

ATRAZINE DISRUPTS REPRODUCTIVE DEVELOPMENT AND FUNCTION ACROSS VERTEBRATE CLASSES

We (40 scientists representing 10 countries, and six continents) have evaluated the existing studies examining atrazine as an endocrine disruptor along with emerging data on this topic. We evaluated over 100 studies that have shown adverse effects of atrazine on primary and secondary sex differentiation, reproductive development, morphology and behavior and sex hormone dependent cancers. Atrazine consistently demasculinizes and feminizes the gonads of exposed males across all vertebrate classes examined. Multiple laboratories have demonstrated that atrazine exposure results in testicular lesions including loss of interstitial cells [1], loss of supporting (Nurse or Sertoli) cells [2], dilation of testicular tubules with increased tubular cellular debris [3-6], and severe loss of germ cells [4-6]. These effects have been demonstrated in peer-reviewed studies in fish [1], amphibians [3], reptiles [6] and mammals [4-5]. Other studies have demonstrated associated demasculinization effects in males including a decrease in laryngeal size and breeding gland size in amphibians [3], decreased penis size in reptiles (Victor-costa, unpublished), and disorders in accessory sex organs in mammals [4]. Also, atrazine partially feminizes the gonads of exposed males in fish [7], amphibians [3, 8-10] and reptiles [11], resulting in the development of testicular oocytes. Other studies show that atrazine induces female-typical coloration [12], feminized larynges [3], and hypertrophied oviducts in amphibians (Hayes, unpublished). Atrazine also completely feminizes fish [13], amphibians [14-15], and reptiles [11, 16] resulting in shifts in sex ratios and confirmed sex reversal in amphibians (development of functional ovaries in genetic males) [3]. Further studies suggest that, among other documented mechanisms, atrazine exposure causes a decline in androgen levels in fish [17], amphibians [3, 8, 12], reptiles and mammals [5, 18-20], consistent with the observed demasculinization effects. In addition, the induction of aromatase and/or subsequent inappropriate estrogen production has been demonstrated now in fish [1, 13, 17], amphibians [3], reptiles [21], and mammals [13, 22-28]. This mechanism is consistent with studies showing feminization of exposed animals across vertebrate classes. Furthermore, functional consequences of endocrine disruption by atrazine have been demonstrated across vertebrate classes as well: Male reproductive behaviors are severely impaired in fish [17], amphibians [3], and mammals [29-30] exposed to atrazine (reptiles have not been examined in this regard). Sperm production and fertility are severely impaired in exposed fish [17], amphibians [3], and mammals (laboratory rodents [29, 31-33]) as well. Finally, impaired fertility and hormone dependent diseases in rodents (prostate [34-35] and mammary cancer [22, 36-41]) and epidemiological studies linking impaired fertility [42], breast [21] and prostate cancer [43] in humans to atrazine exposure are consistent with these experimental observations. These reproductive effects are among many others including impaired mammary development, delayed puberty, induced abortion, and birth defects along with non-reproductive effects such as impaired immune function across vertebrates that are not discussed here.

References

1. Spano, L., et al., *Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (Carassius auratus)*. Aquatic Toxicology (Amsterdam), 2004. **66**(4): p. 369-379.
2. Tavera-Mendoza, L., et al., *Response of the amphibian tadpole (Xenopus laevis) to atrazine during sexual differentiation of the testis*. Environ. Toxicol. Chem., 2002. **21**: p. 527-531.

3. Hayes, T.B., et al., *Atrazine induces complete feminization and chemical castration in male African clawed frogs (Xenopus laevis)*. Proc. Natl. Acad. Sci. USA, 2010. **107** (10): p. 4612-4617.
4. Kniewald, J., et al., *Disorders of male rat reproductive tract under the influence of atrazine*. J. Appl. Toxicol., 2000. **20**: p. 61-68.
5. Victor-Costa, A.B., et al., *Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine*. Reproductive Toxicology, 2010. **29**(3): p. 323-331.
6. Rey, F., et al., *Prenatal exposure to pesticides disrupts testicular histoarchitecture and alters testosterone levels in male Caiman latirostris*. General and Comparative Endocrinology, 2009. **162**(3): p. 286-292.
7. Tillitt, D.E., et al., *Atrazine reduces reproduction in fathead minnow*. Marine Environmental Research, 2008. **66**(1): p. 51-51.
8. Hayes, T.B., et al., *Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses*. Proc. Natl. Acad. Sci. USA, 2002. **99**: p. 5476-5480.
9. Hayes, T.B., et al., *Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (Rana pipiens): Laboratory and field evidence*. Environ. Health Perspect., 2002. **111**: p. 568-575.
10. Hayes, T.B., et al., *Characterization of atrazine-induced gonadal malformations and effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (estradiol 17 β): Support for the demasculinization/feminization hypothesis*. Environ. Health Perspect, 2006. **114** (Suppl. 1): p. 134-141.
11. De Solla, S.R., et al., *Effects of environmentally relevant concentrations of atrazine on gonadal development of snapping turtles (Chelydra serpentina)*. Environmental Toxicology and Chemistry, 2006. **25**(2): p. 520-526.
12. Mckoy, K.A., M.S. Sepulveda, and T. Gross, S. *Atrazine exposure and reproductive system abnormalities in field collected Bufo marinus*. in Soc. Environ. Toxicol. Chem., 23rd Annual Meeting in North America, 2002. Salt Lake City, UT.
13. Suzawa, M. and H. Ingraham, *The herbicide atrazine activates endocrine gene networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells*. PLOS ONE, 2008. **3**: p. 2117.
14. Oka, T., et al., *Effect of atrazine on metamorphosis and sexual differentiation in Xenopus laevis*. Aquatic Toxicology, 2008. **87**(4): p. 215-226.
15. Langlois, V., et al., *Low levels of the herbicide atrazine alters sex ratios and reduces metamorphic success in Rana pipiens tadpoles raised in outdoor mesocosms* Environ Health Perspect, 2009.
16. Willingham, E.J., *The effects of atrazine and temperature on turtle hatchling size and sex ratios*. Frontiers in Ecology and the Environment, 2005. **3**(6): p. 309-313.
17. Moore, A. and C. Waring, *Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (Salmo salar L.) parr*. Pesticide Biochem. Physiol., 1998. **62**: p. 41-50.
18. Friedmann, A., *Atrazine inhibition of testosterone production in rat males following peripubertal exposure*. Reproductive Toxicology., 2002. **16**(3): p. 275-279.
19. Gojmerac, T. and J. Kniewald, *Atrazine biodegradation in rats - a model for mammalian metabolism*. Bulletin of Environmental Contamination and Toxicology, 1989. **43**(2): p. 199-206.
20. Kniewald, J., et al., *Effect of s-triazine compounds on testosterone metabolism in the rat prostate*. J. Appl. Toxicol., 1995. **15**(3): p. 215-218.
21. Kettles, M.A., et al., *Triazine exposure and breast cancer incidence: An ecologic study of Kentucky counties*. Environ. Health Perspect., 1997. **105**(11): p. 1222-1227.
22. Eldridge, J.C., et al., *The mammary tumor response in triazine-treated female rats: A threshold-mediated interaction with strain and species-specific reproductive senescence*. Steroids, 1999. **64**(9): p. 672-678.

23. Sanderson, J., et al., *Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells*. Toxicol. Appl. Pharmacol., 2002. **182**: p. 44-54.
24. Sanderson, J.T., et al., *Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes*. Environ. Health Perspect., 2001. **109**: p. 1027-1031.
25. Sanderson, J.T., et al., *2-chloro-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: A novel mechanism for estrogenicity?* Toxicol. Sci., 2000. **54**: p. 121-127.
26. Heneweer, M., M. van den Berg, and J. Sanderson, *A comparison of human H295R and rat R2C cell lines as in vitro screening tools for effects on aromatase*. Toxicol. Letters, 2004. **146**: p. 183-194.
27. Fan, W., et al., *Herbicide atrazine activates SF-1 by direct affinity and concomitant co-activators recruitments to induce aromatase expression via promoter II*. Biochemical and Biophysical Research Communications, 2007. **355**(4): p. 1012-1018.
28. Fan, W., et al., *Atrazine-induced aromatase expression is SF-1- dependent: Implications for endocrine disruption in wildlife and reproductive cancers in humans*. Environ. Health Perspect., 2007. **115**(5): p. 720-727.
29. Kniewald, Z., B. Simic, and J. Kniewald, *Atrazine inhibits reproductive processes in rats*. Biology of Reproduction, 2008: p. 115-116.
30. Peruzovic, M., et al., *Effect of atrazine ingested prior to mating on rat females and their offspring*. Acta Physiologica Hungarica, 1995. **83**(1): p. 79-89.
31. Abarikwu, S., et al., *Lipid peroxidation, antioxidant enzymes activities in testis and spermatotoxicity of rats during short-term exposure to atrazine*. Journal of Andrology, 2008: p. 43-44.
32. Abarikwu, S.O., et al., *Changes in Sperm Characteristics and Induction of Oxidative Stress in the Testis and Epididymis of Experimental Rats by a Herbicide, Atrazine*. Archives of Environmental Contamination and Toxicology, 2010. **58**(3).
33. Šimić, B., et al., eds. *Effects of Atrazine on Sperm Parameters in Rats*. Current Studies of Biotechnology – vol. II. Environment, ed. Z. Kniewald. 2001, Croatian Soc. of Biotechnology: Zagreb.
34. Pintér, A. and e. al, *Long-term carcinogenicity bioassay of the herbicide atrazine in F344 rats*. Neoplasma, 1980. **37**: p. 533-544.
35. Stanko, J., et al., *Effects of prenatal exposure to a low dose atrazine metabolite mixture on pubertal timing and prostate development of male Long Evans rats*. Reprod. Toxicol., 2010. **Accepted Article Online: Aug 2 2010**
36. Ueda, M., et al., *Possible enhancing effects of atrazine on growth of 7,12-dimethylbenz(a) anthracene induced mammary tumors in ovariectomized Sprague–Dawley rats*. Cancer Sci., 2005. **96**(1): p. 19-25.
37. Eldridge, J., et al., *Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: Hormonal properties, dosage, and animal strain*. Environ. Health Perspect., 1994. **102**(11): p. 29-36.
38. Eldridge, J., L. Wetzel, and L. Tyrey, *Estrous cycle patterns of Sprague-Dawley rats during acute and chronic atrazine administration*. Reprod. Toxicol., 1999. **13**: p. 491-499.
39. Stevens, J., et al., *Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides*. J. Toxicol. Environ. Health., 1994. **43**: p. 139-154.
40. Tennant, M.K., et al., *Chloro-s-triazine antagonism of estrogen action: Limited interaction with estrogen receptor binding*. J. Toxicol. Environ. Health, 1994. **43**: p. 197-211.

41. Wetzel, L.T., et al., *Chronic effects of atrazine on estrus and mammary gland formation in female Sprague-Dawley and Fischer-344 rats*. J. Toxicol. Environ. Health., 1994. **43**: p. 169-182.
42. Swan, S., et al., *Semen quality in relation to biomarkers of pesticide exposure*. Environ. Health Perspect., 2003. **111**(12): p. 1478-1484.
43. Maclennan, P., et al., *Cancer incidence among triazine herbicide manufacturing workers*. JOEM, 2002. **44**(11): p. 1048-1058.