

Supplementary Material of

Toxicity and Risk of Plant-Produced Alkaloids to

Daphnia magna.

Megan R. Griffiths¹, Bjarne W. Strobel¹, Jawameer R. Hama¹, Nina Cedergreen^{1*}.

¹ Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark.

1. Description of the analytical method
2. Table S1: LC-MS/MS parameters for selected alkaloids
3. Table S2: Analytical results
4. Table S3: GUTS-SD-RED parameters
5. Figure S1: Concentration response curves after 96h exposure

LC-MS/MS Analysis

The samples were separated and analyzed on a Waters Acquity UPLC I-Class module, equipped with a 2.1 × 100 mm Acquity UPLC HSS C18 column, particle size 1.8 μm (Waters, Milford). Waters Xevo TQD triple quadrupole mass spectrometer was operated with electrospray ionization in positive ion mode. Mobile phase of A (water + 0.1% formic acid) and B (MeOH + 0.1% formic acid) was used in a gradient program: 0–4 min 10% B, 7 min 20% B, 10 min 50% B, 15 min 90% B, 15–17 min 90% B. The column was equilibrated for 6 min before each run, and the total run time was 23 minutes. For every injection, 2.5 μL standard or sample was injected in a flow rate of 0.20 mL/min on a pre-heated (35°C) column. Detection was performed with a full scan and multiple reaction monitoring (MRM) for all alkaloids. In the MS/MS, the ion traces was obtained for apex retention time (t_R) ± 0.15 min, the corresponding cone voltages (CV) and collision energies (CE) are listed in Table S1. The desolvation temperature was 600°C, the desolvation gas flow 1000 L/h, and the cone gas flow 20 L/h. MassLynx™ version 4.1. (Waters, Milford, USA) was used for data acquisition and processing.

For quantification, the calibration curves were obtained using an internal calibration curve, using seven standard solutions. Curves were obtained by plotting measured analyte peak areas/internal standard peak area against corresponding analyte concentrations/internal standard concentration in the extracted matrix. Linear regressions were performed for each curve. Further details are given in Hama & Strobel (2019).

Table S1. LC-MS/MS parameters for selected alkaloids: retention time (t_R), precursor ion, product ion, CV and CE.

Alkaloids	t_R (min)	Precursor ion (m/z)	Product ion (m/z)	CV (V)	CE (eV)
Gramine	5.62	175.1	130.06	15	15
Sparteine	6.98	235.2	98.09	30	35
Lupinine	4.78	170.2	152.14	25	30
Lupanine	6.14	249.2	136.11	30	35
Heliotrine	6.32	314.2	138.09	20	25
Senecionine	6.95	336.2	138.09	20	30
Monocrotaline	4.19	326.2	138.09	40	40
Monocrotaline N-oxide	4.95	342.2	138.09	35	35

Table S2A. Measured concentrations of the alkaloids in the acute tests at the start of the experiment, and at the end of the 96 h experiments at a minimum of three selected concentrations. Recoveries related to the nominal concentrations are given in percent, as is the recovery of the start concentration at the end of exposure.

<i>Alkaloid</i>	<i>Nominal concentrations (mg/L)</i>	<i>Measured concentrations</i>			<i>Recovery of nominal concentrations (%)</i>		<i>Recovery after 3 days (%)</i>
		Sample time (d)	Fresh media	Old media	Fresh media	Old media	
<i>Sparteine</i>	100	0	21.4		21.4		
	10	0	5.3		53.0		
	1	0	1.0		96.3		
<i>Lupanine</i>	80	0	7.1		8.9		
	40	0	4.7		11.8		
	20	0	2.9		14.5		
<i>Lupinine</i>	80	4		6.8		8.5	96.0
	80	0	7.1		8.9		
	40	0	4.6		11.6		
	20	0	2.6		13.0		
	10	0	1.6		16.5		
	80	4		6.9		8.7	97.2
<i>Gramine</i>	16	0	2.3		14.7		
	8	0	1.4		17.5		
	6	0	1.2		19.4		
	4	0	0.9		21.8		
	1	0	0.3		25.8		
	16	2		2.3		14.5	99.1
	16	2		2.4		14.8	100.8
	16	2		2.3		14.3	97.9
	16	2		2.3		14.4	98.1
<i>Heliotrine</i>	100	0	1.1		1.1		
	17	0	0.6		3.7		
	27	0	0.4		1.4		
	100	4		1.0		1.0	89.0
	100	4		1.1		1.1	105.3
<i>Monocrotaline</i>	100	0	48.0		48.0		
	25	0	1.4		5.8		
	1.56	0	0.5		35.1		
	100	4		5.6		5.6	11.7
	100	4		5.6		5.6	11.6
<i>Monocrotaline N-oxide</i>	100	0	93.2		93.2		
	25	0	24.7		98.8		
	1.6	0	1.3		82.1		
	100	4		97.0		97.0	104.1

	100	4	97.6	97.6	104.7
<i>Senecionine</i>	100	0	28.2	28.2	
	25	0	18.6	74.5	
	1.56	0	1.3	82.8	
	100	4	27.9	27.9	99.3
	100	4	22.1	22.1	78.4

Table S2B. Measured sparteine concentrations in the chronic tests at the start of each media renewal and after three days in selected treatments. Recoveries related to the nominal concentrations are given in percent, as is the recovery of the start concentration at the end of each three-day period between media renewal. Selected samples before and after the addition of food are included to investigate if sparteine sorbed to the food algae.

<i>Nominal concentration (mg/L)</i>	<i>Sample time (d)</i>	<i>Measured concentrations</i>		<i>Recovery of nominal concentrations (%)</i>		<i>Recovery after 3 days (%)</i>
		Fresh media	Three-day old media	Fresh media	Three-day old media	
10	0	525.9		5.3		
10	3	511.6	534.6	5.1	5.3	101.7
10	6	533.3		5.3		
10	9	520.9	527.7	5.2	5.3	98.9
10	12	519.5		5.2		
10	15	527.2	511.3	5.3	5.1	98.4
10	18	542.3		5.4		
10	21		526.1		5.3	97.0
5	0	344.8		6.9		
5	3		339.0		6.8	98.3
5	6	328.6		6.6		
5	9	364.6	347.7	7.3	7.0	105.8
5	12	292.0		5.8		
5	15		341.8		6.8	117.1
5	18	354.4		7.1		
5	21		348.3		7.0	98.3
2.5	0					
2.5	3	256.6	254.2	10.3	10.2	-
2.5	6	228.3		9.1		
2.5	9	242.8	253.8	9.7	10.2	111.2
2.5	12					
2.5	15	249.7	254.7	10.0	10.2	-
2.5	18	248.5		9.9		
2.5	21		251.4		10.1	101.2
1.25	0					
1.25	3		176.9		14.2	-
1.25	6					

1.25	9		204.9		16.4	-
1.25	12	168.5		13.5		
1.25	15	167.9	209.9	13.4	16.8	124.6
1.25	18	150.9		12.1		
1.25	21		206.8		16.5	137.1
<i>Sample time</i>						<i>Recovery after food addition (%)</i>
10	18	525.9	Before food	5.3		95.1
10	18	553.0	After food	5.5		
10	19	527.7	Before food	5.3		100.1
10	19	527.0	After food	5.3		
10	20	526.5	Before food	5.3		96.5
10	20	545.8	After food	5.5		
5	19	349.6	Before food	7.0		100.6
5	19	347.7	After food	7.0		

Table S3. The negative Log-likelihood values (LL), and parameters derived from fitting mobility of daphnids exposed to individual alkaloids and an alkaloid plant extract daily over 96 h to a GUTS-RED-SD model are given together with derived EC₅₀ values after 48 h (standard timeframe for toxicity on *D. magna*) and 96 h. All values are given with their 95% confidence intervals (CI).

	LL	kD_SD /d	bw_SD L/(mg d)	zw_SD mg/L	48h EC ₅₀ mg/L	96h EC ₅₀ mg/L
Gramine	98	7.14 (2.39-144*)	0.196 (0.115-0.305)	3.55 (2.77-3.86)	5.61 (5.06-6.41)	4.52 (4.05-4.91)
Heliotrine		NA	NA	NA	>100	>100
Lupanine	78	0.0016 (0.0016*-0.84)	0.960 (0.004-1.773)	0.034 (0.004-13.7)	240 (130-402)	65 (45-105)
Lupinine	29	2.78 (0.28-144*)	0.00106 (0.00037-0.00290)	10.9 (0.003*-19.5)	414 (191-1216)	193 (96-529)
Monocrotaline	37	1.87 (0.98-5.39)	0.056 (0.027-0.108)	21.2 (17.3-23.3)	34.0 (30.3-39.7)	20.0 (23.2-28.9)
Mono. N-oxide	3.97	1.91 (0.028-144*)	51.8 (0.022-3345*)	83.3 (4.44-99*)	85.5 (29.5-99.2)	83.4 (25.2-99.1)
Senecionine	28	144 (2.82-144*)	0.0139 (0.0082-0.0238)	23.1 (17.9-24.7)	48.1 (39.4-64.9)	35.6 (30.8-43.6)
Sparteine	76	1.80 (0.140-144*)	0.019 (0.010-0.148)	0.000137 (0.000137*-7.12)	24.6 (16.8-38.0)	10.4 (6.2-18.0)
Plant extract	69	0.84 (0.0016*-144*)	0.679 (0.260-223)	0.213 (0.0003*-0.68)	1.38 (0.92-1.79)	0.64 (0.26-1.09)

*edge of 95% parameter CI has run into a boundary

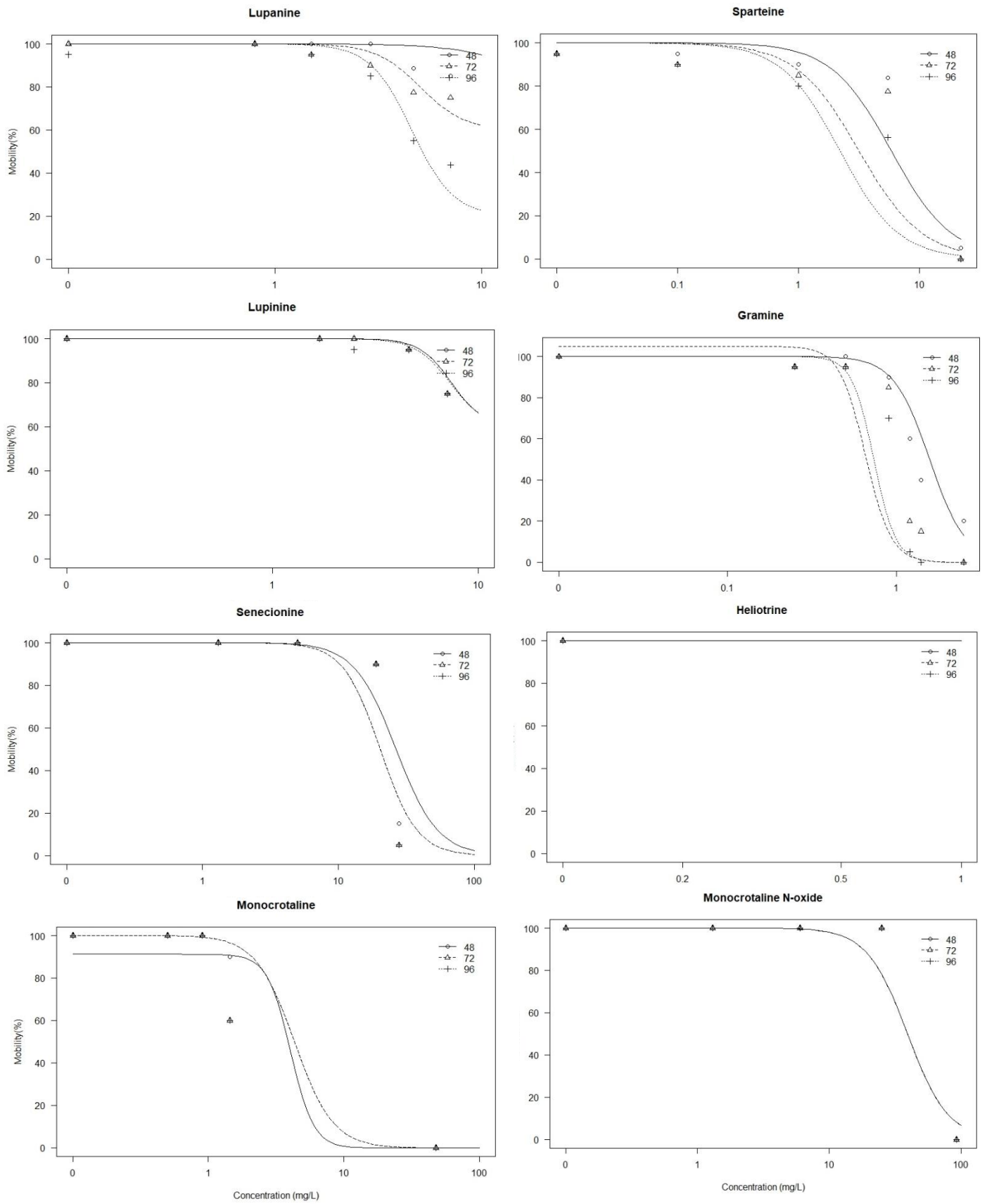


Figure S1. Concentration-response curves for the alkaloids after 96 h of exposure. $N = 4$ replicates per group; 5 daphnids per replicate. Curves obtained from R version 3.5.1.