

Analysis of Developmental Exposure to Imidacloprid on Blood Glucose and Lipid Metabolism in African Clawed Frogs (*Xenopus laevis*)

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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, information on the impacts of widely used neonicotinoid insecticides on amphibian life is scarce.^{2,3}
- Neonicotinoids mimic nicotine and consequently alter the function of the central nervous system.⁴
- As a result of increased neonicotinoid use to control invasive forest insects, aquatic habitats show levels of neonicotinoid contamination that are above what the US EPA deems safe to aquatic life.^{5,6} (Fig. 1)
- Chemical contamination in aquatic environments has been shown to disrupt the endocrine system and stress response in amphibians.⁷

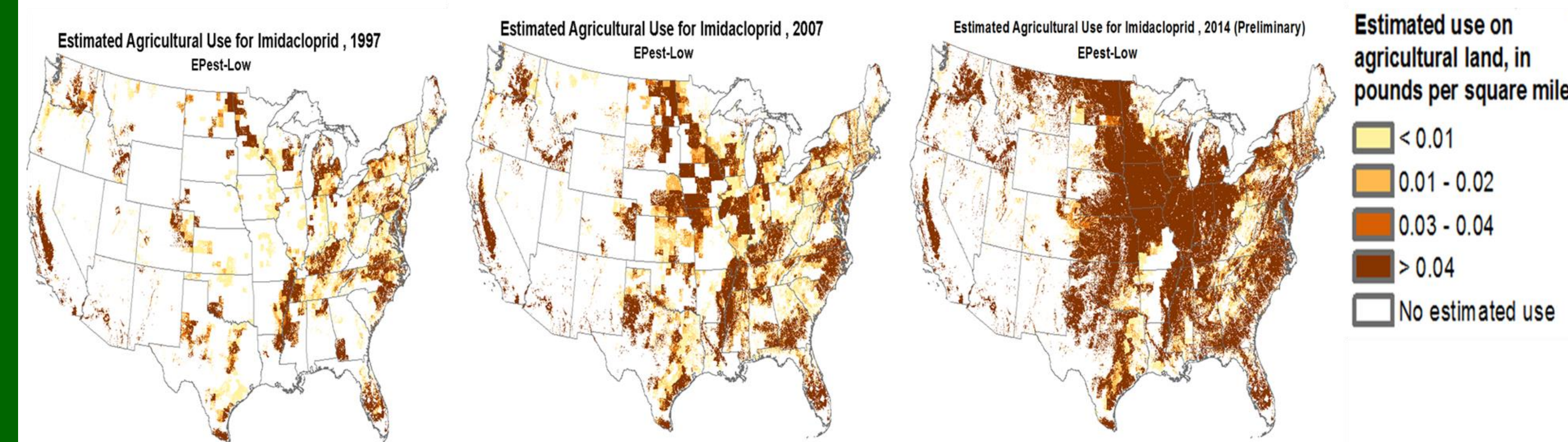


Figure 1. Estimated 20-year imidacloprid use in the US in 1997 (A), 2007 (B) and 2014 (C) (adapted from water.usgs.gov).

- The purpose of this study is to examine the impacts of environmentally relevant levels of imidacloprid exposure on blood glucose and lipid metabolism of African Clawed Frog (*Xenopus laevis*) (Fig.2). It is hypothesized that imidacloprid negatively alters metabolism in these amphibians.

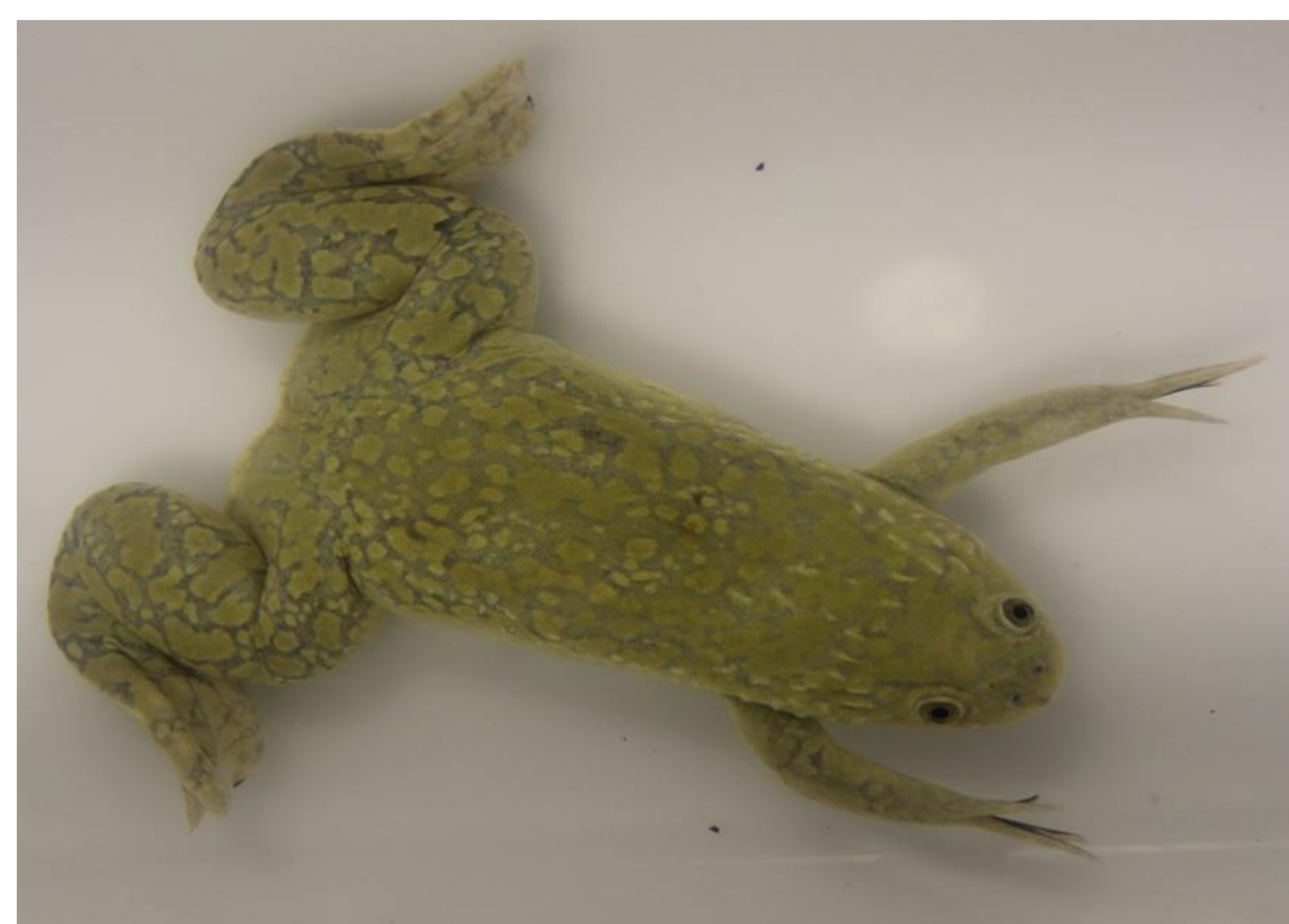


Figure 2. Adult male *Xenopus laevis* from the laboratory of Dr. P. Falso (photo courtesy of P. Falso)

Materials & Methods

- African clawed frogs (*Xenopus laevis*) were exposed to four environmentally relevant concentrations of the neonicotinoid insecticide, imidacloprid, throughout development from hatching through metamorphosis. (Fig.3)
- A subset of the animals was maintained through maturity in the appropriate imidacloprid dose following metamorphosis. (Fig.4)
- Nutrient metabolism will be examined by measuring plasma glucose, triglycerides, and free glycerol.
- Treatment groups:
 - ethanol vehicle (0.2%)
 - 0.3 parts per billion (ppb) imidacloprid
 - 3.0 ppb imidacloprid
 - 30 ppb imidacloprid
 - 300 ppb imidacloprid



Figure 3. Group housed *X. laevis* larvae were exposed to five different environmentally relevant treatment groups throughout metamorphosis. (image courtesy of P. Falso)



Figure 4. Twelve frogs from each treatment were housed individually through sexual maturity in continual exposure to the above levels of imidacloprid. (image courtesy of P. Falso)

- Blood samples were previously collected from these animals at sexual maturity to examine plasma concentrations of endocrine hormones and glucose metabolism.
- **Plasma Glucose Assay:** The glucose assay has been previously validated in the lab of P. Falso and scaled down to accommodate a 96 well plate.
- **Validation of Parallelism:** A standard curve was generated with known concentrations of glucose and dilution of plasma produced a linear relationship relative to the standard curve.
- **Validation of Accuracy:** The accuracy of the glucose assay was tested through the addition of 60 mg/mL glucose to male and female *X. laevis* plasma samples. This kit accurately measures glucose in *X. laevis*.
- **Plate Reading and Analysis:** 96 well plates were incubated at 37 degrees C and absorbances were read at 450 nm by the PerkinElmer Scientific EnSpire plate reader. This data was accordingly analyzed.

Results

We validated the parallelism (Fig. 5) and accuracy (Fig. 6) of the Sigma glucose assay kit with plasma of *X. laevis*. From our standard curve and standard curves of previous researchers in this study, we found that a minimum of 6 μ L of plasma was needed for accuracy. The kit accurately measures glucose in *X. laevis* plasma without interference.

Figure 5. Validation of Parallelism shows that we could use 6 μ L of plasma to get accurate glucose readings.

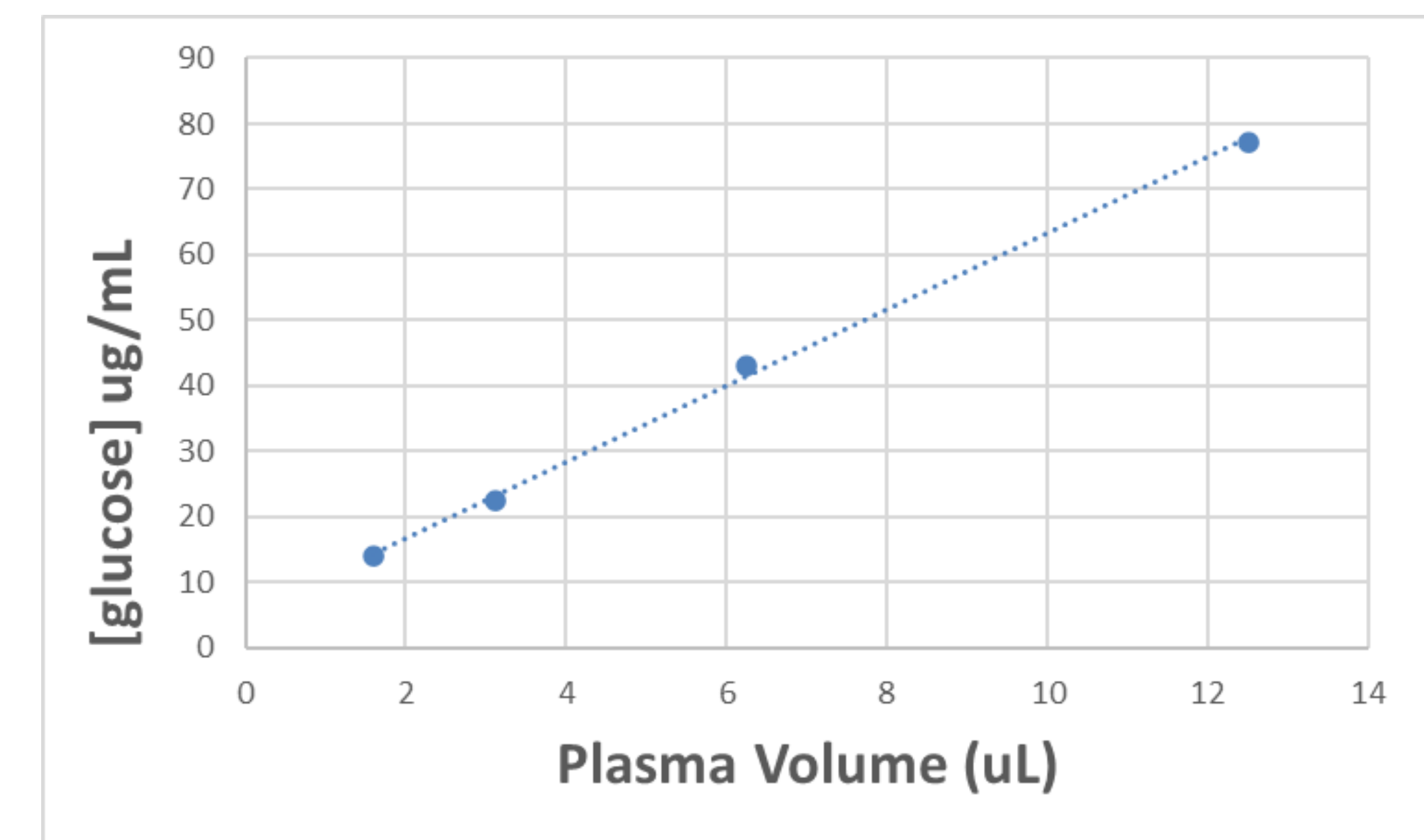
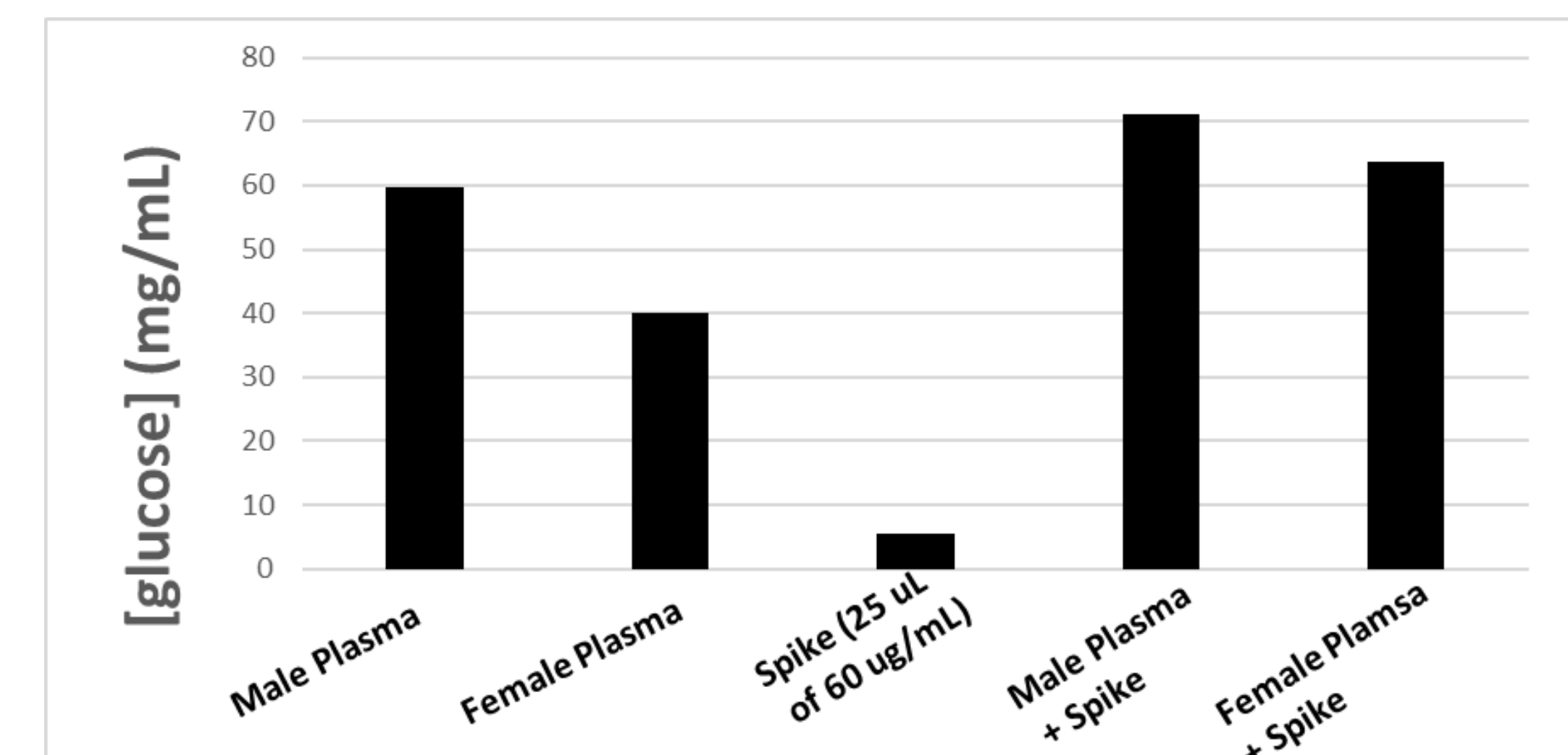


Figure 6. Validation of Accuracy shows that the added male plasma and spike amount to the expected spike. The female plasma with spike is slightly higher than expected and we plan to continue to monitor this difference.



References

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Acknowledgements

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