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Comments from the
NATURAL RESOURCES DEFENSE COUNCIL
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On the EPA review of data relevant to effects of
atrazine on amphibians
in preparation for the SAP meeting in October, 2007

These comments are supported by:

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Beyond Pesticides (Laura Hepting)
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BACKGROUND from the FR Notice:

In October 2007, EPA will make a presentation to the Scientific Advisory Panel (SAP) concerning EPA's evaluation of the effects of atrazine on amphibian gonadal development. The scientific research will include studies that were conducted by Syngenta Crop Protection, Inc. in 2005 and 2006 as well as published open literature studies. The notice identifies the open literature studies that EPA has reviewed and requests public comment to ensure that the list of publications is complete. The studies that have been reviewed focus on testing atrazine alone and only on atrazine's potential effects on amphibian gonadal development.

In this Federal Register Notice, EPA is soliciting public comment on the completeness of its list of open literature studies on the potential effects of atrazine on amphibian gonadal development.

EPA has reviewed the following list of relevant open literature studies in preparation for the October SAP meeting (Syngenta-sponsored papers are identified here by NRDC, based on author associations):

1. ***Syngenta-sponsored:*** Coady K.K., Murphy M.B., Villeneuve D.L., Hecker M., Jones P.D., Carr J.A., Solomon K.R., Smith E.E., Van der Kraak G., Kendall R.J., and J.P. Giesy. 2005. Effects of Atrazine on Metamorphosis, Growth, Laryngeal and Gonadal Development, Aromatase Activity, and Plasma Sex Steroid

Concentrations in Xenopus laevis. Ecotoxicology and Environmental Safety 62:160-173. MRID 458677-04.

2. ***Syngenta-sponsored:*** Coady K.K., Murphy M.B., Villeneuve D.L., Hecker M., Jones P.D., Carr J.A., Solomon K.R., Smith E.E., Van der Kraak G., Kendall R.J., and J.P. Giesy. 2004. Effects of Atrazine on Metamorphosis, Growth, and Gonadal Development in the Green Frog (Rana clamitans). Journal of Toxicology and Environmental Health, Part A, 67: 941-957. MRID 458677- 03.
3. ***Syngenta-sponsored:*** DuPreez L.H., Solomon K.R., Carr J.A., Giesy J.P., Gross C., R. J. Kendall et al. 2005. Population Structure Characterization of Clawed Frog (Xenopus laevis) in Corn-growing Versus Non-corn-growing Areas in South Africa. African Journal of Herpetology. 54: 61 - 68.
4. Freeman, J.L. and A.L. Rayburn. 2005. Developmental Impact of Atrazine on Metamorphing Xenopus laevis as Revealed by Nuclear Analysis and Morphology. Environmental Toxicology and Chemistry 24(7): 1648 - 1653.
5. Forson, D. and A. Storfer. 2005. Effects of Atrazine and Iridovirus Infections on Survival and Life-history Traits of the Long- toed Salamander (Ambystoma macrodactylum). Environmental Toxicology and Chemistry 25(1): 168 - 173.
6. Hayes, T.B. 2004. There is No Denying This: Defusing the Confusion about Atrazine. Bioscience 54: 1138 - 1149.
7. Hayes, T.B. 2005. Comment on ``Gonadal Development of Larval Male Xenopus laevis Exposed to Atrazine in Outdoor Microcosms." Environmental Science and Technology 39(19) 7757-7758.
8. Hayes, T.B. 2005. Welcome to the Revolution: Integrative Biology and Assessing the Impact of Endocrine Disruptors on Environmental and Public Health. Journal of Integrative and Comparative Biology 45: 321- 329.
9. Hayes T.B., Stuart A.A., Mendoza M., Collins A., Noriega N., Vonk A., Johnston W., Liu R., and D. Kpodzo. 2006. Characterization of Atrazine-Induced Gonadal Malformations in African Clawed Frogs (Xenopus laevis) and Comparisons with Effects of an Androgen Antagonist (Cyproterone Acetate) and Exogenous Estrogen (17-[beta]-estradiol): Support for the Demasculinization/Feminization Hypothesis. Environmental Health Perspectives 114: 134 - 141.
10. ***Syngenta-sponsored:*** Jooste A.M., Du Preez L.H., Carr J.A., Giesy J.P., Gross T.S., Kendall R.J., Smith E.E., Van Der Kraak G.J., and K.R. Solomon. 2005. Gonadal Development of Larval Male Xenopus laevis Exposed to Atrazine in Outdoor Microcosms. Environmental Science and Technology 39: 5255- 5261. MRID 458677.
11. ***Syngenta-sponsored:*** Murphy M.B., Hecker M., Coady K.K., Tompsett A.R., Jones,P.D., DuPreez L.H., Solomon K.R., Carr J.A., Smith, E.E., Kendall R.J., van der Kraak G., and J.P. Giesy. 2005. Sediment TCDD-Eq's and EROD and MROD Activities in Ranid Frogs from Agricultural and Non-agricultural Sites in Michigan (USA). Archives of Environmental Contamination and Toxicology 51(3): 467-477. MRID 458677-02.
12. ***Syngenta-sponsored:*** Murphy, M.B, Hecker M., Coady K.K., Tompsett A.R., DuPreez L.H., Everson G.J., Solomon K.R., Carr J.A., Smith E.E., Kendall R.J., van der Kraak G., and J.P. Giesy. 2006. Atrazine Concentrations, Gonadal Gross

Morphology, and Histology in Ranid Frogs Collected in Michigan Agricultural Areas. Aquatic Toxicology 76: 230-245. MRID 458677-02.

13. ***Syngenta-sponsored:*** Murphy, M. B., Hecker M., Coady K.K., Tompsett A.R., Higley E.B., Jones P.D., Du Preez L.H., Solomon K.R., Carr J.A., Smith E.E., Kendall R.J., Van Der Kraak G., and J. P. Giesy. 2006. Plasma Steroid Hormone Concentrations, Aromatase Activities and GSI in Ranid Frogs Collected from Agricultural and Non-Agricultural Sites in Michigan (USA). Aquatic Toxicology 77: 153 - 166.
14. ***Syngenta-sponsored:*** Orton, F., Carr J.A., and R. D. Handy. 2006. Effects of Nitrate and Atrazine on Larval Development and Sexual Differentiation in the Northern Leopard Frog Rana pipiens. Environmental Toxicology and Chemistry 25(1): 65 - 71.
15. ***Syngenta-sponsored:*** Smith E.E., Du Preez L.H., Gentles B.A., Solomon K.R., Tandler B., Carr J.A., Van Der Kraak G.J., Kendall R.J., Giesy J.P. and Gross T.S. 2005. Assessment of Laryngeal Muscle and Testicular Cell Types in Xenopus laevis (Anura Pipidae) Inhabiting Maize and Non-maize Growing Areas of South Africa. African Journal of Herpetology 54(1): 69-76. MRID 458677-10.
16. Sullivan K. B, and K. M. Spence. 2003. Effects of Sublethal Concentrations of Atrazine and Nitrate on Metamorphosis of the African Clawed Frog. Environmental Toxicology and Chemistry 22(3): 627 - 635.

NRDC COMMENTS:

Atrazine levels in rivers and streams does occasionally exceed 20 ppb, whereas concentrations in the wet ditches adjacent to fields, where amphibians may breed, routinely exceed 250 ppb and levels as high as 4,000 ppb have been reported in field run-off water during spring atrazine applications. Even rainfall has detectable levels of atrazine, even in areas far from application sites. Atrazine has an approximate half-life of 146 days in soil and 742 days in water, making it highly likely that breeding and developing amphibians will be exposed. The chemical and its metabolites have been detected in about 75% of stream samples and 40% of ground water samples in agricultural areas across the US, according to government sampling data (USGS, 2006). All the studies cited below report adverse effects from ecologically relevant atrazine exposures. Full citations are provided at the end of these comments.

1. **EPA scientific review fails to fully include scientific evidence of neuroendocrine effects in amphibians associated with atrazine**

Atrazine has been shown by numerous independent laboratories to disrupt neuroendocrine processes in amphibians (Rohr and Palmer, 2005; Hayes et al, 2002, 2003; Larson et al, 1998). Such effects are likely to be a contributing mechanism to observed adverse impacts on population survival. Important scientific papers relevant to this issue that should be included in the EPA and SAP review include but are not limited to:

?? Larval-stage exposure of salamaders to atrazine (40 and 400 ppb) was associated with hyperactivity, fewer water-conserving behaviors, and accelerated dessication 4-8 months after exposure, compared to controls (Rohr and Palmer, 2005).

- Larval salamanders exposed to atrazine (75 ppb, 250 ppb) had elevated plasma thyroxine levels compared with controls. The lower dose treated group (75 ppb) also had reduced levels of corticosterone, compared with both the control and the high dose group. The low dose group developed slower than controls, whereas the high dose group developed at the same time but were smaller and had reduced weights compared with controls (Larson et al, 1998).
- Exposure of *Xenopus laevis* tadpoles during sexual differentiation to atrazine (0, 21 ppb) for 48 hrs resulted in a significant increase in the frequency of secondary oogonia in atrazine-exposed ovaries of females (Tavera-Mendoza et al, 2002a), and a significant reduction in spermatogonial cell nests and nursing cells in atrazine-exposed testis of males (Tavera-Mendoza et al, 2002b), compared with controls. The authors suggest that these effects are likely to impact the reproductive fitness of exposed animals.

2. EPA scientific review fails to fully include scientific evidence of long-term or permanent effects in amphibians associated with atrazine

Atrazine exposure during early developmental stages has been shown to adversely impact later-life outcomes, including susceptibility to infection (Kiesecker, 2002), risk of dessication during adult-hood (Rohr and Palmer, 2005), and long-term survival (Rohr et al, 2006). Such effects are likely to be a contributing mechanism to observed adverse impacts on population survival. Important scientific papers relevant to this issue that should be included in the EPA and SAP review include but are not limited to:

- Relative to control animals, salamanders exposed during larval stages to atrazine at 4 ppb and 40 ppb has statistically significantly reduced survival even 14 months after exposure. These results were identified only when the researchers accounted for toxic effects that persist or appear after the exposure had ended (carryover effects), and the ability of the population to mask harm to individuals by reduced competition among survivors (density-mediated compensation) (Rohr et al, 2006).
- Exposure of salamanders to atrazine (0, 40, 400 ppb) prior to metamorphosis resulted in greater rates of dessication, even as much as 4 and 8 months after the exposure ended, with no sign of recovery, compared with controls (Rohr and Palmer, 2005).
- Wood frog tadpoles exposed to atrazine (0, 3, 30 ppb) for four weeks were then challenged with naturally-occurring trematode infection. Frogs that had been pre-exposed to atrazine at both low and high doses during tadpole-stages had a dose-dependent weakened immune response (reduced eosinophils) and increased infection rate, compared with controls (Kiesecker, 2002).

3. EPA scientific review fails to fully include scientific evidence of nonlinear, nonmonotonic relationships between atrazine concentrations and amphibian responses

Numerous scientific studies report a more severe response to atrazine exposure at low concentrations (= 25 ppb), suggesting that routine environmental exposures during

critical windows of development may be of the most relevant for predicting acute and chronic adverse impacts. Important scientific papers relevant to this issue that should be included in the EPA and SAP review include but are not limited to:

- Tadpoles of four species of frogs--spring peepers (*Pseudacris crucifer*), American toads (*Bufo americanus*), green frogs (*Rana clamitans*), and wood frogs (*Rana sylvatica*)—were exposed during development to atrazine (0, 3, 30, 100 ppb). Survival was significantly lower for all animals exposed to 3 ppb compared with either 30 or 100 ppb, except the late stages of B. americanus and R. sylvatica (Storrs and Kiesecker, 2004).
- Larval-stage exposure of salamaders to atrazine (0, 4, 40, 400 ppb) was associated with hyperactivity, fewer water-conserving behaviors, and accelerated dessication 4-8 months after exposure, compared to controls. The greatest impacts on survival occurred at low exposure concentrations. Relative to control animals, the salamanders exposed to 4 ppb atrazine during early life stages had significantly lower survival 421 days after cessation of exposure, when considering both the exposure and carryover effects. (Rohr et al, 2006).
- Larval salamanders exposed to atrazine (75 ppb, 250 ppb) had elevated plasma thyroxine levels compared with controls. The lower dose treated group (75 ppb) also had reduced levels of corticosterone, compared with both the control and the high dose group. The low dose group developed slower than controls, whereas the high dose group developed at the same time but were smaller and had reduced weights compared with controls (Larson et al, 1998).

4. EPA limits placed on SAP review are likely to bias outcome

<u>EPA scientific review failed to include studies demonstrating adverse endocrine effects of atrazine in mammals:</u>

Even before evidence of hormone disruption activity had emerged in amphibians, EPA scientists and others had been reporting that atrazine disrupts the normal progression of sexual development in rats. Reported findings included: dose-dependent decreased estrogen-induced surges of circulating prolactin and luteinizing hormone levels (Cooper et al, 2000); prostatitis in offspring of dams treated during nursing (Stoker et al, 1999); delayed puberty in males (Stoker et al, 2000) and females (Laws et al, 2000) treated with atrazine by gavage from weaning until puberty; decreased sperm number and motility in adults (Kniewald et al, 2000), and reduced testosterone production by testicular cells of juvenile rats exposed prior to puberty.(Friedmann et al, 2002) The disruption of endocrine pathways is thought to be the cause of observed mammary tumors in one strain of female rats (Sprague-Dawley); while this mechanism may be strain-specific, it is likely to have implications for risks in other species (IARC, 1999; Stevens et al, 1994, 1998, 1999). Important scientific papers relevant to this issue that should be included in the EPA and SAP review include but are not limited to:

- When nursing rats were treated with atrazine the male offspring developed prostate gland inflammation (Stoker et al, 1999).

- ?? Treatment of Wistar male and female rats with atrazine from weaning until puberty resulted in delayed sexual maturity. In the female, oral gavage of 50-200 mg/kg atrazine at postnatal day 22-41 delayed vaginal opening (puberty), in a dose-dependent manner (Laws et al, 2000). In male rats preputial separation was significantly delayed following treatment with 12.5, 50, 100, 150, and 200 mg/kg atrazine administered by gavage (PND 23-53) (Stoker et al, 2000).
- ?? Atrazine reduced sperm motility in exposed Fischer rats. Animals were treated with 60 and 120 mg/kg atrazine administered twice weekly by intraperitoneal (i.p.) injection over a period of 60 days. The authors report that testicular sperm number in atrazine-treated groups increased with the treatment time due to reduced sperm motility (Kniewald et al, 2000).
- ?? Long Evans (LE) rats were exposed in utero to atrazine, followed by challenge with the carcinogen dimethybenz[a]anthracene. Atrazine-exposed pups demonstrated delayed mammary bud outgrowth, followed by an increase in multiplicity and volume of tumors after exposure to the carcinogen, compared to non-atrazine treated controls. In addition, the atrazine-exposed pups showed an increase in organ pathology (adrenal nodules, pituitary foci, large ovarian cysts, lymph node and spleen enlargement), compared with controls. (Birnbaum and Fenton, 2003)
- ?? A mammary tumor response (mammary fibroadenomas and adenocarcinomas) has been consistently observed in Sprague-Dawley (SD) female rats following chronic oral dosing of atrazine and simazine at and above the maximum tolerated dose (400 ppm; based on three 2-year studies] (IARC, 1999)

<u>EPA scientific review failed to include scientific reports of adverse endocrine effects of atrazine in humans:</u>

Relevant human data have been published suggesting that atrazine has been linked to endocrine effects in individuals exposed through their work or home environment. Important scientific papers relevant to this issue that should be included in the EPA and SAP review include but are not limited to:

- ?? A multi-center case-control study of fertile men in U.S. agrarian areas reported a significant association between poor semen quality (reduced sperm concentration and motility) and urinary atrazine metabolite levels above the level of detection (0.1 ?g/g creatinine), compared with men from urban centers (OR=11.3, 95% CI=1.3-98.9).(Swan et al, 2003, 2006)
- ?? It is alarming that far higher urinary metabolite levels have been reported in male farmers who self-applied atrazine, ranging from 0.16 to 5.0 ?g/g creatinine (95% CI=0.33-1.3) (Curwin et al, 2005), suggesting that farmers that apply pesticide themselves (not professionally trained applicators) may represent a population at increased risk of reduced sperm quality from pesticide exposures.

<u>EPA scientific review failed to consider evidence of impacts of mixtures and co-contaminants with atrazine:</u>

The EPA review is artificially constrained to consider exposure to pesticides in isolation; this is extremely likely to artificially underestimate risks from exposure to toxic mixtures that commonly occur in the real world (Chevre et al, 2006; Christin et al, 2004). One assessment of multiple pesticide exposures among men with NHL has reported a suggested superadditive effect of atrazine in combination with the pesticides carbofuran, diazinon, or alachlor (DeRoos et al, 2003). Similarly, the Hayes lab recently examined the effects of atrazine in pesticide mixtures on frog viability and metamorphosis, and reported increased mortality of tadpoles exposed to multiple pesticides at levels that were non-lethal when occurring individually (0.1 ppb) (Hayes et al, 2006). Exposure to multiple pesticides simultaneously is routine for human and wildlife populations; the USGS reported that more than 90% of the time, watershed streams had detections of 2 or more pesticides or metabolites, and about 20% of the time they had detections of 10 or more pesticide contaminants (USGS, 2006). EPA should broaden its charge to the SAP to include consideration of mixtures and co-contaminants with atrazine.

Thank you for the opportunity to provide comments,
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