Food and Chemical Toxicology 107 (2017) 386–394

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Transfer of zearalenone to the reproductive system of female rainbow trout spawners: A potential risk for aquaculture and fish consumers?

Maciej Woźny [a, *], Kazimierz Obremski [b], Tomasz Zalewski [c], Maren Mommens [d], Alicja Łakomiak [a], Paweł Brzuzan [a]

[a] Department of Environmental Biotechnology, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, ul. Słoneczna 45G, 10-709, Olsztyn, Poland
[b] Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 13, 10-950, Olsztyn, Poland
[c] Department of the Salmonid Research in Rutki, Inland Fisheries Institute in Olsztyn, Rutki, 83-330, Żukowo, Poland
[d] AquaGen AS, PO Box 1240, N-7462, Trondheim, Norway

ARTICLE INFO

Article history:
Received 4 February 2017
Received in revised form
12 June 2017
Accepted 4 July 2017
Available online 5 July 2017

Keywords:
Carry over
Food safety
Glucuronides
Metabolism
Mycotoxins
Tissue distribution

ABSTRACT

To investigate whether ZEN transfers from the alimentary tract of fish to the somatic cells of ovaries or the oocytes, mature females of rainbow trout were orally exposed to ZEN at a dose of 1 mg·kg^{-1} body mass. At sampling times of 2, 6, 12, 24, 48, and 96 h, tissues of the fish (intestine, liver, ovaries, oocytes, muscles, and plasma) were extracted to determine the concentration of ZEN and its metabolites using immunoaffinity columns and HPLC-FLD. Our results confirm that ZEN is transferred from the alimentary tract to the reproductive system of the fish, and indicate that the mycotoxin concentrates in the somatic cells of the ovaries. Importantly, ZEN transferred to the fishes' oocytes and muscles only to a limited extent. Our additional survey of fish hatcheries and local stores indicated only trace amounts of ZEN residuals in the samples that were collected in Poland and Norway between 2013 and 2015, which probably reflects good hygienic conditions for the feed used in these hatcheries. Furthermore, our results indicate that the health risk from dietary intake of ZEN from fish roe is negligible. However, the potential of ZEN to transfer to the fish ovaries may be of concern for aquaculture.

1. Introduction

Zearalenone (ZEN) is a mycotoxin produced by some *Fusarium* and *Gibberella* moulds that commonly occur in plant materials. These moulds infect agricultural crops (mainly cereals), resulting in worldwide ZEN contamination of foodstuffs for animals and humans (Zinedine et al., 2007; Rodrigues and Chin, 2012). The most prominent effect of ZEN toxicity is its ability to induce structural disorders or dysfunction in the reproductive system of livestock animals, i.e. pigs, cattle, and poultry (Zinedine et al., 2007; Minervini and Aquila, 2008). ZEN mimics the action of natural hormones (i.e. estrogens), which gives rise to a number of reproductive disorders in exposed livestock mammals, including decreased libido, anovulation, and infertility (Kuiper-Goodman et al., 1987; Fink-Gremmels and Malekinejad, 2007; Metzler et al., 2010).

The activity of ZEN has also been shown to affect fish reproduction. For example, water-borne exposure of zebrafish (*Danio rerio*) to ZEN can reduce spawning frequency (Schwartz et al., 2010) or induce transgenerational changes in fecundity (Schwartz et al., 2013). Although experimental data on the toxicity of ZEN in fish models are constantly increasing, the potential of this mycotoxin to interfere with the reproductive system of economically important fish has been incompletely evaluated (Manning, 2010; Anater et al., 2016; Matejova et al., 2017).

In livestock animals (especially pigs), the toxicokinetics of ZEN have been extensively studied. Once ingested, ZEN is rapidly absorbed from the gut. Then, during the I phase of drug metabolism, it is primarily metabolized in the intestine and liver to its major metabolites α- and β-zearalenol (α- and β-ZEL) by hydroxysteroid dehydrogenases (α- and β-HSD, respectively). In addition, this conversion also takes place in other tissues, e.g. erythrocytes (Chang and Lin, 1984), or ovarian granulosa cells (Malekinejad et al., 2006). In the II phase, the reductive metabolites of ZEN produced in

* Corresponding author.
E-mail address: maciej.wozny@uwm.edu.pl (M. Woźny).

http://dx.doi.org/10.1016/j.fct.2017.07.010
0278-6915/

the I phase are further conjugated, mainly with glucuronic acid by UDP-glucuronyl transferases. After this, the metabolites are excreted in bile and urine (Fink-Gremmels and Malekinejad, 2007; Dänicke and Winkler, 2015).

In fish, however, similar studies describing the toxicokinetics of ZEN are scarce (Laganà et al., 2004; Pietsch et al., 2014). Current knowledge is limited only to information on the concentrations of the two reductive metabolites in the liver and muscular tissue (Laganà et al., 2004; Pietsch et al., 2015; Woźny et al., 2015). The role of glucuronidation and other metabolic pathways in biotransformation of ZEN in fish remains unclear (Pietsch et al., 2014). Moreover, there is a lack of detailed information about possible transfer of ZEN to other organs and their (extrahepatic) contribution to the overall metabolism of ZEN.

The metabolic profile of ZEN is thought to determine the differences in sensitivity to ZEN among livestock animals (Dänicke and Winkler, 2015). A rationale for this statement is that ZEN and its metabolites differ in the way that they interact with estrogen receptors (ERs), which affects the estrogenicity of these compounds. Comparative affinity studies have shown that their estrogenic potency decreases in the following order: α-ZEL > ZEN > β-ZEL (Arukwe et al., 1999; Celius et al., 1999). Thus, reduction of ZEN to α-ZEL is thought to be a major bioactivation pathway, whereas reduction to β-ZEL is considered to be a detoxification pathway (Metzler et al., 2010; Dänicke and Winkler, 2015). More recently, it has been also shown that the estrogenicity of ZEN-glucuronides is even lower than that of their corresponding free (unconjugated) counterparts (Frizzell et al., 2015). Thus, detailed studies on the piscine kinetics and metabolic profile of ZEN are essential in order to understand its biological effects in fish.

ZEN has been found in commercial fish feeds and feed materials (Nácher-Mestre et al., 2013, 2015; Pietsch et al., 2013; Woźny et al., 2013; Sanden et al., 2016). Since contamination of feed with mould is a potential source of ZEN, poor hygienic conditions at fish hatcheries may impair the health and productivity of fish, and thus reduce the profitability of aquaculture (Anater et al., 2016; Gonçalves et al., 2016). Moreover, because fish constitute a large part of some human diets (Renieri et al., 2014), transfer (carry over) of the mycotoxin to fish tissues could pose a threat to consumers. Although there is little transfer of ZEN to fish meat (Nácher-Mestre et al., 2015; Pietsch et al., 2015; Woźny et al., 2015), the mycotoxin also transfers to the ovaries (Woźny et al., 2013), and it is unknown whether it concentrates in the somatic cells of this organ, or in the oocytes. Contamination of these parts of the reproductive system with ZEN could affect the productivity and health of the fish, and contamination of the oocytes with ZEN could also pose a threat to consumers of gourmet foodstuffs (i.e. salted roe or caviar).

Therefore, the aim of this study was to investigate whether ZEN contamination is transferred from the alimentary tract of fish to the somatic cells of the ovaries or the oocytes. For this purpose, mature female rainbow-trout (spawners) were orally exposed to ZEN at a dose of 1 mg kg^{-1} body mass. At sampling times of 2, 6, 12, 24, 48, and 96 h, tissues of the exposed fish (intestine, liver, empty ovaries, oocytes, muscles, and blood plasma) were extracted to determine the concentration of ZEN and its major reductive metabolites (α- and β-ZEL) using immunoaffinity columns and high-performance liquid chromatography with fluorescence detection. Samples extracted from the liver and the ovaries of the exposed fish were also incubated with β-glucuronidase, after which the concentrations of glucuronidated (conjugated) metabolites of ZEN were measured. In addition to the details of ZEN toxicokinetics in female rainbow trout spawners, we were also interested to see whether the potential transfer of ZEN to the ovaries may be an issue for aquaculture and lead to contamination of farmed fish (ovaries and oocytes) and gourmet fish products (salted roe or caviar). To this end, the concentrations of ZEN residuals were measured in additional samples that were collected from fish hatcheries (ovaries and oocytes) or purchased in local grocery stores (salted roe) in Poland and Norway between the years 2013 and 2015.

2. Material and methods

2.1. Fish maintenance and exposure

All procedures related to fish breeding, maintenance and exposure were conducted at the Department of Salmonid Research in Rutki (Inland Fisheries Institute in Olsztyn; Poland). Experimental fish were housed and handled in compliance with widely accepted guidelines of laboratory animal care. The experiment was approved by the Local Ethical Commission (resolution No. 74/2014 of 10th December 2014).

To test the hypothesis that ZEN is transferred from the alimentary tract of fish to their ovaries and/or oocytes (eggs), mature rainbow trout females (*Oncorhynchus mykiss*) were selected for this study (4 + years old spawners; these are so-called "scattering" females, which are ready for spawning). The fish were sorted by their individual masses (~1200 g) and kept in a 3·3 m flow tank supplied with surface water at a flow rate of ~250 L min^{-1} and a natural photoperiod of 10/14 h (light/dark). The fish were fed at least twice a day with a Vitalis A25 diet (Skretting; France) with a reduced feeding procedure, taking into account the water temperature, caloric content of the feed, and fish mass (From and Rasmussen, 1984).

The dose of ZEN (1 mg kg^{-1} of body mass), route of administration (oral), and set of exposure periods (2, 6, 12, 24, 48, 72, 96 h) were based on a study by Laganà et al. (2004). ZEN (purity >98%) was purchased from Santa Cruz Biotechnology (cat. #sc-204943, lot #I0915; Santa Cruz, Texas, USA) and dissolved in corn oil (Sigma-Aldrich; Germany) as a solvent vehicle. The solution contained 1 mg of ZEN per 500 μL (2 mg mL^{-1}) and was given as an oral bolus.

Prior the exposure, the fish were anesthetized by immersion in etomidate solution, weighed, and then given a single oral bolus of the ZEN solution at a dose of 1 mg kg^{-1} of body mass. The bolus was given with the use of a thin silicon hose introduced into the stomach of fish through a glass tube. The volume of the bolus was individually adjusted to the body mass of each fish (0.5 mL kg^{-1} body mass). At each sampling period post administration (p.a.) of the bolus (2, 6, 12, 24, 48, 96 h), three randomly selected individuals were anesthetized, weighed, and measured. The anesthetized fish were stripped to collect eggs and to empty their ovaries. Blood samples were taken from the anesthetized fish and were processed for plasma isolation, according to the procedure described below. Then, the fish were sacrificed with a blow to the head, and their body cavity was opened for visual inspection. The liver with gall bladder, the ovaries (empty, without any eggs), the caudal part of intestine with its content (an ~8 cm section, starting from the anus), and the dorsal part of the white muscles with skin were collected and stored at −20 °C for further extraction and HPLC analysis. Samples from three untreated fish collected at the beginning of the experiment were considered as an control group (sampling period of 0 h). To check for possible contamination of the corn oil that was used as a solvent vehicle, one additional fish received an oral bolus of pure corn oil, and its tissues were collected after 24 h and examined for background contamination.

For blood samples, 1 mL of blood was taken from the heart with a Blood Gas Monovette syringe (Sarstedt, Germany) and a 0.8·30 mm needle. The blood was transferred into 1.3 mL Li-heparin microtubes (Sarstedt), gently mixed, and centrifuged for 10 min at 1250 g using a Hermle Z160M centrifuge (Hermle LaborTechnik; Germany). After centrifugation, 400 μL of