United States Department of the Interior

FISH AND WILDLIFE SERVICE
Washington, D.C. 20240

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In Reply Refer To: FWS/AFHC/DEQ/DCN 49941

Office of Pesticide Programs (OPP) Regulatory Public Docket (7502P)
Environmental Protection Agency
200 Pennsylvania Ave., NW.
Washington, DC 20460–0001

The U.S. Fish and Wildlife Service (Service) appreciates the opportunity to submit the following comments to the U.S. Environmental Protection Agency (EPA) regarding the Petition Requesting Ban on Use and Production of Atrazine Use (EPA DOCKET CONTROL NUMBER OPP–2011–0586; Federal Register Vol. 76, No. 178 /Wednesday, September 14, 2011 /Page 56755). Below is a summary of our comments, which are discussed in detail in the attached technical appendix. Our comments are based on peer reviewed scientific study results and their bearing on Service trust resources. We have specifically focused on recovery of amphibians and other aquatic life listed under the Endangered Species Act (ESA) of 1973, and atrazine exposure and effects to biota on Service managed lands including National Wildlife Refuges (Refuges).

Atrazine is one of the most commonly applied herbicides in North America and is very mobile in the environment. Atrazine and/or its metabolites are virtually ubiquitous in surface and groundwater. With its persistence in aquatic systems, a wide range of aquatic and aquatic-dependent species are chronically exposed to atrazine. Adverse effects from this exposure, including endocrine and olfactory disruption, immunosuppression, organ damage, and impaired reproduction pose a threat to aquatic and aquatic-dependent Service trust resources, including 24 federally listed amphibian species, 145 listed fish, and 72 listed freshwater mussels. In addition, atrazine causes a reversible inhibition of photosynthesis in plants directly affecting phytoplankton, periphyton, and macrophytes, which can then disrupt the entire aquatic community and cause risk to organisms that depend on these food sources at a population level.

Atrazine concentrations found to adversely affect amphibians in laboratory and field assessments have been frequently detected on Refuges. Further, there are no national numeric water quality criteria to protect aquatic life from atrazine and water quality standards for states where atrazine is frequently applied are often non-existent or inadequate. We have previously expressed to EPA that a lack of adequately protective water quality standards for atrazine is likely detrimental to federally listed species.

In summary, the Service is obligated to protect species listed under ESA and the Refuge lands it manages from the harmful effects of contaminants. There is a growing body of evidence that atrazine poses threats to the fitness of Service aquatic trust resources. Therefore, we recommend that EPA fully characterize and assess the ecological risks of atrazine and examine all available lines of evidence in making regulatory decisions regarding its use, particularly for sensitive species or those species protected under the ESA. The environmental risks of continued atrazine use may be more serious than the economic risks of atrazine restrictions. Thus, we request that EPA thoroughly evaluate the extent of atrazine contamination in water and the threats posed to aquatic and aquatic-dependent species when addressing the petitioners' request to ban atrazine.

Thank you again for the opportunity to provide comments. If you have any questions, please contact Dr. Roger Helm, Chief. Division of Environmental Quality at (703) 358-2148.

Sincerely,

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ACTING Assistant Director for Fisheries and Habitat Conservation

Appendix: Atrazine Effects to Aquatic Organisms

Atrazine is a triazine herbicide used to control annual broadleaf and grass weeds. It is one of the most commonly applied herbicides in North America, with nearly 77 million pounds used annually (Eisler 2000; Thelin and Stone, 2010). Atrazine is registered for a variety of crops with 99% of its use in the United States on corn, sorghum, and sugarcane. Atrazine can be used in both pre-emergent and post-emergent applications formulations.

The mobility of atrazine in the environment is problematic from the Service's perspective. Atrazine can enter the atmosphere via volatilization and spray drift and then be aerially deposited (Mast et al. 2003; Hageman et al. 2006; Battaglin 2009). Atrazine is also transported via aerial drift, precipitation, and overland runoff to surface water with further movement into ground water. Atrazine and/or its degradates are virtually ubiquitous in surface and groundwater (Kolpin et al. 1995; Battaglin et al. 2003; Scribner et al. 2005).

The U.S. Environmental Protection Agency (EPA) states that atrazine is practically non-toxic to mammals and birds but moderately-to-highly toxic to fish and aquatic invertebrates (USEPA 2006a). With its persistence in aquatic systems, a wide range of aquatic and aquatic-dependent species are being chronically exposed to atrazine. Specifically, the continued use of atrazine, may pose a variety of risks to non-target aquatic organisms from chronic exposure including reduced primary productivity in plants; reduced populations of aquatic macrophytes, aquatic invertebrates, amphibians, and fish; and loss of sensitive aquatic species (USEPA 2006a). Such effects on aquatic organisms can result in changes to the entire structure and function of the community. Therefore, the Service requests that EPA thoroughly consider the extent of atrazine contamination in water and the effects of atrazine to aquatic and aquatic-dependent species, and review the information provided below regarding concerns to the trust resources of the U.S. Fish and Wildlife Service (Service).

Threatened and Endangered Species

The Service has several aquatic species listed under the Endangered Species Act (ESA), including 24 amphibians, 145 fish, and 72 freshwater mussels. Additionally, there are several amphibians identified as species of concern including 11 frogs, five toads, and 24 salamanders (http://ecos.fws.gov/tess_public/SpeciesReport.do).

Not all of these species will occur in areas where atrazine is applied, but exposure may still occur when atrazine enters a species' habitat from runoff or atmospheric deposition (Battaglin et al. 2009). Exposure and effects from pesticides in general are often identified as potential threats to recovery of listed species. However; atrazine has been specifically identified as a potential threat to the recovery of several federally listed species including the Wyoming Toad (USFWS 1984) and pallid sturgeon (Schwarz et al. 2006). In addition, atrazine and other soluble pesticides were found in spring habitat of the endangered Barton Springs salamander (*Eurycea sosorum*) in downtown Austin, Texas. The U.S. Geological Survey detected atrazine in the spring habitat of the salamander by analyzing filtered water samples during rainfall events. Study results indicate that surface runoff from residential lawns, municipal parks, and golf courses transport atrazine into the salamanders' habitat. Twenty-seven percent of the samples from both spring water and surface water showed atrazine concentrations of at least 0.1 micrograms per liter (µg/L). While it is difficult to quantify effects to the endangered Barton Spring salamander from this exposure, the presence of other environmental stressors may increase its vulnerability and consequences to fitness cannot be ruled out.

National Wildlife Refuges

Atrazine concentrations in waters of National Wildlife Refuges (Refuges) have exceeded concentrations that cause adverse effects in atrazine laboratory and field assessments (see below and Larson et al. 1998; Hayes et al. 2002a, 2003, 2010; Rohr and Palmer 2005; Rohr et al. 2006). For example, atrazine concentrations at Service managed Waterfowl Production Areas in Nebraska were as high as 287 micrograms per liter (µg/L) and frequently exceeded Nebraska's chronic aquatic life water quality criterion of 12 µg/L (NDEQ, 2010). At DeSoto Refuge, Iowa, concentrations of atrazine frequently exceeding afreshwater aquatic life standard of 1.8 µg/L during both pre- and post-application periods (Battaglin et al. 2009). Because atrazine is not applied on the refuge, it most likely originates from groundwater sources or atmospheric deposition (Buske 1991). Additionally, many other pesticides were also detected but the authors state that the relatively high concentrations of atrazine, nicosulfuron, and triclopyr in ditch samples feeding the main lake on the refuge (DeSoto Lake) were high enough that water quality could be adversely affected (Battaglin et al. 2009).

At William L. Finley Refuge in Oregon, atrazine and deethylatrazine were the most frequently detected compounds, found in 76% and 67%, respectively, of the samples collected at six sites on three streams. The highest concentrations were detected in the spring (late March) at 3.0 and 2.2 µg/L, respectively. These two compounds were also detected at the reference site during the rainy spring sampling event as well as throughout the year during both rainy and dry periods. It is suspected that atmospheric transport is the source as the product is not known to be applied upstream (McCarthy 1998).

Data from Loxahatchee Refuge in Florida, revealed that water entering the Refuge from inflow pumping stations had detectable atrazine concentrations (Miller and McPherson, 2008). At one site, atrazine was present in 87 of 88 samples collected from 1996 to 2005 with a maximum concentration of 12.0 µg/L. Between April 1987 and March 2002, atrazine was detected in 57 of 75 samples taken from a second site with a maximum concentration of 12.3 µg/L. Atrazine was also detected in sediment samples from these sites. Because this Refuge is part of the Everglades restoration project, there is the concern that proposed changes to the water flow entering the refuge could result in a further increases in atrazine loads (Miller and McPherson, 2008).

Other sampling events at Refuges have also shown the presence of atrazine in surface water. Atrazine concentrations ranged from 0.01 to 1.5 µg/L and deethylatrazine ranged from 0.01 to 0.13 µg/L in nine samples taken from Rattlesnake Creek, which feeds the Quivira Refuge in Kansas (Christensen 2001). Data collected in the lower Rio Grande Valley and at the Laguna Atascosa Refuge in Texas showed that the triazine herbicides, including atrazine, were detected at six sites with a maximum concentration of 0.8 µg/L (range 0.1 to 0.8 µg/L; Wells et al. 1988).

In Maryland, the Blackwater River feeds the Blackwater Refuge. At four sites along the river atrazine was detected at concentrations up to 1.1 µg/L in surface water (Fleming et al. 2011). Although many of the atrazine concentrations do not exceed the freshwater aquatic life standard of 1.8 µg/L, these water resources are being contaminated on Refuges. Consequently, the aquatic and aquatic-dependent organisms are being chronically exposed to sublethal atrazine levels that can affect many aspects of their survival as discussed below.

Ecological Concerns to Amphibians

The effects of atrazine exposure to aquatic ecological receptors are not obvious. Unlike many chemicals where toxicity parallels the concentration, most effects of atrazine occur sublethally and are the result of chronic exposure. A particularly large body of literature exists demonstrating potential adverse effects to fitness of amphibians.

Reduction of food algae for tadpoles

Algae and other phytoplankton are the primary food source for amphibian larvae. Atrazine is a relatively mobile herbicide that works by producing a reversible inhibition of photosynthesis in plants and can directly affect phytoplankton, periphyton, and macrophytes in aquatic systems (DeNoyelles et al. 1982; Stratton 1984; Solomon et al. 1996). It is classified as moderately persistent to persistent (Briggs 1992) and can be toxic to aquatic plants at concentrations as low as 10 to 20 µg/L (Solomon et al. 1996; Fairchild et al. 1998; USEPA 2006a).

Research on aquatic systems show that the recovery of phytoplankton and periphyton biomass after atrazine exposure typically results in proliferation of atrazine-resistant species (Herman et al. 1986). This can result in increased competition for the remaining food by tadpoles or force the larval anurans to rely on potentially less desirable food source,s thereby slowing amphibian growth and the ability to complete metamorphosis (Dewey 1986; Diana et al. 2000). Overall, exposure to aquatic communities to atrazine can disrupt the entire aquatic community and cause risk to the organisms at a population level (Rohr and Crumrine 2005; USEPA 2006a). This is particularly important with the dramatic declines of amphibians worldwide (Blaustein and Wake, 1990; Griffiths and Beebee, 1992; Corn 1994; Vertucci and Corn, 1996).

Effects to Amphibian Reproduction

Atrazine is classified as an endocrine disruptor (Tillitt et al. 1998; Cooper et al. 2000; Suzawa and Ingraham, 2008). Such endocrine disruptors induce female-skewed sex ratios and alter gonadal development and concentrations of hormones in amphibians (Hayes 2000; McCoy et al. 2008; McCoy and Guillette, 2009). Recent research indicates that atrazine alters the expression of the enzyme CYP19, which can then increase the production of estrogen that alters gonadal tissue (Fan et al. 2007a, 2007b; Gunderson et al. 2011) resulting in the skewed sex ratios. This can occur at ecologically relevant levels of atrazine and the effects to the sexual organs are significant. Even at pulsed exposures of atrazine during development reproductive success could be negatively affected for the amphibian's entire life (Taveras-Mendoza et al. 2002a, 2002b).

Several recent studies have demonstrated these effects. Exposure to 2.5 µg/L atrazine resulted in suppressed mating behavior, reduced spermatogenesis, and decreased fertility of male African clawed frogs (*Xenopus laevis*) as well as complete feminization of some individuals (Hayes et al. 2010). Ten percent of atrazine exposed genetic males developed into functional females that copulated with unexposed males and produced viable eggs (Hayes et al. 2010). Atrazine at concentrations of ≥2 ppb can impair sexual development in larval amphibians (Hayes et al. 2002a, 2002b). Atrazine at concentrations ≥0.1 ppb can induce hermaphroditism and demasculinize the larynges of exposed *X. laevis* in the laboratory. Smaller laryngeal size affects the ability of the frogs to call and therefore their breeding success. When atrazine concentrations reach 25 ppb, testosterone levels decreased 10 times in the frogs (Hayes et al. 2002a, 2002b).

Furthermore, atrazine exposure to *Acris crepitans* and *X. laevis* during embryonic development can cause testicular resorption and the formation of ovotestes (Reeder et al. 1998; Tavera-Mendoza et al. 2002a, 200b). Intersex gonads were found in juvenile stages of *Bufo americanus* and *Hyla versicolor* exposed to atrazine (Storr and Semlitsch, 2008; Storrs-Mendez and Semlitsch, 2009). *Rana pipiens* exposed to >1 ppb atrazine exhibited retarded gonadal development (gonadal dysgenesis) and testicular oogeniesis (intersexed). Additionally, the male frogs that developed slowly were therefore exposed to atrazine for longer periods of time and consequently experienced oocyte growth as both oogenesis and vitellogenesis (Hayes et al. 2003). Developing *R. pipiens* exposed to low atrazine concentrations (1.8 µg/L) in outdoor mesocosms had altered gonadal differentiation and metamorphosis (Langlois et al. 2009).

The likelihood of natural amphibian populations being exposed to these atrazine concentrations used in laboratory studies is quite high, which can result in further declines of already stressed populations (Hayes et al. 2002a). In particular, *R. pipiens* collected from sites across the U.S that were contaminated with atrazine, as well as other agricultural chemicals, commonly exhibited intersex characteristics (Hayes et al. 2003). Specifically, 92% of the male leopard frogs collected (n=20) in the North Platte River near Saratoga, Wyoming, exhibited sex reversal (feminization) where detected atrazine concentrations were 0.20 ppb in river water (Hayes et al. 2002b). The gonadal abnormalities were similar in morphology to those induced by atrazine in leopard frogs in the laboratory (Hayes et al. 2002a, 2002b; Tavera-Mendoza et al. 2002a, 2002b).

Many of the studies on endocrine-related effects of atrazine to amphibians have been summarized by EPA in its 2003 and 2007 "White Papers" (USEPA 2003a; 2007a). In 2003, EPA convened a FIFRA Scientific Advisory Panel (SAP) on Potential Developmental Effects of Atrazine on Amphibians (June 17-20, 2003) which found that the existing lines of evidence support the hypothesis that atrazine interferes with anuran gonadal development at a threshold concentration between 0.01 and 25 µg/L and subsequently requested additional data to further delineate the concentration-response relationship (USEPA 2003b). In 2007, based on the results of these new data, EPA concluded that atrazine does not affect amphibian gonadal development at environmentally relevant concentrations and that no further testing is required to address the issue (USEPA 2007a). However, EPA's 2007 FIFRA SAP on the Potential for Atrazine to Affect Amphibian Gonadal Development (October 9-12, 2007) concluded that current data were not sufficient to refute the hypothesis that atrazine affects amphibian gonadal development at these concentrations (USEPA 2008). Based on the numerous studies outlined above and the continued discovery of effects from laboratory and field studies, the Service agrees with this conclusion and believes that the existing data provide sufficient lines of evidence to suggest that atrazine exposure can result in endocrine disruption in amphibians. The Service also believes that EPA should expand its hypothesis to include potential sublethal effects of atrazine beyond the narrow scope of gonadal development when considering these data.

Effects to Growth and Survival

Corticosteroids and thyroid hormones (T_3 and T_4) are important for growth and development of larval amphibians. Estrogenic chemicals that affect these hormones can result in suppression of the immune system, inhibition of larval growth, and decreased development (Hayes 2000). Low concentrations of atrazine can delay metamorphosis and result in decreased weight, shorter snout to vent length (SVL), and lower hematocrits in amphibian tadpoles (Sullivan and Spence, 2003). Atrazine exposure during the early life stages of amphibians seems to have permanent effects on their activity and delayed effects to their survival (Rohr and Palmer, 2005; Rohr et al. 2006).

Metamorphosis is considered only second to embryogenesis in terms of sensitivity to chemically- and physically-induced damage (Murphy et al. 2000) as hormones regulate the structure of the reproductive organs and there is intense gene expression during this period (Cooke 1981; Hayes 2000). Therefore, one atrazine application can affect amphibian development. Because atrazine is applied during the spring (May and June) when most amphibians are breeding and developing, metamorphosing amphibians are particularly vulnerable to the effects of chemicals that affect hormones and gene expression.

For example, atrazine at high concentrations will decrease growth in *H. versicolor* and increase time to metamorphosis in *X. laevis* (Diana et al. 2000; Sullivan and Spence, 2003). At much lower environmentally realistic concentrations, atrazine in combination with nitrate will reduce growth (both length and weight) in *X. laevis* (Sullivan and Spence, 2003) at metamorphosis.

Chronic exposures to sublethal concentrations were found to diminish *Ambystoma barbouri* growth rates (Rohr et al. 2004). Similarly, *A. tigrinum* exposed to sublethal concentrations of atrazine resulted in smaller sized animal with lower weights. These individuals also took a longer time to metamorphose (Larson et al. 1998). A review by Rohr and McCoy (2010) of various studies on the effect of atrazine on growth and metamorphosis of amphibians found that atrazine can either delay metamorphosis or accelerate it but that atrazine consistently reduced the size of the amphibians at or near metamorphosis. Differences in the timing of metamorphosis are likely the result of species sensitivity and the development stage of the organism when exposure occurs (McCoy et al. 2008; Boone et al. 2009; Gunderson et al. 2011). In nature, species sensitivity may be even more pronounced due to the influence of other environmental stressors (e.g., adverse weather conditions, food shortages, and predation).

Atrazine combined with other chemicals likely found in agricultural setting show similar effects to amphibian growth. Atrazine, in combination with metolachlor, affected amphibians synergistically by retarding growth and likely reducing survival in the longer term by affecting overwintering, fecundity, mate selection, food acquisition, and predator avoidance (Mazanti et al.

2003; Hayes 2006). Toxicity tests using an equal mixture of atrazine and alachlor (96-hour LC50's) for rainbow trout (*Onchoryhnchus mykiss*) and two amphibian species (*Lithobates pipiens and Anaxyrus americanus*) demonstrated that the toxicity of the combination was significantly greater than what would be additively predicted (Howe et al. 1998).

Other studies have shown that atrazine at environmentally realistic levels can affect the motor function which would increase the likelihood of the amphibians succumbing to predation (Rohr et al. 2003). Also, over the long-term, survival may be affected even after the exposure to sublethal concentrations of atrazine have ceased. For example, exposure of *A. barbouri* to $\geq$4 ppb of atrazine resulted in significantly lower survival after 14 months post-exposure than what was found in the control. However, this was evident only when the accumulation of both exposure and carryover effects were considered (Rohr et al. 2006).

Disease and Immunotoxicity

To date, little research has been done to investigate the ability of certain chemicals including atrazine, to compromise the immune system of amphibians, but it is reasonable to suspect that this may be occurring (Crawshaw 2000). Short-term chemical stresses can induce immune suppression that can last for several days and this immune response is mediated through increases in levels of endogenous corticosteroids such as cortisol and corticosterone (Crawshaw 2000). Corticosterone is involved in amphibian metamorphosis and it follows that any disruption during critical times, however short-lived, may alter development as well as affect the immune system (Sheffield et al. 1998).

McCoy and Guillette (2009) suggest that endocrine disrupting chemicals may be attributing to recent outbreaks of infectious disease in amphibians. This is even more likely with the complex mixtures of contaminants that are common at various sites where amphibians reside. Hayes et al. (2006) studied the effects of nine different agricultural pesticides used on cornfields in the midwestern U.S. The researchers exposed *R. pipiens* tadpoles to very low concentrations (0.1 ppb) of each of nine chemical separately or in various combinations and measured differences in growth and development, differentiation of gonads, and immune function. Most single chemical treatments resulted in differences in larval growth and development but the pesticide mixtures had more pronounced effects and included damage to the thymus, which is associated with immunosuppression.

Other studies show that exposure to atrazine increases the susceptibility of amphibians to parasite load. The immune system of *R. sylvatica* was suppressed when exposed to atrazine, malathion, and esfenvalerate. The frogs also had increase parasite loads compared to control animal (Kiesecker 2002). Rohr et al. (2008a) studied lethal and sublethal effects of environmentally relevant concentrations of atrazine, glyphosate, carbaryl, and malathion on the amphibian parasite *Echinostoma trivolvis* and the corresponding parasitic load on *R. pipiens* tadpoles. The pesticides reduced larval survival of the parasite but also increased the susceptibility of the tadpoles to parasitic infections. The reduction of exposure of the tadpoles to the parasite due to pesticide-induced larval mortality was 2.5 times smaller than the pesticide-induced increase in amphibian susceptibility to the parasite. This suggests that environmentally relevant levels of pesticide will elevate the occurrence of amphibian parasitic infections. In a followup study, Rohr et al. (2008b) discovered that atrazine was the best predictor of larval trematode infections in *R. pipiens*, which was consistent across different taxa of trematodes in various wetlands.

When *R. pipiens* tadpoles were exposed in outdoor mesocosms with both atrazine and phosphate, common in agricultural settings, the tadpoles exhibited immune suppressions and elevated parasite loads (Rohr et al. 2008b). Similarly, *R. pipiens* and *X. laevis* exposed to a mixture of environmentally relevant concentrations of pesticides including atrazine, the immune response was altered and increased their susceptibility to infections (Christin et al. 2003; Christin et al. 2004). Likewise, at relatively high levels of atrazine, the immune system of *R. pipiens* was also negatively impacted (Houck and Sessions, 2006). Increased disease susceptibility has also been found in *A. tigrinum* where exposure to atrazine increased their susceptibility to the ranavirus (Forson and Storfer, 2006a, 2006b)

Similarly, in adult frogs (*R. pipiens*) exposed to 21 ppb of atrazine for eight days, the immune system was affected by the suppression of recruitment of white blood cells and by the decreased phagocytic activities of these cells. These results show that the same effective dose that disrupts the immune system also disrupt the endocrine system (Brodkin et al. 2007). In fact, a review by Rohr and McCoy (2010), states that atrazine at environmentally relevant levels was associated with a reduction in 77% immune function endpoints in fish and amphibians. There also was an increase in 13 of 16 infection endpoints in both fish and amphibians, which included elevated parasitic, viral, and bacterial infections (Rohr and McCoy, 2010).

Ecological Concerns to Fish

Atrazine exposure may result in both direct and indirect toxic effects to fish. Direct adverse effects of atrazine exposure to fish include endocrine disruption (Moore and Waring, 1998; Moore and Lower, 2001; Spanó et al. 2004; Suzawa and Ingraham, 2008), altered kidney morphology (Fisher-Scheri et al. 1991; Oulmi et al. 1995), reduced larval growth (Alvarez and Fuiman, 2005), decreased egg production (Tillitt et al. 2010), and altered behavior (Saglio and Trijasse, 1998). Indirect effects to fish that may result from atrazine exposure include habitat modification and decreased availability of prey. For example, atrazine concentrations of 20 μg/L in an experimental pond decreased the number of adult insects emerging and insect diversity by 90 percent and 60 percent, respectively, when compared to untreated ponds (Dewey 1986). Decreased prey availability for fish following atrazine exposure to their habitat has been previously reported (Kettle et al. 1987). Experimental ponds containing 20 μg/L atrazine for 136 days resulted in significantly ($p < 0.001$) fewer invertebrates in bluegill (*Lepomis macrochirus*) stomach contents when compared to control ponds (Kettle et al. 1987). The decreased prey base in the experimental ponds was linked to significantly ($p < 0.01$) lower bluegill reproduction (Kettle et al. 1987).

EPA has completed atrazine effects determinations for federally listed fish species as a component to resolve litigation. Some of these determinations conclude that atrazine is likely to adversely affect (LAA) federally listed fish species including Topeka shiner (*Notropis topeka*), pallid sturgeon (*Scaphirhnchus albus*) and delta smelt (*Hypomesus transpacificus*). For pallid sturgeon, the LAA determination is based on indirect effects to habitat and water quality via direct effects to herbaceous/grassy riparian vegetation (USEPA 2007b), whereas for Topeka shiner atrazine effects included direct chronic effects as well as indirect effects resulting from potential effects to aquatic and terrestrial plants (USEPA 2007c). Not Likely to Adversely Affect determinations were made by EPA for other fish species including the Alabama sturgeon (*S. suttkusi*) and shortnose sturgeon (*Acipenser brevirostrum*). However, we have identified several concerns with EPA's atrazine effects determinations for these species including an inadequate evaluation of direct endocrine and sublethal effects to the species and the decision to limit the assessment to parent atrazine and not atrazine degradates or other structurally similar triazine herbicides (USFWS 2008a).

In Nebraska, atrazine has been frequently detected at high concentrations (i.e., >10 µg/L) in important habitat for pallid sturgeon. Although critical habitat for pallid sturgeon has not been federally designated, the lower Platte River and the Missouri River near the confluence of the Platte River is designated as a Recovery Priority Management Area for pallid sturgeon (Dryer and Sandvol, 1993) and the Elkhorn River also provides important habitat (Lutey 2002). During the spawning period of shovelnose and pallid sturgeon, concentrations of atrazine in the lower Platte River at Louisville and in the Elkhorn River at Waterloo have exceeded 20 µg/L (Frenzel et al. 1998; National Water Information System 2005).

In addition to a decreased invertebrate prey base mentioned above, fish species that are important prey for pallid sturgeon, such as red shiners (*Cyprinella lutrenisis*) and fathead minnows (*Pimephales promelas*) may also may be diminished from exposure to atrazine. Red shiners collected from the lower Platte River and exposed to atrazine concentrations of 10 µg/L at 23 and 30 ^{o}C had a significantly lower Critical Thermal Maximum compared to controls, which may result in decreased survival (Messadd et al. 2000). Female fathead minnows exposed to 0.5, 5, and 50 µg/L atrazine had higher levels of ovarian atresia, as well as a 19 – 39 percent reduction in egg production (Tillitt et al. 2010).

The Service's Nebraska Field Office provided EPA Region 7 two letters (USFWS 2008b and 2009) that explained why Nebraska's water quality standard for atrazine was not protective to federally listed species in Nebraska, including pallid sturgeon and Topeka shiner. In our comment letters we explained that since comments were provided for the 2005 triennial review of water quality (USFWS 2005), additional studies have reported on reproductive effects in fish exposed to atrazine concentrations less than 3 µg/L and we recommended that Nebraska adopt a chronic aquatic life water quality criterion for atrazine of 1 µg/L or less. We also requested that EPA initiate section 7 consultation, pursuant to the ESA, relative to its May 30, 2001, approval of Nebraska's revised chronic aquatic life standard for atrazine that changed the standard from 1 µg/L to a less protective 12 µg/L. In 2003, EPA produced a revised draft atrazine criteria document that abandoned the 12 µg/L criterion and have yet to finalize numeric aquatic life criteria for atrazine (USEPA 2003c).

Ecological Concerns to Freshwater Mussels

There are currently 72 mussel species listed as threatened or endangered under the ESA. Though less information exists regarding effects to these species, atrazine has been show to accumulate in freshwater mussels (Jacomini et al. 2006), and effects of atrazine to bivalves have been documented at environmentally relevant levels. Atrazine disrupted aggregation behavior of the freshwater mussel *Elliptio complanata* when exposed to concentrations of 15 µg/L for 72 hours (Flynn and Spellman, 2009). Aggregation behavior is thought to confer both individual and group advantages and may be related to reproduction. Results were similar to those found in mussels exposed to estradiol, suggesting atrazine has the ability to disrupt endocrine-mediated behaviors in these species. Histological effects to the hepatopancreas, were found in zebra mussels (*Dreissena polymorpha* Pallas) exposed to atrazine concentrations as low as 3 µg/L, with increasing intensity at higher concentrations and durations of exposure (Zupan and Kalafatic, 2003). The hepatopancreas has a significant role in digestion, as well as general metabolism and detoxification processes. Alterations to the ovaries and testes were also seen at concentrations as low as 50 µg/L, including damage to loose connective tissue and interstitial cells in both gonads.

In addition to direct effects of atrazine exposure, mussels may be vulnerable to indirect effects via the adverse effects described above to plant communities and fish. For most mussel species, transformation on a host fish is a required element of their life cycle which cannot be bypassed. Host fish availability and density have been found to be significant factors influencing mussel persistence in particular habitats (Haag and Warren, 1998).

EPA has completed atrazine effects determinations under section 7 of the ESA for federally listed freshwater mussel species as a component to resolve litigation. Some of these determinations conclude that atrazine is likely to adversely affect (LAA) federally listed mussel species based on indirect effects to plant communities (USEPA 2006b, 2007d,e). The Service has identified several concerns with EPA's atrazine effects determinations for these species including the failure to adequately consider sublethal and endocrine effects to bivalves and fish (USFWS 2008a).

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