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Sublethal Effects of Sulfoxaflor Pesticide on Physiology and Behavior of Daphnia magna

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Introduction

As worldwide agriculture grows to meet increasing food demands, crop damage due to insect pest species has become an increasing concern. The consistent application of pesticides used to fend off pest species has caused many insects of concern to develop resistance to the chemicals. In order to address increasing insect resistance to current pesticides, sulfoxaflor was developed by DOW AgroSciences under the name of IsoclastTM Active [1]. It is marketed as a valuable pesticide in rotational use as insects currently resistant to other pesticides showed no signs of resistance to sulfoxaflor in preliminary studies [1]. As sulfoxaflor application protocols require a "drying period" after being applied as a wet spray, rain events are likely the main factor that introduces the pesticide into aquatic environments. Preliminary exposure studies on rodents resulted in neonatal effects in rats and the development of liver tumors in both rats and mice [1]. Slight effects to the growth of the fathead minnow and moderate oral toxicity in birds were also identified [1]. Little research into lethality and sub-lethal effects for aquatic invertebrates has been conducted since its initial approval for use by the EPA, and no studies have been done to examine the presence and concentrations of sulfoxaflor in aquatic environments. This study aims to identify the potential environmental effects of sulfoxaflor on *Daphnia magna* by analyzing various endpoints including lethality, heartrate, and mobility after 48hrs of exposure.

Hypotheses

For mortality assays, we hypothesized that the range of concentrations would not encompass an LD50 for *Daphnia magna*. For behavioral assays, we hypothesized that due to sulfoxaflor's mode of action in insects, daphnia would experience a decreased heart rate and decreased mobility.

Materials and Methods

Organism Selection

- Adult daphnia were used in each of the assays, and were maintained in stock colonies at consistent photothermal conditions (75°F, 16hr : 8hr light : dark). Daphnia were fed a diet of spirulina powder *ad libitum*.

Treatments and Sulfoxaflor Stock Creation

- 10mg of lyophilized sulfoxaflor (CAS #946578-00-3) was dissolved in 10mL of ethanol. 1:10 serial dilutions were then performed to create treatment superstock solutions. A 1:1000 (superstock: H_20) dilution was then performed to create working stock solutions. Treatments were 0 (ethanol control), 0.1, 1.0, 10, 100, and 1000 µg/L.

Mortality Assay

- Daphnia mortality was analyzed after 48hrs of exposure. If no movement was found, the daphnid was placed under a microscope and heart located; death was defined as a lack of heartbeat.

Heartrate Assay

- Daphnia were placed on a well slide with 40 µg of control water and 5 minutes was allowed for acclimation. One minute recordings were then obtained using the ToupView program, with total number of heart contractions quantified over the course of the minute-long recording to determine beats per minute (bpm).

Behavior Assay

- At 3 and 24 hours, individuals were placed in 5 mL of water in a 50 mL beaker, placed directly under a camera with controlled lighting, and allowed to acclimate for 3 minutes. A 3 minute recording was then obtained and later analyzed using the ToxTrac program for various mobility characteristics.

Daphnia Heart Morphology

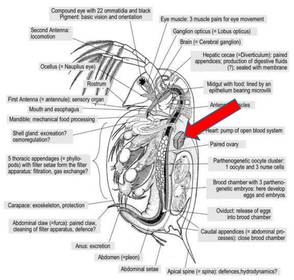

Figure 1: Morphology of *Daphnia magna* in full; arrow indicates location of heart

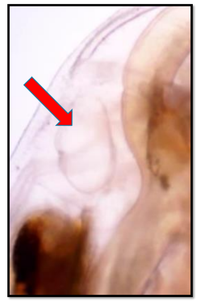

Figure 2: Daphnid with a healthy, properly functioning heart

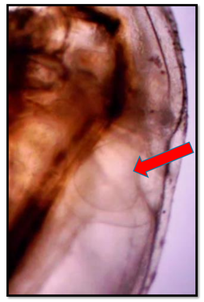

Figure 3: Daphnid with an enlarged heart due to sulfoxaflor exposure

Figure 4: Daphnid with blood adhesion due to sulfoxaflor exposure

Results

Concentration	Mortality (# death events)
0 ug/L	0
0.1 ug/L	0
1 ug/L	0
10 ug/L	0
100 ug/L	1
1000 ug/L	0

Table 1: Total mortality at each sulfoxaflor concentration after the 24-hour mark; n=9

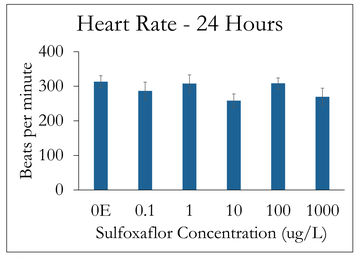

Figure 5: Average heart rate of daphnia per sulfoxaflor concentration; n=9 and error bars are SEM

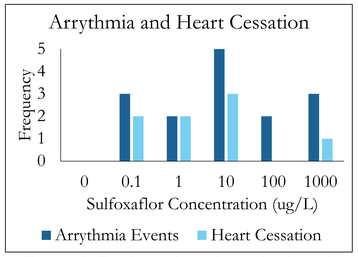

Figure 6: Total number of arrythmia and heart cessation events after 24 hours; n=9

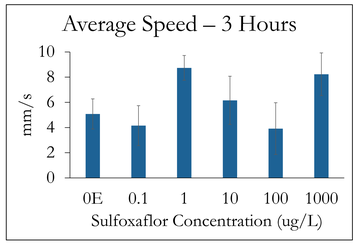

Figure 7: Average movement speed of daphnia after 3 hours; n=9 and error bars are SEM

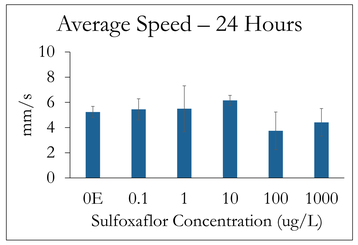

Figure 8: Average movement speed of daphnia after 24 hours; n=9 and error bars are SEM

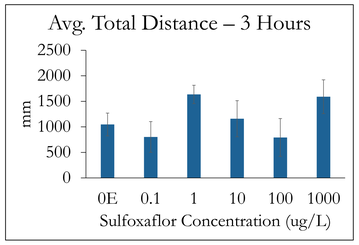

Figure 9: Average total distance traveled of daphnia after 3 hours; n=9 and error bars are SEM

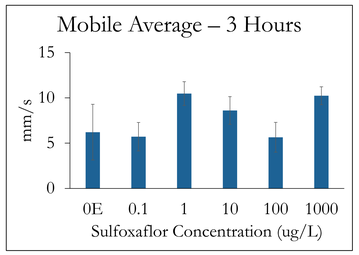

Figure 10: Average acceleration rate of daphnia after 3 hours; n=9 and error bars are SEM

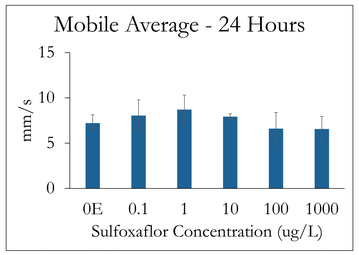

Figure 11: Average acceleration rate of daphnia after 24 hours; n=9 and error bars are SEM

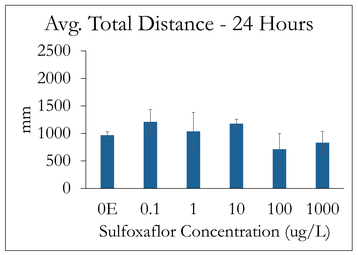

Figure 12: Average total distance traveled of daphnia after 24 hours; n=9 and error bars are SEM

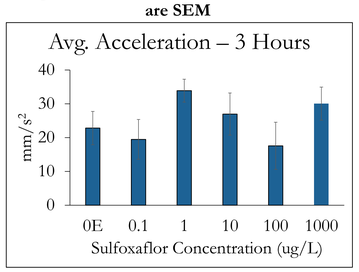

Figure 13: Average acceleration of daphnia after 3 hours; n=9 and error bars are SEM

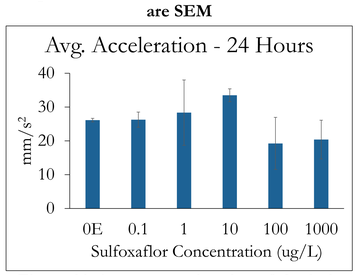

Figure 14: Average acceleration of daphnia after 24 hours; n=9 and error bars are SEM

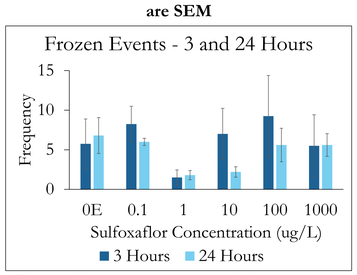

Figure 15: Average number of frozen events of daphnia after 3 hours (n=4) and 24 hours (n=5); error bars are SEM

Discussion

Mortality Assays

- Based on our results presented in Table 1, we found our selected concentrations of sulfoxaflor to be in the sublethal range. Our results support the potential for impacts of sulfoxaflor on non-lethal endpoints in aquatic environments.

Heart Rate Assays

- Based on the data in Figure 5, exposure to certain concentrations of sulfoxaflor resulted in a depressed heartrate trend when compared to the ethanol control. Based on the data in Figure 6, exposure to sulfoxaflor also led to the occurrence of arrythmia and heart cessation events. Figures 3 and 4 also indicate morphological stress responses to sulfoxaflor.

Mobility Assays

- Our results suggest that ToxTrac is an effective method for assessing the impacts of chemical contaminants on *Daphnia magna*. While we did not observe significant differences between treatments, results may have been influenced by our small size (n=9). Based on our results, there is noticeable difference in mobility impacts between 3 and 24 hours of exposure.

Overall Discussion

- Given our results, it can be determined that the detrimental effects of sulfoxaflor extend beyond mortality. Heart rate appears to be the most sensitive endpoint analyzed, given trends in depression and occurrence of arrythmia. These data encourage further exploration into sub-lethal behavioral and morphological effects of sulfoxaflor on aquatic invertebrates.

Conclusions

Based on our results, it appears that sulfoxaflor causes multiple detrimental effects on the endpoints assessed in this study. Our findings suggest that further research is required to elucidate the impacts of sulfoxaflor on aquatic invertebrates and aquatic ecosystems. Although sample sizes for these studies are small, our results indicate that sulfoxaflor may negatively impact the mobility and physiology of *D. magna* at environmentally relevant concentrations, suggesting that further exploration into its potential effects on other aquatic non-target invertebrate species is warranted.

Future Work

Determining the potential generational and reproductive effects of the pesticide require reproductive assays quantifying reproductive maturity and health of offspring. Research into degradation of the chemical in aquatic environments may also give insight into whether exploration of chronic exposures are warranted. As research concerning the effects of sulfoxaflor on non-target species is limited, such assays will aide in the overall understanding of the impacts of sulfoxaflor toxicity beyond mortality in aquatic environments.

References

1. EPA. 2016. Decision to Register the Insecticide Sulfoxaflor with Limited Uses and Pollinator Protective Requirements. Environmental Protection Agency. https://www.epa.gov/ingredients-used-pesticide-products/decision-register-insecticide-sulfoxaflor-limited-uses-and.
2. Sparks, Thomas C.; Watson, Gerald B.; Loso, Michael R.; Geng, Chaoxian; Babcock, Jon M.; Thomas, James D. Sulfoxaflor and the Sulfoximine Insecticides: Chemistry, Mode of Action and Basis for Efficacy on Resistant Insects, *Pesticide Biochemistry and Physiology*. 2013, 107: 1-7.

Acknowledgements

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