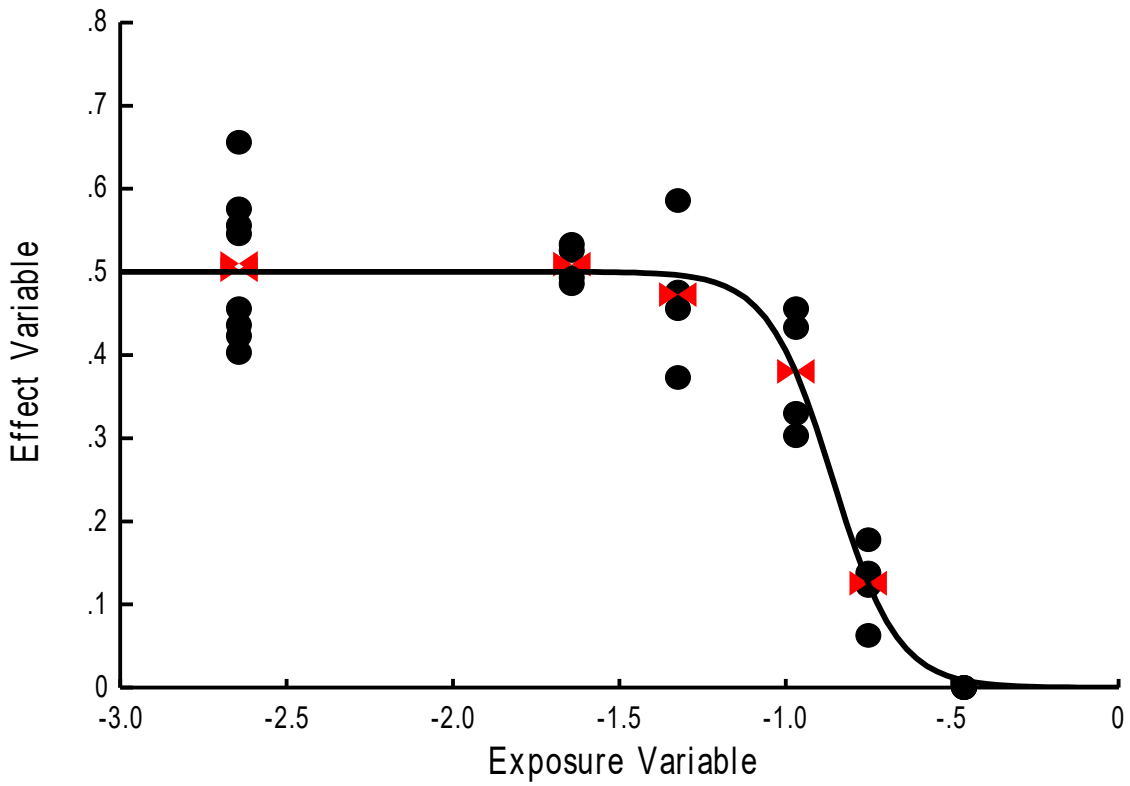
p,p'DDT effects on Hyalella survival



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.7450	0.5000	-1.0450	-0.4450	-0.7280	-0.7737	-0.6823
					0.19	0.17	0.21
logStdDev	0.1869	0.5000	0.0100	0.5000	0.0948	0.0100	0.1896
CtrlSurv	0.9550	0.5000	0.8000	1.0000	0.9550	0.9203	0.9870
logX20					-0.8230	-0.8932	-0.7528
					0.15	0.13	0.18

p,p'DDT effects on Hyalella biomass



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.918	1.000	-1.218	-0.618	-0.859	-0.909	-0.809
X50					0.14	0.12	0.16
Slope	2.205	1.000	1.102	5.000	2.571	1.625	3.947
CtrlVal	0.499	1.000	0.349	0.748	0.500	0.468	0.533
RelStdv	0.061	1.000	0.031	0.122	0.062	0.049	0.083
logX20					-0.993	-1.097	-0.915
X20					0.10	0.08	0.12

S3.2. o,p'DDT

Exposure concentration µg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)
Control	9	10	95%	0.409	0.517
	11	11		0.869	
	9	10		0.429	
	10	10		0.362	
MeOH blank	9	10	98%	0.519	0.703
	10	10		0.902	
	10	10		0.992	
	11	11		0.396	
0.070	10	10	98%	0.403	0.499
	11	11		0.596	
	10	10		1.292**	
	9	10		1.079**	
0.130	10	10	98%	0.382	0.637
	10	10		0.692	
	10	10		0.662	
	9	10		0.809	
0.239	10	10	98%	0.422	0.544
	9	10		0.429	
	10	10		0.552	
	10	10		0.772	
0.443	10	10	95%	0.613	0.458
	10	10		0.492	
	9	10		0.269	
	9	10		1.449**	
0.922	10	10	98%	0.532	0.439
	10	10		0.342	
	9	10		0.269	
	10	10		0.613	
1.61	2	10	13%	-0.014	-0.005
	0	10		0.000	
	0	10		0.000	
	3	10		0.410**	

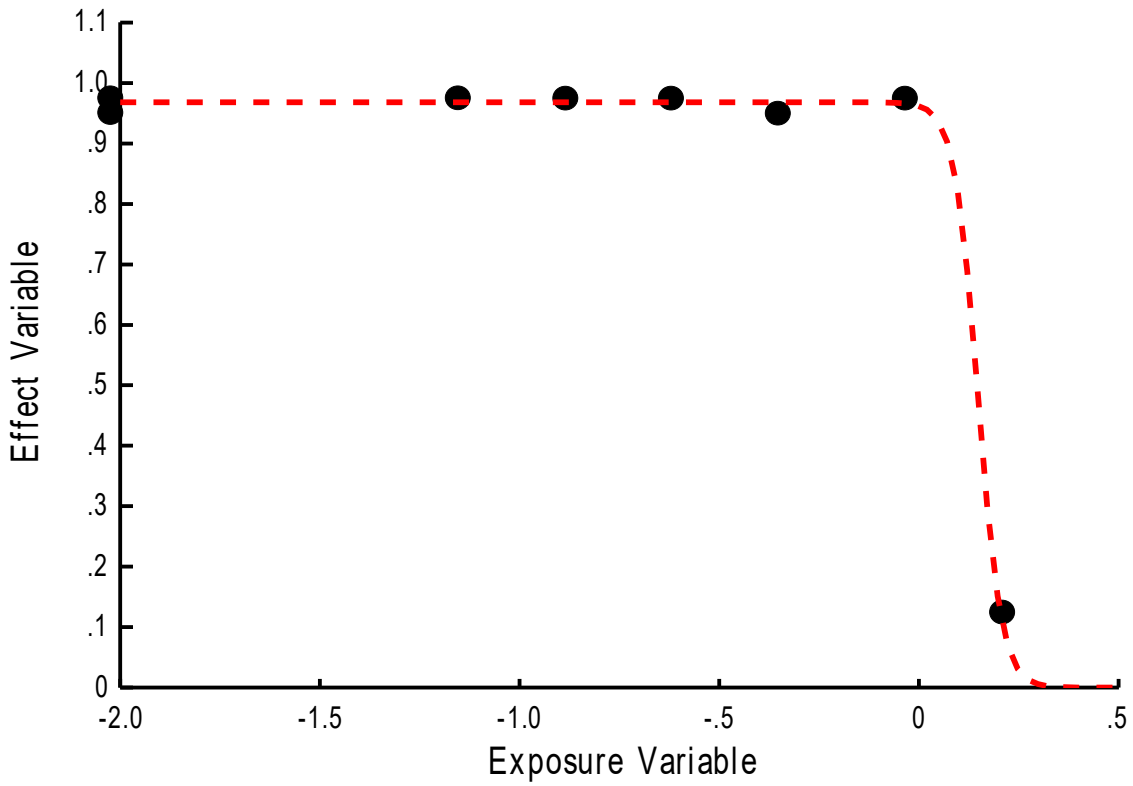
*Correction factor of 0.909 applied to chambers overstocked with 11 organisms

**Technical error; censored from growth analysis

t-test control vs MeOH:

p = 0.360

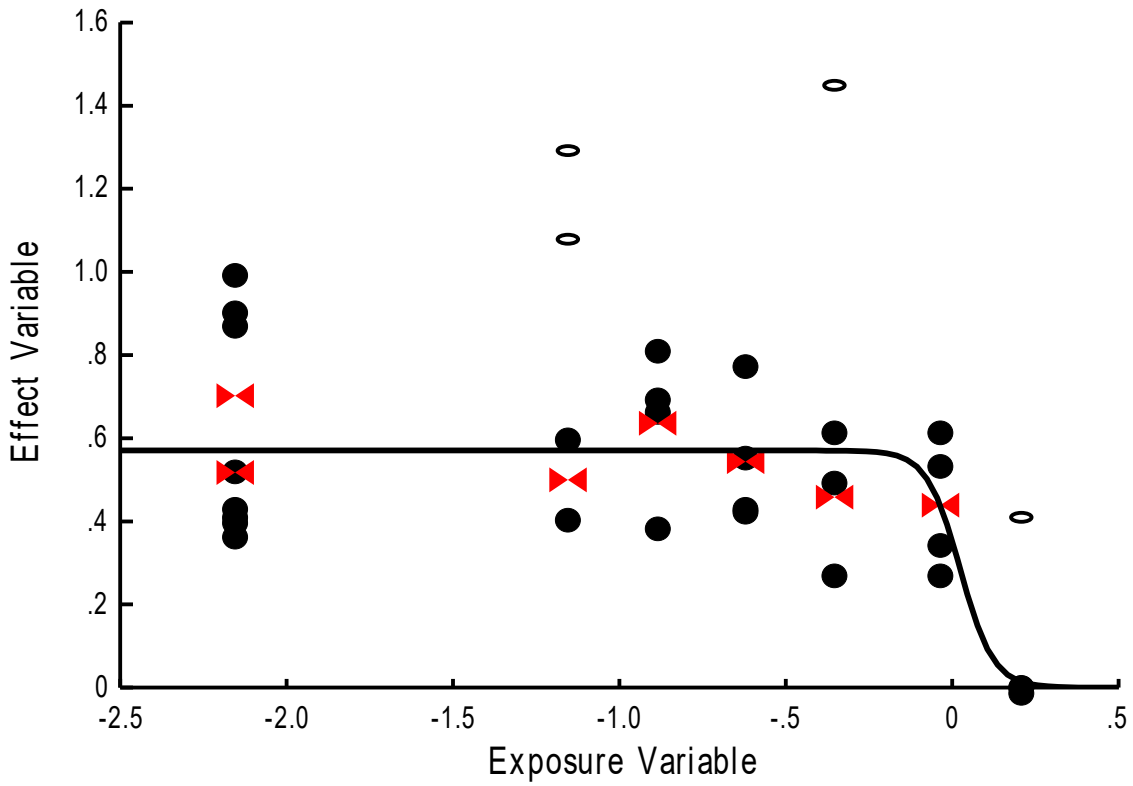
o,p'DDT effects on Hyalella survival



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.1007	0.5000	-0.1993	0.4007	0.1477	0.0931	0.2022
					1.40	1.24	1.59
logStdDev	0.0954	0.5000	0.0100	0.5000	0.0531	0.0100	0.1061
CtrlSurv	0.9682	0.5000	0.8000	1.0000	0.9682	0.9434	0.9849
logX20					0.1091	0.0237	0.1946
					1.29	1.06	1.57

o,p'DDT effects on Hyalella biomass



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.241	1.000	-0.541	0.059	0.024	-0.116	0.059
X50					1.06	0.77	1.15
Slope	2.228	1.000	1.114	5.000	5.000	1.114	5.000
CtrlVal	0.589	1.000	0.412	0.883	0.570	0.491	0.650
RelStdv	0.164	1.000	0.082	0.328	0.180	0.141	0.239
logX20					-0.045	-0.427	0.096
X20					0.90	0.37	1.25

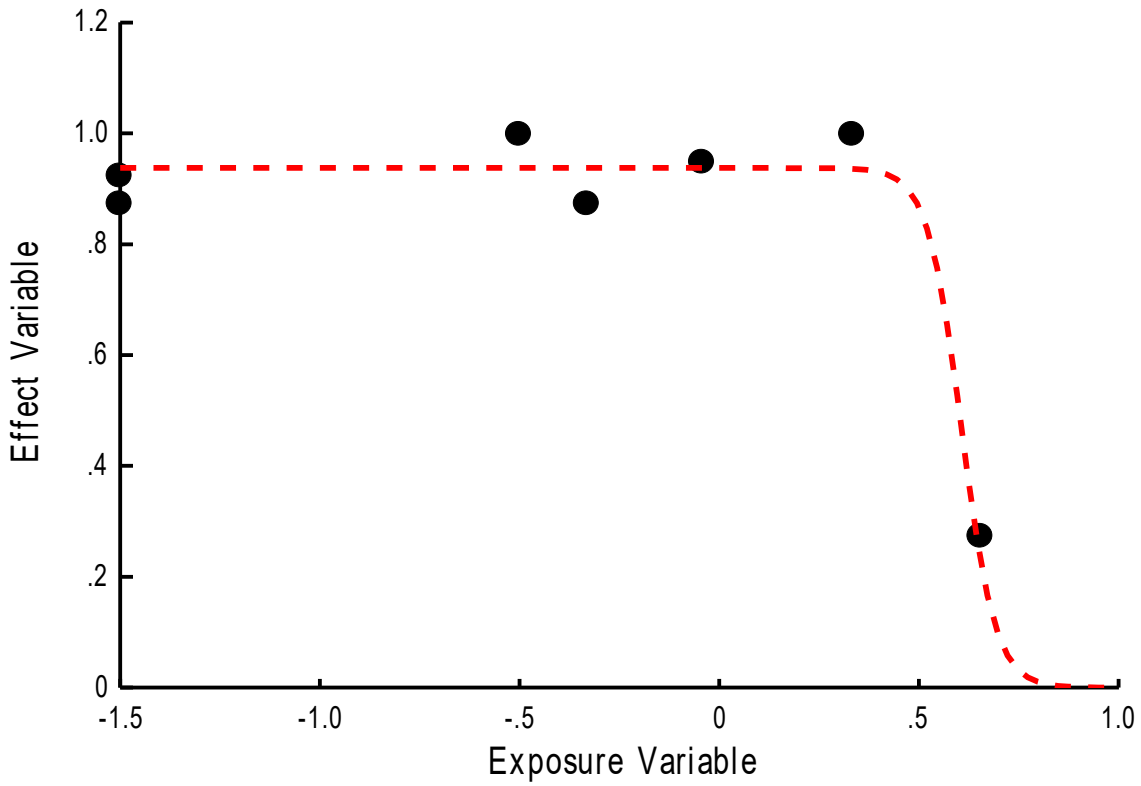
S3.3. p,p'DDE

Exposure concentration µg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)
Control	10	10	88%	0.497	0.450
	8	10		0.447	
	9	10		0.327	
	8	10		0.527	
MeOH blank	9	10	93%	0.617	0.544
	10	10		0.447	
	8	10		0.497	
	10	10		0.617	
0.313	10	10	100%	0.877	0.627
	10	10		0.447	
	10	10		0.697	
	10	10		0.487	
0.463	9	10	88%	0.507	0.555
	10	10		0.677	
	8	10		0.537	
	8	10		0.497	
0.900	10	10	95%	0.547	0.497
	9	10		0.567	
	10	10		0.507	
	9	10		0.367	
2.14	10	10	100%	0.597	0.589
	10	10		0.677	
	10	10		0.627	
	10	10		0.457	
4.48	4	10	28%	0.109	0.067
	3	10		0.079	
	2	10		0.069	
	2	10		0.009	

t-test control vs MeOH:

p = 0.175

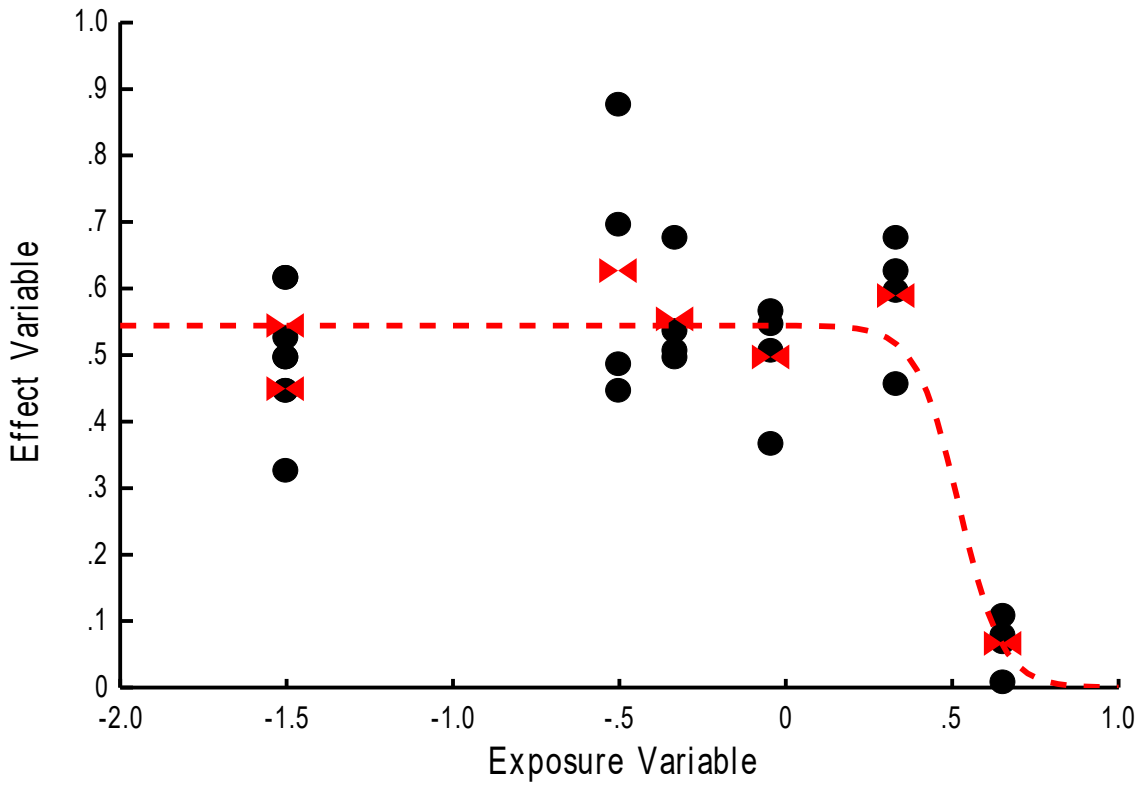
p,p'DDE effects on Hyalella survival



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.5516	0.5000	0.2516	0.8516	0.6065	0.5627	0.6503
					4.04	3.65	4.47
logStdDev	0.1478	0.5000	0.0100	0.5000	0.0771	0.0100	0.1543
CtrlSurv	0.9375	0.5000	0.8000	1.0000	0.9375	0.9022	0.9635
logX20					0.5520	0.4614	0.6427
					3.56	2.89	4.39

p,p'DDE effects on Hyalella biomass



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.525	1.000	0.225	0.825	0.521	0.420	0.622
X50					3.32	2.63	4.19
Slope	5.986	1.000	2.993	5.000	3.868	2.993	5.000
CtrlVal	0.544	1.000	0.381	0.815	0.544	0.493	0.596
RelStdv	0.093	1.000	0.047	0.186	0.116	0.082	0.150
logX20					0.450	0.347	0.553
X20					2.82	2.22	3.57

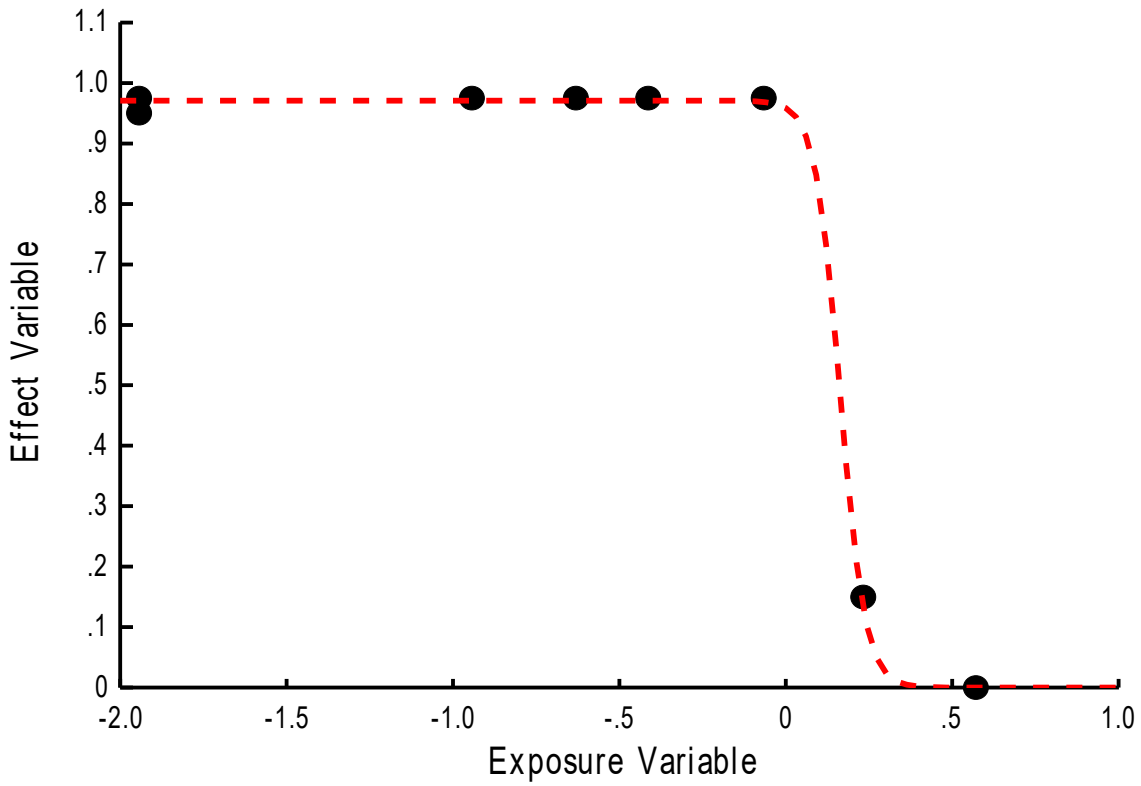
S3.4. p,p'DDD

Exposure concentration µg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)
Control	10	10	98%	0.657	0.559
	9	10		0.561	
	10	10		0.513	
	10	10		0.503	
MeOH blank	10	10	95%	0.603	0.590
	9	10		0.581	
	10	10		0.603	
	9	10		0.571	
0.114	10	10	98%	0.533	0.493
	9	10		0.391	
	10	10		0.593	
	10	10		0.453	
0.234	10	10	98%	0.683	0.550
	10	10		0.593	
	9	10		0.501	
	10	10		0.423	
0.386	10	10	98%	0.553	0.468
	9	10		0.381	
	10	10		0.473	
	10	10		0.463	
0.858	10	10	98%	0.483	0.465
	10	10		0.473	
	10	10		0.423	
	9	10		0.481	
1.71	0	10	15%	0.000	0.018
	0	10		0.000	
	6	10		0.074	
	0	10		0.000	
3.73	0	10	0%	0.000	0.000
	0	10		0.000	
	0	10		0.000	
	0	10		0.000	

t-test control vs MeOH:

p = 0.421

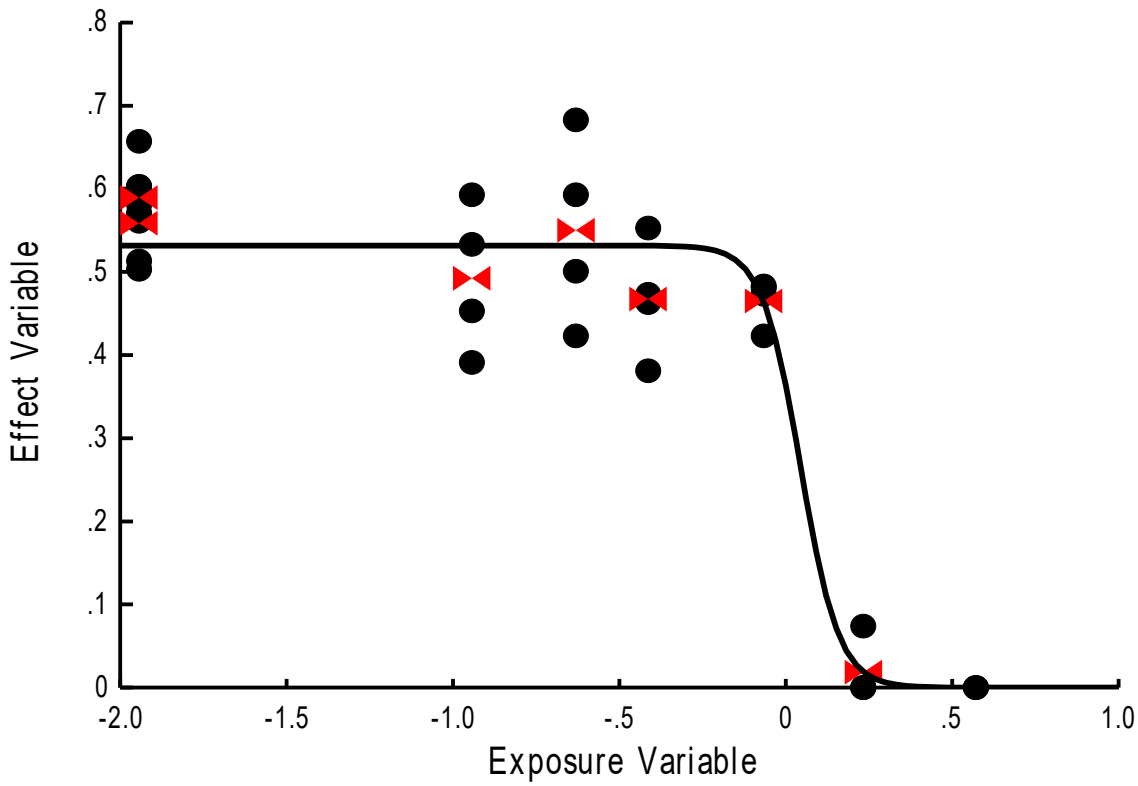
p,p'DDD effects on Hyalella survival



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.2443	0.5000	-0.0557	0.5443	0.1632	0.0986	0.2278
					1.46	1.25	1.69
logStdDev	0.2182	0.5000	0.0100	0.5000	0.0679	0.0100	0.1359
CtrlSurv	0.9708	0.5000	0.8000	1.0000	0.9708	0.9444	0.9874
logX20					0.1145	0.0089	0.2202
					1.30	1.02	1.66

p,p'DDD effects on Hyalella biomass



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	-0.182	1.000	-0.482	0.118	0.044	-0.013	0.118
X50					1.11	0.97	1.31
Slope	2.249	1.000	1.124	5.000	4.357	2.619	5.000
CtrlVal	0.548	1.000	0.383	0.821	0.532	0.501	0.562
RelStdv	0.056	1.000	0.028	0.113	0.067	0.053	0.088
logX20					-0.035	-0.094	0.063
X20					0.92	0.80	1.16

S3.5. o,p'DDD

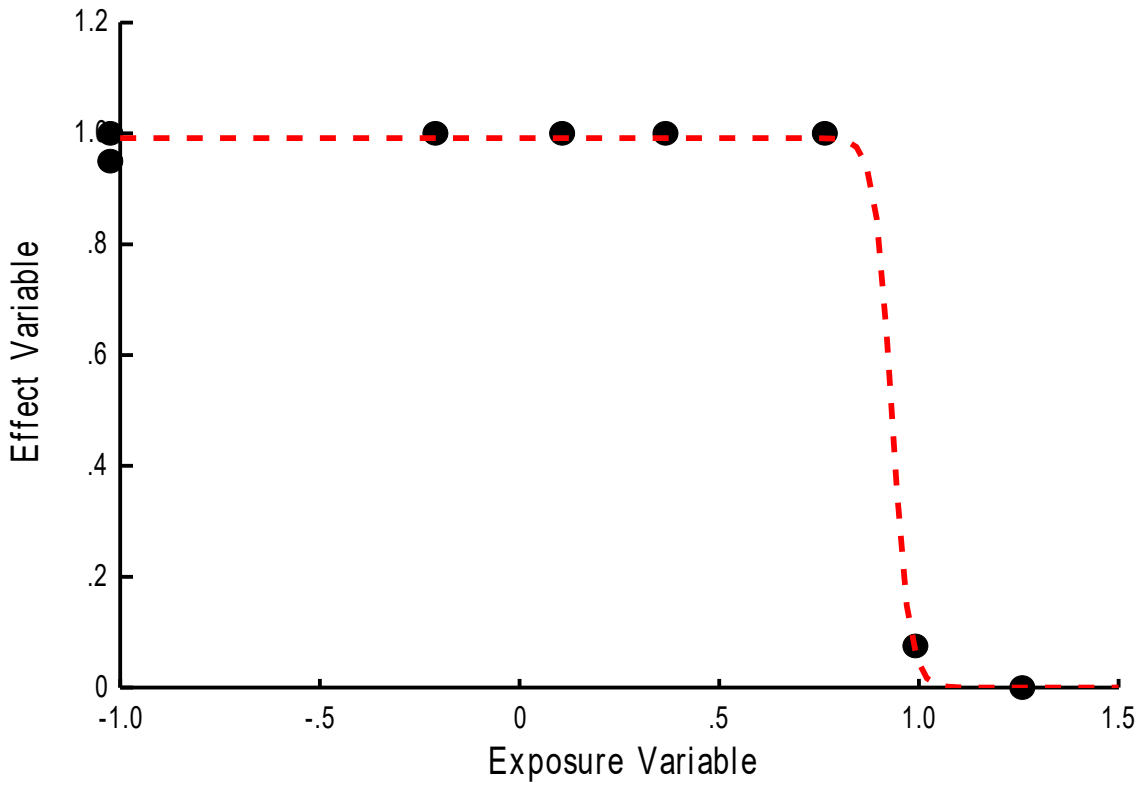
Exposure concentration µg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass gain (mg)*	Treatment average biomass gain (mg)
Control	10	10	95%	0.705	0.592
	10	10		0.665	
	9	10		0.524	
	9	10		0.473	
MeOH blank	10	10	100%	0.675	0.723
	10	10		0.765	
	10	10		0.745	
	10	10		0.705	
0.62	11	11	100%	0.970	0.786
	10	10		0.725	
	10	10		0.593	
	10	10		0.855	
1.28	10	10	100%	0.775	0.698
	10	10		0.435	
	11	11		0.888	
	10	10		0.695	
2.32	10	10	100%	0.655	0.680
	10	10		0.445	
	10	10		0.915	
	10	10		0.705	
5.81	10	10	100%	0.375	0.383
	10	10		0.415	
	10	10		0.315	
	10	10		0.425	
9.81	1	10	8%	-0.019	0.014
	0	10		0.000	
	0	10		0.000	
	2	10		0.073	
18.1	0	10	0%	0.000	0.000
	0	10		0.000	
	0	10		0.000	
	0	10		0.000	

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

t-test control vs MeOH:

p = 0.0684

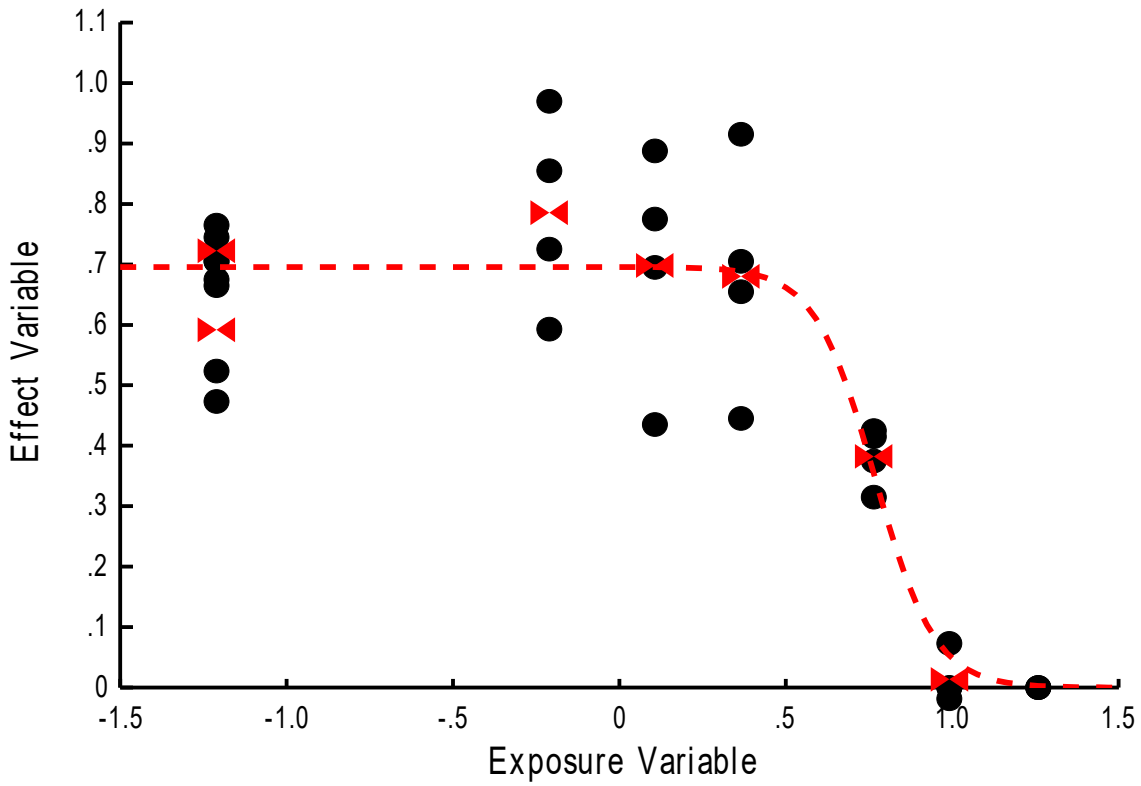
o,p'DDD effects on Hyalella survival



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	1.0116	0.5000	0.7116	1.3116	0.9321	0.8807	0.9834
					8.55	7.60	9.63
logStdDev	0.1648	0.5000	0.0100	0.5000	0.0392	0.0100	0.0784
CtrlSurv	0.9917	0.5000	0.8000	1.0000	0.9917	0.9747	1.0000
logX20					0.9027	0.8297	0.9758
					7.99	6.76	9.46

o,p'DDD effects on Hyalella biomass



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.677	1.000	0.377	0.977	0.767	0.697	0.838
X50					5.85	4.98	6.88
Slope	3.058	1.000	1.529	5.000	2.779	1.545	5.000
CtrlVal	0.696	1.000	0.487	1.043	0.696	0.637	0.754
RelStdv	0.107	1.000	0.054	0.214	0.126	0.093	0.159
logX20					0.623	0.496	0.749
X20					4.19	3.13	5.62

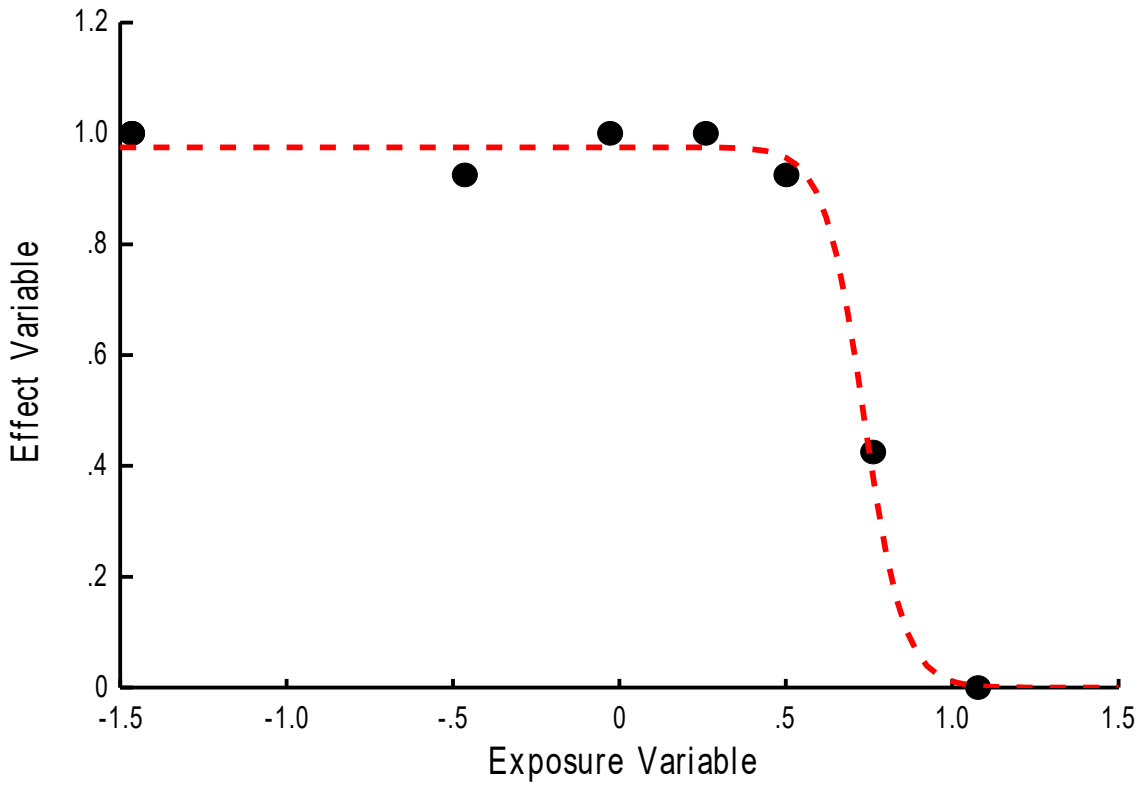
S3.6. p,p'DDMU

Exposure concentration µg/L	Number surviving organisms	Number of original organisms	Treatment average survival	Biomass gain (mg)	Treatment average biomass gain (mg)
Control	10	10	100%	0.725	0.703
	10	10		0.675	
	10	10		0.735	
	10	10		0.675	
MeOH blank	10	10	100%	1.025	0.975
	10	10		1.045	
	10	10		0.925	
	10	10		0.905	
0.343	10	10	93%	0.665	0.608
	8	10		0.606	
	9	10		0.586	
	10	10		0.575	
0.938	10	10	100%	0.925	0.700
	10	10		0.485	
	10	10		0.775	
	10	10		0.615	
1.82	10	10	100%	0.705	0.840
	10	10		0.785	
	10	10		0.995	
	10	10		0.875	
3.18	9	10	93%	0.735	0.623
	9	10		0.695	
	10	10		0.495	
	9	10		0.565	
5.79	3	10	43%	0.088	0.083
	6	10		0.147	
	3	10		0.068	
	5	10		0.027	
12.0	0	10	0%	0.000	0.000
	0	10		0.000	
	0	10		0.000	
	0	10		0.000	

t-test control vs MeOH:

p = 0.000404

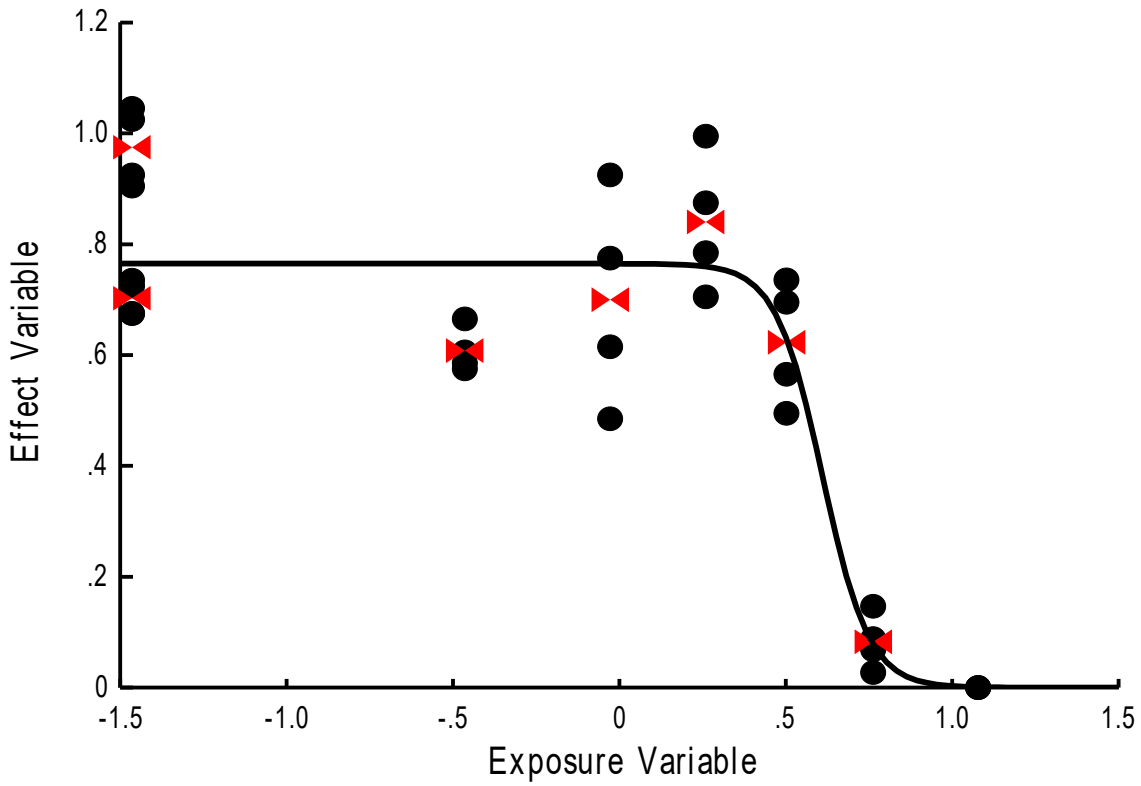
p,p'DDMU effects on Hyalella survival



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.7667	0.5000	0.4667	1.0667	0.7352	0.6758	0.7946
					5.43	4.74	6.23
logStdDev	0.2076	0.5000	0.0100	0.5000	0.1084	0.0100	0.2169
CtrlSurv	0.9750	0.5000	0.8000	1.0000	0.9750	0.9496	0.9976
logX20					0.6500	0.5428	0.7573
					4.47	3.49	5.72

p,p'DDMU effects on Hyalella biomass



Parameter Summary

PName	Guess	Delta	PMin	PMax	PEst	95%LCL	95%UCL
logX50	0.705	1.000	0.405	1.005	0.610	0.535	0.697
X50					4.07	3.42	4.98
Slope	2.682	1.000	1.341	5.000	3.567	2.084	5.000
CtrlVal	0.765	1.000	0.536	1.148	0.765	0.704	0.826
RelStdv	0.085	1.000	0.042	0.170	0.134	0.107	0.170
logX20					0.513	0.425	0.628
X20					3.26	2.66	4.25