Phenanthrene Exposure Affects Cardiovascular Function in African Clawed Frog (*Xenopus laevis*) Embryos

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Introduction

Industrialization and the incomplete combustion of organic matter have caused a significant increase in concentrations of polycyclic aromatic hydrocarbons (PAHs) in the natural environment [1,2]. Major sources of phenanthrene, a tricyclic PAH, include fossil fuel combustion, waste incineration, and weathered crude oil [3]. While phenanthrene has been cited on the Environmental Protection Agency's (EPA) priority pollutant list and the Center for Disease Control's (CDC) toxic substance registry, little is known about its toxicity to humans or wildlife [2,3].

Phenanthrene was found to have cardiotoxic effects in fish including: pericardial edema, cardiac arrhythmia as a 2:1 atrioventricular conduction block, reversible dose-dependent bradycardia (decrease in heart rate), abnormal cardiac morphology, and reduced circulation [4-7]. In previous experiments, we found that phenanthrene exposure also induced bradycardia in *X. laevis* embryos. Here we present evidence that the bradycardic response to phenanthrene was rapid and reversible and that phenanthrene exposure causes cardiac arrhythmia.

Cardiac Morphological event	Stage of development [8]
Specification	12-14
Migration of heart progenitors to ventral midline	26-28
Heart tube formation	31-33
Looping	33-36
Coordinated muscle contraction	35
Chamber differentiation	39-40
Valve formation	41-44
Atrial partitioning	44-45
Mature heart	46

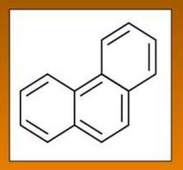
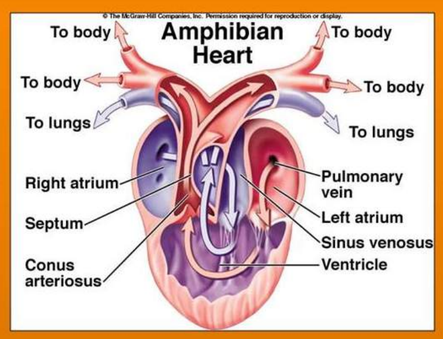


Figure 1. Chemical structure of phenanthrene, a tricyclic polyaromatic hydrocarbon



Methods

Obtaining Embryos
Adults from the Keene State College *Xenopus laevis* breeding colony were bred according to IACUC standards and procedures. Embryos were placed in 10% Holtfreter's at 22ºC in six-well plates up to stage 45 [8], after which they were housed in 250mL beakers.

Preliminary Study (Figure 2.)
Embryos were exposed to 5 ppm phenanthrene (n=12) for 24 or 48 hrs or continuously, with controls in 0.05% DMSO (vehicle). Following acclimation to RT for 1 hr, heart rates were acquired at stages 37, 42 and 45/46 with use of a dissecting microscope complete with Leica® imaging and Screencast-O-Matic® recording software.

Heart Rate and Arrhythmia Studies
Observations were completed using an inverted dissection microscope with Leica® imaging and Camtasia® recording and editing software. Initial heart rates of stage 42 embryos were recorded. Embryos were then exposed to either 0.05% DMSO or 5 ppm phenanthrene (n=5). All individuals were exposed for 10.5 hrs, and heart rates monitored periodically. Embryos were then transferred to 10% Holtfreter's solution and a recovery heart rate was recorded at 5 hrs post-exposure. Arrhythmia was characterized by quantifying interbeat variability [4]. In a subsequent experiment, the heart rates of stage 46 larvae exposed to varying doses of phenanthrene were monitored during exposure and recovery.

Results

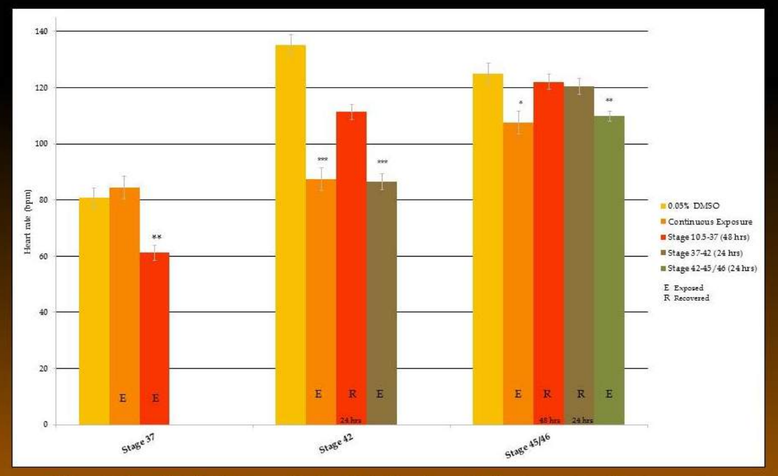


Figure 2. Preliminary study: Heart rates of the same embryos were monitored over three different stages of development. Embryos were either exposed (E) to 5 ppm phenanthrene or 0.05% DMSO for varying periods of time and some were allowed to recover (R).

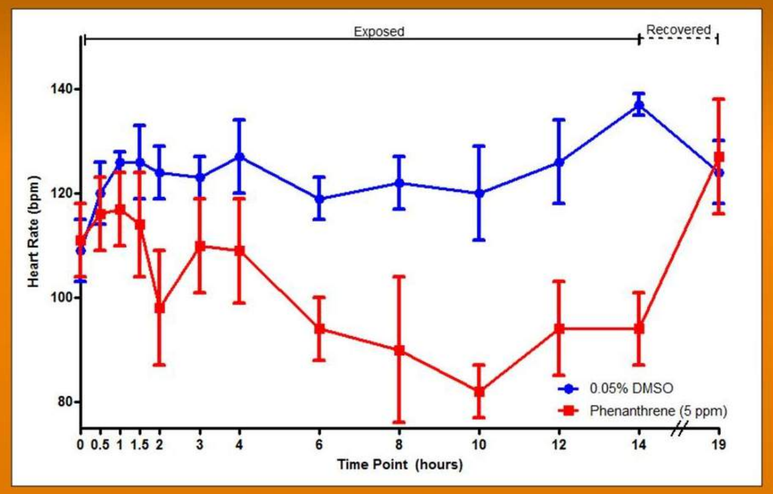


Figure 3. Embryos exposed to 5 ppm phenanthrene at stage 42 demonstrate reversible bradycardia. The heart rates of the treatment and control groups were measured prior to exposure to DMSO and phenanthrene (time 0).

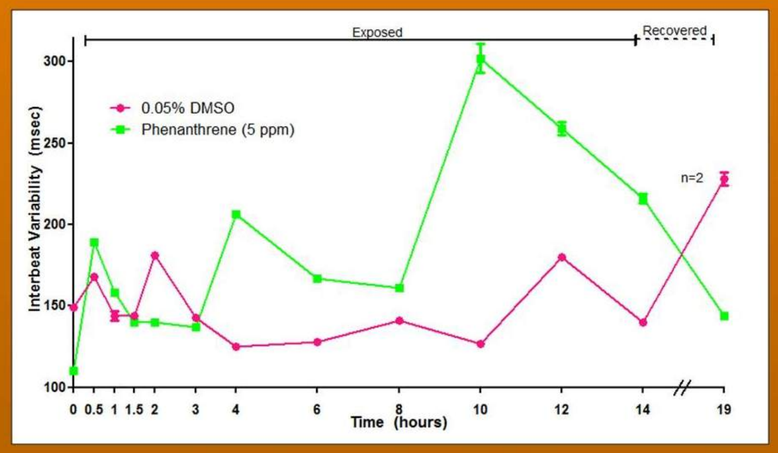


Figure 4. Companion arrhythmia data to experiment illustrated in Fig. 3. Note that interbeat variability is reciprocal to heart rate and that the greatest interbeat variability was observed following 10 hours of exposure, which corresponds to the nadir in heart rate.

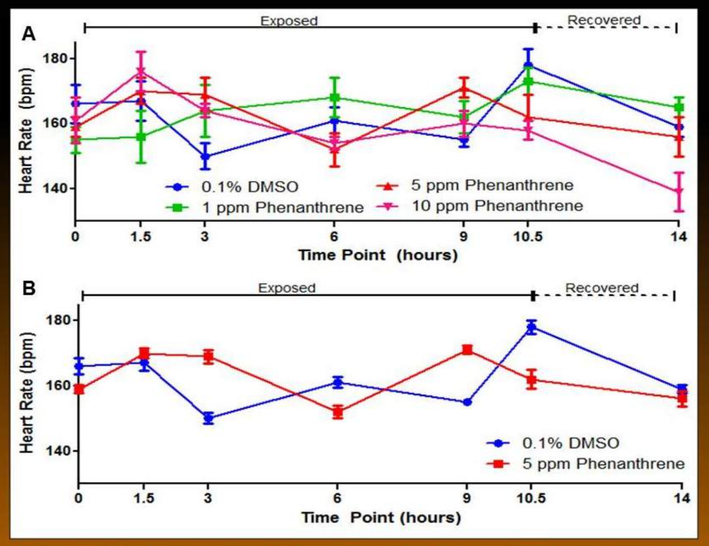


Figure 5. A) Animals were exposed to varying doses of phenanthrene and their heart rates monitored. **B)** In comparison to stage 42, hearts from stage 46 embryos do not demonstrate bradycardia when exposed to 5 ppm phenanthrene.

Figure 6. Video demonstrating normal heart rhythm v. phenanthrene induced arrhythmia

Conclusions

Xenopus laevis embryos exposed to phenanthrene at 5 ppm exhibit significant cardiotoxic effects including bradycardia and arrhythmia. These effects may be stage-dependent, as our preliminary experiments show stage 37 and stage 46 animals do not appear as sensitive to phenanthrene exposure as do stage 42 animals.

Future experiments are underway to assess the cardiac responsiveness to a range of doses of phenanthrene for stage 37 and stage 42 embryos to compare with that of stage 46 animals. In addition, we will be investigating the effects of phenanthrene exposure on circulation and cardiac morphology. Our long-term goal is to understand the mechanism by which phenanthrene exerts its cardiotoxic effects on the developing cardiovascular system.

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Heart photo: http://dtc.pima.edu/~biology/182/lesson11/11step3/11step3images/amphibianheart2.jpg

Acknowledgments: This research was funded by NH-INBRE (Whittemore). Thanks to Dr. Cynthia Hays for statistical help and Dr. Loren Launen for the phenanthrene. Thank you to Liz Richardson and Julia Pinette for helping conduct the research, along with the rest of the Whittemore lab. Krist Hausken is a NH-INBRE research intern.