Monarch chronic clothianidin exposure (Summer 2019)

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Development of monarch butterflies (*Danaus plexippus*) with chronic exposure to clothianidin. Third instar larvae were fed *Asclepias curassavica* foliage treated with 0 ppb control, 10 ppb, 20 ppb, and 40 ppb clothianidin until pupation. Adults were fed untreated sugar syrup. Larval weight, larval survival, and eggs laid per female were taken as averages for each cage; all other parameters measured individual butterflies.

- Combined Replicates: 10 larvae per cage; 6 cages per treatment.
- Following eclosion, adults were consolidated such that Replicate 1 had two cages per treatment, Replicate 2 had one cage per treatment, and Combined Replicates had three cages per treatment.
Chronic monarch Combined Reps

- **Larval weight day 3 (g)**
  - 0 ppb, 10 ppb, 20 ppb, 40 ppb

- **Larval survival (%)**
  - 0 ppb, 10 ppb, 20 ppb, 40 ppb

- **Days to pupation**
  - 0 ppb, 10 ppb, 20 ppb, 40 ppb

- **Pupal weight (g)**
  - 0 ppb, 10 ppb, 20 ppb, 40 ppb

- **Egg lay per female**
  - 0 ppb, 10 ppb, 20 ppb, 40 ppb
Chronic monarch Combined Reps

No. larvae that pupate

- 0 ppb: n = 60
- 10 ppb: n = 62
- 20 ppb: n = 60
- 40 ppb: n = 64

No. pupae that eclose

- 0 ppb: n = 45
- 10 ppb: n = 31
- 20 ppb: n = 29
- 40 ppb: n = 24

No. larvae surviving to adults

- 0 ppb: n = 60
- 10 ppb: n = 62
- 20 ppb: n = 60
- 40 ppb: n = 64

Legend:
- Pupated
- Did not pupate
- Eclosed
- Did not eclose
- Survived
- Did not survive
Chronic Monarch Combined Reps

• **Figure 1.** Mean (+SE) larval weight (F(3,20) = 2.10, p = 0.13), larval survival ($X^2(3) = 1.84$, $p = 0.61$), days to pupation ($X^2(3) = 15.03$, $p = 0.002$), pupal weight ($X^2(3) = 4.92$, $p = 0.18$), and egg lay per female ($X^2(3) = 2.38$, $p = 0.50$) for monarch butterflies (*Danaus plexippus*) exposed to chronic sublethal doses of clothianidin beginning as third instar larvae until pupation. Treatments were 0 ppb control, 10 ppb, 20 ppb, and 40ppb clothianidin. Replicate 1 was started August 21st, 2019; Replicate 2 was started August 26th, 2019.

• **Figure 2.** Number of larvae that pupate ($X^2(3) = 19.22$, $p = 0.0002$), number of pupae that eclose ($X^2(3) = 8.59$, $p = 0.04$), and number of larvae that survive to become adults ($X^2(3) = 3.29$, $p = 0.35$) for monarch butterflies (*Danaus plexippus*) exposed to chronic sublethal doses of clothianidin beginning as third instar larvae until pupation. Treatments were 0 ppb control, 10 ppb, 20 ppb, and 40ppb clothianidin. Replicate 1 was started August 21st, 2019; Replicate 2 was started August 26th, 2019.