Supplementary material

Developmental and reproductive effects of clothianidin exposure in monarch butterflies

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Table S1. Morphological effects of sublethal concentrations of the neonicotinoid insecticide clothianidin on monarch caterpillars (*Danaus plexippus*) reared on swamp milkweed (*Asclepias incarnata*) grown in control (no clothianidin), 15 ng/g (low dose) and 25 ng/g (high dose) soil. Measurements were reported as the mean ± SD (n), except where no data was available (-).

	Treatment	Body length (mm)	Body width (mm)	Body volume (mm^3)	Mass (g)
1st instar	Control	2.2 ± 0.3 (64)	-	-	-
	Low dose	2.3 ± 0.4 (62)	-	-	-
	High dose	2.1 ± 0.5 (56)	-	-	-
3rd instar	Control	11.6 ± 1.4 (65)	2.0 ± 0.3 (66)	38.0 ± 14.0 (65)	-
	Low dose	10.7 ± 1.7 (63)	1.9 ± 0.5 (64)	33.3 ± 18.2 (63)	-
	High dose	9.8 ± 1.8 (52)	1.5 ± 0.2 (51)	18.0 ± 6.3 (51)	-
5th instar	Control	35.8 ± 4.6 (65)	5.3 ± 0.5 (63)	807.9 ± 209.1 (63)	1.3 ± 0.3 (62)
	Low dose	36.3 ± 4.8 (61)	5.0 ± 0.6 (62)	725.6 ± 205.9 (61)	1.3 ± 0.2 (62)
	High dose	40.6 ± 5.8 (52)	3.5 ± 0.5 (53)	397.2 ± 124.2 (52)	1.2 ± 0.3 (52)

Table S2. Effect of sublethal concentrations of the neonicotinoid insecticide clothianidin on the length (mm) of monarch caterpillars (*Danaus plexippus*) reared on swamp milkweed (*Asclepias incarnata*) grown in control (no clothianidin), 15 ng/g (low dose) and 25 ng/g (high dose) soil. Measurements were reported as the mean ± SD (n), except where no data was available (-). Analysis of variance (ANOVA) with Tukey's HSD was calculated for the effect of clothianidin on the length of the 1st, 3rd, and 5th instar caterpillars using the *stats* package (R Core Team 2015a) in R version 3.4.1 (R Core Team 2015b).

1st instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**
2.45	0.09	0.87	0.43	2
Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	0.10	-0.08	0.28	0.39
Control - High dose	-0.07	0.25	0.11	0.62
Low dose - High dose	0.17	-0.01	0.35	0.07
3rd instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**

17.24	< 0.001	92.30	46.16	2
Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	-0.94	-1.62	-0.25	0.004
Control - High dose	-1.78	-2.50	-1.06	< 0.001
Low dose - High dose	0.84	0.12	1.57	0.02
5th instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**
15.26	< 0.001	770.00	385.20	2
Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	0.57	-1.54	2.69	0.80
Control - High dose	4.82	2.61	7.03	< 0.001
Low dose - High dose	-4.25	-6.49	-2.01	< 0.001

Table S3. Effect of sublethal concentrations of the neonicotinoid insecticide clothianidin on the width (mm) of monarch caterpillars (*Danaus plexippus*) reared on swamp milkweed (*Asclepias incarnata*) grown in control (no clothianidin), 15 ng/g (low dose) and 25 ng/g (high dose) soil. Analysis of variance (ANOVA) with Tukey's HSD was calculated for the effect of clothianidin on the width of the 3rd and 5th instar caterpillars using the *stats* package (R Core Team 2015a) in R version 3.4.1 (R Core Team 2015b).

3rd instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**
32.48	< 0.001	7.84	3.92	2
Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	-0.06	-0.21	0.08	0.57
Control - High dose	-0.49	-0.64	-0.34	< 0.001
Low dose - High dose	0.43	0.27	0.58	< 0.001
5th instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**
173.6	< 0.001	105.32	52.66	2

Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	-0.31	-0.55	-0.08	0.005
Control - High dose	-1.81	-2.06	-1.57	< 0.001
Low dose - High dose	1.50	1.26	1.74	< 0.001

Table S4. Effect of sublethal concentrations of the neonicotinoid insecticide clothianidin on the volume of monarch caterpillars (*Danaus plexippus*) reared on swamp milkweed (*Asclepias incarnata*) grown in control (no clothianidin), 15 ng/g (low dose) and 25 ng/g (high dose) soil. Analysis of variance (ANOVA) with Tukey's HSD was calculated for the effect of clothianidin on the volume of the 3rd and 5th instar caterpillars using the *stats* package (R Core Team 2015a) in R version 3.4.1 (R Core Team 2015b).

3rd instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**
30.52	< 0.001	12173.00	6086.00	2
Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	-4.74	-10.64	1.16	0.14
Control - High dose	-20.06	-26.30	-13.81	< 0.001
Low dose - High dose	15.31	9.03	21.60	< 0.001
5th instar				
ANOVA				
F	**P**	**Sum of squares**	**Mean of squares**	**DF**
74.88	< 0.001	5230474.00	2615237.00	2

Tukey HSD				
Treatment	**Difference in observed means**	**Lower**	**Upper**	**P**
Control - Low dose	-82.28	-161.64	-2.92	0.04
Control - High dose	-410.67	-493.44	-327.89	< 0.001
Low dose - High dose	328.39	245.00	411.77	< 0.001

References

R Core Team. 2015a. stats: The R Stats Package [online]. https://stat.ethz.ch/R-manual/R-devel/library/stats/html/stats-package.html

R Core Team. 2015b. R: a language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. Accessed from https://www.r-project.org/ [accessed 6 September 2019].