

### Cytotoxic Effects of Imidacloprid Pesticide on HEK 293T Human Cells

S. M. Herwald, M. J. S. Falso, S. L. Hrizo Slippery Rock University Department of Biology



## Introduction

The purpose of this study is to examine the potential cytotoxic effects of the pesticide imidacloprid. This compound is a neonicotinoid and has been consistently used for insect control on crops in the United States since the late 1980s despite being banned in Europe (1,2).



**Figure 2**. Estimated use of imidacloprid in the US (5).

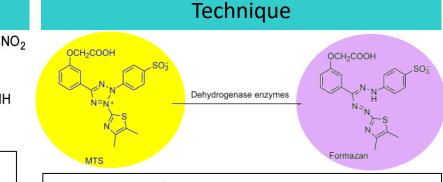
Imidacloprid is potentially dangerous because of its uptake and translocation in plants, unable to be rinsed off and thus exposing humans through intake via consumption. Previous studies using imidacloprid on nonhuman cell types indicate cellular stress and damage on reproductive and nervous system cells, and inflammation in liver

and other organs (3).

Figure 1. The chemical

structure of imidacloprid.

Studies performed on human lymphocytes indicate DNA fragmentation and damage (4). This experiment will utilize HEK 293T human embryonic kidney cells treated with varying concentrations of imidacloprid to determine levels of cell death after 24 hours of exposure.



**Figure 3.** Reaction of MTS to Formazan by dehydrogenase enzymes that occurs in a metabolically active cell. The presence of more metabolically active cells results in a deeper color change that can be quantified by collecting absorbance values.

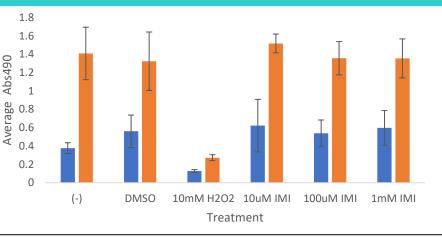
Human HEK 293T cells were cultured in DMEM with L-glutamine, fetal bovine serum, and penicillin + streptomycin media and incubated at 37°C and 5% CO2. For the assay, cells were plated into a 96 well plate with 7,500 cells in 100 $\mu$ L of media per well. After 24 hours, media was aspirated off and cells were treated with 100 $\mu$ L media containing treatment. Designated cells were treated with untreated media, 10 mM H<sub>2</sub>O<sub>2</sub> (positive control), 10  $\mu$ M imidacloprid, 100  $\mu$ M imidacloprid, and 1 mM imidacloprid. A DMSO vehicle control was also included. After 24 hours, 20  $\mu$ L of MTS reagent was added to each well and absorbance values at 490 nm were collected after 1 hour and 2 hours.

#### References

1. Bass, C. Current Biology 28, R772-R773 (2018).

Douglas, M. R. & Tooker, J. F. *Environ Sci Technol* 49, 5088-5097, (2015).
Duzguner, V., & Erdogan, S. *Pest Biochem Phys*, 97, 13-18 (2010).
Costa, C., et al. *Mut Res/Genet Tox Env Mut*, 672(1), 40-44 (2009).
water.usgs.gov

# Results



**Figure 4.** Average absorbance values after 1 hour (blue) and 2 hours (orange) post-MTS reagent addition. Imidacloprid (IMI) treatments show no significant change in cellular metabolic activity upon treatment with varying dosages. (-) is media only. DMSO is the vehicle control. Error bars represent standard deviation among average absorbance values. Higher absorbance values represent more conversion to the formazan end product, indicating more metabolically active cells. n=5

## **Conclusion & Future Directions**

Imidacloprid showed no cytotoxic effects on HEK 293T cells. When compared to the hydrogen peroxide positive control, there is no significant cellular death as represented by absorbance values. Future research directions include treatments exceeding 24 hours, and treatment on other cell lines.