



Lipid Concentrations in Neonate Watersnakes (Colubridae: *Nerodia*) as a Function of *in utero* Exposure to Atrazine



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Abstract

As a component of a commonly-applied herbicide, atrazine is known to disrupt endocrine function in a number of vertebrate species. Recent studies have established links between the intensity of endocrine disruption and the amount of lipid accumulation in various tissues. Viviparous watersnakes provide a model system in which we investigated the relationship between lipid levels in the livers of neonate snakes that had been exposed to atrazine *in utero* via their mothers' diets. We fed fish laced with one of four atrazine concentrations to adult female watersnakes (*Nerodia sipedon*) throughout their gestation. Upon birth, we removed all liver tissue from the euthanized neonates and determined the mass of each sample. We assayed the amount of fat deposition in these tissue samples by exposing them to a chloroform-methanol extraction method. We discuss our results with respect to both the potential for indirect negative impacts of atrazine on non-target organisms, and the importance of examining one of several possible endpoints that indicate the intensity of atrazine exposure.

Introduction

- Multiple endpoints need to be examined to fully understand the effects of endocrine disrupting contaminants (1).
- Atrazine is a commonly-applied herbicide, the exposure to which manifests a variety of endocrine disrupting properties (3,5).
- Many contaminants are either bioaccumulated in lipids, affect lipid accumulation, or affect adipogenesis (8,9).
- The liver is often used for lipid analyses because it is integral in metabolism and detoxification (4).
- The Northern Watersnake (*Nerodia sipedon*) is an appropriate model for examining the endocrine disruption endpoints of atrazine because it is associated with wetland habitat, feeds on aquatic prey, and has a broad geographic distribution (2).

Objective & Hypothesis

The purpose of our study was to establish another endpoint for examining the sublethal endocrine disrupting properties associated with exposure to atrazine. Our null hypotheses were that the presence of atrazine in diet of gravid watersnakes would not affect the total lipid content in the livers of either the females, or the neonates produced at the end of gestation.



Methods

- We collected watersnakes from two lakes (May - June 2009).
- For the duration of gestation, gravid subjects were fed minnows laced with one of the following concentrations of atrazine: 0 (control), 2, 20, and 200 ppb.
- The livers of the neonates were removed and weighed.
- The livers were dried in an oven at 60 °C for 72 h and the dry masses were recorded.
- The livers were homogenized in 800 µl of chloroform:methanol solution, using a plastic grinder for 5 min.
- The samples were dried for 48 h, and the mass of the resulting pellet (extracted lipid) was recorded.

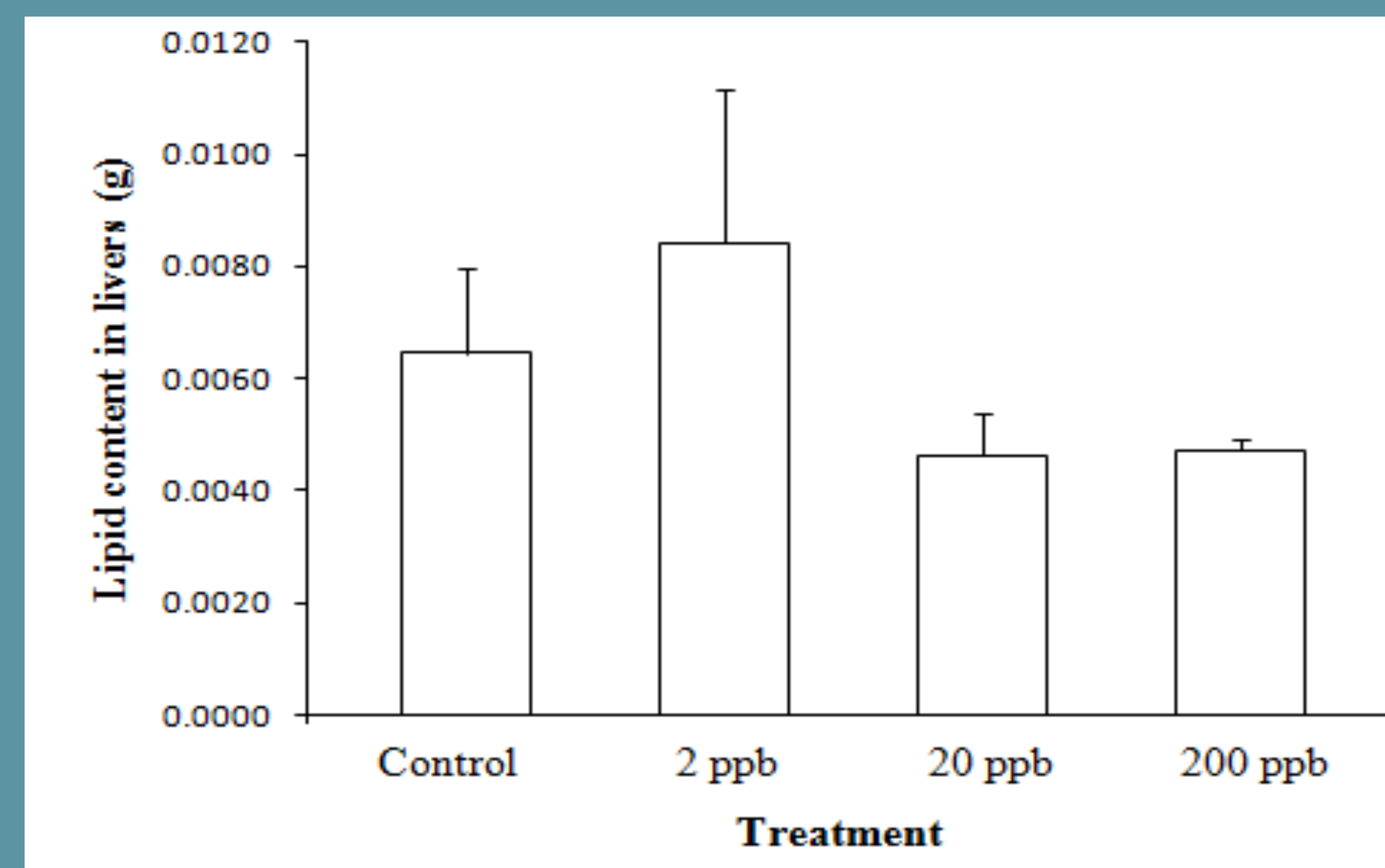


Figure 1. Liver lipid masses of neonate Northern watersnakes (*Nerodia sipedon*) when exposed to atrazine via maternal ingestion. (Control $n = 19$; Low $n = 27$; Medium $n = 15$; High $n = 10$; standard error given in ± 1).

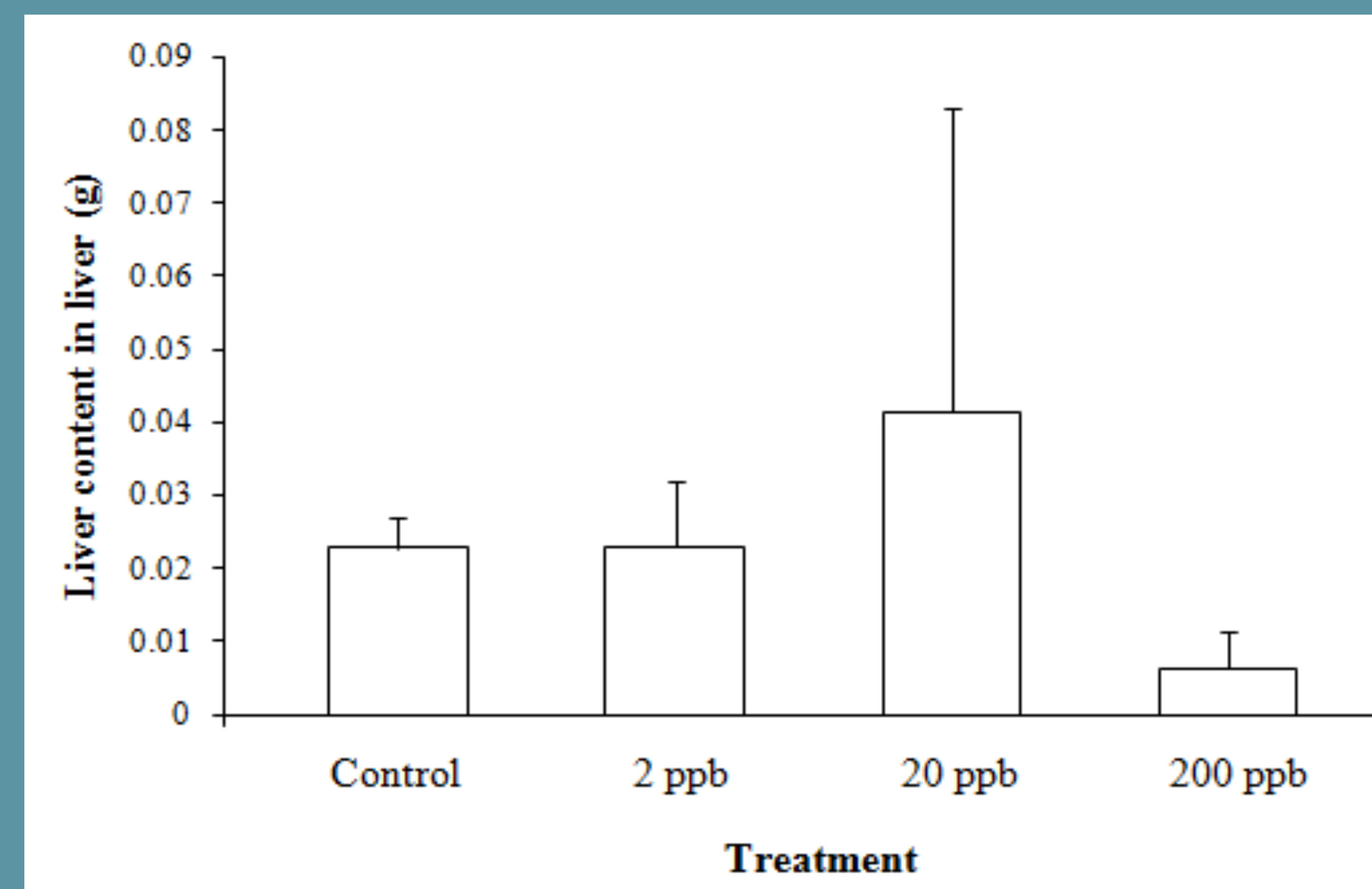


Figure 2. Lipid masses (reported as mean ± 1 SE) in livers of adult female watersnakes after ingesting atrazine for *circa* 3 mo. (sample sizes = 3 (control) 2 (2 ppb), 2 (20 ppb), and 4 (200 ppb), respectively).

Results

- Lipid content of neonate livers varied as a function of the level of *in utero* atrazine exposure ($F_{3,9} = 4.65$, $p = 0.04$, $R^2 = 0.70$; Fig. 1).
 - Neonates exposed to the 2 ppb concentration of atrazine had higher lipid content in their livers.
- Lipid content of adult female livers varied as a function of the level of atrazine received in the diet ($F_{3,3} = 19.52$, $p = 0.02$, $R^2 = 0.97$; Fig. 2).
 - Female subjects exposed to 20 ppb atrazine had higher lipid content in their livers than those exposed to 200 ppb atrazine.

Conclusions

- Atrazine exposure increases lipid levels in the livers of the neonates produced by exposed adult female watersnakes.
 - This change in lipid mass could be caused by either an increase in adipogenesis, or that the affected individuals are less able to effectively break down lipids (4,9).
- Our findings are consistent with research showing that atrazine's effects as an endocrine disruptor can occur at moderate to low doses (6,7).
- In addition to direct contact (via contaminated water), semi-aquatic reptiles can be exposed to atrazine through dietary uptake and, if viviparous, during gestation.
- Future research should examine the long-term (multi-generational) impacts of these exposure effects.

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References

1. Crews et al. 2000. *Quart. Rev. Biol.* 75:243-260.
2. Gibbons & Dorcas 2004. *North American Watersnakes: A Natural History*. Norman: Univ. Oklahoma Press.
3. Hayes et al. 2010. *Proc. Nat. Acad. Sci.* 107:4612-4617.
4. Köhler 1991. *Comp. Biochem. Physiol., C* 100:123-127.
5. McMullin et al. 2004. *Toxicol. Sci.* 79:278-286.
6. Rohr & McCoy 2010. *Environ. Health Perspect.* 118:20-32.
7. Storrs & Kiesecker 2004. *Environ. Health Perspect.* 112:3.
8. Tabb & Blumberg 2006. *Molec. Endocrinol.* 20:475-482.
9. Wada et al. 2007. *J. Pharmacol. Sci.* 105:133-137.

