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Aquatic Toxicology 76 (2006) 230–245

AQUATIC TOXICOLOGY

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Atrazine concentrations, gonadal gross morphology and histology in ranid frogs collected in Michigan agricultural areas

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Received 9 June 2005; received in revised form 24 August 2005; accepted 21 September 2005

Abstract

The triazine herbicide atrazine has been suggested to be a potential disruptor of normal sexual development in male frogs. The goals of this study were to collect native ranid frogs from sites in agricultural and non-agricultural areas and determine whether hypothesised atrazine effects on the gonads could be observed at the gross morphological and histological levels. Juvenile and adult green frogs (*Rana clamitans*), bullfrogs (*R. catesbeiana*) and leopard frogs (*R. pipiens*) were collected in the summers of 2002 and 2003. Atrazine concentrations were below the limit of quantification at non-agricultural sites, and concentrations did not exceed 2 μg/L at most agricultural sites. One concentration greater than 200 μg atrazine/L was measured once at one site in 2002. Hermaphroditic individuals with both male and female gonad tissue in either one or both gonads, were found at a low incidence at both non-agricultural and agricultural sites, and in both adults and juveniles. Testicular oocytes (TO) were found in male frogs at most of the sites, with the greatest incidence occurring in juvenile leopard frogs. TO incidence was not significantly different between agricultural and non-agricultural sites with the exception of juveniles collected in 2003. Atrazine concentrations were not significantly correlated with the incidence of hermaphroditism, but maximum atrazine concentrations

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doi:10.1016/j.aquatox.2005.09.010

were correlated with TO incidence in juvenile frogs in 2003. However, given the lack of a consistent relationship between atrazine concentrations and TO incidence, it is more likely the TOs observed in this study result from natural processes in development rather than atrazine exposure.

Keywords: Triazine herbicides; Amphibian; *Rana clamitans*; Field study; Testicular oocyte; Hermaphrodite

1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a pre-emergent herbicide first approved for use in the US in 1958, where it is used primarily on corn, sorghum and sugar cane (Solomon et al., 1996). Atrazine inhibits electron transport in Photosystem II, which results in a disruption of photosynthesis and in turn leads to death from starvation in broad-leaf plants (Giddings et al., 2004). In 1999, approximately 30,100 metric tonnes of active ingredient were applied in the US, 75% of which was applied to corn (US EPA, 2003). Between 1998 and 2002, 815 tonnes of atrazine were used in Michigan, 99.5% of which was applied to corn (Giddings et al., 2004).

Herbicide application generally occurs in the spring or early summer, a time that coincides with the breeding periods of many amphibian species, some of which breed in aquatic habitats that are often subject to runoff from agricultural fields. Atrazine has low volatility, but its moderate water solubility (33 mg/L at 25 °C) makes it relatively mobile in soil and aquatic environments, where it tends to partition into the water column rather than sorbing to sediments (Giddings et al., 2004). The majority of atrazine breakdown in the environment occurs either through microbial degradation of the parent compound to the hydroxylated metabolite with loss of methyl or ethyl groups or by hydrolysis of the triazine ring (Solomon et al., 1996). Atrazine has been found to have a half-life in soil of from 8 to 99 days, depending on soil and environmental conditions, while the half-life of atrazine in the aquatic environment ranges from 41 to 237 days (Giddings et al., 2004). One study of atrazine biotransformation in anaerobic wetland sediment found that the half-life of the parent compound was 224 days (Chung et al., 1996), while a later study found that the half-life was only 38 days (Seybold et al., 2001). Thus, atrazine can persist in the environment, albeit at relatively small concentrations for much, if not all of the amphibian larval period. Environmental concentrations of atrazine have been reported to usually not exceed 20 μg/L, except in small temporary puddles in fields where peak concentrations can be greater than 200 μg/L for short periods of time after storm events (Solomon et al., 1996; Battaglin et al., 2000).

Exposure to agricultural chemicals, together with other factors, such as habitat fragmentation, introduction of predatory species, wetland losses, UV-B radiation and diseases have been postulated as possible causes for world-wide declines of amphibian populations (Allran and Karasov, 2000, 2001; Blaustein and Kiesecker, 2002). Atrazine, while not acutely toxic to frogs at environmentally relevant concentrations (Allran and Karasov, 2000; Birge et al., 2000; Diana et al., 2000; Coady et al., 2004), has been proposed to contribute to frog population declines because it may disrupt normal sexual development in frogs (Hayes et al., 2002, 2003). The goal of the current study was to determine the incidences of testicular oocytes (TO) and hermaphroditism in ranid frogs collected from agricultural and non-agricultural areas, and to evaluate correlations between measured atrazine concentrations and these incidences.

2. Materials and methods

2.1. Site selection and characterization

Study sites were selected on the basis of potential atrazine exposure and the presence of relatively large populations of ranid frogs. Sites were located in three regions in south-central Michigan: Kalamazoo (KZ), the greater Lansing area (GLA) and Lapeer (LPR) (Fig. 1). Sites adjacent to corn fields were classified as "agricultural", while those in the same general area that did not receive direct runoff from corn fields, either