

ATRAZINE & HEALTH: KEY STUDIES

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Pesticide Action Network North America (PANNA) has compiled and summarized some of the key studies documenting the known and potential health effects of the herbicide atrazine. The categories of health effects explored include Endocrine and Immune System Disruption, Reproductive System Impacts, and Cancer. For each study, we have included the full citation, the author abstract when available, and a “PANNA Summary” describing the findings in lay terms. This compilation is by no means comprehensive.

Endocrine & Immune System Disruption

Hayes, T., 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.

Author abstract: Atrazine is the most commonly used herbicide in the U.S. and probably the world. It can be present at several parts per million in agricultural runoff and can reach 40 parts per billion (ppb) in precipitation. We examined the effects of atrazine on sexual development in African clawed frogs (*Xenopus laevis*). Larvae were exposed to atrazine (0.01–200 ppb) by immersion throughout larval development, and we examined gonadal histology and laryngeal size at metamorphosis. Atrazine (≥ 0.1 ppb) induced hermaphroditism and demasculinized the larynges of exposed males (≥ 1.0 ppb). In addition, we examined plasma testosterone levels in sexually mature males. Male *X. laevis* suffered a 10-fold decrease in testosterone levels when exposed to 25 ppb atrazine. We hypothesize that atrazine induces aromatase and promotes the conversion of testosterone to estrogen. This disruption in steroidogenesis likely explains the demasculinization of the male larynx and the production of

hermaphrodites. The effective levels reported in the current study are realistic exposures that suggest that other amphibian species exposed to atrazine in the wild could be at risk of impaired sexual development. This widespread compound and other environmental endocrine disruptors may be a factor in global amphibian declines.

PANNA Summary: The study authors exposed African clawed frog larvae to atrazine by immersion throughout larval development. Male frogs exposed to atrazine at levels less than 0.1 ppb in the larval stage demonstrated female characteristics as a result of this exposure. At 25 ppb exposure to atrazine, male frogs suffered a 10-fold decrease in testosterone levels. The study authors hypothesized that this demasculinization of male frogs is caused by atrazine-induced conversion of testosterone (the male sex hormone) to estrogen (the female sex hormone). The study highlights the endocrine disrupting impacts of atrazine. The study suggests that amphibian species in the wild may be in danger, since levels of exposure to atrazine in the study are at levels that can realistically be experienced by amphibians in the wild.

Brodin, M., et al., Atrazine is an immune disruptor in adult northern leopard frogs (Rana pipiens). Environ. Toxicol. Chem., 2007. 26(1): p. 80-84.

Author abstract: Atrazine, the most widely used herbicide in the United States, has been shown in several studies to be an endocrine disruptor in adult frogs. Results from this study indicate that atrazine also functions as an immune disruptor in frogs. Exposure to atrazine (21 ppb for 8 d) affects the innate immune response of adult *Rana pipiens* in similar ways to acid exposure (pH 5.5), as we have previously shown. Atrazine exposure suppressed the thioglycollate-stimulated recruitment of white blood cells to the peritoneal cavity to background (Ringer exposed) levels and also decreased the phagocytic activity of these cells. Unlike acid exposure, atrazine exposure did not cause

mortality. Our results, from a dose–response study, indicate that atrazine acts as an immune disruptor at the same effective doses that it disrupts the endocrine system.

PANNA Summary: A study was performed to investigate atrazine’s effects on the immune systems of adult Leopard Frogs. The frogs were subjected to doses known to cause endocrine disruption. The first experiment involved exposing frogs to atrazine contaminated water and then giving them an injection to stimulate an immune system response. The results showed that atrazine was able to reduce the activity of white blood cells in the body. The second experiment involved exposing the frogs to doses of 10, 1, 0.1 and 0.01 ppb. The results showed that the number of highly active white blood cells decreased with increasing atrazine exposure. Although these experiments did not kill the frogs, they proved that atrazine also functions as an immune system disruptor.

Reproductive System Impacts

Swan, S. , et al. Semen quality in relation to biomarkers of pesticide exposure. Environ Health Perspect. 2003 Sep;111(12):1478-84.

Author abstract: We previously reported reduced sperm concentration and motility in fertile men in a U.S. agrarian area (Columbia, MO) relative to men from U.S. urban centers (Minneapolis, MN; Los Angeles, CA; New York, NY). In the present study we address the hypothesis that pesticides currently used in agriculture in the Midwest contributed to these differences in semen quality. We selected men in whom all semen parameters (concentration, percentage sperm with normal morphology, and percentage motile sperm) were low (cases) and men in whom all semen parameters were within normal limits (controls) within Missouri and Minnesota (sample sizes of 50 and 36, respectively) and measured metabolites of eight current-use pesticides in urine samples provided at the time

of semen collection. All pesticide analyses were conducted blind with respect to center and case-control status. Pesticide metabolite levels were elevated in Missouri cases, compared with controls, for the herbicides alachlor and atrazine and for the insecticide diazinon [2-isopropoxy-4-methyl-pyrimidinol (IMPY)]; for Wilcoxon rank test, $p = 0.0007$, 0.012 , and 0.0004 for alachlor, atrazine, and IMPY, respectively. Men from Missouri with high levels of alachlor or IMPY were significantly more likely to be cases than were men with low levels [odds ratios (ORs) = 30.0 and 16.7 for alachlor and IMPY, respectively], as were men with atrazine levels higher than the limit of detection (OR = 11.3). The herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and metolachlor were also associated with poor semen quality in some analyses, whereas acetochlor levels were lower in cases than in controls ($p = 0.04$). No significant associations were seen for any pesticides within Minnesota, where levels of agricultural pesticides were low, or for the insect repellent DEET (N,N-diethyl-m-toluamide) or the malathion metabolite malathion dicarboxylic acid. These associations between current-use pesticides and reduced semen quality suggest that agricultural chemicals may have contributed to the reduction in semen quality in fertile men from mid-Missouri we reported previously.

PANNA Summary: A study was performed to examine the hypothesis that pesticides used commonly in agricultural areas were responsible for poor semen quality in men. The pesticides investigated in this study included atrazine, alachlor, 1-naphthol, and others. Columbia, Missouri, was selected as the study area due to the high level of agricultural activity (corn, sorghum, soybeans, etc) in the region. Minneapolis, Minnesota, was selected as a control area due to it being an urban center, generally devoid of agricultural activity. Subjects were screened to exclude men exhibiting risk factors that would lead to impaired sperm quality (smoking, hereditary infertility, STDs etc). Urine analysis was done to measure the level of pesticide metabolites (biomarker indicators) present in the subjects' systems. Semen was then collected and analyzed for sperm count and quality. Through statistical analysis researchers found that herbicides such

as alachlor and atrazine were associated with decreased semen quality in the Missouri subjects. Another analysis combining exposure to all pesticides showed that exposure was strongly associated with semen quality.

Arbuckle, T.E., Z. Lin, and L.S. Mery. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ. Health Perspect., 2001. 109(8): p. 851-857

Author abstract: The toxicity of pesticides on human reproduction is largely unknown--particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and post-conception (first trimester) windows and for early (< 12 weeks) and late (12-19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1-2.1], triazines (OR = 1.4; 95% CI, 1.0-2.0), and any herbicide (OR = 1.4; 95% CI, 1.1-1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0-2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1-3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0-2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to

more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.

PANNA Summary: Farm residents are subject to the exposure of several different types of pesticides including the family of pesticides known as triazines, which includes the herbicide atrazine. Other studies have shown that occupational exposure to these pesticides during pregnancy is associated with higher rates of fetal death. The authors of the article decided to explore the connection between pesticide exposure and spontaneous abortion (i.e. miscarriage) by surveying families in a farm community in Ontario, Canada. Couples were asked to describe the pesticides used on their farms, medical histories, and complete reproductive history. The study combined this data on exposure with information about the women's gestational period before having spontaneous abortions. Results indicated that preconception exposure to pesticides contributed more to the risk of a spontaneous abortion than exposures during the pregnancy. There was some uncertainty about the potency of triazines themselves due to many members of the population being exposed to several types of pesticides. The authors also indicate that there may be some uncertainty due to the lack of dose information. The article shows that the timing of pesticide exposure can have a large effect on reproductive toxicity, warranting the further study of glyphosate, carbaryl, phenoxy acetic acid, triazines (including atrazine) and thiocarbamate.

Cancer

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.

Author abstract: The incidence of breast cancer in the United States has steadily increased for the past three decades. Exposure to excess estrogen, in both natural and synthetic forms, has been implicated as a risk factor for the development of this disease. Considerable interest has been focused on organochlorines, such as the triazine herbicides, and their possible role in the initiation or promotion of human breast cancer. To explore this relationship, an ecologic study of Kentucky counties was designed. Exposure to triazines was estimated by use of water contamination data, corn crop production, and pesticide use data. A summary index of triazine herbicide exposure was developed to classify counties into low, medium, or high exposure levels. Data on county breast cancer rates were obtained from the state registry. A Poisson regression analysis was performed, controlling for age, race, age at first live birth, income, and level of education. Results revealed a statistically significant increase in breast cancer risk with medium and high levels of triazine exposure [odds ratio (OR) = 1.14, $p < 0.0001$ and OR = 1.2, $p < 0.0001$, respectively]. The results suggest a relationship between exposure to triazine herbicides and increased breast cancer risk, but conclusions concerning causality cannot be drawn, due to the limitations inherent in ecologic study design.

PANNA Summary: An experiment studying the correlation between triazine groundwater contamination and breast cancer incidence was performed in Kentucky. Cancer incidence data was collected through the Kentucky Cancer Registry, surface water contamination data was acquired through the Division of Water of the Kentucky Environmental Protection Cabinet, and groundwater contamination data was acquired through the Kentucky Geological Survey. Other organizations such as the Kentucky College of Agriculture also provided exposure data. Regions tested were categorized into low, medium, and high triazine contamination. The areas with surface water triazine contamination displayed an increased incidence of breast cancer in the earlier years of the study (1991-1992). However, the later years of the study showed a correlation between groundwater contamination and breast cancer (1993-1994). Overall the

results suggest there is a relationship between breast cancer and triazine exposure, but a definite causal relationship cannot be scientifically proven with this study.

MacLennan, Paul A., Delzell, Elizabeth, et al. Cancer incidence among triazine herbicide manufacturing workers. Journal of Occupational and Environmental Medicine. Volume 44(11), 2002, pp 1048-58

and

Sass, Jennifer , Brandt-Rauf, Paul. Cancer Incidence Among Triazine Herbicide Manufacturing Workers. Journal of Occupational and Environmental Medicine. Issue: Volume 45(4), April 2003, pp 343-344

Author abstract: Not available.

PANNA Summary: A cancer-incidence study was conducted on the workers of an atrazine manufacturing facility who were employed there between 1985 and 1997 (McLennan et al, 2002). This study was conducted by evaluating the cancer incidence rates of those working in the manufacturing facility and comparing them to the cancer incidence rates of the general population in the Louisiana industrial corridor. Cancer rates 1.8 times higher than expected were observed in the study; the authors emphasized that there were too many confounding factors to attribute them to triazine exposure. The authors cite the varied availability of cancer screening due to socioeconomic differences and lack of individual-specific exposure data. In conclusion, the authors of the article found no causal relationship between atrazine and cancer.

This article was later contested by senior scientists from the Natural Resources Defense Council (Sass et.al, 2003). The authors of the response article point out

that the 2002 study failed to mention that the authors were consultants paid by Syngenta. In addition, Sass et. al pointed out that the manufacturing facility was owned by Syngenta and that the manufacturing facility's cancer incidence rates were only compared to the industrial corridor's cancer incidence rates, rather than the Louisiana statewide cancer incidence rates. Sass et. al. argue that these factors skewed the results in addition to introducing bias to the study.

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