

## Supplementary Material 2

### Tables and Figures – FACETS

**Fathead minnow exposed to environmentally relevant concentrations of metformin for one life cycle show no adverse effects.**

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Table S1. Liquid chromatography gradient conditions for metformin and guanylyurea analysis in exposure tanks during the fathead minnow life cycle test.

TIME (min)	FLOW (mL/min)	% Water	% Methanol
Metformin			
Initial	0.15	90	10
5	0.15	90	10
12	0.15	0	100
15	0.15	0	100
20	0.15	90	10
35	0.15	90	10
Guanylyurea			
Initial	0.15	90	10
1.2	0.15	0	100
5	0.15	0	100
7	0.15	90	10
20	0.15	90	10

Table S2. The precursor to product transitions monitored for native and labelled metformin and guanylurea. Two ions were chosen so that a ratio between the two could be used as a further confirmation of compound identity.

Electrospray Conditions					
Collision Gas Flow (mL/min)		0.15			
Desolvation Temperature		300 °C			
Gas Flow: Desolvation (L/Hr)		800			
Cone (L/Hr)		150			
Nebuliser(Bar)		7			
Source Voltages: Capillary (KV)		0.94			
Cone (V)		0			
Source Offset (V)		50			
Source Temperature		150 °C			
MS/MS Transitions					
Compound	Precursor (m/z)	Product (m/z)	Dwell Time (sec)	Cone (V)	Collision Energy (V)
Metformin	130.1	60.2	0.25	15	18
Metformin	130.1	71.2	0.25	15	20
D6 Metformin	136.3	77.2	0.25	16	19
D6 Metformin	136.3	119.3	0.25	16	14
Guanylurea	102.87	43.1	0.25	47	14
Guanylurea	102.87	60.5	0.25	38	14
Guanylurea	102.87	86.5	0.25	53	14
<sup>15</sup> N <sub>4</sub> Guanylurea	107.53	63.4	0.25	40	11
<sup>15</sup> N <sub>4</sub> Guanylurea	107.53	89.4	0.25	40	11

Table S3. Feeding chart of fathead minnows over time during the life cycle exposure to metformin.  
Abbreviations: dph = days post-hatch

<b>Age of fish (dph)</b>	<b>24 h-old Newly hatched brine shrimp (<math>\mu\text{L}/\text{fish}/\text{day}</math>)</b>	<b>48 h -old Newly hatched brine shrimp (<math>\mu\text{L}/\text{fish}/\text{day}</math>)</b>	<b>Volume for re-suspending newly hatched brine shrimp (mL)</b>	<b>Frozen brine shrimp (thawed) (<math>\mu\text{L}/\text{fish}/\text{day}</math>)</b>	<b>Milestones</b>
1-9	10	-	20	-	
9-16	60	-	20	-	9 - 17 dph; release from egg cups
16-22	-	120	50	-	
22-30	-	180	50	-	
30-35	-	120	50	100	30 dph; cull to 20 fish per tank
35-45	-	60	50	120	
45-50	-	50	50	150	
50-65	-	50	50	200	60 dph; cull to 15
65-72	-	-	-	300	
72-84	-	-	-	500	75 dph - cull to 12 - add breeding tiles
84-110	-	-	-	600	90 dph; cull and sexing, depending on development
110+	-	-	-	700	

Table S4. Water quality data in exposure aquaria during the life cycle exposure of fathead minnows to metformin.

Tank #	Metformin (µg/L)	n	Temperature (°C)	Conductivity (µS/cm)	pH	Dissolved oxygen (mg/L)	Free ammonia (mg/L)
1	Controls	25	24.9	334.9	8.20	7.92	0.00040
3	Controls	25	24.9	335.0	8.17	7.84	0.00000
7	Controls	25	25.0	334.8	8.15	7.80	0.00000
10	Controls	25	25.0	335.0	8.16	7.80	0.00040
11	Controls	25	25.0	335.6	8.18	7.84	0.00000
13	Controls	25	25.0	334.8	8.20	7.86	0.00000
16	Controls	25	25.0	335.2	8.22	7.88	0.00000
19	Controls	25	25.0	335.3	8.22	7.88	0.00000
4	3.0	25	25.0	335.3	8.15	7.80	0.00000
9	3.0	25	25.0	334.3	8.23	7.89	0.00000
15	3.0	25	25.0	335.7	8.18	7.83	0.00208
17	3.0	25	25.1	335.3	8.21	7.87	0.00000
5	31	25	25.0	335.4	8.15	7.78	0.00042
8	31	25	25.0	335.5	8.21	7.86	0.00083
12	31	25	25.0	335.7	8.20	7.87	0.00042
20	31	25	25.1	335.3	8.18	7.82	0.00042
2	322	25	25.0	335.6	8.19	7.84	0.00000
6	322	25	25.0	336.0	8.15	7.79	0.00042
14	322	25	25.0	335.3	8.18	7.81	0.00042
18	322	25	25.0	335.2	8.23	7.87	0.00250
	max		25.1	336.0	8.23	7.92	0.00250
	min		24.9	334.3	8.15	7.78	0.00000
	mean		25.0	335.3	8.19	7.84	0.00042
	s.d.		0.1	0.4	0.03	0.04	0.00069

Table S5. Water quality data in incoming dilution water to the aquatic life research facility during the life cycle exposure of fathead minnows to metformin. Each parameter was measured 8 times, so the means and standard deviation (s.d.) are calculated from 8 measurements.

Analyte	Units	Mean	s.d.
Alkalinity, Total (CaCO <sub>3</sub> )	mg/L	91.1	3.8
Ammonia as N	mg/L	0.006	0.002
Calcium	mg/L	36.6	1.4
Carbon, Dissolved Inorganic	mg/L	21.9	0.8
Carbon, Dissolved Organic	mg/L	1.10	0.23
Carbon, Particulate Organic	mg/L	0.11	0.41
Chloride	mg/L	34.0	4.0
Fluoride	mg/L	0.65	0.03
Hardness	mg/L	128.7	5.1
Magnesium	mg/L	8.98	0.32
Nitrate/Nitrite as N	mg/L	0.500	0.163
Nitrogen, Particulate Organic	mg/L	0.022	0.057
pH	pH	7.69	0.22
Phosphorus, Total	mg/L	0.0013	0.0015
Potassium	mg/L	1.77	0.08
Silica	mg/L	1.25	0.29
Sodium	mg/L	18.1	2.1
Specific conductance (25°C)	µS/cm	347.3	20.0
Sulfate	mg/L	27.0	0.9

Table S6. Raw data for metformin concentrations ( $\mu\text{g/L}$ ) measured every 2 weeks in exposure water during the life cycle exposure of fathead minnows to metformin. nd = not detected (so assigned a value of 0.004423  $\mu\text{g/L}$ ) \*\*duplicate sample

dph	-4	-4	9	23	37	51	65	79	93	107	121	135	149
tank		**											
<b>Controls</b>				all nd	all nd	all nd	all nd	all nd	all nd	all nd	all nd	all nd	all nd
1	0.004423	0.004423	0.025	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
3	0.004423	0.004423	0.027	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
7	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
10	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
11	0.083	0.095	0.042	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
13	0.004423	0.004423	0.024	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
16	0.035	0.033	0.028	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
19	0.0022116	0.004423	0.042	spilled	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423	0.004423
<b>3.0 <math>\mu\text{g/L}</math> Metformin</b>	-4	9	23	37	51	65	79	93	107	121	135	149	
4	2.25	2.22	2.4	2.38	2.22	2.62	3.59	2.71	2.88	2.41	2.36	1.61	2.76
9	2.66	2.61	2.73	4.34	2.78	2.78	3.15	3.02	4.16	2.03	2.25	2.21	2.1
15	3.06	2.95	3.1	3.31	2.81	3.54	3.61	3.57	3.93	3.26	3.56	3.8	4.07
17	2.86	2.87	3.13	3.51	2.99	3.4	3.68	3.37	4.08	2.87	3.41	3.56	3.17
<b>30 <math>\mu\text{g/L}</math> Metformin</b>	-4	9	23	37	51	65	79	93	107	121	135	149	
5	30.6	26.1	30.6	44.6	37.8	30.3	37.7	36.4	34.4	28.6	27.7	38.8	32
8	31.9	26.5	28.5	38.2	31.5	27.9	39.9	25.2	30	28	18.1	30	30.5
12	27.6	25.3	26.6	29.8	33.9	26.2	32.8	30.1	31.4	25.8	38.1	31.4	32.1
20	32.5	28.3	27.6	38.9	30.6	28.9	31.2	26.7	23.2	26.3	33.6	31.4	30.9
<b>300 <math>\mu\text{g/L}</math> Metformin</b>	-4	9	23	37	51	65	79	93	107	121	135	149	
2	276	275	309	390	306	302	335	349	303	307	322	312	329
6	262	267	276	317	308	285	304	372	365	296	278	323	293
14	281	280	331	370	399	334	435	379	400	408	410	412	305
18	280	280	266	313	319	287	393	304	295	284	293	328	312

Table S7a. Statistical analysis of survival over time data with the two tanks included (tank 9 and 19) where dripping water killed some fish. ANOVA p values assessing differences in survival over time in each treatment during the life cycle exposure of fathead minnows to metformin.

Time point (dph = days post-hatch)	ANOVA p value	controls		3 µg/L metformin		31 µg/L metformin		322 µg/L metformin	
		Mean survival (%)	standard deviation	Mean survival (%)	standard deviation	Mean survival (%)	standard deviation	Mean survival (%)	standard deviation
Hatch (0 dph)	0.910	94.6	2.5	94.2	4.1	95.8	4.2	95.0	3.3
9 dph	0.808	90.4	10.3	91.8	3.1	95.0	3.3	91.7	6.4
16 dph	0.711	90.4	10.3	90.1	4.5	95.0	3.3	93.3	3.8
30 dph	0.725	88.3	9.1	89.3	4.9	93.3	2.7	90.8	7.9
45 dph	0.292	88.3	9.1	76.4	23.3	92.2	4.7	90.8	7.9
64 dph	0.238	87.8	8.9	75.4	22.8	92.2	4.7	89.6	7.0
78 dph	0.238	87.8	8.9	75.4	22.8	92.2	4.7	89.6	7.0
92 dph	0.238	87.8	8.9	75.4	22.8	92.2	4.7	89.6	7.0
106 dph	0.143	87.8	8.9	73.3	22.2	92.2	4.7	89.6	7.0
120 dph	0.143	87.8	8.9	73.3	22.2	92.2	4.7	89.6	7.0
141 dph	0.143	87.8	8.9	73.3	22.2	92.2	4.7	89.6	7.0
End of test	0.311	86.3	8.8	73.3	22.2	92.2	4.7	89.6	7.0





Table S8. Raw data for growth of randomly-culled fish over time in each tank during the life cycle exposure of fathead minnows to metformin.

Metformin concn (µg/L)	Tank	n	Weight (mg)	s.d.	Total Length (mm)	s.d.	Tail Length (mm)	s.d.	Condition Factor	s.d.
30 dph										
Controls	1	8	73.1	27.7	21.1	2.7	4.0	0.7	0.745	0.055
Controls	3	7	68.7	29.6	20.4	2.8	3.9	0.6	0.757	0.084
Controls	7	7	63.6	28.0	19.6	3.1	3.6	0.6	0.788	0.090
Controls	10	4	48.3	21.0	18.6	2.5	3.3	0.9	0.712	0.042
Controls	11	8	63.6	29.7	20.0	3.4	3.6	0.7	0.706	0.113
Controls	13	6	46.9	26.6	18.2	3.1	3.3	1.0	0.698	0.116
Controls	16	7	54.9	11.1	19.5	1.2	3.6	0.5	0.732	0.033
Controls	19	0								
3.0	4	8	70.1	18.0	21.0	1.6	3.9	0.6	0.743	0.044
3.0	9	8	70.5	35.9	20.4	3.5	3.6	0.7	0.766	0.084
3.0	15	5	70.9	20.8	21.2	1.8	4.0	0.7	0.725	0.039
3.0	17	4	39.6	30.3	17.5	3.3	3.0	0.8	0.625	0.167
31	5	9	64.0	20.3	20.7	2.0	3.7	0.4	0.701	0.048
31	8	8	59.0	13.9	20.4	1.1	3.6	0.4	0.677	0.081
31	12	7	75.8	19.4	21.6	1.8	3.7	0.3	0.733	0.038
31	20	6	90.8	23.5	22.8	1.6	4.2	0.3	0.747	0.068
322	2	8	62.1	23.9	20.1	2.2	3.7	0.6	0.734	0.047
322	6	9	58.9	25.7	19.9	3.0	3.8	0.6	0.685	0.108
322	14	4	63.1	23.8	20.3	2.4	3.4	0.6	0.725	0.055
322	18	6	56.4	39.5	19.0	3.8	3.3	0.8	0.682	0.150
60 dph										
Controls	1	4	425.1	99.7	36.9	2.6	7.3	0.6	0.837	0.048
Controls	3	4	561.8	206.8	38.9	3.8	7.5	0.6	0.916	0.108
Controls	7	5	378.5	121.8	35.3	3.6	7.0	0.9	0.827	0.083
Controls	10	5	399.1	157.4	35.3	4.9	6.3	1.1	0.869	0.053
Controls	11	5	437.2	48.5	36.8	1.5	6.6	0.7	0.875	0.022
Controls	13	7	398.9	165.3	35.6	3.9	6.6	0.8	0.835	0.072
Controls	16	5	325.0	136.6	33.0	3.6	6.3	0.7	0.857	0.092
Controls	19	5	464.3	117.9	37.1	3.2	7.2	0.8	0.896	0.079
3.0	4	4	400.0	146.4	35.3	3.3	6.8	0.5	0.880	0.091
3.0	9	0								
3.0	15	4	341.1	183.6	32.5	5.2	6.6	1.1	0.926	0.084
3.0	17	5	522.4	108.1	38.0	2.2	7.2	0.6	0.941	0.054
31	5	5	340.7	169.5	32.9	6.2	6.2	0.9	0.892	0.095

31	8	5,4,4,4	490.4	174.1	35.8	3.9	6.5	0.4	0.926	0.049
31	12	4	425.3	81.3	37.0	2.9	7.4	0.5	0.834	0.040
31	20	3	487.8	125.2	38.2	3.7	7.2	0.3	0.866	0.065
322	2	5	483.1	173.3	37.3	4.4	7.3	0.8	0.884	0.097
322	6	4	500.8	78.2	37.6	1.9	7.3	0.9	0.936	0.066
322	14	4	445.3	78.5	36.8	2.1	6.9	0.3	0.890	0.042
322	18	5	434.5	139.6	35.8	3.8	6.2	0.8	0.913	0.089
78 dph										
Controls	1	3	746.5	56.3	42.7	0.6	7.5	0.5	0.960	0.035
Controls	3	3	758.9	78.7	43.3	0.6	7.8	0.8	0.931	0.068
Controls	7	3	554.2	263.5	40.0	5.6	7.8	1.0	0.819	0.061
Controls	10	3	637.6	156.1	41.3	3.2	7.8	0.8	0.892	0.046
Controls	11	3	585.5	188.2	40.0	4.0	7.5	0.5	0.889	0.048
Controls	13	3	682.5	252.7	41.2	4.9	7.5	1.0	0.948	0.061
Controls	16	3	453.3	194.1	37.0	4.9	6.3	1.3	0.847	0.079
Controls	19	3	513.1	199.3	38.0	4.6	6.8	0.8	0.898	0.049
3.0	4	3	663.3	45.1	39.8	0.3	7.0	0.5	1.049	0.049
3.0	9	0								
3.0	15	3	605.4	43.5	40.2	1.3	7.7	0.3	0.936	0.077
3.0	17	3	723.2	271.1	41.7	5.5	7.3	1.5	0.966	0.098
31	5	2	526.7	115.2	40.0	2.8	7.8	0.4	0.816	0.007
31	8	3	714.2	117.7	43.0	2.0	8.0	0.5	0.893	0.046
31	12	3	614.5	166.5	40.7	2.5	7.3	0.6	0.896	0.088
31	20	3	585.9	124.7	38.7	2.1	6.7	0.3	1.004	0.103
322	2	3	783.8	125.2	43.2	2.0	7.3	0.6	0.969	0.019
322	6	3	592.6	123.6	38.8	3.2	6.5	0.5	1.009	0.127
322	14	3	557.4	97.7	39.3	2.3	7.3	0.8	0.912	0.069
322	18	3	605.7	230.1	40.0	4.4	7.5	0.5	0.910	0.098

Table S9. Raw data for percent mature fish over time in each tank during the life cycle exposure of fathead minnows to metformin. Fish were assessed every 2-3 weeks at the indicated day post-hatch (dph). Mean number of females per tank over time is shown in the last column.

		day post-hatch (dph)			
Tank	Metformin (µg/L)	64	64 (after cull)	78	78 (after cull)
1	Controls	0.0	0.0	13.3	16.7
3	Controls	5.3	6.7	13.3	16.7
7	Controls	0.0	0.0	26.7	33.3
10	Controls	5.0	6.7	13.3	16.7
11	Controls	0.0	0.0	6.7	8.3
13	Controls	4.5	6.7	6.7	8.3
16	Controls	0.0	0.0	0.0	0.0
19	Controls	0.0	0.0	6.7	8.3
4	3.0	5.0	6.7	13.3	16.7
9	3.0	0.0	0.0	12.5	12.5
15	3.0	0.0	0.0	46.7	58.3
17	3.0	5.0	6.7	20.0	25.0
5	31	0.0	0.0	7.1	8.3
8	31	0.0	0.0	13.3	16.7
12	31	0.0	0.0	6.7	8.3
20	31	0.0	0.0	13.3	16.7
2	322	5.0	6.7	0.0	0.0
6	322	5.3	6.7	6.7	8.3
14	322	5.0	6.7	13.3	16.7
18	322	5.0	6.7	13.3	16.7
		day post-hatch (dph)			
Tank	Metformin (µg/L)	92	92 (after cull)	106	106 (after cull)
1	Controls	50.0	60.0	80.0	77.8
3	Controls	33.3	40.0	70.0	66.7
7	Controls	50.0	60.0	90.0	88.9
10	Controls	50.0	60.0	60.0	55.6
11	Controls	50.0	60.0	90.0	88.9
13	Controls	58.3	70.0	90.0	88.9
16	Controls	41.7	50.0	60.0	60.0
19	Controls	58.3	70.0	100.0	100.0
4	3.0	66.7	80.0	100.0	100.0

9	3.0	75.0	75.0	75.0	75.0
15	3.0	66.7	80.0	100.0	100.0
17	3.0	58.3	70.0	80.0	75.0
5	31	58.3	70.0	80.0	75.0
8	31	25.0	30.0	80.0	80.0
12	31	66.7	80.0	100.0	100.0
20	31	25.0	30.0	60.0	55.6
2	322	41.7	50.0	60.0	55.6
6	322	25.0	30.0	70.0	70.0
14	322	33.3	40.0	30.0	30.0
18	322	58.3	70.0	90.0	90.0
		day post-hatch (dph)			
Tank	Metformin ( $\mu\text{g/L}$ )	120	120 (after cull)	141	141 (after cull)
1	Controls	77.8	75.0	100.0	100.0
3	Controls	80.0	87.5	87.5	85.7
7	Controls	100.0	100.0	100.0	100.0
10	Controls	60.0	66.7	88.9	85.7
11	Controls	90.0	100.0	100.0	100.0
13	Controls	100.0	100.0	100.0	100.0
16	Controls	70.0	66.7	88.9	85.7
19	Controls	100.0	100.0	100.0	100.0
4	3.0	100.0	100.0	100.0	100.0
9	3.0	87.5	85.7	85.7	85.7
15	3.0	100.0	100.0	100.0	100.0
17	3.0	66.7	77.8	100.0	100.0
5	31	80.0	100.0	100.0	100.0
8	31	100.0	100.0	100.0	100.0
12	31	100.0	100.0	100.0	100.0
20	31	70.0	75.0	87.5	85.7
2	322	70.0	75.0	87.5	83.3
6	322	90.0	87.5	100.0	100.0
14	322	63.6	66.7	77.8	71.4
18	322	100.0	100.0	100.0	100.0

Tank	Metformin ( $\mu\text{g/L}$ )	day post-hatch (dph)			
		172	Mean number of females per tank		
1	Controls	100.0	2.87		
3	Controls	71.4	2.14		
7	Controls	100.0	3.91		
10	Controls	100.0	2.53		
11	Controls	100.0	4.47		
13	Controls	100.0	5.16		
16	Controls	100.0	2.87		
19	Controls	100.0	5.49		
4	3.0	100.0	5.16		
9	3.0	85.7	2.89		
15	3.0	100.0	5.00		
17	3.0	100.0	3.53		
5	31	100.0	4.10		
8	31	100.0	5.53		
12	31	100.0	5.14		
20	31	85.7	2.51		
2	322	83.3	1.91		
6	322	100.0	3.87		
14	322	71.4	1.82		
18	322	100.0	5.22		





Metformin (µg/L)	Tank	n	Tubercle index	Liver-somatic index	Gonadosomatic index	Carcass weight (g)	
Controls	1	3	2.40	1.82	1.41	3.03	
Controls	3	4	1.65	2.38	1.47	2.99	
Controls	7	3	0.20	1.90	1.05	2.70	
Controls	10	4	1.03	3.17	1.38	2.93	
Controls	11	3	1.57	2.07	1.18	2.57	
Controls	13	3	1.83	2.68	1.12	2.29	
Controls	16	4	0.33	2.89	1.31	2.81	
Controls	19	3	0.80	2.93	1.44	2.18	
	Mean	Mean	1.23	2.48	1.29	2.69	
	s.d.	s.d.	0.77	0.51	0.16	0.32	
3.0	4	3	0.37	2.69	1.37	2.55	
3.0	9	3	2.30	2.20	1.05	2.66	
3.0	15	3	0.17	2.63	1.02	2.40	
3.0	17	3	1.17	2.03	0.99	2.62	
	Mean	Mean	1.00	2.39	1.11	2.56	
	s.d.	s.d.	0.97	0.32	0.17	0.12	
31	5	3	1.40	2.91	1.62	2.75	
31	8	3	3.43	1.92	1.34	2.31	
31	12	3	3.03	3.26	1.16	3.33	
31	20	3	1.83	3.41	1.40	3.26	
	Mean	Mean	2.43	2.87	1.38	2.92	
	s.d.	s.d.	0.96	0.67	0.19	0.48	
322	2	3	1.83	2.47	1.39	2.72	
322	6	3	0.87	2.49	1.06	2.51	
322	14	3	2.60	1.63	0.94	3.19	
322	18	3	1.07	2.20	1.04	2.03	
	Mean	Mean	1.59	2.20	1.11	2.61	
	s.d.	s.d.	0.79	0.40	0.20	0.48	





Metformin (µg/L)	Tank	n	Ovipositor area (mm <sup>2</sup> )	Liver-somatic index	Gonado-somatic index	Carcass weight (g)	
Controls	1	3	1.47	4.00	11.96	1.19	
Controls	3	1	1.47	4.71	10.55	1.07	
Controls	7	3	1.22	3.72	12.93	1.29	
Controls	10	3	1.10	3.80	11.73	1.16	
Controls	11	4	1.05	3.51	14.74	1.26	
Controls	13	4	1.25	4.42	9.78	1.06	
Controls	16	3	1.32	3.23	15.44	1.33	
Controls	19	5	1.34	3.13	19.79	1.40	
	Mean	Mean	1.28	3.82	13.37	1.22	
	s.d.	s.d.	0.16	0.55	3.23	0.12	
3.0	4	5	1.13	3.51	15.94	1.12	
3.0	9	3	1.46	2.88	16.83	1.11	
3.0	15	4	1.51	3.72	14.69	1.04	
3.0	17	3	1.69	3.87	13.62	1.32	
	Mean	Mean	1.45	3.50	15.27	1.15	
	s.d.	s.d.	0.23	0.44	1.41	0.12	
31	5	5	1.17	2.79	12.97	1.20	
31	8	5	1.13	4.08	13.61	1.07	
31	12	5	1.21	3.18	13.87	1.11	
31	20	3	1.01	3.61	13.11	1.34	
	Mean	Mean	1.13	3.41	13.39	1.18	
	s.d.	s.d.	0.09	0.56	0.42	0.12	
322	2	2	1.54	3.59	18.20	0.99	
322	6	4	1.57	4.10	16.30	1.13	
322	14	2	1.48	3.86	16.97	1.12	
322	18	5	1.34	3.79	14.70	1.11	
	Mean	Mean	1.48	3.83	16.54	1.09	
	s.d.	s.d.	0.10	0.21	1.46	0.07	

Table S12. Reproductive summary of each tank during the life cycle exposure of fathead minnows to metformin. Time to first breeding, total eggs per tank, and eggs per female is shown.

Metformin (µg/L)	Tank	Day of first egg production (dph)	Average # of females/tank	Total # of eggs/tank	Eggs per female
Controls	1	99	2.9	5529	1924
Controls	3	89	2.1	3594	1680
Controls	7	89	3.9	9262	2368
Controls	10	95	2.5	5052	1996
Controls	11	106	4.5	12043	2695
Controls	13	96	5.2	18672	3615
Controls	16	97	2.9	6759	2352
Controls	19	99	5.5	14755	2686
	Ave		<b>3.7</b>	<b>9458</b>	<b>2415</b>
	s.d.		1.3	5294	604
3.0	4	107	5.2	10012	1939
3.0	9	103	2.9	8852	3067
3.0	15	93	5.0	18343	3669
3.0	17	99	3.5	9288	2630
	Ave		<b>4.1</b>	<b>11624</b>	<b>2826</b>
	s.d.		1.1	4505	729
31	5	110	4.1	5792	1412
31	8	104	5.5	15144	2738
31	12	103	5.1	13341	2596
31	20	108	2.5	4557	1818
	Ave		<b>4.3</b>	<b>9709</b>	<b>2141</b>
	s.d.		1.4	5311	632
322	2	107	1.9	1205	630
322	6	100	3.9	9868	2548
322	14	120	1.8	2617	1436
322	18	94	5.2	18090	3469
	Ave		<b>3.2</b>	<b>7945</b>	<b>2021</b>
	s.d.		1.6	7755	1245

Table S13. Table of egg quality for each tank during the life cycle exposure of fathead minnows to metformin. Time to first breeding, total eggs per tank, and eggs per female is shown.

Tank	Metformin (µg/L)	Clutch size (eggs/clutch)	Mean # clutches	Total # normal eggs	Total # unfertilized eggs	Total # dead eggs	Total # mutant eggs
1	Controls	178	31	3478	399	536	825
3	Controls	178	20	1924	51	481	533
7	Controls	166	55	6831	228	670	1218
10	Controls	160	31	3751	154	392	352
11	Controls	218	55	8521	694	885	1289
13	Controls	327	57	13366	540	1325	2312
16	Controls	192	35	4252	355	361	1636
19	Controls	305	48	9818	1330	1592	1306
4	3.0	180	55	7499	761	528	911
9	3.0	191	46	6395	1006	429	696
15	3.0	247	74	13598	603	1113	1969
17	3.0	265	35	6559	620	412	1176
5	31	162	35	4274	234	407	655
8	31	268	56	10863	923	1081	1859
12	31	282	47	9997	365	790	1796
20	31	174	26	3359	104	265	701
2	322	146	8	764	55	73	199
6	322	200	49	6941	1178	707	810
14	322	200	13	2131	30	133	286
18	322	285	63	13430	436	876	2315
Tank	Metformin (µg/L)	Total # eggs laid	Total # eggs broken during handling	Total # eggs into egg cup to assess hatch	Total # egg cups per tank	Average % normal eggs	Average % unfertilized eggs
1	Controls	5508	290	1487	17	68	9
3	Controls	3560	601	1257	14	62	2
7	Controls	9116	297	2655	30	75	4
10	Controls	4970	393	1865	20	75	5
11	Controls	11993	648	2573	27	67	8
13	Controls	18636	1119	3448	36	77	3
16	Controls	6711	148	1597	18	69	4
19	Controls	14625	692	2856	29	65	17
4	3.0	9878	301	2375	24	77	7
9	3.0	8801	321	2313	25	76	12
15	3.0	18283	1053	4134	43	77	5
17	3.0	9271	520	1873	19	77	7

5	31	5685	218	1737	19	79	4
8	31	14998	410	3536	37	73	8
12	31	13277	392	2700	27	74	7
20	31	4533	128	1119	12	71	2
2	322	1167	108	502	6	74	6
6	322	9813	223	2134	23	65	17
14	322	2605	36	878	9	79	1
18	322	17986	1030	4046	42	78	3
Tank	Metformin (µg/L)	Average % dead eggs	Average % mutant eggs	Total % normal eggs	Total % unfertilized eggs	Total % dead eggs	Total % mutant eggs
1	Controls	10	13	67	8	10	16
3	Controls	14	22	65	2	16	18
7	Controls	7	14	77	3	8	14
10	Controls	11	9	82	3	9	8
11	Controls	10	15	75	6	8	11
13	Controls	7	13	76	3	8	13
16	Controls	4	24	65	5	6	25
19	Controls	7	10	70	10	11	9
4	3.0	5	10	78	8	6	10
9	3.0	5	7	75	12	5	8
15	3.0	6	13	79	4	6	11
17	3.0	4	12	75	7	5	13
5	31	6	10	78	4	7	12
8	31	7	12	74	6	7	13
12	31	7	12	78	3	6	14
20	31	10	18	76	2	6	16
2	322	6	14	72	5	7	19
6	322	9	10	72	12	7	8
14	322	5	15	83	1	5	11
18	322	5	15	79	3	5	14

Table S14. Hatch success of F1 offspring from parent fathead minnows exposed to metformin for a life cycle.

Metformin (µg/L)	Tank	% Hatchability	% Hatch success	% Severely deformed	% Mild-moderate deformed	Time to hatch (days)
Controls	1	95.9	93.7	2.1	2.4	5.1
Controls	3	97.8	95.7	2.1	2.1	5.4
Controls	7	96.2	94.3	1.9	2.0	5.0
Controls	10	97.0	96.0	1.0	1.0	5.4
Controls	11	95.3	92.2	3.0	4.2	5.1
Controls	13	97.1	95.7	1.2	1.4	5.3
Controls	16	94.9	91.9	3.2	3.7	5.1
Controls	19	97.3	93.1	4.3	6.3	5.4
	<b>Mean</b>	<b>96.4</b>	<b>94.1</b>	<b>2.3</b>	<b>2.9</b>	<b>5.2</b>
	<b>s.d.</b>	<b>1.0</b>	<b>1.6</b>	<b>1.1</b>	<b>1.7</b>	<b>0.2</b>
3.0	4	98.4	95.1	3.3	4.1	5.1
3.0	9	97.9	90.9	7.0	8.8	5.2
3.0	15	97.9	97.2	0.6	0.6	5.1
3.0	17	95.2	92.4	2.6	3.3	5.1
	<b>Mean</b>	<b>97.4</b>	<b>93.9</b>	<b>3.4</b>	<b>4.2</b>	<b>5.1</b>
	<b>s.d.</b>	<b>1.5</b>	<b>2.8</b>	<b>2.7</b>	<b>3.4</b>	<b>0.1</b>
31	5	97.6	95.4	2.0	2.1	5.3
31	8	94.8	89.3	5.9	8.5	5.1
31	12	95.5	92.7	3.0	3.6	5.4
31	20	95.9	92.8	3.2	3.6	5.0
	<b>Mean</b>	<b>96.0</b>	<b>92.5</b>	<b>3.5</b>	<b>4.4</b>	<b>5.2</b>
	<b>s.d.</b>	<b>1.2</b>	<b>2.5</b>	<b>1.7</b>	<b>2.8</b>	<b>0.2</b>
322	2	90.3	88.5	1.7	1.8	5.2
322	6	93.1	89.2	4.3	6.1	5.4
322	14	95.5	93.1	2.3	2.4	4.9
322	18	97.1	95.6	1.5	1.5	5.2
	<b>Mean</b>	<b>94.0</b>	<b>91.6</b>	<b>2.4</b>	<b>3.0</b>	<b>5.2</b>
	<b>s.d.</b>	<b>3.0</b>	<b>3.3</b>	<b>1.3</b>	<b>2.1</b>	<b>0.2</b>

Table S15. Survival and growth of F1 larvae from parent fathead minnows exposed to metformin for a life cycle.

Metformin (µg/L)	Tank	N	Survival from hatch to 9 dph (%)	Mean wet weight (mg)	Mean total length (mm)	Mean condition factor	Mean tail length (mm)
Controls	1	4	97.5	5.75	10.02	0.566	1.22
Controls	3	3	90.0	3.76	9.13	0.486	1.00
Controls	7	4	92.5	3.31	8.60	0.478	0.98
Controls	10	4	90.0	3.96	9.02	0.537	1.00
Controls	11	4	100.0	3.77	8.91	0.512	1.00
Controls	13	4	97.5	4.63	9.46	0.532	1.07
Controls	16	4	100.0	4.56	9.63	0.507	1.10
Controls	19	4	100.0	4.53	9.57	0.501	1.06
	Mean		95.9	4.28	9.29	0.515	1.06
	s.d.		4.4	0.76	0.46	0.029	0.08
3.0	4	4	95.0	4.24	9.59	0.478	1.14
3.0	9	4	97.5	4.74	9.71	0.505	1.06
3.0	15	4	100.0	4.49	9.32	0.545	1.09
3.0	17	4	97.5	4.28	9.20	0.536	1.06
	Mean		97.5	4.44	9.45	0.516	1.09
	s.d.		2.0	0.23	0.24	0.031	0.03
31	5	4	97.5	4.54	9.48	0.533	1.08
31	8	4	97.5	5.58	10.01	0.549	1.24
31	12	4	97.5	4.49	9.36	0.540	1.05
31	20	4	97.5	3.92	9.28	0.488	0.97
	Mean		97.5	4.63	9.53	0.527	1.09
	s.d.		0.0	0.69	0.33	0.027	0.11
322	2	4	97.5	4.77	9.82	0.487	1.23
322	6	4	95.0	3.75	9.19	0.487	1.01
322	14	4	95.0	3.83	9.06	0.494	0.97
322	18	4	97.5	3.70	9.02	0.500	0.97
	Mean		96.3	4.01	9.27	0.492	1.04
	s.d.		1.4	0.51	0.37	0.006	0.13
Metformin (µg/L)	Tank	n	Survival from 9 to 16 dph (%)	Mean wet weight (mg)	Mean total length (mm)	Mean condition factor	Mean tail length (mm)
Controls	1	4	100.0	19.60	13.62	0.770	2.43
Controls	3	3	93.3	14.63	12.70	0.670	2.17
Controls	7	4	100.0	16.57	13.06	0.720	2.12
Controls	10	4	100.0	12.35	12.24	0.664	2.04
Controls	11	4	95.0	14.09	12.61	0.691	2.07

Controls	13	4	100.0	14.39	12.65	0.706	2.17
Controls	16	4	100.0	16.39	13.17	0.706	2.18
Controls	19	4	100.0	17.53	13.75	0.669	2.25
	Mean		98.5	15.69	12.98	0.699	2.18
	s.d.		2.7	2.28	0.52	0.035	0.12
3.0	4	4	97.5	15.21	12.94	0.699	2.23
3.0	9	4	100.0	16.97	13.47	0.691	2.27
3.0	15	4	100.0	15.05	12.86	0.705	2.20
3.0	17	4	100.0	14.63	12.59	0.728	2.15
	Mean		99.4	15.46	12.97	0.706	2.21
	s.d.		1.3	1.03	0.37	0.016	0.05
31	5	4	100.0	14.20	12.85	0.663	2.19
31	8	4	100.0	18.01	13.86	0.671	2.43
31	12	4	95.0	15.82	13.15	0.693	2.28
31	20	4	100.0	13.24	12.54	0.669	2.06
	Mean		98.8	15.32	13.10	0.674	2.24
	s.d.		2.5	2.09	0.56	0.013	0.15
322	2	4	100.0	15.54	13.13	0.683	2.26
322	6	4	100.0	12.18	12.23	0.660	2.03
322	14	4	95.0	15.43	12.94	0.702	2.14
322	18	4	100.0	12.83	12.35	0.676	2.05
	Mean		98.8	13.99	12.66	0.680	2.12
	s.d.		2.5	1.74	0.44	0.017	0.10



Table S16. Vitellogenin in mature male and female fish at the end of the life cycle exposure of fathead minnows to metformin. Note different units for VTG in males (ng/mL) and in females ( $\mu\text{g/mL}$ ). Some fish are missing as a blood sample was not obtainable or the capillary tube shattered on centrifugation. n is the number of fish in each tank in which plasma VTG was measured.

Sex	Tank	Metformin ( $\mu\text{g/L}$ )	Fish #	Plasma VTG (ng/mL)	Log plasma VTG (log ng/mL)	n
Male	1	Controls	1	1268	3.103119	3
Male	1	Controls	3	822	2.914872	
Male	1	Controls	5	14284	4.15485	
Male	3	Controls	14	412	2.614897	4
Male	3	Controls	15	802	2.904174	
Male	3	Controls	17	256	2.40824	
Male	3	Controls	19	2438	3.387034	
Male	7	Controls	43	1481	3.170555	3
Male	7	Controls	45	124	2.093422	
Male	7	Controls	47	1961	3.292478	
Male	10	Controls	65	3411	3.532882	4
Male	10	Controls	67	185	2.267172	
Male	10	Controls	68	313	2.495544	
Male	10	Controls	69	3459	3.538951	
Male	11	Controls	72	435	2.638489	3
Male	11	Controls	74	810	2.908485	
Male	11	Controls	76	2329	3.367169	
Male	13	Controls	88	454	2.657056	3
Male	13	Controls	90	470	2.672098	
Male	13	Controls	92	1714	3.234011	
Male	16	Controls	111	1261	3.100715	4
Male	16	Controls	112	136	2.133539	
Male	16	Controls	114	5203	3.716254	
Male	16	Controls	116	935	2.970812	
Male	19	Controls	132	1401	3.146438	2
Male	19	Controls	134			
Male	19	Controls	136	19291	4.285355	
Male	4	3.0	20	3887	3.589615	2
Male	4	3.0	22			
Male	4	3.0	24	565	2.752048	
Male	9	3.0	58	203	2.307496	3
Male	9	3.0	60	222	2.346353	
Male	9	3.0	62	659	2.818885	
Male	9	3.0	64			

Male	15	3.0	102	164	2.214844	3
Male	15	3.0	104	2351	3.371253	
Male	15	3.0	107	3231	3.509337	
Male	17	3.0	117	965	2.984527	2
Male	17	3.0	119			
Male	17	3.0	121	543	2.7348	
Male	5	31	28	313	2.495544	3
Male	5	31	30	1412	3.149835	
Male	5	31	32	1427	3.154424	
Male	8	31	50	32797	4.515834	2
Male	8	31	52			
Male	8	31	54	2922	3.46568	
Male	12	31	80	393	2.594393	3
Male	12	31	82	5976	3.776411	
Male	12	31	84	3456	3.538574	
Male	20	31	140	131	2.117271	3
Male	20	31	142	2645	3.422426	
Male	20	31	144	1685	3.2266	
Male	2	322	7	4360	3.639486	3
Male	2	322	9	1215	3.084576	
Male	2	322	11	1238	3.092721	
Male	2	322	12			
Male	6	322	36	7756	3.889638	2
Male	6	322	38			
Male	6	322	40	568	2.754348	
Male	14	322	95	614	2.788168	3
Male	14	322	96			
Male	14	322	97	79	1.897627	
Male	14	322	98			
Male	14	322	100	1883	3.27485	
Male	18	322	124	3239	3.510411	2
Male	18	322	126			
Male	18	322	128	14065	4.14814	
Sex	Tank	Metformin ( $\mu\text{g/L}$ )	Fish #	Plasma VTG ( $\mu\text{g/mL}$ )	Log plasma VTG (log $\mu\text{g/mL}$ )	n
Female	1	Controls	2	9597	3.982135	3
Female	1	Controls	4	12354	4.091808	
Female	1	Controls	6	12189	4.085968	
Female	3	Controls	13	13073	4.116375	1

Female	3	Controls	16			
Female	3	Controls	18			
Female	7	Controls	44	22146	4.345295	3
Female	7	Controls	46			
Female	7	Controls	48	13973	4.14529	
Female	7	Controls	49	14891	4.172924	
Female	10	Controls	66	14049	4.147645	3
Female	10	Controls	70	11866	4.074304	
Female	10	Controls	71	11456	4.059033	
Female	11	Controls	73	13519	4.130945	4
Female	11	Controls	75	16468	4.216641	
Female	11	Controls	77	12572	4.099404	
Female	11	Controls	78	9975	3.998913	
Female	11	Controls	79			
Female	13	Controls	89	8947	3.951677	3
Female	13	Controls	91	9963	3.99839	
Female	13	Controls	93			
Female	13	Controls	94	6152	3.789016	
Female	16	Controls	110	14441	4.159597	2
Female	16	Controls	113			
Female	16	Controls	115	13904	4.14314	
Female	19	Controls	133			3
Female	19	Controls	135			
Female	19	Controls	137	16735	4.223626	
Female	19	Controls	138	17318	4.238498	
Female	19	Controls	139	16262	4.211174	
Female	4	3.0	21			3
Female	4	3.0	23	15092	4.178747	
Female	4	3.0	25	13870	4.142076	
Female	4	3.0	26	16735	4.223626	
Female	4	3.0	27			
Female	9	3.0	59	14050	4.147676	2
Female	9	3.0	61	14529	4.162236	
Female	9	3.0	63			
Female	15	3.0	103	9649	3.984482	3
Female	15	3.0	105	8176	3.912541	
Female	15	3.0	106			
Female	15	3.0	108	12621	4.101094	
Female	15	3.0	109			
Female	17	3.0	118	11661	4.066736	4
Female	17	3.0	120	14178	4.151615	

Female	17	3.0	122	11141	4.046924	
Female	17	3.0	123	15352	4.186165	
Female	5	31	29	8835	3.946207	5
Female	5	31	31	6860	3.836324	
Female	5	31	33	11213	4.049722	
Female	5	31	34	12500	4.09691	
Female	5	31	35	10279	4.011951	
Female	8	31	51	13116	4.117801	3
Female	8	31	53	10950	4.039414	
Female	8	31	55			
Female	8	31	56	14927	4.173973	
Female	8	31	57			
Female	12	31	81	17423	4.241123	3
Female	12	31	83	16747	4.223937	
Female	12	31	85	13361	4.125839	
Female	12	31	86			
Female	12	31	87			
Female	20	31	141	8570	3.932981	3
Female	20	31	143	14927	4.173973	
Female	20	31	145			
Female	20	31	146	10825	4.034428	
Female	2	322	8	8484	3.928601	2
Female	2	322	10	16087	4.206475	
Female	6	322	37	12183	4.085754	4
Female	6	322	39	11120	4.046105	
Female	6	322	41	10388	4.016532	
Female	6	322	42	16395	4.214711	
Female	14	322	99			1
Female	14	322	101	12252	4.088207	
Female	18	322	125	13099	4.117238	4
Female	18	322	127	13998	4.146066	
Female	18	322	129	15850	4.200029	
Female	18	322	130	15691	4.195651	

Table S17a. Gonad histology data for mature male fish at the end of the life cycle exposure of fathead minnows to metformin.

Sex	Fish	Tank	Metformin (µg/L)	Rep.	Teste Stage	# of Slides
Male	1	1	Controls	A	2	21
Male	3	1	Controls	A	2	27
Male	5	1	Controls	A	3	28
Male	14	3	Controls	A	3	34
Male	15	3	Controls	A	3	25
Male	17	3	Controls	A	3	23
Male	19	3	Controls	A	2	28
ImmMale	(IMM) 16	3	Controls	A	.	.
ImmMale	(IMM) 18	3	Controls	A	1	11
Male	43	7	Controls	B	2	19
Male	45	7	Controls	B	2	20
Male	47	7	Controls	B	3	24
Male	65	10	Controls	B	2	18
Male	67	10	Controls	B	3	28
Male	68	10	Controls	B	3	22
Male	69	10	Controls	B	3	23
Male	72	11	Controls	C	2	18
Male	74	11	Controls	C	2	21
Male	76	11	Controls	C	2	17
Male	88	13	Controls	C	2	21
Male	90	13	Controls	C	3	20
Male	92	13	Controls	C	2	15
Male	111	16	Controls	D	3	19
Male	112	16	Controls	D	2	20
Male	114	16	Controls	D	3	28
Male	116	16	Controls	D	2	24
Male	132	19	Controls	D	3	25
Male	134	19	Controls	D	3	15
Male	136	19	Controls	D	3	19
Male	20	4	3.0	A	2	22
Male	22	4	3.0	A	2	27
Male	24	4	3.0	A	3	28
Male	58	9	3.0	B	2	16
Male	60	9	3.0	B	2	25
Male	62	9	3.0	B	3	21
ImmMale	(IMM) 64	9	3.0	B	3	27
Male	102	15	3.0	C	2	16

Male	104	15	3.0	C	3	15
Male	107	15	3.0	C	3	14
Male	117	17	3.0	D	2	21
Male	119	17	3.0	D	2	22
Male	121	17	3.0	D	2	11
Male	28	5	31	A	3	18
Male	30	5	31	A	2	24
Male	32	5	31	A	2	32
Male	50	8	31	B	3	28
Male	52	8	31	B	2	21
Male	54	8	31	B	2	16
Male	80	12	31	C	2	24
Male	82	12	31	C	3	31
Male	84	12	31	C	2	27
Male	140	20	31	D	3	27
Male	142	20	31	D	2	17
Male	144	20	31	D	3	34
ImmMale	(IMM) 145	20	31	D	2	15
Male	7	2	322	A	3	21
Male	9	2	322	A	2	28
Male	11	2	322	A	3	24
ImmMale	(IMM) 12	2	322	A	3	25
Male	36	6	322	B	2	22
Male	38	6	322	B	3	21
Male	40	6	322	B	2	19
Male	95	14	322	C	3	28
Male	97	14	322	C	2	22
Male	100	14	322	C	3	20
ImmMale	(IMM) 96	14	322	C	1.5	15
ImmMale	(IMM) 98	14	322	C	3	27
Male	124	18	322	D	2	17
Male	126	18	322	D	3	13
Male	128	18	322	D	3	17

Table S17b. Gonad histology data for mature female fish at the end of the life cycle exposure of fathead minnows to metformin.

<b>Females</b>									
Fish	Tank	Metformin (µg/L)	Total # oocytes counted	# Primary	# Cortical Alveolar	# Early Vitellogenic	# Late Vitellogenic	# Atretic	Atretic Score (0 to 3)
2	1	Controls	259	157	25	31	46	0	0
4	1	Controls	262	163	48	18	32	1	1
6	1	Controls	309	166	68	20	55	0	0
13	3	Controls	275	168	44	15	47	1	1
44	7	Controls	326	194	63	18	51	0	0
46	7	Controls	304	256	15	0	0	33	3
48	7	Controls	198	138	23	17	20	0	0
49	7	Controls	619	440	81	81	16	1	1
66	10	Controls	285	159	60	26	40	0	0
70	10	Controls	294	199	49	46	0	0	0
71	10	Controls	262	153	53	56	0	0	0
73	11	Controls	184	83	30	31	39	1	1
75	11	Controls	215	87	53	26	48	1	1
77	11	Controls	233	137	29	20	44	3	1
78	11	Controls	310	187	40	36	47	0	1
89	13	Controls	249	173	26	19	31	0	0
91	13	Controls	342	254	40	45	0	3	1
93	13	Controls	228	166	9	14	39	0	0
94	13	Controls	365	245	65	53	2	0	0
110	16	Controls	263	135	40	27	61	0	0
113	16	Controls	154	86	25	12	30	1	1
115	16	Controls	277	112	55	49	60	1	1
133	19	Controls	404	239	27	62	60	16	3
135	19	Controls	170	64	20	31	55	0	0
137	19	Controls	155	69	23	18	44	1	1
138	19	Controls	838	747	39	11	41	0	1
139	19	Controls	271	124	29	34	84	0	0
21	4	3.0	222	121	40	14	47	0	0
23	4	3.0	341	184	69	29	59	0	2
25	4	3.0	252	131	55	22	44	0	3
26	4	3.0	221	96	43	34	46	2	1
27	4	3.0	241	132	38	18	53	0	0
59	9	3.0	171	59	30	22	51	9	2
61	9	3.0	321	238	47	19	10	7	2
63	9	3.0	207	139	21	10	37	0	0

103	15	3.0	298	191	48	20	39	0	0
105	15	3.0	294	162	53	34	43	2	1
106	15	3.0	312	172	53	21	64	2	1
108	15	3.0	554	431	68	52	1	2	1
118	17	3.0	403	337	52	13	0	1	2
120	17	3.0	309	185	60	19	45	0	0
122	17	3.0	224	108	41	34	40	1	1
123	17	3.0	224	123	31	20	50	0	0
29	5	31	245	160	26	27	32	0	0
31	5	31	341	199	38	35	69	0	0
33	5	31	175	73	26	28	31	17	3
34	5	31	320	209	44	30	35	2	1
35	5	31	240	141	33	19	46	1	1
51	8	31	50	7	12	24	7	0	3
53	8	31	172	74	42	17	39	0	0
55	8	31	216	135	25	17	39	0	0
56	8	31	252	157	42	16	36	1	1
57	8	31	178	100	29	36	13	0	0
81	12	31	277	109	63	32	72	1	1
83	12	31	289	203	34	25	27	0	0
85	12	31	242	133	46	13	50	0	0
86	12	31	469	356	70	26	17	0	0
87	12	31	473	331	63	29	49	1	1
141	20	31	546	353	102	57	1	33	3
143	20	31	301	176	43	36	45	1	1
146	20	31	374	249	43	24	58	0	0
8	2	322	143	72	27	16	25	3	1
10	2	322	273	185	25	30	33	0	0
37	6	322	287	171	53	22	39	2	1
39	6	322	236	121	49	17	47	2	1
41	6	322	150	89	16	20	25	0	0
42	6	322	325	178	48	40	59	0	0
99	14	322	176	93	18	22	43	0	0
101	14	322	419	233	80	42	62	2	1
125	18	322	325	154	61	72	37	1	1
127	18	322	273	176	57	13	26	1	1
129	18	322	237	119	42	25	49	2	1
130	18	322	173	96	31	20	26	0	0
131	18	322	461	327	63	24	47	0	0



Table S18. List of the ten statistically-significant differences observed in the life cycle exposures of fathead minnows to metformin. Sex of fish, time point, and concentration where difference was seen, as well as p values (compared to controls), are shown. Abbreviations are dph = days post-hatch, con = controls, M = males, F = females.

Number	<u>Statistically significant differences</u>			<u>Concentration of metformin (<math>\mu\text{g/L}</math>)</u>			p value
	Endpoint & Difference	Sex	Time-point	3.0	31	322	
1	Greater length (21.4 mm compared to control 19.6 mm)	immature	30 dph		X		0.038
2	Higher condition factor (0.906 compared to controls 0.864)	immature	64 dph			X	0.033
3	More mature fish compared to controls	M and F	92 dph	X			0.005
4	Delayed mean post-hatch breeding start (delay = roughly 10 days)	Breeding groups	~97 to 107 dph		X		0.003
5	Larger relative ovary size (GSI of 16.5 vs. con 13.4 %)	F	End of exposure			X	0.041
6	Slightly larger ovipositors	F	End of exposure			X	0.023
7	Slightly increased secondary sex characteristics	M	End of exposure		X		0.045
8	Higher percentage of normal eggs (76.7 vs. con 69.7 %)	F	F1	X			0.008
9	Lower percentage of dead eggs (5.4 vs. con 9.4 %)	F	F1	X			0.012
10	Lower percentage of dead eggs (6.2 vs. con 9.4 %)	F	F1		X		0.034

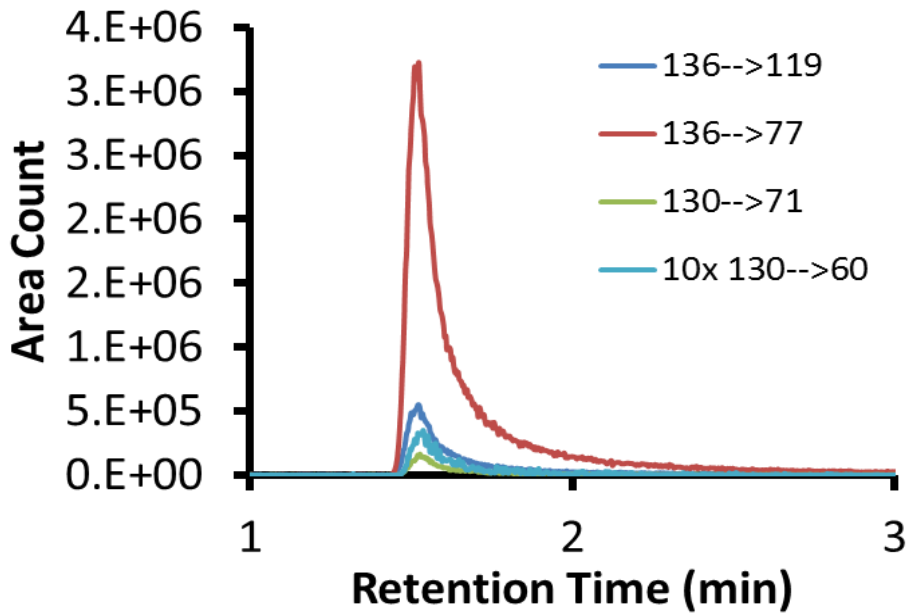


Figure S1. Area count versus retention time for metformin.

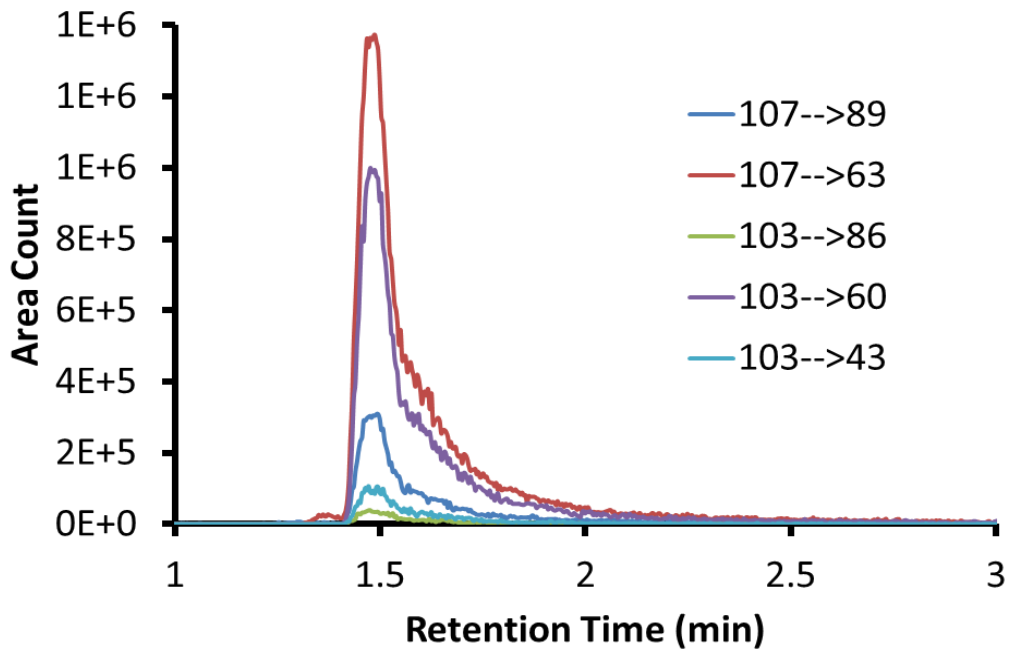


Figure S2. Area count versus retention time for guanylyurea.

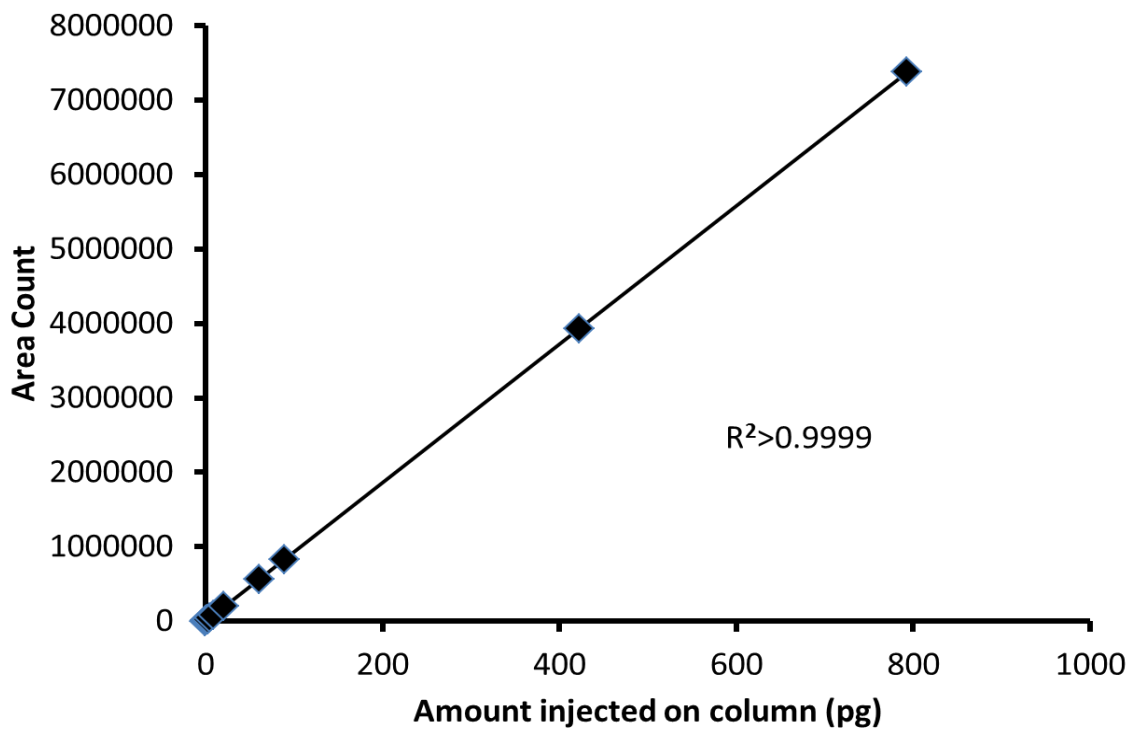


Figure S3. Area count versus amount injected on column for metformin.

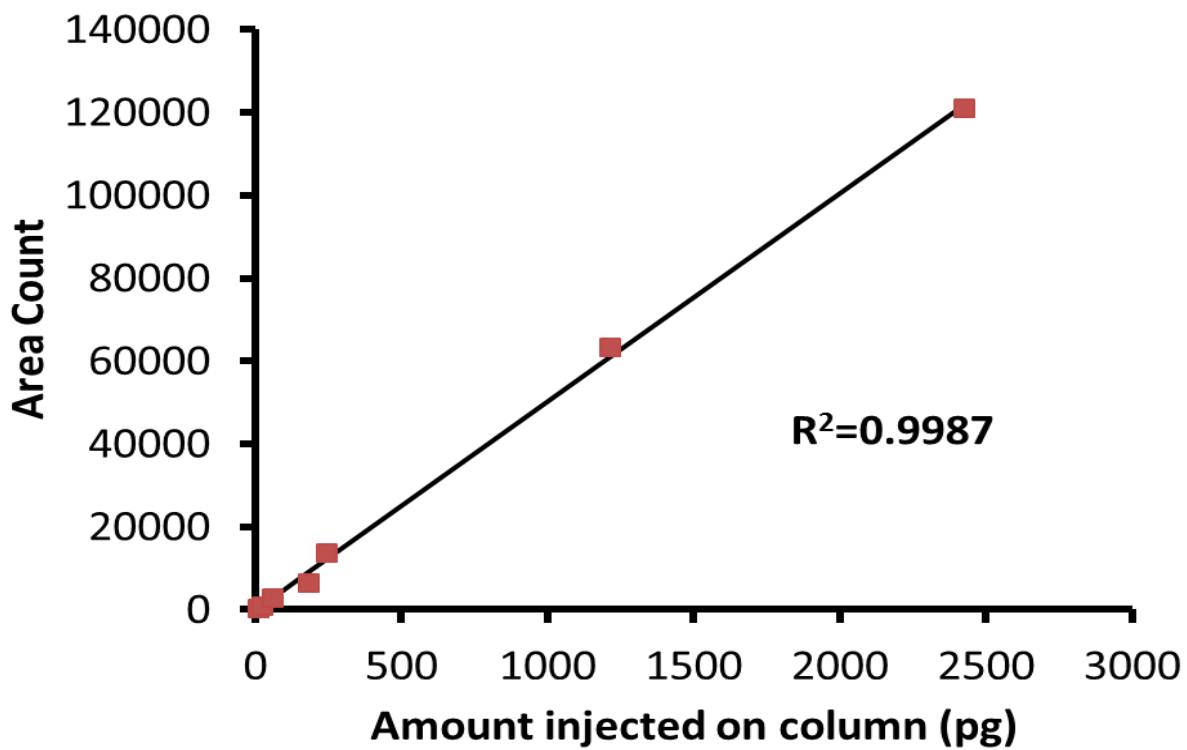


Figure S4. Area count versus amount injected on column for guanylyurea.

1	2	3	4	5	6	7	8	9 leak dripped in from above	10
11	12	13	14	15	16	17	18	19 leak dripped in from above	20

Figure S5. Set-up of aquaria during the life cycle exposure of fathead minnows to metformin. Leaks from the header tank (containing clean water) dripped over dusty shelves and into tanks 9 and 19 killing some of the fish.