Atrazine exposure produces same major metabolites as mammals along with adverse developmental effects

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Abstract

Atrazine (ATZ) is a triazine herbicide that is the second most commonly used in the United States and heavily applied to crops in the Midwestern US. ATZ is highly mobile in soils and contaminates potable water sources. Due to contamination concerns, the US EPA has set the maximum contaminant level in potable water sources at 3 parts per billion (ppb) or less. Depending on the time of year and sampling location, water sources often exceed this limit. ATZ has a long half-life and has also been implicated as an endocrine disrupting chemical in multiple species. The current study used a biomedically relevant model, the zebrafish, to test the hypothesis that developmental ATZ exposure generates metabolites similar to those found in mammals and alters morphology and behavior in developing larvae. Adult AB zebrafish were bred, embryos were collected, and exposed to 0, 0.3, 3, or 30 ppb ATZ from 1 to 120 hours post fertilization (hpf). Targeted metabolomics analysis found that zebrafish produce the same major metabolites as mammals: desethyl atrazine, deisopropyl atrazine, and diminochloroatrazine with ATZ exposure. Morphology measurements do not show significant differences in total length, head length, head width, and brain length in any treatment group at 120 hpf (p>0.05). The visual motor response test detected hyperactivity in the 0.3 ppb treatment group and hypoactivity for the 30 ppb treatment group in comparison to control larvae (p<0.05). These findings suggest that a single ATZ exposure during early development generates metabolite profiles similar to mammals and leads to behavioral alterations supporting ATZ as a developmental toxicant.

Introduction

• ATZ is an herbicide used on agricultural crops in the Midwest and throughout the globe, and its metabolites can be detected in human urine.
• Maternal exposure is associated with small-for-gestational-age and birth defects.
• Embryonic ATZ exposure in zebrafish affects neural and reproductive systems development in larval and adult zebrafish.

Hypothesis

Developmental exposure elicits similar metabolite profiles found in mammals leading to morphological and behavioral alterations.

Methods

ATZ zebrafish exposure

Ab wildtype embryos were mixed, and embryos were collected at 1 hour post fertilization (hpf). For metabolomics, embryos were exposed to a range of concentrations to limit detection of equipment with the following concentrations: 0, 0.3, 3, or 30 ppb (µg/L). Zebrafish were exposed to atrazine from 1 hpf-120 hpf, and to cease exposure, larvae were rinsed for all experiments.

Results

Same major metabolites as those seen in mammals

No differences in morphology parameters assessed

Dose dependent hyper- or hypo- activity

Discussion

• High accumulation of the metabolite DACT in fish tissue agrees with higher ratios of DACT detected in human urine.
• No morphological changes occurred at 120 hpf, but previous studies in our lab show increased head length at 72 hpf.
• Behavioral hyperactivity was seen in 0.3 ppb treatment group in total distance, center-point movement and the first light phase of total distance moved within phase. 30 ppb treatment group hypoactivity in the second and third dark phase agrees with hypoactivity in a previous lab study done at 72 hpf.

Acknowledgements

References

5. Winibsky et al. 2015. Toxicology. 333: 156-67