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The Role of Pyrethroids on Cell Cycle Regulation and Craniofacial Development in Xenopus laevis

Kylee Hockaday

Deborah Howton

The Effect of Pyrethroids on Orofacial Development

VIRGINIA COMMONWEALTH UNIVERSITY
College of Humanities and Sciences

Kylee Hockaday, Deborah Howton, & Amanda Dickinson

Department of Biology, Virginia Commonwealth University.

Abstract

Pyrethroids are synthetic insecticides that are found in many household items such as pet flea medication. Pyrethroids are used more so than organophosphate pesticides due to increasing concerns about non-target effects. However, there is some concern with pyrethroid exposure resulting in teratogenic effects including craniofacial defects. Using a Xenopus model, three pesticides (AMDRO Quick Kill, Pyrethrum, and Cypermethrin) were tested for their effect on craniofacial development. Exposure to AMDRO Quick Kill, pyrethrum, and cypermethrin resulted in development of craniofacial defects in Xenopus. Following exposure, various measurements were collected. Cypermethrin exposure led to a decrease in face width in embryos. Results from pH3 immunohistochemistry and preliminary acridine orange experiments suggests that cell proliferation and death may be affected by pesticides. Results from qRT-PCR suggest that pesticide exposure results in an increase in p53 expression.

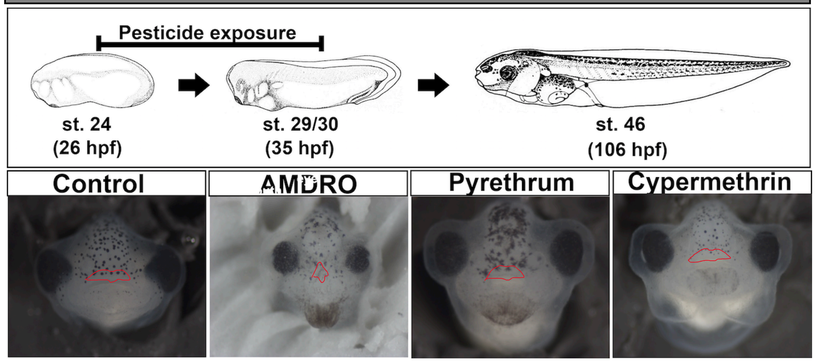
Craniofacial Defects

- Deformities of the head and facial bones, usually present at birth
- Most common: cleft lip/palate
  - 1-2 babies per 1000 each year are born with cleft lip or palate in the US
  - Can have great impact on how a child socializes
- Known or potential causes:
  - Genetics/family history
  - Vitamin/folic acid deficiency
  - Environment → Pesticides?

Pyrethroids

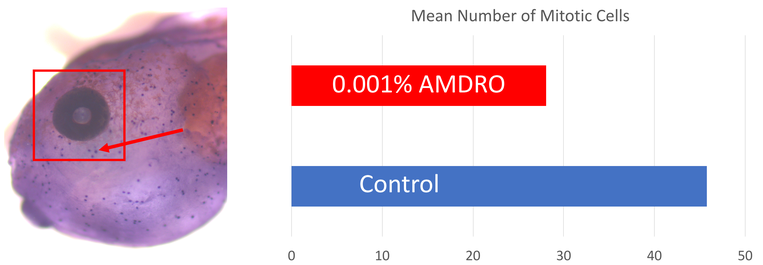
- Pyrethroids are synthetic chemical insecticides, commonly found in household products
  - Flea & tick shampoo/medication
  - Store-bought insecticides
- Currently considered to have low-moderate acute toxicity towards non-target species
- Arising concerns of toxicity to more susceptible populations (i.e. children) (DeMicco et. al 2009)
- Study on pyrethroid effect on zebrafish resulted in craniofacial defects and edema following exposure (Insecticides in Zebrafish Embryos)

Pyrethroid exposure causes craniofacial defects



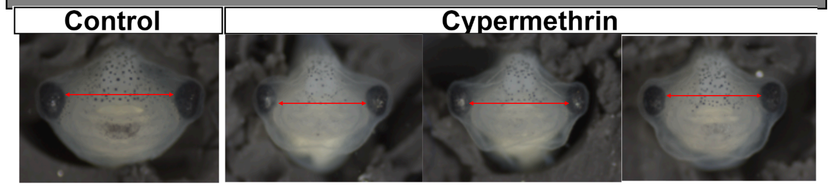
Xenopus embryos were treated with AMDRO Quick Kill, Pyrethrum, or Cypermethrin between stage 24 and stages 29/30. At stage 46, embryos were washed and fixed in 4% PBT. Craniofacial abnormalities were seen in all treatment groups. Cleft palates, indicated by a triangular-shaped mouth, developed in AMDRO Quick Kill-treated embryos. Edemas developed in all treatment groups.

Pyrethroid exposure may affect cell proliferation and death

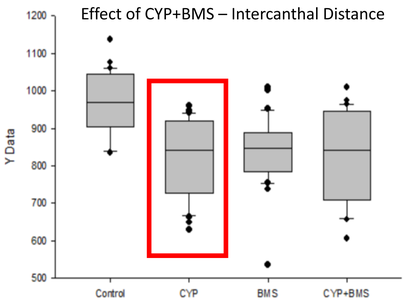


Embryos were treated with AMDRO Quick Kill before being fixed in 4% Paraformaldehyde. Using a pH3 antibody, immunohistochemistry techniques were applied to stain mitotic cells a deep purple color. Following the staining, images were taken of the embryo heads from a lateral angle. Uniformly sized boxes were placed onto each image, centered on the eye. Mitotic cells, the dark purple spots, within the boxes were counted for each embryo within each group and analyses revealed a significant difference in the number of mitotic cells in AMDRO treated embryos and control embryos. Preliminary acridine orange experiments suggest pyrethroids affect cell death.

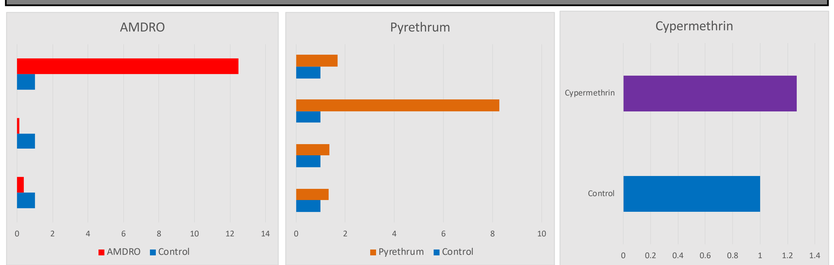
Cypermethrin exposure results in decreased face width and death



Embryos were treated with Cypermethrin then imaged using light microscopy. Measurements of intercanthal distance of embryos, control and treated, were taken. Analyses revealed a significant difference in intercanthal distance of control embryos and those treated with Cypermethrin.



Pyrethroid exposure causes an increase in p53 protein expression



Embryos were treated with a pyrethroid (AMDRO Quick Kill, Pyrethrum, or Cypermethrin) before RNA was extracted. qRT-PCR was then performed on each using a p53 primer and a housekeeping primer (ef1-alpha). All treatment groups showed an average increase in p53 protein expression compared to controls.

Future Directions

- More extensive morphometric analyses would help to further our understanding of pyrethroid effect on facial development

References

DeMicco, A., Cooper, K. R., Richardson, J. R., & White, L. A. (2009). Developmental Neurotoxicity of Pyrethroid Insecticides in Zebrafish Embryos. Toxicological Sciences,113(1), 177-186. doi:10.1093/toxsci/kfp258

https://www.stanfordchildrens.org/en/topic/default?id=overview-of-craniofacial-anomalies-90-P01830