US EPA ARCHIVE DOCUMENT

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Atrazine: A Tale Of Two Species (ED MYP)

Lesley Mills, Ruth Gutjahr-Gobell, Gerald Zaroogian, Saro Jayaraman, Doranne Borsay Horowitz, Mark Cantwell, Richard Pruell, Susan Laws (NHEERL, RTD)

Agency Problem

Endocrine disrupting chemicals (EDCs) are synthetic and naturally occurring chemicals that mimic or block the actions of hormones and can disrupt the normal functioning of an organism's endocrine system, resulting in developmental or reproductive problems.

The issues associated with EDCs intersect with EPA's requirements through several environmental laws including, the Toxic Substances Control Act (TSCA), the Food Quality Protection Act (FQPA), the Safe Drinking Water Act Amendments and the Clean Water Act (CWA).

ORD has developed an Endocrine Disruptors MYP (ED MYP) to help identify 1) chemicals that are EDCs and their mechanisms of action,
2) appmaches needed to assess risk to humans and wildlife from EDCs, and 3) effects that are occurring in wildlife populations due to EDC exposure.

This research supports several EPA offices, including the Office of Science Policy, the Office of Prevention, Pesticides and Toxic Substances, and the Office of Water.

Research Goals

To gain a better understanding of:

) the extent to which organism-level effects can be extrapolated across species

) the population-level ramifications of EDC exposure

Approach

The herbicide atrazine was selected as a model EDC for a case study using two species, a rat and a fish. Atrazine has been shown to change the activity of the steroidogenic enzyme aromatase, which is the enzyme complex that catalyzes the conversion of androgens to estrogens. Atrazine is known to disrupt normal reproductive processes in rats, fish and frogs. Our focus at AED was on atrazine effects in the marine fish cunner. AED scientists developed a strong collaboration with scientists at the Reproductive Toxicology Division in Research Triangle Park, NC, where rat studies were conducted.

Impacts and Outcomes

Through this research, we have identified a number of differences between species that indicate there are significant uncertainties involved in atrazine risk assessment across diverse species. This knowledge will help the Agency formulate accurate risk assessment approaches for EDCs in the environment, thereby minimizing the adverse ecological effects of EDCs on diverse wildlife species.

hemical of Interest: Atrazine

Widely-used broad-leaf herbicide

oncentrations in aquatic environments peak during spring and summer due to un-off from agricultural applications

n Texas estuaries, spring time water levels measured as high as 2000 ng/L, ith yearly average of 920 ng/L - Pennington *et al*, 2001, *J Environ Sci Health*

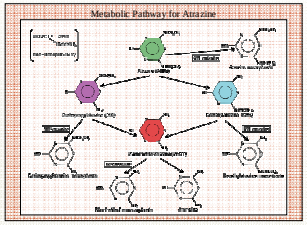
.S. EPA criterion to protect saltwater aquatic life from chronic exposure is 17000 g/L

considered an endocrine-disrupting chemical (EDC), *i.e.*, interacts with the ndocrine signaling pathway s in an organism

Atrazine: Mode of Action

- Atrazine does not interact with steroid hormone receptors - Connor et al., 1996, *Fundam Appl Toxicol*; Roberge *et al.*, 2004, *Toxicol Letters*
- In cell cultures, atrazine modulates the activity of aromatase, an enzyme complex that converts androgens to estrogens - Sanderson *et al.*, 2000, *Toxicol Sci*; Keller and McClellan- Green, 2004, *Mar Environ Res*
- Atrazine blocks conversion of cAMP to 5'-AMP by phosphodiesterase - Roberge *et al.*, 2004, *Toxicol Letters*
- cAMP levels affect the state of phosphorylation of steroidogenic enzymes like aromatase, which in turn causes enzyme activation or inactivation - Haider, 2003, *Comp Biochem Physiol Part A*

Metabolic Pathway for Atrazine



Reported Impacts of Atrazine Exposure

In Rats:

- Mammary gland tumors
- Disrupted cyclicity
- Delayed puberty
- Altered lactation
- Pregnancy loss
- Inflammation of the prostate gland

In Fish:

- Developmental abnormalities in zebrafish embryos
- Altered swimming behavior in red drum, zebrafish and goldfish
- Reduced responsiveness to female pheromone in male salmon
- Changes in social behavior in goldfish

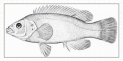
HYPOTHESIS #1

Atrazine will have no effect on the reproductive success of exposed fish.

APPROACH: Measure reproductive endpoints in spawning cunner (*Tautogolabrus adspersus*) in the laboratory both before and after treatment with atrazine.

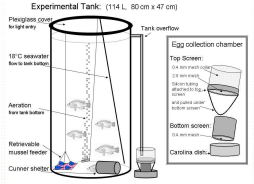
Cunner:

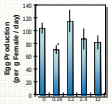
- Common inshore in estuarine and saltwater areas near sheltered rock substrates, wharves, wrecks and reefs
- Spawn daily for 4 - 6 week period in the spring
- Paired courtship behavior or group spawning, both ending in a rapid vertical run to the water's surface and simultaneous release of gametes

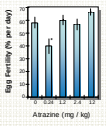


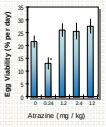
Tautogolabrus adspersus

Experimental Tank: (114 L 80 cm x 47 cm)









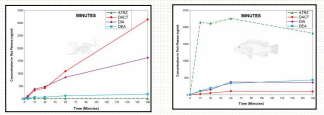
Fish treated with the lowest concentration of atrazine had significantly decreased egg production, egg fertility and egg viability.

HYPOTHESIS #2

Assimilation, metabolism and clearance of atrazine is similar in rats and fish exposed to the same doses by the same method.

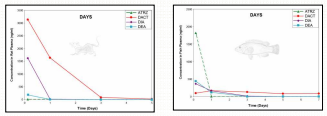
APPROACH: Expose rats and fish to identical doses of atrazine delivered by gavage and analyze plasma samples for atrazine and metabolites over time.

Short-term
50 mg/kg atrazine & metabolites



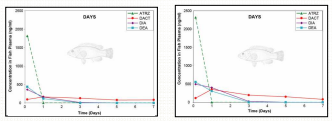
Rats metabolize atrazine quickly while, in contrast, fish retain high circulating concentrations of atrazine over the first few hours after exposure.

Long-term
50 mg/kg atrazine & metabolites



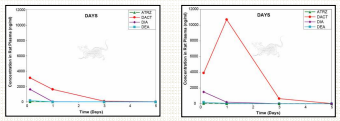
Over a period of days, metabolism and clearance of atrazine differs between rats and fish. However, in both species, DACT was retained the longest.

Long-term
50 vs 200 mg/kg atrazine & metabolites



Increasing the dose of atrazine delivered to cunner by gavage does not notably change circulating levels of atrazine and metabolites, suggesting uptake from the gut is a limiting factor.

Long-term
50 vs 200 mg/kg atrazine & metabolites



Increasing the dose of atrazine delivered to rats by gavage notably increases the circulating level of DACT, suggesting differences in up-take or clearance compared to fish.

HYPOTHESIS #3

Impacts on reproductive endpoints in both rats and fish due to atrazine exposure can be linked to modulation of aromatase activity in both organisms.

APPROACH: Measure tissue-specific aromatase activity in fish and rats exposed to atrazine.

Aromatase

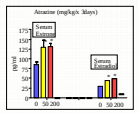
- Member of the cytochrome P450 family of enzymes (CYP19 gene)
- Converts androgens to estrogens during steroidogenesis
- Found in all vertebrates, including mammals, fish, reptiles and amphibians
- Highest concentrations found in the gonads and brains of vertebrates
- One type in most mammals; two types in fishes
  - P450aromA (encoded by CYP19a/CYP19A1) predominates in fish ovaries
  - P450aromB (encoded by CYP19b/CYP19A2) predominates in fish brains

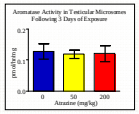
Aromatase Gene Expression

- Aromatase isoforms in fish are structurally and functionally distinct, deriving from separate genes
- Transcription of these aromatase isoforms in fish are controlled by distinct regulatory mechanisms
  - CYP19a/CYP19A1 (gonad aromatase)
    - Conserved binding site for SF-1
    - Multiple cAMP responsive elements (CREs)
    - Others: Fox1.2; SRY/SOX; AhR/Arnt
  - CYP19b/CYP19A2 (brain aromatase)
    - Estrogen responsive elements (EREs)
    - Others: CRE; PPARα/RXRα; NGFI-B/Nur77; SRY/SOX

In Rats:

Although atrazine increases serum estrogens in rats, there is no evidence that atrazine alters aromatase activity or gene expression

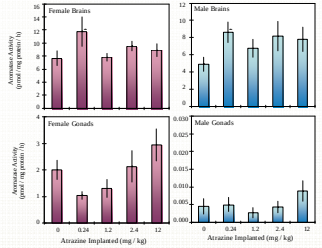




- No change: aromatase activity in testes
- No change: CYP19 expression in brain, testes or adipose

In Fish:

Fish from the same atrazine treatment that had reduced reproductive success also showed a significant elevation in brain aromatase activity.



- Both male and female cunner showed altered brain aromatase activity in the atrazine treatment
- Aromatase activity in testes was very low compared to other tissues.

Conclusions

- Egg production, fertility and viability was reduced in cunner implanted with a low concentration of atrazine, but not higher concentrations.
- Rats and fish assimilate and metabolize atrazine differently even when treated by the same method (gavage) and with the same doses.
- Brain aromatase activity was increased in fish treated with a low concentration of atrazine, but atrazine did not alter aromatase in rats.
- Results suggest that accurate interspecies extrapolation is a process complicated by differences in physiology and metabolism between organisms, even when a chemical's mode of action is established.

Future Directions

- Are effects of low concentrations of atrazine on fish reproductive success and brain aromatase activity environmentally relevant?
  Approach: expose cunner to environmentally relevant concentrations of atrazine by gavage and measure reproductive endpoints and aromatase activity.
- Can differences in assimilation and metabolism of atrazine between rats and fish explain the different responses in the two species?
  Approach: compare the efficacy of atrazine and major metabolites at eliciting responses in the two species.

EPA Atlantic Ecology Division Narragansett, Rhode Island