Birke and Scholz:

Zebrafish Embryo and Acute Fish Toxicity Test Show Similar Sensitivity for Narcotic Compounds

Supplementary Data

Index

Details on animals

Zebrafish embryo acute toxicity test

Zebrafish of the strain UFZ-OBI (generation F13 and F14) were cultured and used for the production of embryos as described previously (Fetter et al., 2015). Husbandry and experimental procedures were performed in accordance with the German animal protection standards and were approved by the Government of Saxony, *Landesdirektion Leipzig*, Germany (DD24-5131/25/7). The ZFET was conducted according to the OECD TG 236 with two minor modifications: Ten embryos were used per concentration instead of 20 but therefore experiments were replicated with a modified range of concentrations to improve modelling of concentration response curves. 24-well plates (Costar®, Polystyrene, Corning Incorporated, Kennebunk ME, USA), with an exposure volume of 2 ml per embryo and well) were used. Mortality was identified as described in the OECD TG 236, i.e. by coagulation, a non-detached tail or lack of heart beat (OECD, 2013). Mortality was assessed at 24, 48, 72, and 96 hpf (hours post fertilization). In all exposure solutions, the pH and oxygen levels during the testing period were in the range of acceptance criteria for the OECD TG 236 (dissolved oxygen concentration in the negative control and highest test concentration ≥ 80% of saturation; pH did not vary more than 1.5 units and was within a range of pH 6.5 and 8.5). Mortality in controls did not exceed 10%, mortality in the positive controls (31.7 µM 3,4-dichloroaniline) was at least 30% at the end of the test. Exposure was static for 4-chloroaniline, 3-iodo-2-propynyl-N-butylcarbamate, aniline, acetochlor and pyraclostrobin. A 12-h renewal interval was used for folpet in order to compensate at least partially for the short half-life of the compound. Stock solutions were prepared in exposure medium (OECD, 2013) or dimethyl sulfoxide (DMSO) in order to accelerate solubilization (pyraclostrobin) or to avoid degradation of the exposure chemical (folpet) prior to the start of the exposure. If exposure solutions were prepared using DMSO stock solutions, the DMSO concentration was 0.1% (v/v) for all treatments and appropriate solvent controls. No solvents were used for all other compounds.

Details on materials and methods

Exposure chemicals

Exposure chemicals were obtained from the following suppliers: 4-chloroaniline (purity > 99%, Merck, Darmstadt, Germany), 3-iodo-2-propynyl-N-butylcarbamate (purity ≥ 98.5%, Sigma Aldrich, Seelze, Germany), aniline (purity 99%, Merck), acetochlor (purity 96%, Sigma Aldrich), folpet (purity 99.9%, Sigma Aldrich), pyraclostrobin (Sigma Aldrich, analytical grade). The known or potential mode of action (MoA) of each compound had been obtained previously based on a literature review and structural alert analysis (Sobanska et al., 2018).

Chemical analysis

The exposure concentrations of chemicals were analyzed by reversed phase HPLC-MS/MS at the beginning of the exposure, the end of the experiment (i.e., after 96 h of exposure) or before and after a 12-h renewal interval of exposure solutions in case of treatments with folpet. Chemical analysis was performed for all concentrations. Due to sensitivity issues, chemical analysis of folpet could only be conducted for samples of concentrations of 0.26 µmol/l and above. Detailed information on the analytical method parameters and equipment are given in Table S1.

Sample aliquots were diluted to match the range of the calibration curves. Folpet containing samples were stabilized with 80/20/0.2% (v/v) acetonitrile/exposure medium/formic acid to prevent further hydrolysis. Samples containing pyraclostrobin were stored in silanized sample vials containing 50% (v/v) methanol to prevent adsorption. All samples were stored at -18°C prior to analysis. Sample matrix effects were taken into account by diluting the calibration solutions in exactly the same dilution medium as the appropriate sample solutions. Linear calibration curves with a correlation coefficient of > 0.99

doi:10.14573/altex.1808101s1

ALTEX 36(1), SUPPLEMENTARY DATA

for all substances were obtained. The calibration range started with at least 80% of the lowest analyzed sample concentration and ended with at least 120% of the highest analyzed sample concentration. The half-life of folpet was estimated by modelling the exponential decay (equation 1) using the software R and the package drc (R Core Team, 2015). C refers to the concentration at a given time t, C_0 represents the initial concentration and *a* represent the exponential time constant obtained by curve fitting.

$$C = C_0 * e^{(-\frac{t}{a})} \quad (1)$$

Concentration-response modelling, calculation of baseline toxicity and toxic ratios

Concentration-response curves for mortality in the ZFET were fitted to the data using the Hill-slope equation (equation 2) and used to estimate LC50 values and corresponding confidence intervals.

$$y = Min + \frac{Max-Min}{1+(\frac{x}{LC50})^{-p}} \quad (2)$$

The parameters Max and Min were set to 100% and 0%, respectively, and the slope (p) was estimated. The independent variable *x* represents the nominal or – in case of deviations by more than 20% from nominal – the measured exposure concentration [µM] and y the percentage of survival. The software R and the package drc (R Core Team, 2015) embedded into a KNIME workflow were used to model concentration-response curves (Berthold et al., 2008). ZFET baseline toxicity of the test compounds was calculated according to the QSAR described by Klüver et al. (2016). The toxicity ratio (TR, equation 3, (ratio of baseline fish embryo LC50 and experimentally observed LC50) was calculated as an indicator of baseline toxicity.

$$TR = \frac{LC50_{baseline\ toxicity}}{LC50_{exp}} \quad (3)$$

Origin of physico-chemical compound properties and comparative fish embryo acute toxicity and acute fish toxicity data

Existing data of fish embryo acute toxicity data (96-h exposure, conducted similar to OECD TG 236) and acute fish toxicity data were obtained from the meta-analysis of Sobanska et al. (2018). Physico-chemical data (log K_{ow}, water solubility) represented values estimated with ECOSAR (Clements and Nabholz, 1994). None of the selected compounds were indicated as volatile (log K_{aw} < -4). ZFET data stemming from a previous meta-analysis but originally published by Truong et al. (2014) and Padilla et al. (2012) refer to values based on a reanalysis of raw data for mortality (Sobanska et al., 2018).

Tab. S1: Details on methods used for the analysis of exposure media
All compounds were analyzed by HPLC-MSMS. HFo, formic acid; NH_4Fo, ammonium formate

Compound	HPLC-MSMS device	Analytical column	HPLC eluent	Flow rate, gradient	Further settings	Detection
3-Iodo-2-propynyl-butylcarbamate	Agilent 1200 HPLC with a 6410 triple quadrupole MS detector	Agilent Zorbax Eclipse Plus C18 50 x 2.1 mm, 1.8 µm	A: H_2O with 0.1% HFo, 5 mM NH_4Fo B: MeOH with 0.1% HFo, 5 mM NH_4Fo	0.350 ml/min, 0.0 min 30% B, 4.5 min 100% B, Replicate time 6.0 min; post time 3.5 min	Column temperature: 40°C; injection volume: 5 µl	ESI positive, m/z: 282→165 (Quantifier), 282→127, 282→100 (Qualifier 1 and 2); retention time: 3.8 min LOD < 0.01 µmol/l
4-Chloroaniline		Phenomenex-Kinetex PFP, 100 x 2.1 mm; 2.6 µm core shell	A: H_2O with 0.1% HFo B: Acetonitrile with 0.1% HFo	0.300 ml/min, isocratic with 80% A, Replicate time 5 min	Column temperature: 40°C; injection volume: 1 µl	ESI positive, SIM m/z: 128 (Quantifier), 130, 111, 93; retention time: 2.1 min LOD < 0.85 µmol/l
Acetochlor		Agilent Zorbax Eclipse Plus C18 50 x 2.1 mm, 1.8 µm	A: H_2O with 0.1% HFo, 5 mM NH_4Fo B: MeOH with 0.1% HFo, 5 mM NH_4Fo	0.350 ml/min, 0.0 min 50% B, 6.0 min 100% B, Replicate time 8.0 min, Post time 3.5 min	Column temperature: 40°C; injection volume: 10 µl	ESI positive, m/z: 270→224 (Quantifier), 270→148, 270→132; retention time: 1.1 min LOD < 0.02 µmol/l
Aniline		Phenomenex-Kinetex PFP, 100 x 2.1 mm; 2.6 µm core shell	A: H_2O with 0.1% HFo B: Acetonitrile with 0.1% HFo	0.300 ml/min, isocratic with 80% A Replicate time: 5 min	Column temperature: 40°C; injection volume: 1 µl	ESI positive, m/z: 94→77 (Quantifier), 94→51, 94→50 (Qualifier 1 and 2); retention time: 1.1 min LOD < 0.6 µmol/l
Pyraclostrobin		Agilent Zorbax Eclipse Plus C18 50 x 2.1 mm, 1.8 µm	A: H_2O with 0.1% HFo, 5 mM NH_4Fo B: MeOH with 0.1% HFo, 5 mM NH_4Fo	0.350 ml/min, 0.0 min 30% B, 4.5 min 100% B, Replicate time: 6.0 min, post time 3.5 min	Column temperature: 40°C; injection volume: 10 µl	ESI positive, m/z: 388→163 (Quantifier), 388→133, 388→104 (Qualifier 1 and 2); retention time: 4.2 min LOD < 0.001 µmol/l
Folpet	Agilent 1200 HPLC with a 6470 triple quadrupole MS detector	Agilent Zorbax Eclipse Plus C18 50 x 2.1 mm, 1.8 µm	A: H_2O with 0.1% HFo, 5 mM NH_4Fo B: MeOH with 0.1% HFo, 5 mM NH_4Fo	0.300 ml/min, 0.0 min 20% B, 4.0 min 100% B, Replicate time 8.0 min, post time 3.5 min	Column temperature: 35°C; injection volume: 20 µl	ESI positive, m/z: 313→260 (Quantifier), 315→130 (Qualifier); retention time: 4.5 min LOD = 0.26 µmol/l

Tab. S2: Physico-chemical properties of exposure chemicals and measured concentration of exposure solutions
Data of individual replicates and concentrations are given in Table S3. Start and end of exposure refer to a 96-h static exposure except for the semistatic exposure of folpet, for which the concentration was analyzed at the start and end of each 12-h exposure renewal interval.
* This value refers to the highest test concentration of 10.1 µmol/l and a detection limit of 0.12 µmol/l.

Compound	CAS-No.	Log K_{ow}	Log K_{aw}	Water solubility (µM)	MoA	Concentration range for chemicals analysis (mmol/l)	Average percent of nominal concentrations (start/end of exposure)
4-Chloroaniline	106-47-8	1.72	-4.24	$2.02*10^4$	Narcosis	9.07-13.1	107/105
Acetochlor	34256-82-1	3.37	-6.04	176	Narcosis	0.34-0.48	102/109
Aniline	62-53-3	1.08	-4.11	$2.24*10^5$	Narcosis	4.69-8.71	99/96
3-Iodo-2-propynyl-butylcarbamate	55406-53-6	2.45	-6.44	450	Unknown	0.15-1.33	98/89
Pyraclostrobin	175013-18-0	5.45	-13.33	3.67	Narcosis	0.02-0.32	54/49
Folpet	133-07-3	2.84	-5.50	160	Narcosis	0.008-2.02	119/< 0.12*

Tab. S3: Data of chemical analysis of exposure solution for individual replicates
Start and end refer to the beginning and end of a 96-h static exposure interval. In case of folpet start and end refer to the beginning and end of a 12-h exposure interval. C = control, SC = solvent control containing 0.1% (v/v) dimethylsulfoxide

3-Iodo-2-propynyl-N-butylcarbamate

Treatment; sampling time Replicate 1	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]	Treatment; sampling time Replicate 2	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	< LOD	-	1 (C); test start	0.000	< LOD	-
1 (C); test end	0.000	< LOD	-	1 (C); test end	0.000	< LOD	-
2; test start	0.834	0.826	99%	2; test start	0.303	0.345	114%
2; test end	0.834	0.726	87%	2; test end	0.303	0.246	81%
3; test start	1.668	1.582	95%	3; test start	0.454	0.508	112%
3; test end	1.668	1.382	83%	3; test end	0.454	0.384	85%
4; test start	3.335	3.102	93%	4; test start	0.681	0.721	106%
4; test end	3.335	2.974	89%	4; test end	0.681	0.562	83%
5; test start	6.670	6.341	95%	5; test start	1.022	0.838	82%
5; test end	6.670	6.262	94%	5; test end	1.022	1.078	105%
6; test start	13.34	12.68	95%	6; test start	1.533	1.622	106%
6; test end	13.34	12.52	94%	6; test end	1.533	1.317	86%
7; test start	26.68	24.42	92%	7; test start	2.299	2.294	100%
7; test end	26.68	27.07	101%	7; test end	2.299	1.861	81%
8; test start	53.36	47.33	89%	8; test start	3.449	3.306	96%
8; test end	53.36	51.85	97%	8; test end	3.449	2.879	83%

4-Chloroaniline

Treatment; sampling time Replicate 1	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]	Treatment; sampling time Replicate 1	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.00	< LOD	-	1 (C); test start	0.000	< LOD	-
1 (C); test end	0.00	< LOD	-	1 (C); test end	0.000	< LOD	-
2; test start	18.83	19.01	101%	2; test start	90.68	94.54	104%
2; test end	18.83	19.15	102%	2; test end	90.68	88.86	98%
3; test start	37.65	38.64	103%	3; test start	117.9	123.5	105%
3; test end	37.65	39.24	104%	3; test end	117.9	119.7	102%
4; test start	75.30	79.32	105%	4; test start	153.2	164.0	107%
4; test end	75.30	80.63	107%	4; test end	153.2	153.1	100%
5; test start	150.6	169.7	113%	5; test start	199.2	212.5	107%
5; test end	150.6	182.9	113%	5; test end	199.2	212.5	107%
6; test start	301.2	263.3	87%	6; test start	259.0	303.8	117%
6; test end	301.2	360.3	120%	6; test end	259.0	285.3	110%
7; test start	602.4	666.6	111%	7; test start	336.7	379.8	113%
7; test end	602.4	656.5	109%	7; test end	336.7	364.5	108%
8; test start	1205	1303	108%	8; test start	437.7	484.9	111%
8; test end	1205	1338	111%	8; test end	437.7	354.0	81%

Acetochlor

Treatment; sampling time Replicate 1	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	< LOD	-
1 (C); test end	0.000	< LOD	-
2; test start	0.684	0.720	105%
2; test end	0.684	0.767	112%
3; test start	1.710	1.749	102%
3; test end	1.710	1.999	117%
4; test start	4.276	4.250	99%
4; test end	4.276	4.846	113%
5; test start	10.69	11.23	105%
5; test end	10.69	11.50	108%
6; test start	26.72	28.38	106%
6; test end	26.72	29.76	111%
7; test start	66.80	71.15	107%
7; test end	66.80	70.10	105%
8; test start	167.0	188.3	113%
8; test end	167.0	188.7	113%

Treatment; sampling time Replicate 2	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	< LOD	-
1 (C); test end	0.000	< LOD	-
2; test start	9.273	9.633	104%
2; test end	9.273	9.633	104%
3; test start	13.91	14.34	103%
3; test end	13.91	14.61	105%
4; test start	20.86	20.84	100%
4; test end	20.86	22.34	107%
5; test start	31.30	31.31	100%
5; test end	31.30	34.58	111%
6; test start	46.94	47.04	100%
6; test end	46.94	51.09	109%
7; test start	70.41	68.41	97%
7; test end	70.41	76.01	108%
8; test start	105.6	93.77	89%
8; test end	105.6	110.3	104%

Aniline

Treatment; sampling time Replicate 1	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	< LOD	-
1 (C); test end	0.000	< LOD	-
2; test start	310.5	316.5	102%
2; test end	310.5	305.2	98%
3; test start	620.9	525.7	103%
3; test end	620.9	540.8	106%
4; test start	1242	1197	96%
4; test end	1242	1221	98%
5; test start	2484	2431	98%
5; test end	2484	2409	97%
6; test start	4967	4723	95%
6; test end	4967	4192	84%
7; test start	9935	9630	97%
7; test end	9935	9673	97%
8; test start	19869	18870	95%
8; test end	19869	19347	97%

Treatment; sampling time Replicate 2	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	< LOD	-
1 (C); test end	0.000	< LOD	-
2; test start	361.1	368.2	102%
2; test end	361.1	342.3	95%
3; test start	469.4	485.3	103%
3; test end	469.4	499.2	106%
4; test start	610.2	507.7	83%
4; test end	610.2	641.3	105%
5; test start	793.3	790.3	100%
5; test end	793.3	736.9	93%
6; test start	1031	1051	102%
6; test end	1031	847	82%
7; test start	1341	1355	101%
7; test end	1341	1365	102%
8; test start	1743	1779	102%
8; test end	1743	1432	82%

Folpet

Treatment Replicate 1	Sampling time, start/end	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Reco-very [%]
1 (C)	0 h start	0.000	< LOD	-
1 (C)	12 h end	0.000	< LOD	-
1 (C)	12 h start	0.000	< LOD	-
1 (C)	24 h end	0.000	< LOD	-
1 (C)	24 h start	0.000	< LOD	-
1 (C)	36 h end	0.000	< LOD	-
1 (C)	36 h start	0.000	< LOD	-
1 (C)	48 h end	0.000	< LOD	-
1 (C)	48 h start	0.000	< LOD	-
1 (C)	60 h end	0.000	< LOD	-
1 (C)	60 h start	0.000	< LOD	-
1 (C)	72 h end	0.000	< LOD	-
1 (C)	72 h start	0.000	< LOD	-
1 (C)	84 h end	0.000	< LOD	-
1 (C)	84 h start	0.000	< LOD	-
1 (C)	96 h end	0.000	< LOD	-
2 (SC)	0 h start	0.000	< LOD	-
2 (SC)	12 h end	0.000	< LOD	-
2 (SC)	12 h start	0.000	< LOD	-
2 (SC)	24 h end	0.000	< LOD	-
2 (SC)	24 h start	0.000	< LOD	-
2 (SC)	36 h end	0.000	< LOD	-

Treatment Replicate 2	Sampling time, start/end	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Reco-very [%]
1 (C)	0 h start	0.000	< LOD	-
1 (C)	12 h end	0.000	< LOD	-
1 (C)	12 h start	0.000	< LOD	-
1 (C)	24 h end	0.000	< LOD	-
1 (C)	24 h start	0.000	< LOD	-
1 (C)	36 h end	0.000	< LOD	-
1 (C)	36 h start	0.000	< LOD	-
1 (C)	48 h end	0.000	< LOD	-
1 (C)	48 h start	0.000	< LOD	-
1 (C)	60 h end	0.000	< LOD	-
1 (C)	60 h start	0.000	< LOD	-
1 (C)	72 h end	0.000	< LOD	-
1 (C)	72 h start	0.000	< LOD	-
1 (C)	84 h end	0.000	< LOD	-
1 (C)	84 h start	0.000	< LOD	-
1 (C)	96 h end	0.000	< LOD	-
2 (SC)	0 h start	0.000	< LOD	-
2 (SC)	12 h end	0.000	< LOD	-
2 (SC)	12 h start	0.000	< LOD	-
2 (SC)	24 h end	0.000	< LOD	-
2 (SC)	24 h start	0.000	< LOD	-
2 (SC)	36 h end	0.000	< LOD	-

Treatment Replicate 1	Sampling time, start/end	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Reco-very [%]	Treatment Replicate 2	Sampling time, start/end	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Reco-very [%]
2 (SC)	36 h start	0.000	< LOD	-	2 (SC)	36 h start	0.000	< LOD	-
2 (SC)	48 h end	0.000	< LOD	-	2 (SC)	48 h end	0.000	< LOD	-
2 (SC)	48 h start	0.000	< LOD	-	2 (SC)	48 h start	0.000	< LOD	-
2 (SC)	60 h end	0.000	< LOD	-	2 (SC)	60 h end	0.000	< LOD	-
2 (SC)	60 h start	0.000	< LOD	-	2 (SC)	60 h start	0.000	< LOD	-
2 (SC)	72 h end	0.000	< LOD	-	2 (SC)	72 h end	0.000	< LOD	-
2 (SC)	72 h start	0.000	< LOD	-	2 (SC)	72 h start	0.000	< LOD	-
2 (SC)	84 h end	0.000	< LOD	-	2 (SC)	84 h end	0.000	< LOD	-
2 (SC)	84 h start	0.000	< LOD	-	2 (SC)	84 h start	0.000	< LOD	-
2 (SC)	96 h end	0.000	< LOD	-	2 (SC)	96 h end	0.000	< LOD	-
3	0 h start	0.041	< LOD	-	3	0 h start	0.888	0.858	97%
3	12 h end	0.041	< LOD	-	3	12 h end	0.888	< LOD	-
3	12 h start	0.041	< LOD	-	3	12 h start	0.888	0.848	95%
3	24 h end	0.041	< LOD	-	3	24 h end	0.888	< LOD	-
3	24 h start	0.041	< LOD	-	3	24 h start	0.888	0.833	94%
3	36 h end	0.041	< LOD	-	3	36 h end	0.888	< LOD	-
3	36 h start	0.041	< LOD	-	3	36 h start	0.888	0.793	89%
3	48 h end	0.041	< LOD	-	3	48 h end	0.888	< LOD	-
3	48 h start	0.041	< LOD	-	3	48 h start	0.888	0.800	90%
3	60 h end	0.041	< LOD	-	3	60 h end	0.888	< LOD	-
3	60 h start	0.041	< LOD	-	3	60 h start	0.888	0.731	82%
3	72 h end	0.041	< LOD	-	3	72 h end	0.888	< LOD	-
3	72 h start	0.041	< LOD	-	3	72 h start	0.888	0.713	80%
3	84 h end	0.041	< LOD	-	3	84 h end	0.888	< LOD	-
3	84 h start	0.041	< LOD	-	3	84 h start	0.888	0.734	83%
3	96 h end	0.041	< LOD	-	3	96 h end	0.888	< LOD	-
4	0 h start	0.104	< LOD	-	4	0 h start	1.332	1.077	81%
4	12 h end	0.104	< LOD	-	4	12 h end	1.332	< LOD	-
4	12 h start	0.104	< LOD	-	4	12 h start	1.332	1.211	91%
4	24 h end	0.104	< LOD	-	4	24 h end	1.332	< LOD	-
4	24 h start	0.104	< LOD	-	4	24 h start	1.332	1.195	90%
4	36 h end	0.104	< LOD	-	4	36 h end	1.332	< LOD	-
4	36 h start	0.104	< LOD	-	4	36 h start	1.332	1.223	92%
4	48 h end	0.104	< LOD	-	4	48 h end	1.332	< LOD	-
4	48 h start	0.104	< LOD	-	4	48 h start	1.332	1.330	100%
4	60 h end	0.104	< LOD	-	4	60 h end	1.332	< LOD	-
4	60 h start	0.104	< LOD	-	4	60 h start	1.332	1.239	93%
4	72 h end	0.104	< LOD	-	4	72 h end	1.332	< LOD	-
4	72 h start	0.104	< LOD	-	4	72 h start	1.332	1.135	85%
4	84 h end	0.104	< LOD	-	4	84 h end	1.332	< LOD	-
4	84 h start	0.104	< LOD	-	4	84 h start	1.332	1.228	92%
4	96 h end	0.104	< LOD	-	4	96 h end	1.332	< LOD	-
5	0 h start	0.259	0.277	107%	5	0 h start	1.998	1.923	96%
5	12 h end	0.259	< LOD	-	5	12 h end	1.998	< LOD	-
5	12 h start	0.259	0.267	103%	5	12 h start	1.998	1.886	94%
5	24 h end	0.259	< LOD	-	5	24 h end	1.998	< LOD	-
5	24 h start	0.259	0.251	97%	5	24 h start	1.998	1.895	95%
5	36 h end	0.259	< LOD	-	5	36 h end	1.998	< LOD	-
5	36 h start	0.259	0.246	95%	5	36 h start	1.998	1.773	89%
5	48 h end	0.259	< LOD	-	5	48 h end	1.998	< LOD	-
5	48 h start	0.259	0.252	97%	5	48 h start	1.998	1.951	98%
5	60 h end	0.259	< LOD	-	5	60 h end	1.998	< LOD	-
5	60 h start	0.259	0.255	98%	5	60 h start	1.998	1.828	91%
5	72 h end	0.259	< LOD	-	5	72 h end	1.998	< LOD	-
5	72 h start	0.259	0.257	99%	5	72 h start	1.998	1.941	97%
5	84 h end	0.259	< LOD	-	5	84 h end	1.998	< LOD	-
5	84 h start	0.259	0.260	100%	5	84 h start	1.998	2.014	101%
5	96 h end	0.259	< LOD	-	5	96 h end	1.998	< LOD	-
6	0 h start	0.647	0.694	107%	6	0 h start	2.997	3.111	104%
6	12 h end	0.647	< LOD	-	6	12 h end	2.997	< LOD	-
6	12 h start	0.647	0.646	100%	6	12 h start	2.997	2.789	93%
6	24 h end	0.647	< LOD	-	6	24 h end	2.997	< LOD	-
6	24 h start	0.647	0.728	113%	6	24 h start	2.997	3.289	110%
6	36 h end	0.647	< LOD	-	6	36 h end	2.997	< LOD	-

Treatment Replicate 1	Sampling time, start/end	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Reco-very [%]
6	36 h start	0.647	0.699	108%
6	48 h end	0.647	< LOD	-
6	48 h start	0.647	0.686	106%
6	60 h end	0.647	< LOD	-
6	60 h start	0.647	0.672	104%
6	72 h end	0.647	< LOD	-
6	72 h start	0.647	0.548	85%
6	84 h end	0.647	< LOD	-
6	84 h start	0.647	0.674	104%
6	96 h end	0.647	< LOD	-
7	0 h start	0.619	1.857	115%
7	12 h end	0.619	< LOD	-
7	12 h start	0.619	1.869	115%
7	24 h end	0.619	< LOD	-
7	24 h start	0.619	1.687	104%
7	36 h end	0.619	< LOD	-
7	36 h start	0.619	1.831	113%
7	48 h end	0.619	< LOD	-
7	48 h start	0.619	1.900	113%
7	60 h end	0.619	< LOD	-
7	60 h start	0.619	1.902	113%
7	72 h end	0.619	< LOD	-
7	72 h start	0.619	2.000	119%
7	84 h end	0.619	< LOD	-
7	84 h start	0.619	1.925	115%
7	96 h end	0.619	< LOD	-
8	0 h start	4.047	4.103	101%
8	12 h end	4.047	< LOD	-
8	12 h start	4.047	4.598	114%
8	24 h end	4.047	< LOD	-
8	24 h start	4.047	4.243	105%
8	36 h end	4.047	< LOD	-
8	36 h start	4.047	4.080	101%
8	48 h end	4.047	< LOD	-
8	48 h start	4.047	3.993	99%
8	60 h end	4.047	< LOD	-
8	60 h start	4.047	3.915	97%
8	72 h end	4.047	< LOD	-
8	72 h start	4.047	4.071	101%
8	84 h end	4.047	< LOD	-
8	84 h start	4.047	3.859	95%
8	96 h end	4.047	< LOD	-
9	0 h start	10.12	1.173	116%
9	12 h end	10.12	< LOD	-

Treatment Replicate 2	Sampling time, start/end	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Reco-very [%]
6	36 h start	2.997	2.771	92%
6	48 h end	2.997	< LOD	-
6	48 h start	2.997	2.607	87%
6	60 h end	2.997	< LOD	-
6	60 h start	2.997	2.939	98%
6	72 h end	2.997	< LOD	-
6	72 h start	2.997	2.699	90%
6	84 h end	2.997	< LOD	-
6	84 h start	2.997	2.767	92%
6	96 h end	2.997	< LOD	-
7	0 h start	4.496	4.230	94%
7	12 h end	4.496	< LOD	-
7	12 h start	4.496	3.835	85%
7	24 h end	4.496	< LOD	-
7	24 h start	4.496	4.275	95%
7	36 h end	4.496	< LOD	-
7	36 h start	4.496	4.206	94%
7	48 h end	4.496	< LOD	-
8	0 h start	6.774	7.488	111%
8	12 h end	6.774	< LOD	-
8	12 h start	6.774	6.479	96%
8	24 h end	6.774	< LOD	-
9	0 h start	10.12	9.928	98%
9	12 h end	10.12	< LOD	-
9	12 h start	10.12	10.02	99%
9	24 h end	10.12	< LOD	-

Sample analytics of Pyraclostrobin (Replicate 1)

Treatment; sampling time Replicate 1	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Geometric mean [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	<LOD	<LOD	<LOD
1 (C); test end	0.000	<LOD		<LOD
1 (SC); test start	0.000	<LOD	<LOD	<LOD
1 (SC); test end	0.000	<LOD		<LOD
3; test start	0.040	0.020	0.018	49%
3; test end	0.040	0.016		40%
4; test start	0.081	0.037	0.033	45%
4; test end	0.081	0.029		36%
5; test start	0.161	0.091	0.082	56%
6; test end	0.161	0.073		46%
6; test start	0.322	0.179	0.178	56%
7; test end	0.322	0.178		55%
7; test start	0.645	0.393	0.367	61%
8; test end	0.645	0.342		53%
8; test start	1.289	0.829	0.821	64%
5; test end	1.289	0.813		63%
9; test start	2.578	1.879	1.835	73%
9; test end	2.578	1.791		69%

Treatment; sampling time Replicate 2	Nominal conc. [µmol/l]	Analyzed conc. [µmol/l]	Geometric mean [µmol/l]	Recovery [% of nominal]
1 (C); test start	0.000	<LOD	<LOD	<LOD
1 (C); test end	0.000	<LOD		<LOD
1 (SC); test start	0.000	<LOD	<LOD	<LOD
1 (SC); test end	0.000	<LOD		<LOD
3; test start	0.068	0.029	0.027	42%
3; test end	0.068	0.025		37%
4; test start	0.102	0.046	0.042	45%
4; test end	0.102	0.038		37%
5; test start	0.153	0.071	0.067	46%
6; test end	0.153	0.063		41%
6; test start	0.230	0.112	0.106	49%
7; test end	0.230	0.100		44%
7; test start	0.345	0.176	0.172	51%
8; test end	0.345	0.167		48%
8; test start	0.517	0.284	0.278	55%
5; test end	0.517	0.272		53%
9; test start	0.775	0.449	0.453	58%
9; test end	0.775	0.456		59%

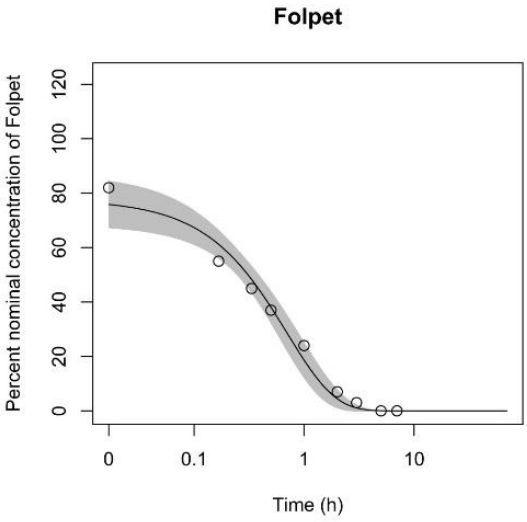
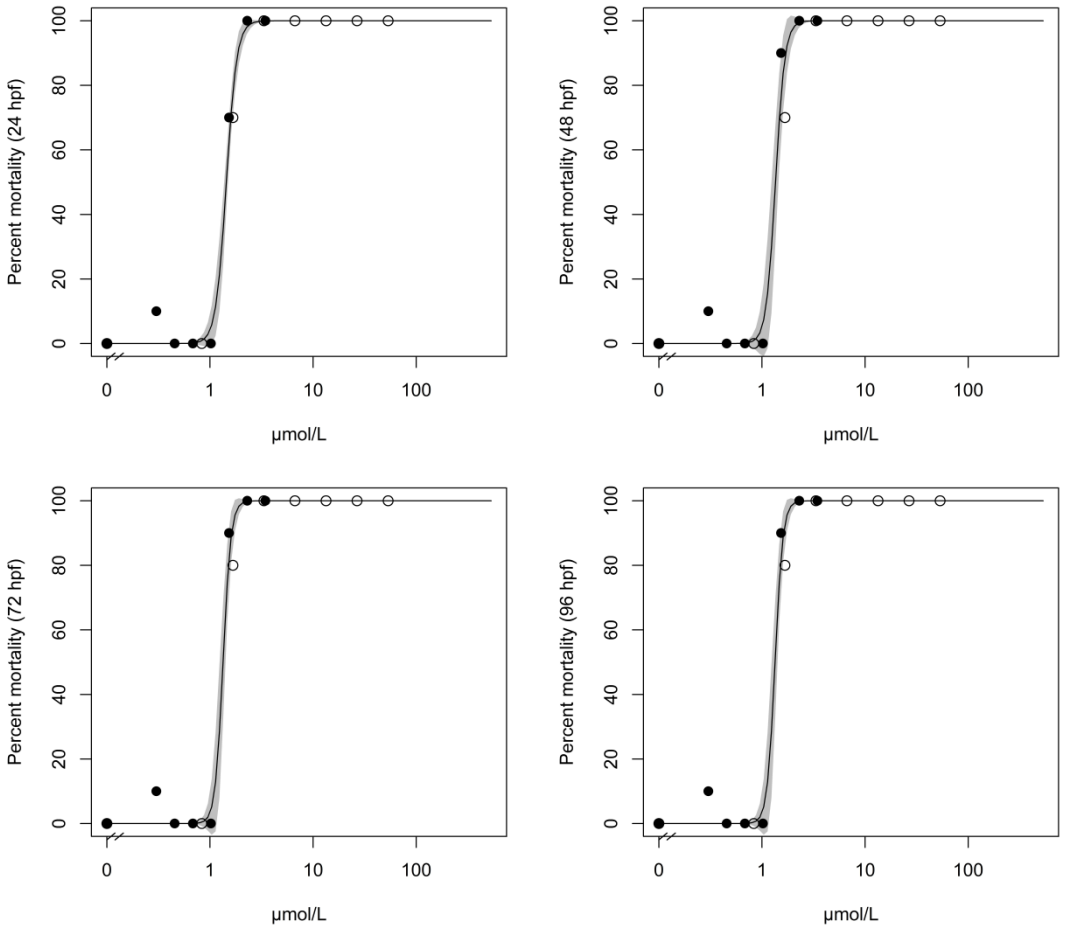


Fig. S1: The stability of Folpet in exposure medium
The concentration of folpet in aqueous exposure medium was monitored for a concentration of 10.12 µM over a period of 7 h. The decline in concentration was modelled using an exponential decay function (concentration = $100*e^{-t/a}$). Given that the initial concentration may already deviate from the nominal concentration, it was not set to 100 but also estimated using the exponential decay model.

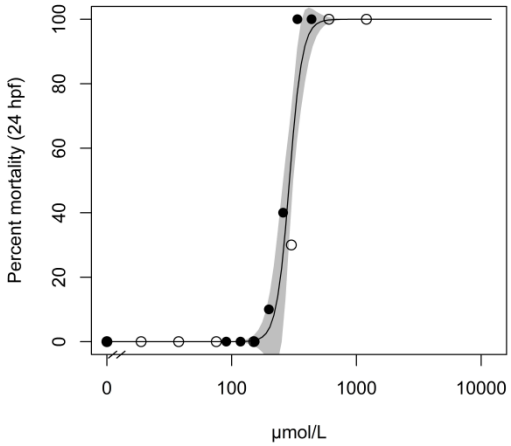
Fig. S2: Concentration-response curves and raw data of mortality analysis for all time points (24, 48, 72, and 96 hpf)
Different symbols in concentration-response plot refer to independent replicates.

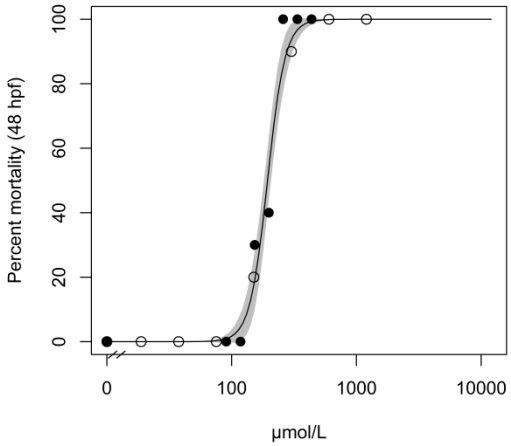
3-Iodo-2-propynyl-N-butylcarbamate

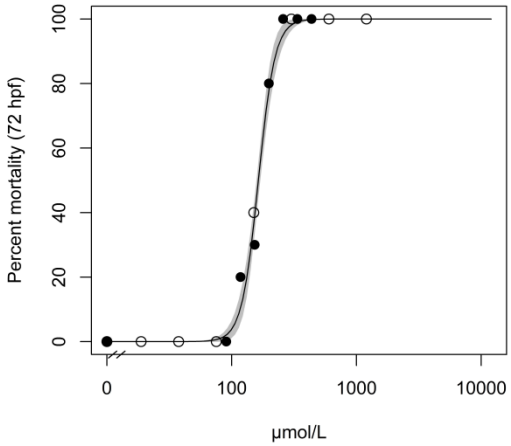


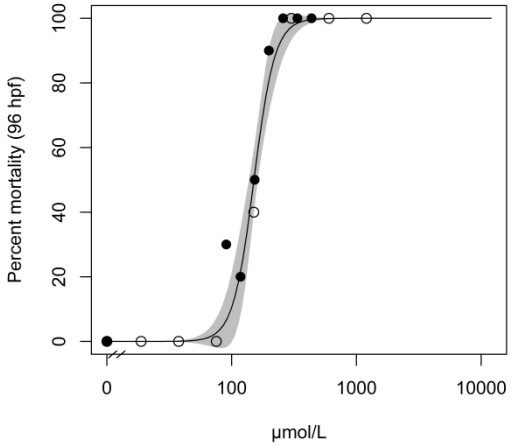
Concentration [µmol/l]	Replicate	Mortality [%]			
		24 hpf	48 hpf	72 hpf	96 hpf
0.000	1	0	0	0	0
0.000	2	0	0	0	0
0.302	2	10 (10)	10 (10)	10 (10)	10 (10)
0.455	2	0	0	0	0
0.683	2	0	0	0	0
0.832	1	0	0	0	0
1.021	2	0	0	0	0
1.533	2	70 (70)	90 (90)	90 (90)	90 (90)
1.668	1	70 (70)	70 (70)	80 (70)	80 (70)
2.298	2	100 (100)	100 (100) (100) (100) (100) (100)	100 (100)	100 (100)
3.333	1	100 (100)	100 (100)	100 (100)	100 (100)
3.447	2	100 (100)	100 (100)	100 (100)	100 (100)
6.670	1	100 (100)	100 (100)	100 (100)	100 (100)
13.34	1	100 (100)	100 (100)	100 (100)	100 (100)
26.68	1	100 (100)	100 (100)	100 (100)	100 (100)
53.36	1	100 (100)	100 (100)	100 (100)	100(100)

4-Chloroaniline



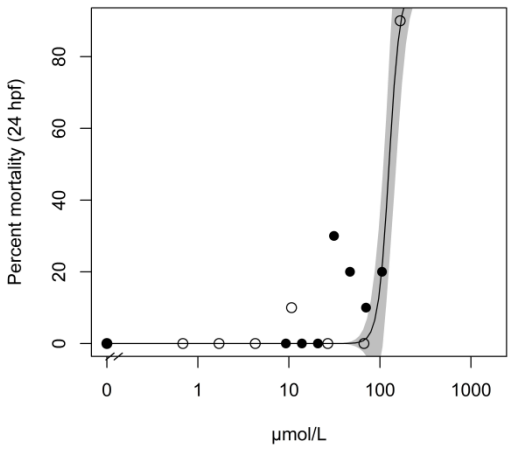


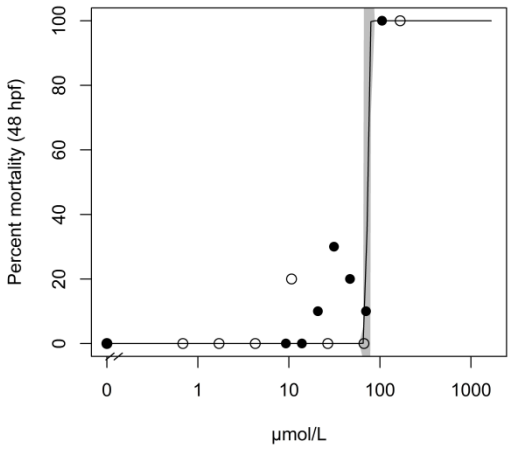


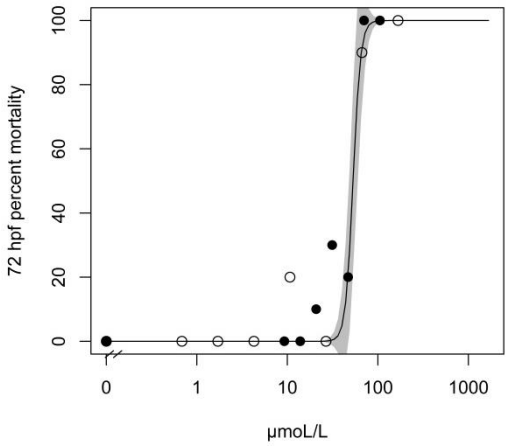


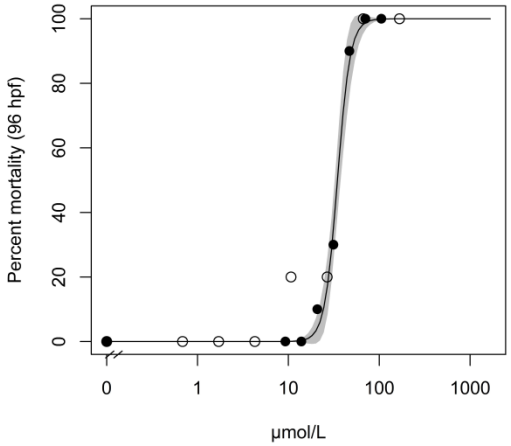
Concentration [µmol/l]	Replicate	Mortality [%]			
		24 hpf	48 hpf	72 hpf	96 hpf
0.000	1	0	0	0	0
0.000	2	0	0	0	0
18.83	1	0	0	0	0
37.65	1	0	0	0	0
75.30	1	0	0	0	0
90.68	2	0	0	0	30 (0)
117.9	2	0	0	20 (0)	20 (0)
150.6	1	0	20 (0)	40 (0)	40 (0)
153.2	2	0	30 (30)	30 (30)	50 (30)
199.2	2	10 (10)	40 (40)	80 (50)	90 (50)
259.0	2	40 (40)	100 (90)	100 (90)	100 (90)
301.2	1	30 (30)	90 (30)	100 (90)	100 (90)
336.7	2	100 (100)	100 (100)	100 (100)	100 (100)
437.7	2	100 (100)	100 (100)	100 (100)	100 (100)
602.4	1	100 (100)	100 (100)	100 (100)	100 (100)
1205	1	100 (100)	100 (100)	100 (100)	100 (100)

Acetochlor



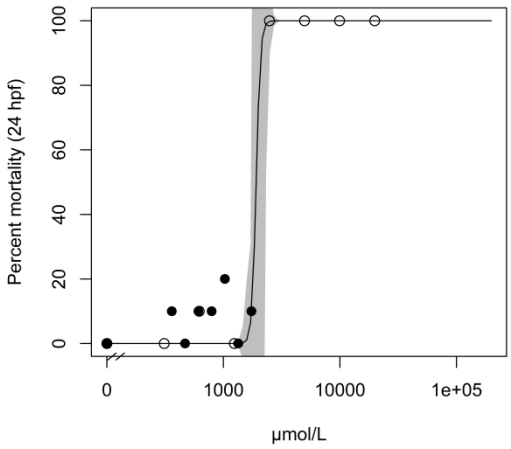


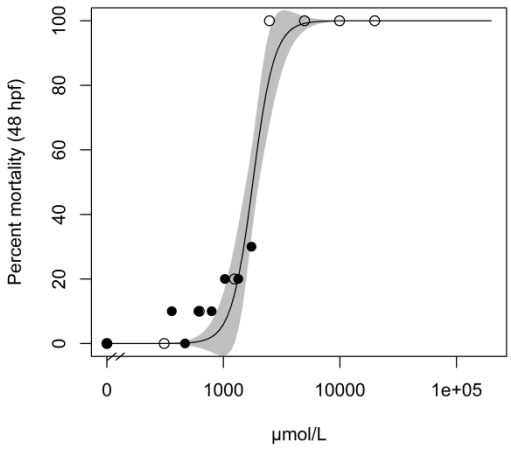


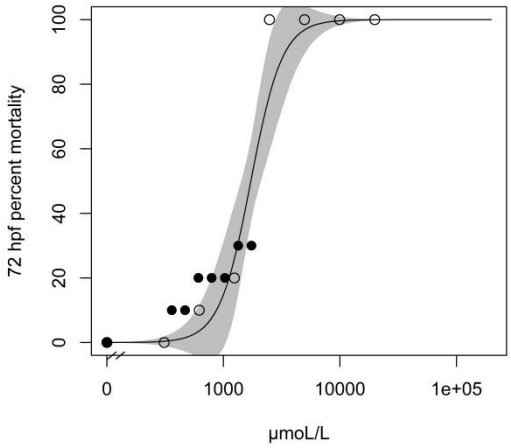


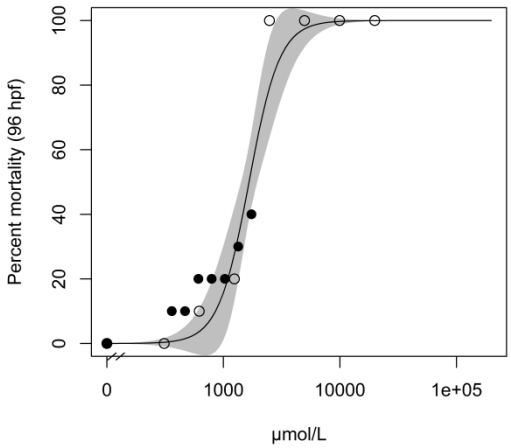
Concentration [µmol/l]	Replicate	Mortality [%]			
		24 hpf	48 hpf	72 hpf	96 hpf
0.000	1	0	0	0	0
0.000	2	0	0	0	0
0.684	1	0	0	0	0
1.710	1	0	0	0	0
4.275	1	0	0	0	0
9.273	2	0	0	0	0
10.69	1	10 (10)	20 (10)	20 (20)	20 (20)
13.91	2	0	0	0	0
20.86	2	0	10 (10)	10 (10)	10 (10)
26.72	1	0	0	0	20 (0)
31.30	2	30 (30)	30 (30)	30 (30)	30 (30)
46.94	2	20 (20)	20 (20)	20 (20)	90 (20)
66.81	1	0	0	90 (0)	100 (0)
70.41	2	10 (10)	10 (10)	100 (10)	100 (10)
105.6	2	20 (20)	100 (20)	100 (20)	100 (20)
167.0	1	90 (10)	100 (10)	100 (10)	100 (10)

Aniline



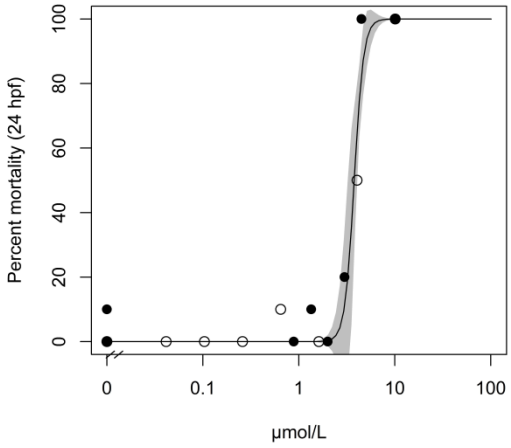


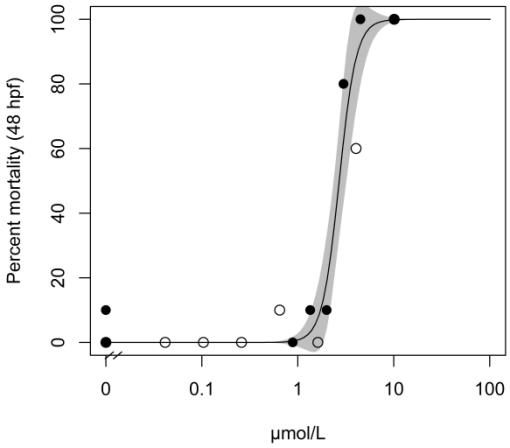


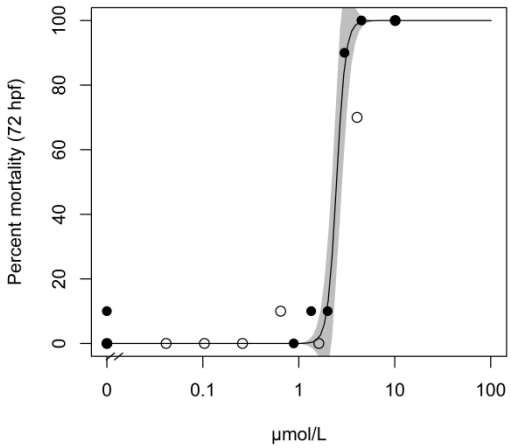


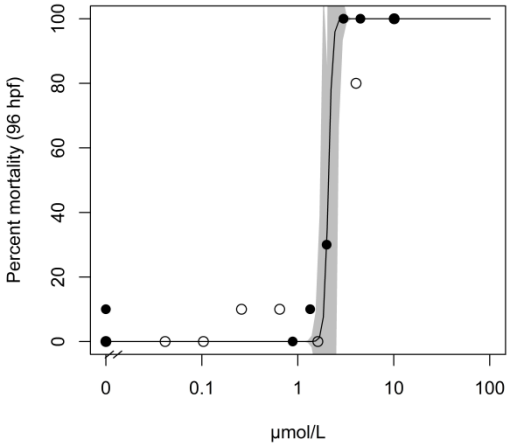
Concentration [µmol/l]	Replicate	Mortality [%]			
		24 hpf	48 hpf	72 hpf	96 hpf
0.000	1	0	0	0	0
0.000	2	0	0	0	0
310.4	1	0	0	0	0
361.1	2	10 (10)	10 (10)	10 (10)	10 (10)
469.5	2	0	0	10 (0)	10 (0)
610.2	2	10 (10)	10 (10)	20 (10)	20 (10)
621.0	1	10 (10)	10 (10)	10 (10)	10 (10)
793.3	2	10 (10)	10 (10)	20 (10)	20 (10)
1031	2	20 (20)	20 (20)	20 (20)	20 (20)
1242	1	0	20 (0)	20 (0)	20 (0)
1341	2	0	20 (0)	30 (0)	30 (0)
1743	2	10 (10)	30 (20)	30 (20)	40 (20)
2484	1	100 (100)	100 (100)	100 (100)	100 (100)
4967	1	100 (100)	100 (100)	100 (100)	100 (100)
9935	1	100 (100)	100 (100)	100 (100)	100 (100)
19,865	1	100 (100)	100 (100)	100 (100)	100 (100)

Folpet



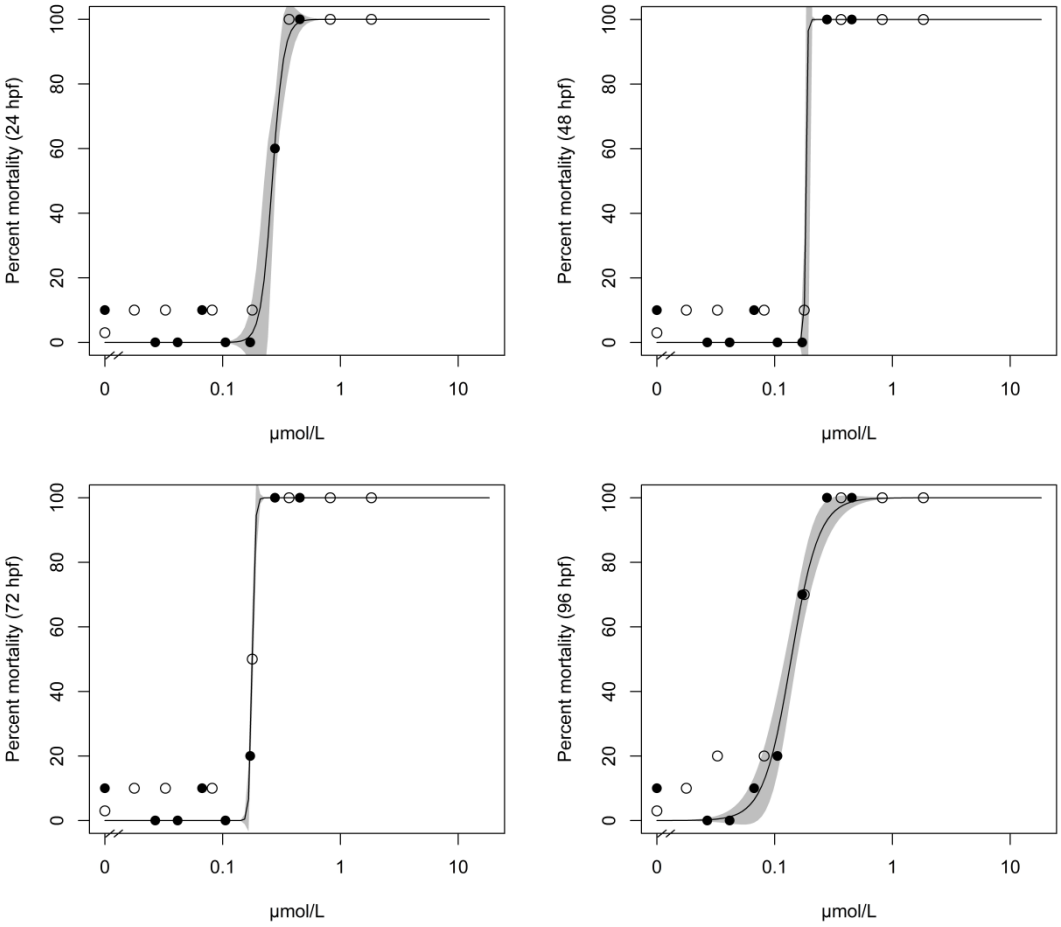






Concentration [µmol/l]	Replicate	Mortality [%]			
		24 hpf	48 hpf	72 hpf	96 hpf
0.000	1	0	0	0	0
0.000	1	0	0	0	0
0.000	2	0	0	0	0
0.000	2	10 (10)	10 (10)	10 (10)	10 (10)
0.041	1	0	0	0	0
0.104	1	0	0	0	0
0.259	1	0	0	0	10 (0)
0.647	1	10 (10)	10 (10)	10 (10)	10 (10)
0.887	2	0	0	0	0
1.349	2	10 (10)	10 (10)	10 (10)	10 (10)
1.619	1	0	0	0	0
2.000	2	0	10 (10)	10 (10)	30 (10)
2.998	2	20 (20)	80 (80)	90 (80)	100 (80)
4.047	1	50 (50)	60 (60)	70 (70)	80 (70)
4.495	2	100 (100)	100 (100)	100 (100)	100 (100)
10.12	1	100 (100)	100 (100)	100 (100)	100 (100)
10.12	2	100 (100)	100 (100)	100 (100)	100 (100)

Pyraclostrobin



Concentration [µmol/l]	Replicate	Mortality [%]			
		24 hpf	48 hpf	72 hpf	96 hpf
0.000	1	3 (3)	3 (3)	3 (3)	3 (3)
0.000	2	10 (10)	10 (10)	10 (10)	10 (10)
0.000	1	10 (10)	10 (10)	10 (10)	10 (10)
0.000	2	10 (10)	10 (10)	10 (10)	10 (10)
0.018	1	10 (10)	10 (10)	10 (10)	10 (10)
0.027	2	0	0	0	0
0.033	1	10 (10)	10 (10)	10 (10)	20 (10)
0.042	2	0	0	0	0
0.067	2	10 (10)	10 (10)	10 (10)	10 (10)
0.081	1	10 (10)	10 (10)	10 (10)	20 (10)
0.106	2	0	0	0	20 (10)
0.178	1	10 (10)	10 (10)	50 (30)	70 (30)
0.172	2	0	0	20 (0)	70 (0)
0.278	2	60 (60)	100 (60)	100 (70)	100 (70)
0.366	1	100 (20)	100 (50)	100 (50)	100 (50)
0.453	2	100 (10)	100 (10)	100 (60)	100 (60)
0.821	1	100 (100)	100 (100)	100 (100)	100 (100)
1.835	1	100 (100)	100 (100)	100 (100)	100 (100)

References

Berthold, M. R., Cebron, N., Dill, F. et al. (2008). Knime: The Konstanz Information Miner. In C. Preisach, H. Burkhardt, L. Schmidt-Thieme and R. Decker (eds.), *Data Analysis, Machine Learning and Applications: Proceedings of the 31st Annual Conference of the Gesellschaft für Klassifikation e.V., Albert-Ludwigs-Universität Freiburg, March 7-9, 2007*. Berlin, Heidelberg, Germany: Springer. doi:10.1007/978-3-540-78246-9_38

Fetter, E., Baldauf, L., Fonte, D. F. et al. (2015). Comparative analysis of goitrogenic effects of phenylthiourea and methimazole in zebrafish embryos. *Reprod Toxicol 57*, 10-20. doi:10.1016/j.reprotox.2015.04.012

Klüver, N., Vogs, C., Altenburger, R. et al. (2016). Development of a general baseline toxicity QSAR model for the fish embryo acute toxicity test. *Chemosphere 164*, 164-173. doi:10.1016/j.chemosphere.2016.08.079

OECD (2013). Test No. 236: Fish Embryo Acute Toxicity (FET) Test. *OECD Guidelines for the Testing of Chemicals, Section 2*. OECD Publishing, Paris. doi:10.1787/9789264203709-en

Padilla, S., Corum, D., Padnos, B. et al. (2012). Zebrafish developmental screening of the ToxCast™ Phase I chemical library. *Reprod Toxicol 33*, 174-187. doi:10.1016/j.reprotox.2011.10.018

R Core Team (2015). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2013. http://www.r-project.org

Sobanska, M., Scholz, S., Nyman, A.-M. et al. (2018). Applicability of the fish embryo acute toxicity (FET) test (OECD 236) in the regulatory context of REACH. *Environ Toxicol Chem 37*, 657-670. doi:10.1002/etc.4055

Truong, L., Reif, D. M., St Mary, L. et al. (2014). Multidimensional in vivo hazard assessment using zebrafish. *Toxicol Sci 137*, 212-233. doi:10.1093/toxsci/kft235