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Effect of pyrethroid treatment against sea lice in salmon farming regarding consumers' health

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ABSTRACT

Pyrethroids are the most popular drug against sea lice in salmon farming. Although they are more toxic to insects, they have toxic effects in mammals. Pyrethroids were detected in 100% of farmed salmon with a mean concentration of 1.31 ± 1.39 ng g^{-1} ww and in 50% of wild salmon with a mean of 0.02 ± 0.03 ng g^{-1} ww. Cypermethrin and deltamethrin, the active ingredients of anti-sea lice formulations, represented 77 ± 27% of the total contamination of farmed salmon. Although farmed salmon had higher concentrations than wild salmon, the daily intake of pyrethroids through salmon consumption was several orders of magnitude below the accepted daily intake (ADI). Thus, the pyrethroids treatment on salmon does not pose a threat on the health of the consumers.

1. Introduction

Sea lice (Copepoda: Caligidae) have been the most widespread pathogenic marine parasite in the three decades of salmon farming industry. In the second part of this period, pathogenic infestations on other farmed fish and wild salmonids have increased notoriously (Ragias et al., 2004; Costello, 2006). The impact of sea lice on the host ranges from mild skin damage to mortality induced by stress, including epidemics in wild fish populations in Europe and British Columbia (Costello, 2006). A non-comprehensive list of other effects would include epithelium loss, increased mucus discharge, bleeding, tissue necrosis and consequent exposure to secondary infections; reduced appetite, growth and food-conversion efficiency; anaemia and reduced lymphocytes (Tully and Nolan, 2002; Johnson et al., 2004; Costello, 2006).

Pyrethroids became the most popular drug against sea lice around 1995, substituting organophosphates, which had previously been the preferred compounds (Grave et al., 2004). The anti-sea lice pesticide formulations AlphaMax® and Excis® are emulsifiable concentrates containing 1% of the synthetic pyrethroids deltamethrin or cypermethrin as the active ingredient, respectively. Both pesticides are effective against all attached stages of sea lice including adults (Haya et al., 2005; Burridge et al., 2010). Treatment of salmon is either a 40-min bath with AlphaMax® at a target concentration of 2.0 μg/L deltamethrin (SEPA, 2008) or a 1-h bath with Excis® at a target concentration of 5.0 μg/L cypermethrin (Van Geest et al., 2014).

Pyrethroid insecticides are applied for household, commercial and farming purposes, in medicine against lice and scabies and to control malaria in tropical countries by impregnating mosquito nets with them (Bradberry et al., 2005). In salmon farming pyrethroids are used against the sea lice, which parasite the fish (Haya et al., 2005). Pyrethroids were considered ideal insecticides because they were thought not to be persistent in the environment and to be metabolised by mammals instead of accumulated (Casida et al., 1975; Leng et al., 1997). Therefore, their popularity grew during the 1970s and pyrethroids substituted other banned pesticides (Ridgway et al., 1978). Nowadays, they account for 25% of the insecticides used worldwide, which equals about 100 tons of pyrethroids a year (Casida and Quistad, 1998; Shafer et al., 2005).

Their toxic effects include disruption of the function of the neurons' sodium channels as they provoke repetitive after-

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discharges in neurons and muscle cells that produce repeated stimulation (Narahashi et al., 1998; Pollack et al., 1999). At high concentrations of pyrethroids, the sodium intake may block conduction, causing paralysis. Lethal concentration 50 (LC_{50}) of pyrethroids have been reported for some fish. LC_{50} values cover a wide range, e.g. 0.06 μg l^{-1} for tefluthrin on trout, 19 μg l^{-1} for allethrin on trout and 150 μg l^{-1} for bifenthrin on trout (Lewis et al., 2016). Toxicity of pyrethroids is 2250 times higher to insects than mammals, since insects have more sensitive sodium channels, smaller bodies and lower body temperatures. On the other hand, several acute and chronic effects on humans have been reported (IARC, 1991; Muller-Mohnssen and Hahn, 1995; Kolaczinski and Curtis, 2004; Bradberry et al., 2005; EPA, 2015).

Because of their toxicity, exposure of aquatic organisms to pyrethroids has always caused concern (Mauck and Olson, 1976). Used on the land or for domestic purposes as vector control, pyrethroids can enter the aquatic environment through atmospheric deposition, river runoff or municipal treatment discharges. They associate with sediments and then benthic organism become exposed to pyrethroids by ingestion or contact of sediment particles or from interstitial water. Fish are exposed to pyrethroids through diet or gill absorption due to the lipophilicity of these compounds (Edwards et al., 1987).

Although pyrethroids are believed to be converted to non-toxic metabolites in mammals by hydrolysis and oxidation (Abernath et al., 1973; Casida et al., 1975), our research group found evidence that they bioaccumulate in marine mammals from Brazil (Alonso et al., 2012) and Spain (Aznar-Alemany et al., 2017). These insecticides have also been detected in human breast milk (Zehringer and Herrmann, 2001; Corcellas et al., 2012). Due to their aforementioned toxic effects and the evidence of their accumulation in mammals, the World Health Organisation (WHO) has reported a no-observed-adverse-effect level (NOAEL) and acceptable daily intake (ADI) for the individual pyrethroids expressed in quantity of the compound per kilogram of a person's body weight (bw) per day (WHO, 2005). NOAELs for the pyrethroids in this work are between 1 and 5 mg (kg bw)$^{-1}$ day^{-1} and maximum ADIs are between 0.02 and 0.05 mg (kg bw)$^{-1}$ day^{-1}.

Pyrethroids derive from allethrin, type I pyrethroids contain a carboxylic ester and type II pyrethroids have an additional cyano group; most of the pyrethroids contain a cyclopropane, too (Bradberry et al., 2005). Because of these groups, type I pyrethroids possess 2 chiral centres and type II possess 3 of them. This means that type I and type II pyrethroids have 2 and 4 diastereoisomers (therefore enantiomer pairs), respectively, which could show different toxicity and accumulation (Jin et al., 2012). This is relevant as isomeric composition is an important toxicological parameter for a number of compounds (Zhao et al., 2010; Sun et al., 2016; Wang et al., 2016).

The aim of this work was to compare the occurrence of 10 pyrethroid compounds in farmed salmon to wild salmon and assess the effect of the pyrethroids baths against sea lice. Additionally, comparisons of farmed salmon's pyrethroid levels were performed, first between places of origin of the samples and second between processed (i.e. marinated or smoked) and non-processed samples. The risk on consumer's health was assessed comparing an estimated daily intake of pyrethroids through consumption of salmon to the ADI. Finally, enantiomer selective accumulation for different species was studied.

2. Materials and method

2.1. Sampling

Samples of salmon (51), including farmed (39) and wild (12) salmon, were purchased at retail stores and fishmongers from Barcelona (Spain) between February 2014 and February 2016. Salmon from 6 different species, farmed in 8 different countries and sold in 5 different presentations were collected. The farming locations of the samples included Alaska, Chile, Denmark, France, Norway, the Pacific Ocean, Scotland and Spain. The presentations available were fresh, frozen, marinated, refrigerated and smoked. The species of salmon included *Oncorhynchus gorbuscha*, *Oncorhynchus keta*, *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, *Oncorhynchus nerka* and *Salmo salar*. See Table 1 for more details. Previous to freeze-drying, the skin was removed. Muscle of all samples was freeze-dried, homogenised and stored frozen.

2.2. Standards and reagents

Bifenthrin, λ-cyhalothrin, fluvalinate, resmethrin and a mixture of pyrethroids containing cyfluthrin, cypermethrin, deltamethrin, fenvalerate, permethrin and teramethrin were used as analytical standards. Internal standards were d_6-*trans*-permethrin and d_6-*trans*-cypermethrin. All of them were certified pyrethroids standards purchased from Dr. Ehrenstorfer (Augsburg, Germany). Pesticide grade organic solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions were prepared in ethyl acetate. Solid phase extraction (SPE) C18 (2 g/15 ml) and basic alumina (5 g/25 ml) cartridges were obtained from Isolute Biotage and Interchim, respectively.

2.3. Sample preparation

Sample preparation was carried out according to Feo et al., 2012. Salmon meat (0.1 g dry weight (dw)) was spiked with deuterated internal standards (d_6-*trans*-permethrin and d_6-*trans*-cypermethrin). The sample was stirred and extracted by sonication with 20 ml of hexane:dichloromethane (2: 1) and centrifuged twice. Both organic phases were transferred to one vial and evaporated. The remaining fat was re-dissolved with 20 ml of acetonitrile and underwent a clean-up by filtering the extract through basic alumina and C18 SPE cartridges in tandem. The eluate was evaporated and re-dissolved with 100 μl of ethyl acetate.

To determine the lipid content of the samples, 1 g dw of sample was also extracted with 20 ml of hexane:dichloromethane (2: 1) twice and evaporated. Then the lipid content was determined gravimetrically.

2.4. Instrumental analysis

The pyrethroid analysis was performed with an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) according to Feo et al., 2011. Chromatographic conditions were as follows: injection volume was 3 μl; inlet temperature was 270 °C; DB-5ms capillary column (15 m × 0.25 mm, 0.1 μm film thickness) containing 5% methyl phenyl siloxane; carrier gas was He at 1 ml/min, and temperature was 100 °C for the first minute, then raised from 100 to 230 °C for 8 min, then from 230 to 310 °C for 8 min and, finally, was constant for 2 min. Transfer line temperature was 275 °C, the ion source temperature for negative ion chemical ionisation in tandem mass spectroscopy (NICI-MS/MS) was 250 °C and the reagent gas was ammonia at 2×10^{-4} torr. Run time for each sample was 17 min.

Selective reaction monitoring (SRM) mode was used with two transitions monitored for each compound. The most intense transition was used for quantification and the second transition provided a confirmation comparing the SRM_1/SRM_2 ratio calculated for the samples with the ratio found in the standards (Supplementary