

Report for 2003KY21B: Effects of a waterborne herbicide, Atrazine, on the auditory physiology of fish

- Conference Proceedings:
 - Anraku, Kazuhiko, N.V. Lintecum, and H.Y. Yan, 2004, Effects of water acidification on chemoreception ability of fathead minnow (*Pimephales Promelas*), in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 47-48.
 - Lintecum, N.V., and H.Y. Yan, 2004, Effect of acidification on fish olfactory response to alarm substance, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 49-50.

Report Follows

Problems and Research Objectives

Atrazine (2-chloro-4-ethylamino-6-isopropylamine-1,3,5-triazine) is a major agricultural herbicide that has been in use worldwide for more than 40 years. It is the primary pesticide used on corn. In Kentucky, about 423,000 kg of atrazine was used on 1,562,000 acres of farmland in 1999. Concentrations of atrazine in runoff from more than 1,543,000 acres exceeded the 3 ppb maximum contaminant level set by United States Environmental Protection Agency. Recent studies raise concern for all aquatic life in atrazine-contaminated field runoff.

This project investigates the neurotoxicological effects of atrazine exposure on the auditory physiology of a cyprinid fish, the fathead minnow (*Pimephales promelas*), that is widely distributed in the waterways of Kentucky. Atrazine can alter thyroid function of animals. Animals with compromised thyroid glands may suffer great loss of auditory function through altered calcium channels which play an important role in auditory physiology. Hearing is a major sensory modality used by fish for survival in the wild (predator evasion, prey detection, mate recognition, conspecific and heterospecific communication). A compromised mechanosensory system may render fish unable to survive in the wild even though their reproductive system may not be affected by atrazine. Since fathead minnow is a cosmopolitan species, it can serve as a biological indicator and the results of neurotoxicological assays can be applied to the majority of fish fauna in Kentucky.

Methodology

A total of 160 young fathead minnows were used in the experiment. Each 10 fish were randomly selected as a group and housed in individual 10-gallon glass tanks (with aeration but no filtration—to avoid atrazine being filtration by the system). Each tank received one of the following combinations: exposure concentration: 3 ppb, 0.3 ppb, 0.03 ppb or 0 ppb (the control); exposure duration: 2, 4, 6 or 8 weeks. Such a 4x 4 design allowed detailed investigation on how atrazine exposure concentration and duration impact auditory responses of fish. At the end of each exposure interval, fish were subjected to auditory brainstem response (ABR) recording to document changes of hearing threshold over the experimental period (and compared to the baseline group). At the end of each ABR recording, select fish from each exposure regimen were perfused with physiological saline followed with histological fixative and their lower jaws were processed for histological examination of changes in their thyroid gland follicles.

Principal findings and Significance

The hearing thresholds for the 3 ppb group started showing significant elevation by 4 weeks of exposure. However, no significant increase in mortality was observed (as compared to the control group). For the 0.3 ppb group, significant changes only showed after 8 weeks of exposure. Less than 8 weeks exposure at 0.3 ppb apparently did not exert any significant physiological impacts to the endocrine systems that regulate the hearing ability of these fish. For the 0.03 ppb exposure group, no hearing threshold

changes could be observed in any exposure duration group. We are currently conducting a long term (6 months) low concentration (0.03 ppb) exposure experiment to delineate possible chronic exposure effects.

We are still in the process of analyzing the histological sections of thyroid glands of all exposed groups. Based on the data so far collected, exposure to the highest concentration (3 ppb) is associated with signs of hypothyroidism (i.e., reduction of secretion of thyroxin as indicated by shrinkage of follicle cells from the sections). The analysis of histological sections is a rather tedious and time consuming process. However, the completed histological analysis will add great power for the explanation of the hearing threshold data collected in this investigation.