Chemosphere 205 (2018) 126–136

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Extended fish short term reproduction assays with the fathead minnow and Japanese medaka: No evidence of impaired fecundity from exposure to atrazine

Richard A. Brain [a, *], Suzanne Z. Schneider [b], Julie C. Anderson [c], Loren D. Knopper [d], Jeffrey C. Wolf [e], Mark L. Hanson [f]

[a] Syngenta Crop Protection, Greensboro, NC, USA
[b] EAG, Easton, MD, USA
[c] Stantec Consulting Ltd., Winnipeg, Manitoba, Canada
[d] Stantec Consulting Ltd., Stoney Creek, Ontario, Canada
[e] Experimental Pathology Laboratories Inc., Sterling, VA, USA
[f] Department of Environment and Geography, University of Manitoba, Winnipeg, Manitoba, Canada

HIGHLIGHTS

- Extended fish short-term reproduction assays were conducted with 2 species of fish.
- Mature fathead minnows and Japanese medaka were exposed to atrazine.
- Exposures were at environmentally relevant concentrations: 1–100 or 10–250 μg/L.
- No adverse effects on survival, body size, GSI, VTG, or fecundity in both species.
- No adverse effects on gonad histology in either species tested.

ARTICLE INFO

Article history:
Received 11 December 2017
Received in revised form
12 April 2018
Accepted 13 April 2018
Available online 16 April 2018

Handling Editor: James Lazorchak

Keywords:
Atrazine
Hypothalamus-pituitary-gonad axis
Gonadosomatic index (GSI)
Fecundity
Fertility
Histopathology

ABSTRACT

Short-term reproduction assays were conducted with fathead minnow (*Pimephales promelas*) and Japanese medaka (*Oryzias latipes*) to evaluate responses from atrazine exposure at environmentally relevant concentrations and above. Breeding groups of fish with multiple males and females were exposed to atrazine under flow-through conditions. Fathead minnows were exposed to mean measured concentrations of 1.0, 10, 26, 52, and 105 μg atrazine/L for 28 days. Medaka were exposed to mean measured concentrations of 9.4, 48, 74, 97, and 244 μg atrazine/L for 28 or 29 days. Fish were evaluated for survival, fecundity, fertility, total length, wet weight, secondary sex characteristics, gonadosomatic index (GSI) (*P. promelas* only), plasma or hepatic vitellogenin (VTG), and histopathology of gonads. General observations of health and behaviour were also conducted. There were no statistically significant effects (i.e., $p < 0.05$) of atrazine on survival, size, reproduction, behaviour, GSI, VTG, or secondary sex characteristics in either species at any exposure level. In fathead minnows, there were no histopathological findings associated with atrazine exposure in male fish, but there was an increased proportion of Stage 4.0 ovaries accompanied by an increase in proportion of Grade 3 post-ovulatory follicles in females of the 105 μg/L treatment group. Without a concomitant increase in oocyte atresia, neither of these findings are considered adverse for the health of the fish. In medaka, there were no significant effects of atrazine exposure on histopathology in either sex. These data support current weight-of-evidence assessments that atrazine does not cause direct adverse effects on fish reproduction at environmentally realistic concentrations.

1. Introduction

The herbicide atrazine (2-chloro-4-ethylamino-6-

* Corresponding author.
E-mail address: richard.brain@syngenta.com (R.A. Brain).

https://doi.org/10.1016/j.chemosphere.2018.04.068
0045-6535/

isopropylamino-s-triazine) has been used widely in agricultural practice in the United States since its initial registration in 1958, primarily to control broadleaf and grassy weeds in corn, sorghum, and sugar cane (US EPA, 2006a). Atrazine has the potential to translocate to surface waters, primarily through run-off events (Andrus et al., 2013, 2015), where non-target organisms may experience pulse exposures. Due to its solubility and relatively long aquatic half-life, atrazine is relatively frequently detected in streams, rivers, and ponds, though rarely at concentrations of 100 μg/L or greater (e.g., Giddings et al., 2005). As a result of its wide use, presence in the environment, and its potential for effects on a range of aquatic receptors, atrazine has been scrutinized extensively, both by regulators (e.g., US Environmental Protection Agency (US EPA) scientific advisory panels (US EPA, 2003; 2007a, 2007b; 2012) and by others (e.g., see reviews by Solomon et al., 2008; Boffetta et al., 2013; Van Der Kraak et al., 2014; Wirbisky and Freeman, 2015). Some of these reviews have highlighted responses in freshwater fish as being of concern, particularly those that involve reproduction and development.

A recent weight-of-evidence (WOE) risk assessment for atrazine was conducted that included studies with 31 species of fish (Van Der Kraak et al., 2014). In the WOE approach, studies were scored for both the strength of their methods (i.e., how well the studies were performed) and the relevance of responses observed (i.e., at environmentally relevant concentrations, as well as biological relevance). Among the endpoints evaluated in these reviews, many are related to reproduction, including sexual differentiation, sex ratio, gonad–somatic index (GSI), gonadal histopathology, and production of sperm and eggs. Overall, Van Der Kraak et al., 2014 concluded that exposure to atrazine does not result in statistically significant adverse effects on fish reproduction at environmentally relevant concentrations (≤100 μg/L).

A study by Papoulias et al. (2014) examined reproduction in Japanese medaka (*Oryzias latipes*) following atrazine exposure (0.5, 5.0, and 50 μg/L) for 35 days and reported statistically significant adverse effects along the hypothalamus–pituitary–gonad axis in the form of non–dose-dependent reductions in mean weekly rate of egg production and cumulative mean number of eggs per tank. Van Der Kraak et al. (2014) noted that Papoulias et al. (2014) differed substantially from Organization for Economic Co-operation and Development (OECD) guidelines for fish short-term reproduction assays (OECD, 2012) in several areas. One of the most important, which is fundamental to the performance of the assay, was the ratio of males to females used in the study. Papoulias et al. (2014) used one male and four females per replicate, whereas the OECD recommends equal numbers, specifically three of each sex for *O. latipes* (OECD, 2012). Because the Papoulias et al. (2014) study had fewer males available (5–6 total per treatment sampled over two time points), it is possible that the control animals failed to represent the full range of normal morphologic and physiological variation. Thus, for example, normal males in the treatment groups that differed from the controls were scored as altered in response to the treatment. Tillitt et al. (2010), examined responses in fathead minnow at nominal concentrations of 0.5, 5, and 50 μg/L for 14 or 30 days to assess potential reproductive effects of atrazine. After 17–20 days of exposure, reduced cumulative egg production (19–39% reductions) was reported for atrazine-exposed fish, as well as gonadal abnormalities in both male and female fish. The study was based on one male with two females as opposed to the recommended two males and four females (OECD, 2012; US EPA, 2009). Van Der Kraak et al. (2014) concluded that the use of a single male per enclosure for these studies could be a source of divergent results between studies with different designs, because the results relied on the performance of single male fish per replicate, which can increase the significance of group-wise findings such as spawning success. In 2017, Hosmer et al. (2017) published on a 35-day reproduction assay with Japanese medaka exposed to measured concentrations of atrazine at 0.6, 5.5, and 52 μg/L following standard protocols with multiple males per enclosure under good laboratory practice (GLP). They observed no statistically significant effects of atrazine exposure observed on survival, growth, or hepatic vitellogenin (VTG). In addition, cumulative and daily egg production, secondary sex characteristics (i.e., anal fin papillae), and sexual developmental stage were unaffected by exposure to atrazine up to 52 μg/L.

To further contribute to WOE determinations of atrazine effects on fish, two new guideline-based GLP fish short-term reproduction assays were conducted with adult fathead minnow (*Pimephales promelas*) and Japanese medaka. These tests were performed, in part, to address uncertainties identified by Van Der Kraak et al. (2014), and to demonstrate the reproducibility of Hosmer et al. (2017). In addition, these new studies were developed to capture a wider range of exposure concentrations using the accepted standard protocol with multiple males per enclosure. The test duration was also expanded to address concerns that a 21-day assay is insufficient to detect effects (Tillitt et al., 2010). The resulting data from the current studies will be of value to risk assessors in terms of understanding the potential toxicity posed by atrazine.

2. Methods

2.1. Fathead minnow study

2.1.1. General experimental design

A 28-day reproduction assay was conducted with adult fathead minnows at the EAG Laboratories aquatic toxicology facility (Eastland, MD). The study was conducted according to protocols based on the US EPA Series 890 – Endocrine Disruptor Screening Program Test Guidelines, OPPTS Number 890.1350 (US EPA, 2009), and the OECD Guidelines for Testing of Chemicals, Guideline 229 (OECD, 2012).

2.1.2. Test solutions

Minnows were exposed to analytical-grade atrazine (97.5% w/w, Syngenta, Batch ID 13095) at nominal concentrations of 1.0, 10, 25, 50, and 100 μg/L, plus a negative control. Stock solutions (nominal concentrations of 1000 μg/L) were prepared 18 times during the experiment by mixing test substance (0.1200 g) into reverse osmosis water (120 L). Target concentrations were achieved by pumping the stock into diluter mixing chambers assigned to each treatment group, then test solutions were pumped into test chambers at a target rate of 44 mL/min. The negative control received dilution water only. No solvents were used to dissolve the atrazine.

Dilution water was obtained from a freshwater well located on the EAG Laboratories site. Prior to use, the moderately-hard water was filtered to 0.45 μm to remove fine particles and passed through an ultraviolet sterilizer. Dilution water quality parameters, including pH, alkalinity, hardness, specific conductance, and total organic carbon (TOC) were measured weekly over the 4-week period preceding test initiation, and water was screened for pesticides, organic contaminants, and metals.

2.1.3. Test organisms and husbandry

Fathead minnows were obtained from Osage Catfisheries (Osage Beach, MO) and housed in mixed cultures in the testing facility (16:8-h light:dark photoperiod, 25 °C) for an acclimation period of approximately 8 weeks. Fish were fed live brine shrimp nauplii (*Artemia* sp.) *ad libitum* two or three times daily to promote active reproduction and maintenance of body condition. At the start of the