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Tissue distribution and elimination of deoxynivalenol and ochratoxin A in dietary-exposed Atlantic salmon (*Salmo salar*)

Aksel Bernhoft^a, Helga R. Høgåsen^a, Grethe Rosenlund^b, Lada Ivanova^a, Marc H. G. Berntssen^c, Jan Alexander^d, Gunnar Sundstøl Eriksen^a and Christiane Kruse Fæste^a

^aNorwegian Veterinary Institute, Oslo, Norway; ^bSkretting Aquaculture Research Centre AS, Stavanger, Norway; ^cNational Institute of Nutrition and Seafood Research, Bergen, Norway; ^dNorwegian Institute of Public Health, Oslo, Norway

ABSTRACT

Post-smolt Atlantic salmon (*Salmo salar*) were fed standard feed with added 2 or 6 mg kg⁻¹ pure deoxynivalenol (DON), 0.8 or 2.4 mg kg⁻¹ pure ochratoxin A (OTA), or no added toxins for up to 8 weeks. The experiments were performed in duplicate tanks with 25 fish each per diet group, and the feed was given for three 2-h periods per day. After 3, 6 and 8 weeks, 10 fish from each diet group were sampled. In the following hours after the last feeding at 8 weeks, toxin elimination was studied by sampling three fish per diet group at five time points. Analysis of DON and OTA in fish tissues and plasma was conducted by liquid chromatography-mass spectrometry and high-pressure liquid chromatography with fluorescence detection, respectively. DON was distributed to the liver, kidney, plasma, muscle, skin and brain, and the concentrations in liver and muscle increased significantly from 3 to 8 weeks of exposure to the high-DON diet. After the last feeding at 8 weeks, DON concentration in liver reached a maximum at 1 h and decreased thereafter with a half-life ($t_{1/2}$) of 6.2 h. DON concentration in muscle reached a maximum at 6 h and was then eliminated with a $t_{1/2} = 16.5$ h. OTA was mainly found in liver and kidney, and the concentration in liver decreased significantly from 3 to 8 weeks in the high-OTA group. OTA was eliminated faster than DON from various tissues. By using Norwegian food consumption data and kinetic findings in this study, we predicted the human exposure to DON and OTA from fish products through carryover from the feed. Following a comparison with tolerable daily intakes, we found the risk to human health from the consumption of salmon-fed diets containing maximum recommended levels of these toxins to be negligible.

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Introduction

Deoxynivalenol (DON) and ochratoxin A (OTA) are mycotoxins that may affect both animal and human health. DON, a trichothecene frequently occurring in *Fusarium*-infected cereals in temperate climates, is the most prevalent mycotoxin in Norway (VKM 2013; Bernhoft et al. 2016). In farm animals DON may cause feed refusal, vomiting and immunotoxic reactions, of which the latter two may also occur in humans. OTA is produced by mould of the genera *Penicillium* and *Aspergillus*, which may infect suboptimally stored feed and food commodities worldwide. OTA can induce kidney damage in mammals (VKM 2013). Feed ingredients of plant origin, including cereals, are increasingly used in fish farming, and DON and OTA have also been shown to occur in such ingredients and in fish feed (Pietsch et al. 2013; VKM 2013; Nacher-Mestre et al. 2015; Sanden et al.

2015). Fish feeds to carnivorous species such as Atlantic salmon have traditionally been based on fish meal and oil, but this supply is limited. Marine ingredients have therefore to a large extent been replaced with plant ingredients to support the demand for sustainable feed ingredients to the rapidly growing aquaculture sector (Ytrestoyl et al. 2015). The shift from marine to more plant-based feeds introduces new potential contaminants that before have not been associated with the farming of marine fish species such as Atlantic salmon (Nacher-Mestre et al. 2015). In this context two issues are important: possible adverse effects in the fish and carryover to fish products consumed by humans.

Knowledge on the possible toxic effects and toxicokinetics of DON and OTA in fish is very sparse. Toxicokinetic information is important both for understanding the possible adverse effects in fish

and for evaluating a possible accumulation of the parent mycotoxin or its metabolites in fish tissues, which could result in carryover to the edible part of the fish and potential health risks for consumers. In species other than fish, such as birds, mammalian animals and humans, DON is readily metabolised and eliminated. Exposure of human consumers to residues of DON and its metabolites through consumption of food products from birds and mammals is not considered to be of concern (reviewed by EFSA 2004a, 2013; VKM 2013). Corresponding toxicokinetic data of DON in fish and an assessment of the carryover potential from feed to fish products are lacking. However, results from two studies of DON residues in fish are available. In a study primarily investigating potential toxic effects on the innate immune responses in carp (*Cyprinus carpio*) fed with DON up to approximately 1 mg kg⁻¹ feed for 4 weeks, Pietsch et al. (2014) reported only marginal concentrations of DON in muscle (up to 1.3 µg kg⁻¹ dried weight) when sampled 12 h after last feeding. Nacher-Mestre et al. (2015) could not detect DON in the muscle of Atlantic salmon or in gilthead sea bream (*Sparus aurata*) fed plant-based replacement diets with natural trace (0.02–0.08 mg kg⁻¹) concentrations of DON for 7 or 8 months.

In various non-fish species and depending on species, OTA is retained to some extent in blood and other tissues due to variations in absorption, binding affinity to serum proteins and the extent of enterohepatic recirculation (reviewed by EFSA 2004b, 2006). Transfer to edible animal products may occur, in particular to those containing blood or liver from monogastric mammals such as pigs. In a comparative study in carp, rodents, monkey and quail, it was found that the oral bioavailability was considerably lower in fish (1.6%) than in mammals and birds (44–97%). Moreover, the elimination half-life was shorter in fish (Hagelberg et al. 1989). In another study, ¹⁴C-labelled OTA was administered intravenously with a single dose

in rainbow trout, and the tissue affinity was studied during an 8-day period by whole-body autoradiography (Fuchs et al. 1986). The radioactivity disappeared rapidly from the blood and almost no distribution to muscle tissue was detected, whereas accumulation was detected in the posterior kidney and the bile duct.

Atlantic salmon is the most important species in Norwegian fish farming. To our knowledge, toxicokinetic studies of DON and OTA in Atlantic salmon have not been conducted so far. The objectives of the present study were to examine tissue distribution and elimination as well as to assess possible accumulation of DON and OTA in post-smolt Atlantic salmon during and after 8 weeks of toxin exposure through feed. Measured levels in fish fillets were used to assess the potential health risk for humans consuming salmon fed diets containing cereals.

Materials and methods

Study design

The fish study was conducted at Skretting ARC, Lerang Research Station, Stavanger, Norway. It was part of a larger study on health effects and toxicokinetics in fish fed with different feed concentrations of DON or OTA. The study on health effects will be published separately.

The toxicokinetic study included two dosage groups per toxin and a control group. These were a low DON group (2 mg kg⁻¹ feed), a high DON group (6.0 mg kg⁻¹ feed), a low OTA group (0.8 mg kg⁻¹ feed) and a high OTA group (2.4 mg kg⁻¹ feed), and control group receiving feed without added toxins.

Juvenile post-smolt salmon (*Salmo*Breed, 12 months old, both genders) were randomly distributed into 10 circular tanks (100 L) supplied with flow-through seawater at 12°C and exposed to 24 h of light. Each tank received 25 fish with an initial average weight as presented in Table 1. All experiments were performed in duplicate with two tanks

Table 1. Body weights (g) of Atlantic salmon (means ($n = 10$) with standard deviations (SD)) after 0, 3, 6 and 8 weeks.

Diet	0 weeks	3 weeks	6 weeks	8 weeks
Low DON, 2.0 mg kg ⁻¹	57.6 (5.4)	70.2 (8.6)	95.6 (21.6)	114.8 (20.8)
High DON, 6.0 mg kg ⁻¹	58.8 (6.3)	63.7 (9.0)	72.1 (13.0)	80.2 (7.2)
Low OTA, 0.8 mg kg ⁻¹	57.8 (6.3)	70.3 (9.1)	88.3 (25.0)	120.1 (19.8)
High OTA, 2.4 mg kg ⁻¹	57.7 (5.5)	78.5 (9.9)	103.3 (17.1)	132.7 (28.8)
Control, 0 mg kg ⁻¹	57.7 (5.7)	75.2 (6.0)	99.1 (9.8)	123.2 (23.4)

per dose group and toxin. Two tanks were used to secure the experiment in case of technical problems with one of the tanks. The order of tanks was also randomised. After 1 day of acclimatisation the fish were fed with the experimental diets for 8 weeks. Feeding was done automatically during three 2-h periods per day (08:00–10:00, 12:00–14:00 and 22:00–24:00 hours). The amount of feed was adjusted to the number and size of the fish, and the daily feed ration given was in slight excess of the expected voluntary feed intake. Feed pellets fell through the water column in the tank for approximately 1 min. The not-ingested spilled rest was collected on a sieve above the drain for effluent water at the bottom of the tank. This feed rest was removed after each 2-h feeding period, dried, weighed and subtracted from the total daily feed supply of each tank to calculate actual feed intake per tank.

Chemicals

Deoxynivalenol (Biopure standard DON; lot #06221Z) and ochratoxin A (Biopure standard OTA; lot #S07301Z) were purchased from Romer Labs Diagnostic GmbH (Tulln, Austria). Chloroform, hydrochloric acid, phosphoric acid, sodium bicarbonate, phosphate-buffered saline (PBS), acetic acetate, ammonium acetate and ammonium formate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water (Optima, LC-MS grade), methanol and acetonitrile (MeOH and MeCN, Optima, LC-MS grade) were obtained from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA).

Experimental fish feed

The feed was a standard Atlantic salmon feed (Spirit 3 mm, Skretting, Stavanger, Norway) containing 48% crude protein and 25% crude fat. The pellets were coated with DON or OTA with the aim to provide diets with relatively low or high content of mycotoxin while not causing acute toxic effects in the fish. The respective goals were 2.0 or 6.0 mg DON kg⁻¹ feed, or 0.8 or 2.4 mg OTA kg⁻¹ feed, which was in the same range as the levels in European Union and Norwegian guideline levels for fish feedingstuff, which are for DON 5 and

2 mg kg⁻¹, respectively, and for OTA 0.25 and 1 mg kg⁻¹, respectively (European Commission 2006; Norwegian Food Safety Authority 2015).

DON and OTA were dissolved in 50 ml 70% ethanol (DON: 10 and 30 mg/50 ml; OTA: 4 and 12 mg/50 ml; control: 50 ml without toxin) and coated on 5 kg of feed pellets by carefully spraying the feed while moving it in a drum. Subsequently, the pellets were coated with 25 g fish oil (0.5%) for stabilisation while still rotating for another 3 min. The amount of ethanol in the pellets was 0.7%, of which a major part may have evaporated during storage of the feed before trial start. The amount of ethanol left in the pellet is unlikely to influence the fish health or the kinetics of the toxins. The control feed was treated with the same quantities of ethanol and fish oil as the toxin-treated feed. Release of toxins from the coated pellets during the seconds of falling through the water column was not tested, but was considered as unlikely. Three representative samples (250 g) of each feed preparation were collected for analysis of DON or OTA content by LC-MS/MS or HPLC, respectively. The fortified feeds were produced 3 weeks prior to trial start and stored at 4°C.

Sample preparation of feed for analyses of DON and OTA

DON- and OTA-coated feed samples and control feed samples (three samples of 250 g per dosage level and type) were milled (Grindomix GM 200, Retsch GmbH, Haan, Germany) for 1 min at RT. For DON-coated and control feed samples, aliquots of 25 g were extracted with 125 ml 84% acetonitrile (MeCN) for 60 min at RT under shaking (200 shakes/min). After filtration (WhatmanTM, 595 ½, 150 mm), 5 ml of the supernatant were loaded onto a Mycosep 225 column (Romer Labs), and 3 ml from the top of the column were evaporated to dryness under a nitrogen stream at 60°C before derivatisation by using pentafluoropropionic anhydride (100 µl) in 500 µl of 0.4 M imidazole in toluene/MeCN (85:15, v/v). Finally, samples were analysed by GC-MS according to Langseth et al. (2010). The measured DON concentrations (mean ± standard error of the mean (SEM), $n = 3$) in low and high DON diets were 2.42 ± 0.05 and 5.53 ± 0.06 mg kg⁻¹ feed, respectively. The control

Table 2. Sampling time points of five Atlantic salmon per tank at 3, 6 and 8 weeks. Three daily feeding periods are 08:00–10:00, 12:00–14:00 and 22:00–24:00 hours.

Tank number	Diet	Sampling, 3 weeks	Sampling, 6 weeks	Sampling, 8 weeks
7	Low DON	13:15–13:40 day 1	12:20–12:40 day 1	12:18–12:40 day 1
20	Low DON	10:05–10:17 day 2	09:59–10:17 day 2	10:04–10:23 day 2
1	High DON	08:15–08:55 day 1	08:00–08:20 day 1	08:00–08:30 day 1
10	High DON	15:02–15:33 day 1	13:55–13:40 day 1	13:57–14:23 day 1
2	Low OTA	09:00–09:30 day 1	08:25–08:45 day 1	08:43–09:02 day 1
9	Low OTA	14:27–14:54 day 1	13:20–13:44 day 1	13:15–13:48 day 1
3	High OTA	09:35–10:05 day 1	09:05–09:25 day 1	09:10–09:31 day 1
16	High OTA	07:55–08:10 day 2	07:48–08:05 day 2	07:59–08:20 day 2
8	Control	13:54–14:17 day 1	12:56–13:15 day 1	12:51–13:08 day 1
17	Control	08:20–08:48 day 2	08:20–08:42 day 2	08:30–08:50 day 2

feed did not contain any quantifiable DON (LOQ < 20 $\mu\text{g kg}^{-1}$).

For OTA-coated and control feed samples, aliquots of 25 g were extracted with 125 ml 60% MeCN for 60 min at RT under shaking (175 shakes/min). After filtration (WhatmanTM, 595 $\frac{1}{2}$, 150 mm), 5 ml of the filtered sample were diluted to 55 ml with PBS (pH 7.4) before application onto an Ochraprep[®] immunoaffinity column (R-Biopharm Rhône, Glasgow, UK). The subsequent steps are described below. The measured OTA concentrations (mean \pm SEM, $n = 3$) in low and high OTA diets were 0.72 ± 0.02 and 2.00 ± 0.17 mg kg^{-1} feed, respectively. The control feed did not contain any quantifiable OTA (LOQ < 0.015 $\mu\text{g kg}^{-1}$).

Sampling of fish

After 3, 6 and 8 weeks, five fish were collected from each tank, in total 10 fish for each diet group and sampling point. The fish were subsequently anaesthetised with 10 ml Fiquel 40 g l^{-1} in 5 L water. They were weighed and their lengths measured before they were killed and blood and relevant tissues sampled, and an autopsy was performed. The blood (heparinised) was centrifuged, and plasma, muscle and different organs were stored at -20°C until analysis.

In DON- and OTA-exposed fish, liver and muscle were collected after 3 and 6 weeks, and liver, muscle, posterior kidneys and skin after 8 weeks. In the control group, liver, muscle, kidneys and plasma were sampled at corresponding time points and used for the preparation of matrix-matched DON and OTA standards for LC-MS/MS or HPLC analysis. The same sampling procedure was followed at each time point, always observing the same order of fish tanks and the same time

schedule. Each sampling was executed on 2 consecutive days, from morning to afternoon on the first day, and from morning to noon of the second day, independent of feeding times (Table 2). The sampling of fish was performed according to a carefully designed schedule designed to reduce the variability within each group. Therefore, the 10 fish receiving the same dose were considered together although they were kept in two separate tanks.

After the last feeding time point at 8 weeks a study of toxin elimination was conducted. Plasma, liver, kidney, muscle and brain were sampled at 0, 1, 3, 6 and 24 h from DON-fed fish and at 2, 4, 8, 18 and 48 h from OTA-fed fish, using three fish per diet group.

Sample preparation of DON-containing tissues and plasma

Tissue samples (0.15–0.45 g) were homogenised with 10 ml 84% MeCN using an Ultra-Turrax T25 blender (Janke & Kunkel, Staufen, Germany) for 1 min. After centrifugation (AllegraX-30R; Beckman Coulter, Inc., Brea, CA, USA) with 3000g for 10 min at RT, supernatants were transferred to the new 15 ml glass tubes and washed with 10 ml hexane. An aliquot (5 ml) of the lower phase was loaded onto a Mycosep 225 column (Romer Labs), and 3 ml from the top of the column were evaporated to dryness under nitrogen stream at 60°C . The residue was redissolved in 800 μl 10% MeCN, centrifuged for 1 min at 10,000g (Centrifuge 5424, Eppendorf, Hamburg, Germany) through a 0.22- μm nylon filter (Costar Spin-X[®] 0.22 μm Nylon filter; Corning, Inc., Corning, NY, USA), and 200 μl from the eluate were analysed by LC-MS/MS.

Plasma samples were precipitated with ice-cold MeCN (1:3, v/v) and centrifuged at 3000g and 4°C

for 10 min (AllegraX-30R). The supernatant was transferred to a glass tube and evaporated to dryness using a gentle stream of nitrogen (45°C). The dry residues were reconstituted in 500 µl 10% MeCN, vortexed for 30 s, centrifuged for 1 min at 10,000g (Eppendorf) through a 0.22-µm nylon filter (Costar Spin-X) and analysed by LC-MS/MS.

Sample preparation of OTA-containing tissues and plasma

Tissue samples (0.5 g) were homogenised with 10 ml chloroform, 0.15 g diatomaceous earth (Celite-545, Merck Millipore, Darmstadt, Germany) and 0.15 ml 8.7 M H₃PO₄ using an Ultra-Turrax T25 blender for 3 min. After centrifugation at 3000g (AllegraX-30R) for 10 min at RT, 5 ml of the chloroform phase were extracted with 5 ml of 0.5 M NaHCO₃ for 2 min. The aqueous phase (2.5 ml) was diluted with 30 ml PBS (pH 7.4), acidified by adding 350 µl 1 M HCl and cleaned by application onto an Ochraprep® immunoaffinity column (R-Biopharm Rhône) at a flow rate of about 1 drop/second. The column was washed with 5 ml PBS solution, followed by 15 ml distilled water and dried by applying vacuum for 5–10 s. The affinity-bound OTA was slowly eluted with 3 ml methanol containing CH₃COOH (98:2, v/v). The eluted extract was evaporated under nitrogen stream at 60°C, redissolved with 100 µl MeCN and 200 µl 0.1 M H₃PO₄, and centrifuged for 1 min at 10,000g (Eppendorf) through a 0.22-µm nylon filter (Costar Spin-X) prior to analysis by HPLC.

Plasma samples (0.5 ml) were mixed with 0.4 ml 1 M HCl, 4 ml distilled water and 4 ml chloroform, homogenised using an Ultra-Turrax T25 blender for 1 min, placed on a horizontal shaker at 175 shakes/min for 60 min at RT, and centrifuged at 3000g (AllegraX-30R) for 10 min. The organic phase (2 ml) was evaporated to dryness under nitrogen stream at 60°C, and the residue was redissolved with 100 µl MeCN and 200 µl 0.1 M H₃PO₄. After centrifuging for 1 min at 10,000g (Eppendorf) through a 0.22-µm nylon filter (Costar Spin-X) the sample was analysed by HPLC.

DON analyses by use of LC-MS/MS

Tissue and plasma samples were analysed using a TSQ Quantum Access triple-stage quadrupole MS with a heated-electrospray ionisation (H-ESI) probe (Thermo

Fisher Scientific, Inc., Waltham, MA, USA) coupled to an Accela fast LC system (Thermo Fisher). Samples (5 µl) were separated on a Waters XBridge C18 column (100 × 2.1 mm i.d., 3.5 µm) with a flow rate of 0.3 ml min⁻¹ and a linear gradient ranging from 10% methanol (eluent A) and 90% water containing 0.1% NH₄COOH (eluent B) to 100% eluent B in 9 min after a 1 min lag at start. The eluent composition was held for 5 min, returned to the initial conditions in 0.1 min and the column was equilibrated for 3.9 min. The MS was operated in negative-ionisation mode with a collision energy of 13 eV. Two parent ion-product ion transitions (*m/z* 295.08 → 247.06 and 295.08 → 265.11) were used for SRM with the following parameters: capillary spray voltage, 4.0 kV; capillary temperature, 260°C; ion source discharge current, 4.0 A; collision gas, argon at 15 psi; Q1/Q3 peak width, 0.7 Da. The DON contents in tissue and plasma samples were determined by using measured peak areas and a linear calibration curve ranging from 25 to 150 ng ml⁻¹ DON prepared in matched matrices. The LOQ for DON in tissues was 3 µg kg⁻¹ and in plasma was 0.1 µg kg⁻¹.

OTA analyses by use of HPLC with fluorescence detection

Feed, tissue and plasma samples were analysed on a Waters Alliance 2695 HPLC (Waters Corp., Milford, MA, USA) coupled to a Dionex RF-2000 fluorometric detector (Dionex Corp., Sunnyvale, CA, USA). OTA was measured using 330 nm for excitation and 466 nm for emission. The sample injection volume was 75 µl and chromatographic separation was achieved on a Symmetry C18 Waters column (3.9 × 150 mm; 5 µm) at a flow rate of 1 ml min⁻¹. The system was run isocratically with a mobile phase consisting of methanol and 0.01 M H₃PO₄ (65:35 v/v). The OTA contents in feed, tissues and plasma were determined using peak heights and a linear calibration curve ranging from 0.067 to 26.805 ng ml⁻¹ OTA in eluent. The LOQ for OTA was 0.015 µg kg⁻¹ in feed, 0.09 µg kg⁻¹ in tissues and 0.004 µg kg⁻¹ in plasma.

Preparation of liver samples for DON-metabolite analysis

For the analysis of DON-related metabolites, liver tissue samples (0.1 g) obtained from salmon fed for

8 weeks were homogenised in 1 ml 80% MeOH (Ultra-Turrax T25 blender) for 1 min. Following centrifugation at 3000g for 10 min (AllegraX-30R) the supernatants (600 µl) were extracted with hexane (1:2, v/v). After 30 s of mixing and 1 min of centrifugation, 500 µl of the lower phase were transferred to new tubes and evaporated to dryness using a gentle stream of nitrogen (60°C). The dried residue was reconstituted in 100 µl 50% MeCN, vortexed for 15 s, centrifuged at 10,000g (Eppendorf) for 1 min, transferred to HPLC vials (Thermo Fisher Scientific) and analysed by HPLC-HRMS.

HPLC-HRMS method for the determination of DON and DON metabolites

High-pressure liquid chromatography-high-resolution mass spectrometry (HPLC-HRMS) analysis of fish liver samples was performed on a Q-Exactiv™ Hybrid Quadrupole-Orbitrap MS equipped with a heated electrospray ion source (HESI-II) and coupled to a UHPLC Dionex Ultimate 3000 system (Thermo Fisher Scientific). The HESI-II interface was operated at 300°C in negative-ionisation mode, and the parameters were adjusted as follows: spray voltage 4 kV, capillary temperature 250°C, sheath gas flow rate 35 l min⁻¹, auxiliary gas flow rate 10 l min⁻¹ and S-lens RF level 55. Data were acquired in the full-scan monitoring (FS)/data-dependent MS² (dd-MS²) mode targeting the [M + acetate]⁻ ions for DON and deepoxy-DON (*m/z* 355.1387 and 339.1438, respectively) and the [M – H]⁻ ions for DON-glucuronides (*m/z* 471.1497) with a quadrupole isolation width of 2 *m/z* and a mass resolution of 70,000 full width half-maximum (FWHM) at *m/z* 200 for FS. The presence of a target ion above a threshold intensity of 5 × 10³ triggered a MS² scan for analyte verification (dd-MS²) using normalised collision energy set to 17 and 25 for DON/DON-glucuronides and deepoxy-DON, respectively. The mass resolution during dd-MS² was set to 17,500 FWHM. The automatic gain control (AGC) target was set to 5 × 10⁵ ions including a maximum injection time (IT) of 250 ms during FS, whereas for dd-MS² the AGC target was 1 × 10⁴ and the IT was 200 ms. Chromatographic separation was performed at 30°C on a 150 × 2.1 mm i.d. Accucore™ C18 LC column (2.6 µm; Thermo Scientific, Waltham, MA, USA) with guard column 2.6 µm AQ 10 × 2.1 mm (Thermo). The flow rate of the mobile phase was 0.4 ml min⁻¹ and the injection volume

was 1 µl. Eluent A was water (containing 0.5% acetic acid) and eluent B was 95% MeCN (containing 5 mM ammonium acetate and 0.1% acetic acid). Gradient elution was employed starting at 100% A for 1 min, linearly increasing to 15% B in 15 min and to 95% B in 0.5 min. After flushing the column for 2.5 min with 95% B, the mobile phase composition was returned to the initial conditions and the column was eluted isocratically for 3.0 min. DON-glucuronides in salmon liver extracts were identified by comparison with standard material. The LOQs for DON, DON-3-glucuronide (DON-3glc), DON-15-glucuronide (DON-15glc) and deepoxy-DON were, respectively, 37, 81, 101 and 201 µg kg⁻¹ in liver matrix. The LOQ for DON-8-glucuronide (DON-8glc) in liver was according to previous data probably comparable with that of DON-15glc. A standard calibration curve in liver matrix was not constructed in the present experiment due to little availability of the in-house produced DON-8glc standard. This well-described substance (Uhlig et al. 2016) was, however, used to generate the standard chromatogram (Figure 3) for the identification of DON metabolites in fish.

Statistical analysis

Statistical analyses were performed using JMP® Pro 11.0.0. (ANOVA, 2013 SAS Institute Inc., Cary, NC, USA). Results from laboratory analyses below the LOQs were replaced by 0.5 LOQs to allow the inclusion in the calculations. The two tanks per dosage group allowed checking for variation between the tanks, which was insignificant compared with the inter-individual and treatment variations. Therefore, the data from the two replicates were pooled.

An all pairs Tukey–Kramer HSD (honestly significant difference) test was used for the pairwise comparisons of the means of different dose groups and time points within a dose group. Significant changes in toxin concentration by time points were also tested by linear regression. Pearson correlation coefficients (*r*) were used to quantify the linear relationship between the concentrations in different salmon tissues from fish fed low- and high-DON or low- and high-OTA.

Determination of toxicokinetic parameters

Toxicokinetic parameters for DON and OTA in salmon were derived from the plasma or tissue concentration–time curves obtained after the last feeding in

the high-dose groups. The respective t_{\max} and c_{\max} were directly determined from the curves. Elimination constants (k_{el}) were determined by curve regression ($c(t) = c_0 * e^{(k_{\text{el}} * t)}$) in Microsoft Excel 2010 (Windows 7). The elimination half-life is the time necessary to half the concentration, resulting in $t_{1/2} = \ln 2/k_{\text{el}}$. For bi-exponential curves two $t_{1/2}$ were determined ($t_{1/2\lambda_1}$ and $t_{1/2\lambda_2}$). The area under the plasma concentration–time curve (AUC) was approximated by integration using the trapezoid rule for numeric summarisation of individual areas in semi-logarithmic scale: $\text{AUC}_{\text{total}} = \sum \text{AUC}_{\text{individual}} = \sum [(c_1 + c_2)/2 * (t_2 - t_1)]$.

Estimation of human health risk from the consumption of DON-containing salmon fillet

The potential exposure of humans through the consumption of fillet from salmon fed with DON-containing feed was calculated by using the measured DON concentrations in fish muscle for both dose groups, and the mean and 95th percentile of the dietary intake of farmed salmon for adults and 2-year-old children based on consumption data acquired by the Norwegian Scientific Committee for Food (VKM) (2013, 2014). According to these surveys, the average consumption of farmed salmon is 12 g day⁻¹ in adults and 1 g day⁻¹ in 2-year-old children. The 95th percentile is 83 g day⁻¹ in adults and 4 g day⁻¹ in 2-year-old children (VKM 2013, 2014), here defined as high consumption. The standard body weight of adults was considered to be 77.5 kg, and that of 2-year-old children 12.8 kg (VKM 2013, 2014).

The estimated intake was compared with the established TDI (= 1 µg kg⁻¹ bw day⁻¹) (JECFA 2001). It was also compared with the normal DON exposure from cereals, which was estimated to be 0.27–0.45 µg kg⁻¹ bw day⁻¹ in adults and 1.1–2.0 µg kg⁻¹ bw day⁻¹ in 2-year-old children (VKM 2013).

Results

Exposure of salmon to DON and OTA in feed

The fish weight gain was monitored during the 8-week study (Table 1). Except for the high-DON group (6 mg kg⁻¹), all other diets, control and with toxin, led to a doubling of body weights during 8 weeks. Salmon receiving the high-DON diet had a weight gain of about 60%.

Feed intake per tank was determined daily, showing some day-to-day variations in the consumption (data not shown). Therefore, the mean daily feed intake was calculated by averaging the intake in the week before the respective samplings as shown in Table 3. The mean daily mycotoxin dose per fish calculated from the measured DON and OTA concentrations in feed and the mean daily feed consumption per fish at 3, 6 and 8 weeks are also shown in Table 3.

Tissue concentrations in salmon fed DON- and OTA-containing feed

The DON and OTA concentrations in several tissues were determined after 3, 6 and 8 weeks of exposure (Table 4).

The DON concentrations in liver increased significantly (about 200%; $p < 0.01$) in the high-DON group from 3 to 8 weeks at which the mean concentration was 28.6 µg kg⁻¹ (Figure 1(a) and Table 4). In the low-DON group the mean concentrations in liver at 3 and 8 weeks were 12.2 and 18.1 µg kg⁻¹, and not significantly different. The measured concentrations (µg toxin kg⁻¹) were normalised by the corresponding daily doses (µg toxin g⁻¹ bw fish), using the mean daily mycotoxin dose per fish divided by the corresponding fish weights (g). The increase of the corresponding dose-normalised DON liver concentrations was about 110% and 40%, respectively, in the high- and low-DON group (Table 4).

The measured DON concentrations in muscle, the edible fish fillet, showed a significant increase (about

Table 3. Daily mean feed intake (g) and mycotoxin dose (µg) per fish in weeks 3, 6 and 8.

	Weeks	Low DON	High DON	Low OTA	High OTA	Control
Feed intake	3	0.80	0.49	0.80	0.94	0.90
Mycotoxin dose		1.93	2.69	0.58	1.87	n.d.
Feed intake	6	1.02	0.63	0.93	1.10	1.05
Mycotoxin dose		2.48	3.47	0.67	2.20	n.d.
Feed intake	8	1.41	0.86	1.45	1.51	1.52
Mycotoxin dose		3.41	4.78	1.04	3.02	n.d.

n.d.: not determined.

Table 4. Measured effective and dose-normalised DON and OTA concentrations (means ($n = 10$) with standard deviations (SD)) in different fish tissues after 3, 6 and 8 weeks.

Diet	Week	Liver	Muscle	Kidney	Skin
		c (SD)/ c_{norm} ($\mu\text{g kg}^{-1}$)/(g bw kg^{-1})	c (SD)/ c_{norm} ($\mu\text{g kg}^{-1}$)/(g bw kg^{-1})	c (SD)/ c_{norm} ($\mu\text{g kg}^{-1}$)/(g bw kg^{-1})	c (SD)/ c_{norm} ($\mu\text{g kg}^{-1}$)/(g bw kg^{-1})
Low DON	3	12.2 (4.1)/444	5.6 (2.8)/204	n.s.	n.s.
	6	12.8 (7.9)/463	8.5 (3.7)/326	n.s.	n.s.
	8	18.1 (10.5)/610	6.0 (2.3)/201	12.3 (8.5)/414	n.s.
High DON	3	9.6* (8.8)/227	10.3* (7.9)/244	n.s.	n.s.
	6	20.2* (22.0)/420	17.3* (10.0)/359	n.s.	n.s.
	8	28.6* (11.4)/480	18.6* (5.8)/311	16.8 (7.6)/282	20.8 (6.8)/350
Low OTA	3	1.86 (1.01)/227	< LOQ	n.s.	n.s.
	6	1.53 (1.17)/201	< LOQ	n.s.	n.s.
	8	1.01 (0.72)/116	< LOQ	0.16 (0.16)/18.9	n.s.
High OTA	3	4.81* (2.69)/201	< LOQ	n.s.	n.s.
	6	3.27* (1.35)/154	< LOQ	n.s.	n.s.
	8	2.61* (1.04)/115	< LOQ	1.03 (0.86)/45.4	< LOQ

Notes: n.s., Not sampled; < LOQ, below the limit of quantification.

*Significant differences ($p < 0.05$) in a tissue within a dose group as determined by the all-pairs Tukey–Kramer test.

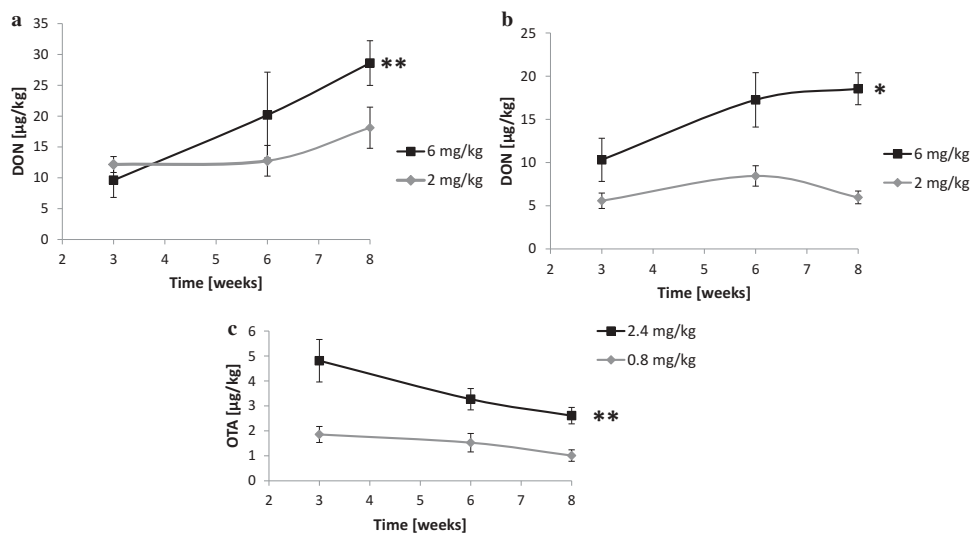


Figure 1. Concentrations ($\mu\text{g kg}^{-1}$) of (a) deoxynivalenol (DON) in liver, (b) DON in muscle and (c) ochratoxin A (OTA) in liver of Atlantic salmon fed with, respectively, 2 and 6 mg kg^{-1} DON or 0.8 and 2.4 mg kg^{-1} OTA for 8 weeks. Mean and SEMs for 10 fish per sampling point are shown. Significance levels of linear regression fitting are shown with one ($p < 0.05$) or two ($p < 0.01$) asterisks.

80%, $p < 0.05$) to a mean concentration of $18.6 \mu\text{g kg}^{-1}$ at 8 weeks in the high-DON group. In the low-DON group, the mean DON concentration in muscle at 8 weeks was $6.0 \mu\text{g kg}^{-1}$ and had not significantly changed from study week 3 (Figure 1(b) and Table 4). The same curve shapes were observed for both dose groups and both profiles had an intermediate top in week 6. The similarity between the normalised DON concentrations of both dosage groups indicated dose linearity.

Kidney and skin samples were analysed after 8 weeks of exposure. The measured kidney concentration was higher in the high-DON than in the low-DON group, but the opposite was observed for the

dose-normalised concentrations. The DON concentration in skin after high-DON exposure for 8 weeks was comparable with the concentration in muscle (Table 4). Positive linear correlations were found for DON concentrations in liver and kidney ($r = 0.55$; $p < 0.05$) and in muscle and skin ($r = 0.59$; $p = 0.072$) (Table 5).

The measured OTA concentrations in liver in both dose groups decreased during the study (Table 4 and Figure 1(c)). The levels after 8 weeks in both OTA groups were about 50% of the level at 3 weeks. The reduction was statistically significant ($p < 0.01$) for the high-dose group. The dose-normalised concentrations were comparable for both

Table 5. Pearson correlation coefficients (r) of DON or OTA concentrations in different tissues of Atlantic salmon sampled after 8-week exposure to DON or OTA ($n = 20$, except for $n = 10$ in combinations with DON in skin).

	Liver	Kidney	Muscle
<i>DON</i>			
Kidney	0.55*		
Muscle	0.40	0.13	
Skin	0.00	-0.19	0.59
<i>OTA</i>			
Kidney	0.82*		
Muscle	n.d.	n.d.	
Skin	n.d.	n.d.	n.d.

Notes: n.d., Not determined due to the low number of quantifiable concentrations.

*Statistically significant linear correlation ($p < 0.05$).

dose groups, indicating dose linearity (Table 4). The concentrations in liver were about 2.5 (high-OTA group) to 10 times (low-OTA group) higher than in kidney, and far higher than in muscle and skin where OTA was only measurable ($> \text{LOQ}$) in some fish from the high-OTA group. The liver and kidney concentrations of OTA were significantly correlated ($r = 0.82$; $p < 0.05$) (Table 5).

Elimination of DON and OTA from salmon plasma and tissues

The elimination of DON after the last feeding from various tissues and plasma in fish exposed to high-DON feed for 8 weeks is presented in Figure 2(a). The profiles were similar for the low-DON group (data not shown). The DON concentration in liver reached a maximum ($c_{\text{max,liver}} = 42.3 \mu\text{g kg}^{-1}$) at 1 h ($t_{\text{max,liver}}$) after the end of feeding (time zero) and decreased thereafter with a half-life ($t_{1/2,\text{liver}}$) of 6.2 h (four time points considered, $r^2 = 0.985$). In kidney, $c_{\text{max,kidney}} = 47.0 \mu\text{g kg}^{-1}$ was reached at $t_{\text{max,kidney}} = 6$ h and was eliminated with $t_{1/2,\text{kidney}} = 13.3$ h (two time points considered). The DON concentration in muscle reached $c_{\text{max,muscle}} = 23.9 \mu\text{g kg}^{-1}$ at $t_{\text{max,muscle}} = 6$ h and was then eliminated with $t_{1/2,\text{muscle}} = 16.5$ h (two time points considered). In brain, $t_{\text{max,brain}}$ was also 6 h ($c_{\text{max,brain}} = 20.3 \mu\text{g kg}^{-1}$), and the elimination $t_{1/2,\text{brain}} = 13.3$ h (two time points considered). The DON plasma concentration was highest at time zero ($c_{\text{max,plasma}} = 5.7 \mu\text{g kg}^{-1}$) and DON was eliminated with $t_{1/2,\text{plasma}} = 15.1$ h (five time points considered, $r^2 = 0.996$). Thus, the $c_{\text{max,kidney}}$ was slightly higher than $c_{\text{max,liver}}$ whereas $c_{\text{max,muscle}}$ and $c_{\text{max,brain}}$ were about half of this level. The area under the plasma concentration–time

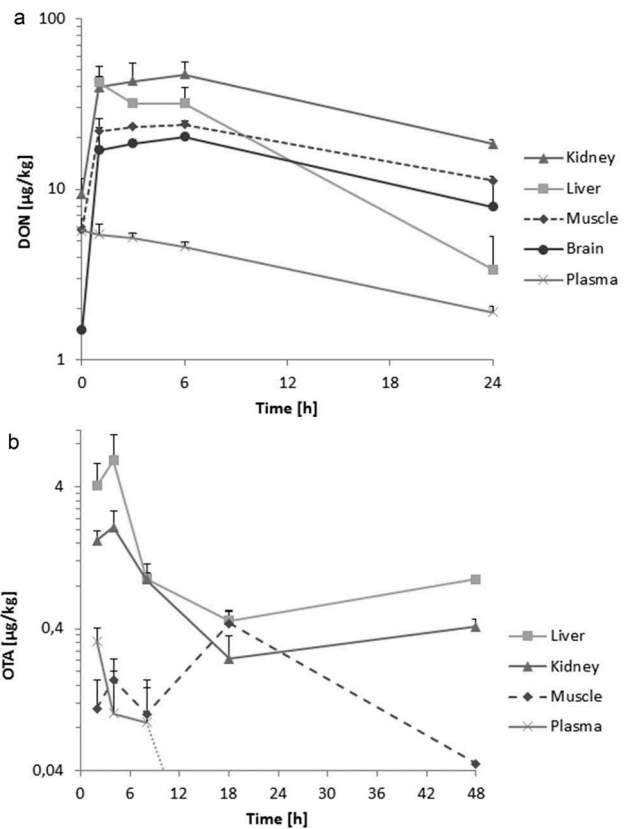


Figure 2. Elimination profiles from different tissues in the hours after the final feeding in the 8-week study with Atlantic salmon fed with (a) 6 mg DON kg^{-1} diet or (b) $2.4 \text{ mg OTA kg}^{-1}$ diet. Mean and SEMs for three fish per sampling point are shown.

curve (AUC) (Figure 2(a)) was approximately $\text{AUC}_{\text{po,plasma}} = 89.4 \mu\text{g}^*\text{h l}^{-1}$, or dose normalised by the mean daily dose in week 8 in the fish receiving 6 mg DON kg^{-1} feed (about $60 \mu\text{g kg}^{-1}$ bw) $\text{AUC}_{\text{po,plasma, norm}} = 1.5 \text{ kg}^*\text{h l}^{-1}$ (Table 6).

OTA was eliminated faster than DON from plasma and various tissues after the last feeding in the 8-week study with high-OTA feed (Figure 2(b)). The profiles were similar (in liver) or unquantifiable ($< \text{LOQ}$) in other tissues for the low-OTA group (data not shown). The maximum OTA concentration ($c_{\text{max,liver}} = 6.1 \mu\text{g kg}^{-1}$) in liver was reached after 4 h ($t_{\text{max,liver}}$) and subsequently decreased in two phases (bi-exponentially) with $t_{1/2\lambda 1,\text{liver}} = 1.4$ h and $t_{1/2\lambda 2,\text{liver}} = 10.2$ h (two time points considered each) before the concentration increased again slightly between 18 and 48 h, indicating redistribution to two or more body compartments. In kidney, $c_{\text{max,kidney}} = 2.1 \mu\text{g kg}^{-1}$ was reached at $t_{\text{max,kidney}} = 4$ h. OTA was eliminated with $t_{1/2,\text{kidney}} = 4.7$ h (three time points considered, $r^2 = 0.984$), but the concentration increased slightly between 18 and 48 h. The

Table 6. Toxicokinetic parameters of DON and OTA in salmon.

		Plasma	Liver	Kidney	Muscle	Brain
DON						
C_{max}	$\mu\text{g kg}^{-1}$	5.7	42.3	47.0	23.9	20.3
t_{max}	h	0	1	6	6	6
$t_{1/2}$	h	15.1	6.2	13.3	16.5	13.3
AUC_{po}	$\mu\text{g}^*\text{h l}^{-1}$	89.4	n.d.	n.d.	n.d.	n.d.
$AUC_{po,norm}$	$\text{kg}^*\text{h l}^{-1}$	1.5	n.d.	n.d.	n.d.	n.d.
OTA						
C_{max}	$\mu\text{g kg}^{-1}$	0.3	6.1	2.1	0.4	< LOQ
t_{max}	h	2	4	4	18	n.d.
$t_{1/2\lambda 1}$	h	1.2	1.4	4.7	n.d.	n.d.
$t_{1/2\lambda 2}$	h	19.8	10.2	n.d.	n.d.	n.d.
AUC_{po}	$\mu\text{g}^*\text{h l}^{-1}$	n.d.	n.d.	n.d.	n.d.	n.d.
$AUC_{po,norm}$	$\text{kg}^*\text{h l}^{-1}$	n.d.	n.d.	n.d.	n.d.	n.d.

Notes: ^aDerived from the plasma and tissue concentration curves after the last feeding (see Figure 2(a, b)).
n.d., Not determined.

OTA profile in muscle peaked twice at comparably low concentrations, at 4 h and at $t_{max,muscle} = 18$ h ($C_{max,muscle} = 0.4 \mu\text{g kg}^{-1}$), which was either due to compound redistribution or analytical bias. The plasma concentrations ($C_{max,plasma} = 0.3 \mu\text{g kg}^{-1}$, $t_{max,plasma} = 2$ h) were low and decreased bi-exponentially with $t_{1/2\lambda 1,plasma} = 1.2$ h and $t_{1/2\lambda 2,plasma} = 19.8$ h (two time points considered each), thus the $AUC_{po,plasma}$ was not determined (Table 6). The concentrations in brain were all < LOQ.

Detection of DON-glucuronide in salmon liver

Livers from salmon fed with DON for 8 weeks were analysed by HRMS for contents of DON-glucuronides. Only DON-3glc, but not DON-15glc or DON-8glc, was detected in the liver extracts (Figure 3).

Predicted human exposure to DON from the consumption of fillet from fish exposed to low- and high-DON feed

The maximal mean DON concentration observed in salmon muscle after exposure to low-DON feed was $8.5 \mu\text{g kg}^{-1}$ (week 6) (Table 4). Considering the data

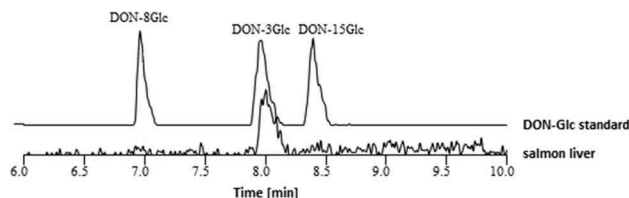


Figure 3. HPLC-HRMS target ion spectra (m/z 471.1497, $[M - H]^{-}$) of a liver extract from Atlantic salmon exposed to DON for 8 weeks and of a standard solution containing the purified DON-glucuronides such as DON-3glc, DON-15glc and DON-8glc.

on intake of farmed salmon from Norwegian food consumption surveys, the corresponding DON exposure to adults eating average amounts of fillet would be $1.3 \text{ ng kg}^{-1} \text{ bw day}^{-1}$, whereas those consuming high amounts (95th percentile) would be exposed to $9.1 \text{ ng kg}^{-1} \text{ bw day}^{-1}$. Two-year-old children consuming average amounts would be exposed to $0.7 \text{ ng kg}^{-1} \text{ bw day}^{-1}$, whereas those consuming high amounts (95th percentile) would be exposed to $2.7 \text{ ng kg}^{-1} \text{ bw day}^{-1}$ (Table 7).

The maximal mean DON concentration observed in salmon muscle after exposure to high-DON feed was $18.6 \mu\text{g kg}^{-1}$ (week 8) (Table 4). Adults eating average amounts of fillet would thus be exposed to $2.9 \text{ ng kg}^{-1} \text{ bw day}^{-1}$ (95th percentile: $19.9 \text{ ng kg}^{-1} \text{ bw day}^{-1}$), and 2-year-olds would be exposed to $1.5 \text{ ng kg}^{-1} \text{ bw day}^{-1}$ (95th percentile: $5.8 \text{ ng kg}^{-1} \text{ bw day}^{-1}$) (Table 7). In high consumers the intake of DON from high-DON fish would be close to 2% of the TDI in adults, and to 0.6% of the TDI in 2-year-olds. Such exposure would represent 4.4–7.4% of the estimated DON intake from cereals in adults and 0.3–0.5% of the estimated DON intake from cereals in 2-year-olds.

Discussion

In the present study, the carryover from feed to various tissues including the edible parts of farmed salmon was investigated for the commonly occurring mycotoxins DON and OTA. Growing salmon were fed three times a day with an artificially contaminated diet with the aim to achieve relatively constant exposure to DON and OTA.

Table 7. Predicted DON exposure by the consumption of salmon fillets with the experimental levels of DON.

	Daily consumption of salmon (g)	Daily intake of DON		High-DON exposure compared with ...		
		Low-DON fillet (ng kg ⁻¹ bw day ⁻¹)	High-DON fillet (ng kg ⁻¹ bw day ⁻¹)	TDI (%)	DON from cereals (%)	
Adult (77.5 kg)	Average	12	1.3	2.9	0.3	0.6–1.1
	High (95%)	83	9.1	19.9	2.0	4.4–7.4
2-year-old (12.8 kg)	Average	1	0.7	1.5	0.2	0.1–0.1
	High (95%)	4	2.7	5.8	0.6	0.3–0.5

Toxicokinetics of DON

DON residues were detected in all analysed tissues of Atlantic salmon exposed to low- and high-DON feed, 2 and 6 mg DON kg⁻¹ feed, respectively, with a mean daily intake for up to 8 weeks of 1.1% or 0.9% of the bw, respectively. Normalisation of the measured concentrations by the average daily doses (µg DON g⁻¹ bw fish) allowed comparison across doses and study weeks by considering the increasing salmon body weights and feed intake, and indicated approximate dose linearity in liver and muscle, but not in kidney. Nevertheless, positive correlation was found between DON levels in liver and kidney, as well as between muscle and skin for the higher dose.

Furthermore, DON was detectable in the muscle of fish at all sampling time points for both doses, and up to 24 h after the last high-DON feeding with a maximum concentration of 24 µg kg⁻¹ after 6 h. DON appeared to be able to readily penetrate the blood–brain barrier in salmon since the concentrations in brain were comparable with those in muscle.

Interestingly, in salmon fed with high-DON feed, DON accumulated significantly in muscle and liver as a result of a high systemic uptake and relatively slow elimination. In the elimination experiments at the end of the study the half-lives in plasma and muscle were estimated to be higher than 15 h, whereas the plasma $t_{1/2}$'s were between 0.5 and 5 h in rodents, birds, pigs, sheep and cows (EFSA 2004a; Goyarts & Dänicke 2006; Wu et al. 2010; VKM 2013). Accordingly, DON tissue accumulation has not been found in these species. This finding implied that the elimination of DON in Atlantic salmon, and possibly also in other fish species (Pietsch et al. 2014a), could be considerably slower than in terrestrial vertebrates. In the Atlantic salmon, the half-life in liver was notably shorter than that in the other tissues, which showed almost identical elimination profiles, indicating distribution into the tissues after liver first-pass.

The calculated $AUC_{po,plasma,norm} = 1.5 \text{ l (h*kg)}^{-1}$ of DON in Atlantic salmon illustrated a considerable systemic uptake when compared with data from other species. In both chicken (Osselaere et al. 2013) and turkey (Devreese et al. 2015) the DON absorption was low as reflected by the $AUC_{po,plasma,norm} = 0.03 \text{ l (h*kg)}^{-1}$. On the contrary, the absorption appears to be higher in piglets (Prelusky et al. 1988) and pigs (Goyarts & Dänicke 2006) as shown by $AUC_{po,plasma,norm} = 4.4$ and $2.3 \text{ l (h*kg)}^{-1}$, respectively. Hence, DON toxicokinetics in fish apparently differ to some extent from mammalian and bird species.

The analysis of the salmon livers for contents of known DON biotransformation products revealed the presence of DON-3glc, a major metabolite found in *in vitro* metabolism studies with liver microsomes from rat, carp, rainbow trout, chicken, pig, cow and humans (Maul et al. 2012, 2015; Uhlig et al. 2013, 2016). The present study shows for the first time that DON-3glc is also produced *in vivo* in salmon fed with DON-containing feed. The other two known DON-glucuronides, DON-15glc and DON-8glc, were not detected.

Toxicokinetics of OTA

The results of the present study on the tissue distribution and elimination of OTA in Atlantic salmon confirmed previous findings of OTA toxicokinetics in rainbow trout and carp (Fuchs et al. 1986; Hagelberg et al. 1989). The low plasma concentrations detected in the final elimination experiment after 8 weeks in fish exposed to approximately 2.4 mg OTA kg⁻¹ feed indicated a rather low bioavailability, similar to the low bioavailability observed in carp (1.6%). The low bioavailability in fish differ substantially from those observed in terrestrial vertebrates, which is commonly > 50% (Galtier et al. 1981). Plasma binding of OTA is a relevant factor

for both distribution and elimination since only free, unbound molecules can be transported through membranes, metabolised by biotransformation enzymes and excreted. The 100-fold higher fraction of unbound OTA in fish (carp) ($f_{u,plasma} = 22\%$) as compared with rodents, birds, pigs and primates ($f_{u,plasma} < 0.2\%$) can be regarded as an important factor for the observed diverging kinetic characteristics (Stojković et al. 1984; Hageberg et al. 1989).

Common to all species including humans, and also confirmed for Atlantic salmon in the present study, is, however, the two-phase elimination profile suggesting the applicability of a two-compartment open model with blood as the central compartment and all other tissues as the peripheral compartment (Galtier et al. 1981; Li et al. 1997; Studer-Rohr et al. 2000; Dietrich et al. 2005). A further common feature appears to be the presence of a secondary peak in the OTA plasma concentrations, observed in rats, mice and cows, and now also in salmon muscle, kidney and liver, which was explained by a potential enterohepatic recirculation (Fuchs et al. 1986; Fuchs & Hult 1992; Li et al. 1997). OTA is hydroxylated by liver enzymes to a number of known products such as OTA- α , 4-OH-OTA and 10-OH-OTA (Wu et al. 2011) that could be conjugated to glucuronides, excreted via the biliary route, split in the gastrointestinal tract and reabsorbed. In the current study, OTA concentrations were highest in liver, so a similar mechanism in salmon could be assumed. Biotransformation products of the hepatic metabolism in salmon have, however, not been investigated yet. The significant reduction of OTA concentrations in liver during the 8-weeks study indicates an increasing ability of the fish to eliminate the compound, e.g., through induced metabolism.

The persistence of OTA in kidney as observed in rainbow trout (Fuchs et al. 1986) has been attributed to renal reabsorption and is thought to explain kidney toxicity, a major effect in rodents and pig (Dietrich et al. 2005). This might also be true for fish considering the specific distribution pattern in kidney found in a study with ^{14}C -labelled OTA (Fuchs et al. 1986; Fuchs & Hult 1992) and also the elevated OTA concentrations in salmon kidney in the present study.

Predicted human exposure to DON and OTA from edible fish products from fish exposed via feed

Human exposure to DON following high consumption (95th percentile) of fillets from the high-DON

group ($6 \text{ mg kg}^{-1} \text{ feed}$) after 8 weeks feeding was predicted to be 2% of the TDI and 4.4–7.3% of the estimated normal DON intake from cereals in adults ($0.27\text{--}0.45 \text{ } \mu\text{g kg}^{-1} \text{ bw day}^{-1}$). In 2-year-old children, corresponding data were 0.6% of the TDI and 0.3–0.5% of the estimated normal DON intake from cereals ($1.1\text{--}2.0 \text{ } \mu\text{g kg}^{-1} \text{ bw day}^{-1}$) (VKM 2013). Cereal consumption is considered to contribute the most to the daily intake of DON and might come close or even exceed the established TDI of $1 \text{ } \mu\text{g kg}^{-1} \text{ bw day}^{-1}$ (VKM 2013).

The maximum recommended levels of DON in the current legislation on animal feed in the European Union and Norway (European Commission 2006; Norwegian Food Safety Authority 2015) are respectively 5 and 2 mg kg^{-1} . Actual exposure of farmed salmon is therefore likely to be lower than the high dose of the present study. Also, reduced growth in high-DON fish further supports that accidentally high levels of DON in fish feed would be detected. Although some uncertainty remains regarding the distribution of DON in adult salmon in comparison with the post-smolts used in this study, the present findings indicate that consumption of farmed salmon fed diets containing cereals is unlikely to contribute significantly to the total human exposure to DON.

OTA residues in muscle were only detectable at very low levels in the high-OTA group (approximately $2.4 \text{ mg OTA kg}^{-1} \text{ feed}$). Previous observations in rainbow trout similarly showed no radioactivity in the muscle in a whole-body autoradiography experiment with radiolabelled OTA (Fuchs et al. 1986). The actual guidance levels for OTA in fish feed in the European Union and Norway are, respectively, 0.25 and $1 \text{ mg kg}^{-1} \text{ feed}$ (European Commission 2006; Norwegian Food Safety Authority 2015) meaning that the doses used in the present study are likely to be higher than those found in aquaculture feed. Human exposure to OTA via the consumption of salmon fed with OTA-contaminated feed is most likely negligible.

Conclusions

DON was evenly distributed in the liver, kidney, muscle, skin and brain of Atlantic salmon fed with contaminated feed for 8 weeks. In fish fed with the

highest DON concentration (6 mg kg⁻¹ feed) a significant tissue accumulation of DON was shown during the 8-week exposure. OTA was mainly distributed to liver and kidney with only trace or non-detectable concentrations in other tissues. OTA was rapidly eliminated, and in fish fed with the highest OTA concentration (approximately 2.4 mg kg⁻¹ feed) a significant tissue reduction of OTA was shown from 3 to 8 weeks exposure, indicating induced elimination mechanisms.

In spite of accumulated DON concentrations in edible tissues of the fish the health risk to consumers of salmon fillet would be negligible as long as toxin levels in fish feed comply with the present regulation. Human exposure to OTA via the consumption of salmon fed with OTA-contaminated feed is most likely negligible.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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