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Distribution and persistence of the anti sea-lice drug teflubenzuron in wild fauna and sediments around a salmon farm, following a standard treatment

Ole B. Samuelsen [a,*], Bjørn T. Lunestad [b], Rita Hannisdal [b], Raymond Bannister [a], Siri Olsen [a], Tore Tjensvoll [b], Eva Farestveit [a], Arne Ervik [a]

[a] Institute of Marine Research, P.O. Box 1870 Nordnes, N-5817 Bergen, Norway
[b] National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, N-5817 Bergen, Norway

HIGHLIGHTS

- Distribution of teflubenzuron was studied in the marine environment after medication
- Teflubenzuron containing organic particles were collected 1100 m from the farm
- Teflubenzuron persists in the sediment for a prolonged time
- Residues were found in nearly all species of biota collected
- Residues found in saith and crab exceeded the MRL value given for farmed salmon

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ABSTRACT

The salmon louse (*Lepeoptheirus salmonis*) is a challenge in the farming of Atlantic salmon (*Salmo salar*). To treat an infestation, different insecticides are used like the orally administered chitin synthetase inhibitor teflubenzuron. The concentrations and distribution of teflubenzuron were measured in water, organic particles, marine sediment and biota caught in the vicinity of a fish farm following a standard medication. Low concentrations were found in water samples whereas the organic waste from the farm, collected by sediment traps had concentrations higher than the medicated feed. Most of the organic waste was distributed to the bottom close to the farm but organic particles containing teflubenzuron were collected 1100 m from the farm. The sediment under the farm consisted of 5 to 10% organic material and therefore the concentration of teflubenzuron was much lower than in the organic waste. Teflubenzuron was persistent in the sediment with a stipulated halflife of 170 days. Sediment consuming polychaetes had high but decreasing concentrations of teflubenzuron throughout the experimental period, reflecting the decrease of teflubenzuron in the sediment. During medication most wild fauna contained teflubenzuron residues and where polychaetes and saith had highest concentrations. Eight months later only polychaetes and some crustaceans contained drug residues. What dosages that induce mortality in various crustaceans following short or long-term exposure is not known but the results indicate that the concentrations in defined individuals of king crab, shrimp, squat lobster and Norway lobster were high enough shortly after medication to induce mortality if moulting was imminent. Considering food safety, saith and the brown meat of crustaceans contained at first sampling concentrations of teflubenzuron higher than the MRL-value set for Atlantic salmon. The concentrations were, however, moderate and the amount of saith fillet or brown meat of crustaceans to be consumed in order to exceed ADI is relatively large.

1. Introduction

The copepod ecto-parasite salmon louse (*Lepeoptheirus salmonis*, Krøyer 1837) is the major challenge in the production of Atlantic salmon (*Salmo salar*) in Europe and North America. Infestation with the parasite reduces the general welfare of the farmed fish and causes economic loss due to reduced growth, increased mortality and downgrading of fish quality (Burka et al., 2012; Costello, 2006, 2009; MacKinnon, 1997; Pike and Wadsworth, 2000). Furthermore, salmon lice derived from aquaculture sources may negatively impact the wild populations of sea trout and migrating wild post-smolts of Atlantic salmon (Costello, 2009; Wagner et al., 2008).

* Corresponding author.

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Commonly salmon louse infestations are treated using antiparasitic agents, and a number of insecticides have been approved for this purpose. The antiparasitic agents are either used for bath treatment or administered orally to the fish via the feed. In Norway, flubenzurons (teflubenzuron and diflubenzuron) were used in the 1990s till 2001. From 2002, emamectin-benzoate and the pyrethroids, cypermethrin and deltamethrin, were the drugs of choice, but due to increasing problems with reduced sensitivity for these compounds, the flubenzurons were reintroduced in 2009 with a total yearly consumption of 3441, 2919, 730, 2362 and 4968 kg active compounds respectively, from 2009 to 2013 (statistic provided at http://www.fhi.no/artikler/?id=109432). Flubenzurons are orally administered agents that are transmitted to the parasite via the mucus, skin and blood of the host. They act by interference with the synthesis of chitin in the salmon lice and are effective against all stages of the parasite that undergo moulting, including the larval and pre-adult stages (Branson et al., 2000; Campbell et al., 2006a, 2006b; Ritchie et al., 2002). Teflubenzuron is administered as medicated pellets containing 2 g teflubenzuron per kg feed and the recommended dosing regimen for Atlantic salmon is 10 mg teflubenzuron per kg of fish daily for 7 days.

When pharmaceuticals are administered orally as medicated feed, they either enter the environment as uneaten pellets or as eliminated parent compound from the fish via urine or faeces (Dempster et al., 2009; Overland et al., 2009; Skretting, 2010). Pharmacokinetic studies have shown that the absorption of teflubenzuron from the gastrointestinal tract of Atlantic salmon is poor, with only around 10% of the administered dose being retained by the fish and 90% being directly released (SEPA, 1999). Furthermore, as the metabolism of teflubenzuron in Atlantic salmon is minor most of the absorbed drug will be released as parent compound. The main excretion pathway for teflubenzuron is via the bile to the intestine, and due to low water solubility (0.0094 mg/L at 20 °C) and high lipophilicity (Log $K_{ow} = 5.39$), the drug will be released from the fish associated to faecal particles (Langford, 2011; Langford et al., 2014; Marsella et al., 2000; Selvik et al., 2002).

Uneaten medicated pellets serve as food for wild fish in particular, as shown in previous studies administering pellets enriched with antibacterial agents, and both medicated pellets and faecal material from the farmed fish were food sources for sessile and benthic organisms (Ervik et al., 1994b; Samuelsen et al., 1992a).

In conjunction with medication, organic materials containing the pharmaceutical may contaminate the sediment under and close to the farms (Langford, 2011; Langford et al., 2014; Samuelsen et al., 1992a; Selvik et al., 2002) and may persist in the sediment for a prolonged period (Samuelsen et al., 1992b). In a screening survey published by the Norwegian Climate and Pollution Agency (Langford, 2011) residues of flubenzurons were found in samples of water, bottom sediment, in particulate matter and in biota after medication. In this study, three farms were investigated and each location sampled once. The persistence of the drugs in sediment and biota over time was therefore not investigated. Flubenzuron residues dissolved in water were found at distances up to 900 m from the farms (Langford, 2011; Langford et al., 2014). Selvik et al. (2002) found diflubenzuron in the sediment under a farm, but the distribution was less than 20 m from the net pen edge. Fifteen months later, no residues could be found. This farm was however located in a protected bay with low water current and the depth at the site was 40 m. Today fish farms are generally located at more exposed sites and usually at greater depths that may influence the area of distribution of the drug. Furthermore, in order to study the persistence of drugs in sediment and biota, multiple samplings over time are necessary.

While flubenzurons are relatively non-toxic to marine species like fish, algae and shellfish (Burridge et al., 2010; Eisler, 1992; Fischer and Hall, 1992; Haya et al., 2005), they are potentially highly toxic to any species that undergo moulting within their life cycle. LC_{50} values have been established for insects and crustaceans when exposed to flubenzurons dissolved in water (summarised in Langford, 2011). Studies involving effects of orally administered drugs to non-target marine crustaceans are, on the other hand, sparse but in a recent published paper Samuelsen et al. (2014) showed that under controlled laboratory conditions, oral intake of teflubenzuron induced mortality and deformities in juvenile European lobster (*Homarus gammarus*) during ecdysis. Langford et al. (2014) found minor residues of diflubenzuron and teflubenzuron (<11.3 ng/g) in deep water scrimps (*Pandalus borealis*) collected by trawl 1 to 5 km away from the farms, and up to 538 ng/g in brown crabs (*Cancer pagurus*) caught between 100 and 300 m from the farms. Samples of brown crabs were a homogenate of white and brown tissues and hepatopancreas. With respect to deep water shrimps data from individuals caught closer to the farm is deemed necessary and to register any changes in concentrations over time.

In order to sustain the safety of consumers, teflubenzuron has been given an Acceptable Daily Intake (ADI) value of 0.01 mg/kg body weight/day and a Maximum Residue Limit value (MRL-value) in farmed Atlantic salmon of 500 μg/kg (Anonymous, 1999). Langford et al. (2014) found no residues of flubenzurons in Atlantic cod (*Gadus morhua*) caught in the vicinity of the farms. However, since a number of fish species are shown to aggregate around fish farms displaying a wide range of feeding strategies, a more thorough study of the concentrations of therapeutics in wild fish is deemed necessary (Dempster et al., 2009, 2011; Ervik et al., 1994b). Such data is needed for a toxicological evaluation of flubenzurons when present in food for human consumption.

Given this background, a field investigation was carried out to examine the distribution of teflubenzuron in wild fish and bottom dwelling organisms, following a standard medication at a commercial fish farm. The distribution and persistence of teflubenzuron in bottom sediments, the presence of drug containing organic particles and the concentration of teflubenzuron in water were also examined.

2. Material and methods

2.1. Farm site

After observing salmon lice in numbers on cultivated Atlantic salmon that necessitated treatment at a salmon farm in Matre north of Bergen, Norway, medicated feed was given to the fish with teflubenzuron in the period 2nd to 8th of February, 2012. The biomass in the farm was approximately 116,000 kg and following a standard medication regime (10 mg/kg fish for 7 consecutive days) the total consumption of teflubenzuron was 8120 g. The depth at the site was 25 m close to shore, increasing to 60 m at the end of the farm to more than 300 m in the middle of the fjord. The bottom under the farm consisted alternately of rock, sand and shellsand and a minor area enriched with organic material (Rådgivende Biologer, 2008). The dominating current direction of surface water at the farm is west (Fig. 1). The farmed fish were slaughtered during the first weeks of May (2012), followed by a fallowing period of 5 months. This farm has no history of using flubenzurons and the distance to the nearest salmon farm is approximately 6 km. Furthermore, no farms in the area used flubenzurons in the experimental period.

2.2. Sampling

2.2.1. Sediment traps and current metres

In order to study the distribution of teflubenzuron residues associated to slow descending organic particles, duplicate cylindrical sediment traps (9.6 cm diameter, 80 cm distance between the duplicates) were deployed at the farm and at distances of 250, 700 and 1100 m west from the farm (Fig. 1) between the 1st and 23rd of February. At the farm, the traps were deployed 10 m from the bottom whereas the distant stations had traps at both 50 m depth and 10 m from the bottom. In order to measure the current speed and direction, a SD-6000 MINI Current metre (Sensor Data, AS) was mounted together with the sediment traps at the farm and at 50 m depth at the distant stations 700