



## Atrazine: A Tale Of Two Species (ED MYP)

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- 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 environ mental laws including, the Toxic Substances Control Act (TSCA), the Food Quality Protection Act (FQPA), the Safe Drinking Water Act Amendments and the Cleam 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) approaches 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

1) the extent to which organism-level effects can be extrapolated across species 2) the population-level ramifications of EDC exposure

The herbicide attrazine was selected as a model EDC for a case study using two species, a rat and a fish. Attrazine has been shown to change the activity of the strenkingsgenic eary are aromatus, which is the earyne complex that catalyses the conversion of androgens to estingeness. Attractive is known to disempt round reproductive processes in ant, fish and fine). Our flows at AED was on attractive effects in the marine fish cunner. AED scientists developed a strong collaboration with scientists at the Reproductive Toxicology Division in Research Trangle Fast. No, there in studies were conducted.

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

## Chemical of Interest: Atrazine

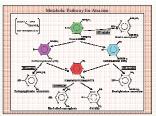
## Widely-used broad-leaf herbicide

- In cell culture, strazine modulates the activity of aromatase, an enzyme complex that converts androgens to extrogens
   Sandrason et al., 2000, Toxicol Seri, Keller and McClellas-Green, 2004, Mar Environ Res In Texas estuaries, spring time water levels measured as high as 2000 ng/L, with yearly average of 920 ng/L. Pennington et al., 2001, J Environ Sci Hea U.S. EPA criterion to protect saltwater aquatic life from chronic exposure is 17000 net.
  - Atrazine blocks conversion of cAMP to S'-AMP by phosphodiesterase Roberge et al., 2004, Toxical Letters

Atrazine: Mode of Action

Atrazine does not interact with seroid hormone receptors - Connor et al., 1996, Fundam Appl Toxicol; Roberge et al., 2004, Toxicol Letterx

cAMP levels affect the state of phosphorylation of steroidogenic enzy like aromatase, which in turn causes enzyme activation or inactivation - Haider, 2003, Comp Biochem Physiol Part A



### Reported Impacts of Atrazine Exposure In Fish: In Rats: Developmental abu Altered swimming behavior in red drum, zebrafish · Altered lactation

### HYPOTHESIS#1 Atrazine will have no effect on the reproductive success

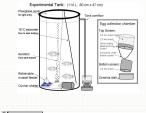
of exposed fish. APPROACH: Measure reproductive endpoints in spawning cunner

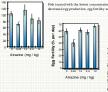
## (Tautogolabrus adspersus) in the laboratory both before and after treatment with atrazine. Cunner:

· Inflammation of the prostate pland

- Spawn daily for 4 6 week period in the spring









## 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

## 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 e

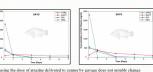
50 mg/kg atrazine & metabolites

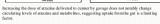




Over a period of days, metabolis mand clearance of atrazine differs betweer rats and fish. However, in both species, DACT was retained the longest.

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





DACT DACT DACT DACT

# DAGT DAGT DIA DIA DEA

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

In Rats:

- Member of the cytochrome P450 family of enzymes (CYP19 gene)
- 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

## Aromatase Gene Expression

- Conserved binding site for SF-1

- Others: FoxL2: SRY/SOX: AhR/Arnt - CYP19b/CYP19A2 (brain arom
- Others CRE-PPARGRANG NGELBNir77-SRV/SOX

In Fish:





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

- Rats and fish assimilate and metabolize atrazine differently even when treated by the same method (gavage) and with the same do:

- Results suggest that accurate interspecies extrapolation is a process complicated by differences in physiology and metabolis m between organisms, even when a chemical's mode of action is established.

Are effects of low concentrations of atrazine on fish reproductive success and brain aro matase activity environmentally relevant?
 Approach: expose cunner to environmentally relevant concentra of atrazine by gavage and measure reproductive endpoints and

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 as eliciting responses in the two species.

