Plasma Metabolite Profiling in an Amphibian Following Exposure to a Neonicotinoid Pesticide

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Abstract

Globally, many amphibian populations are in decline and some populations face the potential of extinction. Previous research supports that localized amphibian population declines may be attributed to chemical contaminant exposure. However, the connection between contaminant exposure and changes in individual or population success may be more difficult to discern in the absence of overt toxicity or mortality. We examined the sublethal influence of exposure to a common contaminant of surface waters on metabolic changes in adult African clawed frogs (*Xenopus laevis*). Adult male (*X. laevis*) were exposed to environmentally relevant concentrations of the commonly used neonicotinoid insecticide, imidacloprid, by immersion for 48 days. Following exposure, we determined plasma glucose, glycerol, and triglycerides from samples collected in baseline and handling-stressed conditions. This research will contribute to our knowledge of the factors influencing amphibian decline by providing insight into sublethal influences of chemical exposure on amphibian metabolism.

Introduction

- Globally there has been a decline in amphibian populations and over one-third of amphibian species are threatened with extinction.[1]
- While many studies focus on the toxicity of other high use chemicals, relatively less information is available on the impacts of widely used neonicotinoid insecticides on amphibian life. [2,3]
- Imidacloprid is the most commonly used neonicotinoid.[3]
- Neonicotinoids mimic nicotine by binding to nicotinic acetylcholine receptors in an irreversible mechanism and consequently alter the function of the central nervous system of insects. [4]
- Neonicotinoids are most commonly applied as an agricultural preplant seed coating, notably in corn and soybean, but are also used in forested areas to control invasive insects.[5,6,7]
- Increased application has led to levels of neonicotinoid contamination in aquatic habitats that are above what the US EPA deems safe to aquatic life. [5,6,8] (Fig. 1)
- Chemical contamination in aquatic environments has been shown to disrupt the endocrine system and alter the stress response in amphibians.[9]
- The vertebrate stress response is associated with changes in metabolism, including glucose and triglyceride levels in the blood.[10]
- The purpose of this study is to examine the impacts of environmentally relevant levels of imidacloprid exposure on blood glucose and lipid metabolism of adult male African Clawed Frogs (*Xenopus laevis).*

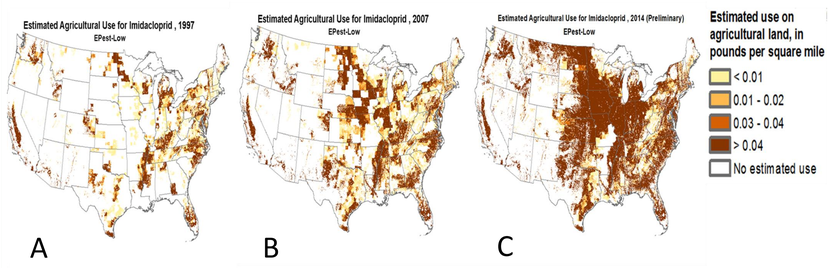


Figure 1. Estimated 20-year imidacloprid use in the US in 1997 (A), 2007 (B) and 2014 (C) (adapted from water.usgs.gov).

Materials & Methods

- Adult male *X. laevis* (Fig. 2) were housed individually and exposed to environmentally relevant imidacloprid concentrations for 48 days via immersion (Fig. 3).

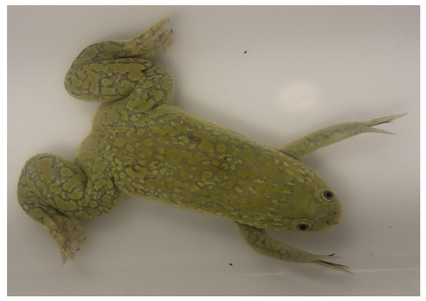


Figure 2. Adult male *Xenopus laevis*

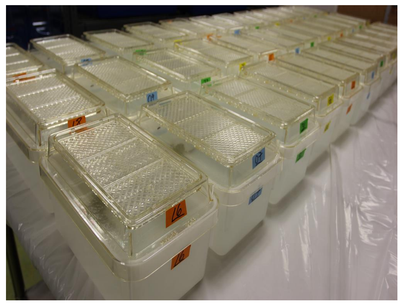
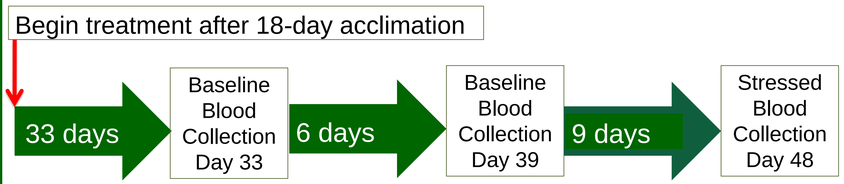


Figure 3. Frogs were exposed to imidacloprid via immersion

- Imidacloprid treatments were based on concentrations found in surface water prior to this study. [5]
  - Control = ethanol vehicle (0.02%)
  - 0.3 parts per billion (ppb)
  - 3.0 ppb
  - 300 ppb



- Plasma samples were collected from adult male *X. laevis* (n=9 per treatment) on day 33 to establish the baseline response to imidacloprid treatment. Additional blood samples were collected at day 39 (baseline treatment) and day 48 (handling stress + treatment).
- **Plasma Glucose Assay**: Glucose was determined via colorimetric assay. The glucose assay quantitatively and enzymatically determines glucose levels[11] in the plasma and has been scaled down to accommodate a 96-well plate.
- **Glycerol and Triglyceride Assays:** Glycerol and triglycerides were measured via colorimetric assays. Protocols (adapted from Guglielmo et al. 2004 and Fokidis et al. 2012) used a commercially available reagent and were validated for *X. laevis* and scaled down to accommodate a 96-well plate. Free glycerol was measured directly while total triglyceride is an estimate following enzymatic removal of glycerol from triglycerides by hydrolysis.[12, 13] Triglycerides serve as a fat reserve (deposition) and under stress may be mobilized into free glycerol.[13] Thus, levels of free glycerol versus triglycerides may potentially serve to indicate the response to an environmental stressor.
- All measurements were analyzed by use of appropriate ANOVA.

Acknowledgments

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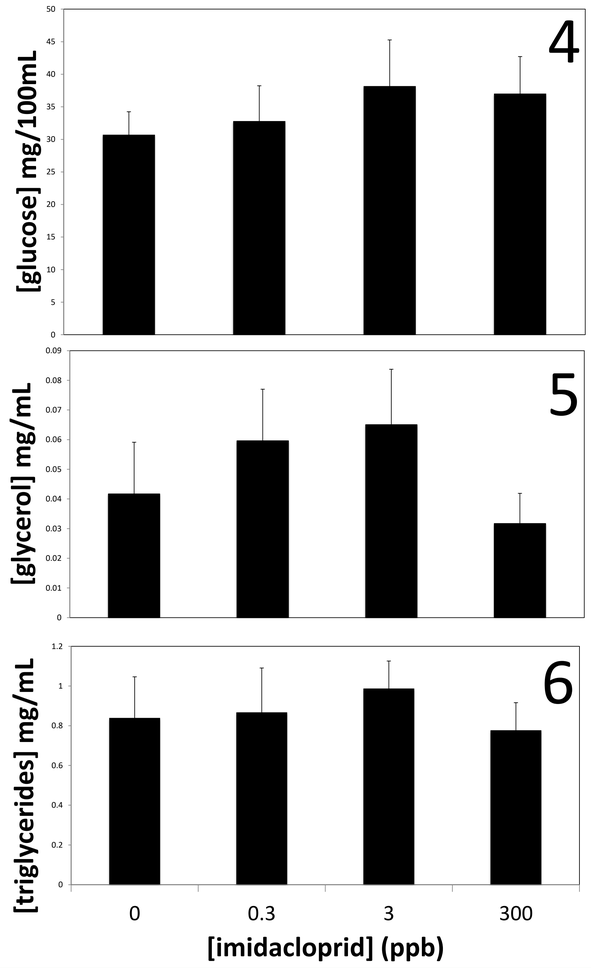
Results

- Plasma glucose (Fig. 4) free glycerol (Fig. 5) and total triglyceride (Fig. 6) levels were not significantly altered ($p>0.05$) by imidacloprid exposure at day 33 in preliminary analysis of adult male *X. laevis.*



Discussion

- Imidacloprid exposure generally, though not significantly, increased plasma glucose levels at day 33.
- Additional samples collected on day 33, as well as days 39 and 48, are yet to be analyzed and will provide a more complete metabolite profile of long-term imidacloprid exposure in adult male *X. laevis*.

References

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