



Position Statement

Response to:

“The Herbicide Atrazine Activates Endocrine Gene Networks Via Non-Steroidal NR5A Nuclear Receptors in Fish and Mammalian Cells”

Suzawa, M. and Ingraham, H. A. The Herbicide Atrazine Activates Endocrine Gene Networks Via Non-Steroidal NR5A Nuclear Receptors in Fish and Mammalian Cells. PLoS ONE 3 (5): 1-11 (2008).
<http://www.plosone.org/article/info:doi%2F10.1371%2Fjournal.pone.0002117>

There were two parts to this study: 1) dosing of zebrafish with atrazine and 2) incubation of human cell lines with atrazine. In the zebrafish portion, the authors reported an up-regulation of aromatase and an increase in the ratio of females to males. In the cell line portion, the authors reported that atrazine “turns on” aromatase genes via a series of cellular interactions.

- 1) Similar animal studies have been conducted before, and there is NO previous evidence for an effect of atrazine on aromatase in any whole animal.**
 - a) Other researchers have conducted this same experiment in zebrafish and did NOT find effects on aromatase at atrazine concentrations up to 100 ppb¹.
 - b) In another study, atrazine had no effect on goldfish gonads or aromatase at concentrations up to 1,000 ppb².
 - c) Atrazine concentrations up to 100 ppb did not cause any effects on aromatase activity in *Xenopus laevis* frogs³.
 - d) In the most extensive study to date with more than 3,000 frogs dosed from 0.01-100 ppb atrazine, no sexual malformations were seen. If aromatase were up-regulated, it would have had an effect on gonadal development, and it did not.
 - e) It has also been shown that atrazine does not affect aromatase in rats⁴.

- 2) Effects reported *in vitro* (with human cell lines) already have been noted in previous studies, but no physiological consequence of these effects has ever been seen in whole animals.**
 - a) Sanderson et al. (2001) previously reported an increase in aromatase enzyme activity in the same cell line (JEG3) and at similarly high concentrations."⁵

¹ Kazeto, Y. et al. (2004). Effects of endocrine-disrupting chemicals on the expression CYP19 genes in zebrafish (*Danio rerio*) juveniles. *Aquatic Toxicology*, 69:25-34.

² Nadzialek, S. et al. (2008). High doses of atrazine do not disrupt activity and expression of aromatase in female gonads of juvenile goldfish (*Carassius auratus* L.). *Ecotoxicology* (DOI: 10.1007/s10646-008-0198-9).

³ Hecker et al. (2005). Effects of atrazine on CYP19 gene expression and aromatase activity in testis and on plasma sex steroid concentrations of male African-clawed frogs (*Xenopus laevis*). *Tox. Sci.* 86(2):273-280; Hecker et al. (2004). Plasma sex steroid concentrations and gonadal aromatase activities in African-clawed frogs (*Xenopus laevis*) from South Africa. *Env. Tox. & Chem.* 23(8): 1996-2007 ; Coady et al. (2005). Effects of atrazine on metamorphosis, growth, laryngeal and gonadal development, aromatase activity, and sex steroid concentrations in *Xenopus laevis*. *Ecotox. & Env. Safety* 62:160-173; Oka, et al. (2008). Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*. *Aquatic Toxicology* (DOI:10.1016/j.aquatox.2008.02.009).

⁴ Modic, M. (2004). The role of testicular aromatase in the atrazine mediated changes of estrone and estradiol in the male wistar rat. Thesis submitted to North Carolina State University.

- 3) Effects of atrazine on aromatase gene expression in zebrafish are unreliable.**
- a) There were 15 fish in a 500 mL beaker at each dose level. Three groups of five fish were ground up for the assay and treated as independent samples. All 15 fish should be considered as one statistical “unit,” and multiple replicates, typically a minimum of five, should have been used.
 - b) This approach imparted artificial “tightness” to the data, which in turn generated artificial statistical significance when treated groups were compared to controls.
- 4) The sex-ratio graph cannot be verified.**
- a) Fish were not sexually differentiated at the beginning of the study in either the control or the treatment beaker, so the authors would not know how many males vs. females there were.
 - b) The study reports that females increased in proportion by 400%--a 4x change—relative to the control. If there were an even number of female and male fish in each beaker, (e.g., 7 or 8 of each) at the start of the exposure, a 4x change would mean there were 28-32 females in each beaker by the end of the exposure.
 - c) The authors do not report how many fish they counted – only the percentage.
 - d) The early life stage acute toxicity value (LC₅₀) for atrazine is 890 ppb. Exposure of 2157 ppb would be expected to kill some of the fish, but no mortality was reported. Since neither the total number of fish counted nor the actual number of male or female fish is reported, it’s unknown how many males and females were actually observed.
- 5) All *in vitro* (human cell line) effects are seen only at high atrazine concentrations (>216 ppb) that are not environmentally relevant.**
- a) All effects on cells were observed at >1 uM (216 ppb), and most were observed at the excessively high concentration of 10 uM (or 2157 ppb). These do not represent real-world concentrations or exposures.

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⁵ Sanderson, J. et al. (2001). Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin productions in male carp hepatocytes. *Environ. Health Perspect.* 109:1027-1031.