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Tittel- Title
Kartlegging av veterinærlegemidler brukt i akvakultur – diflubenzuron og teflubenzuron
Environmental screening of veterinary medicines used in aquaculture – diflubenzuron and teflubenzuron
(NIVA-rapport 6133-2011)

Sammendrag
Kartleggingen undersøkte forekomsten av de to benzoylurea-kitinsynteseinhiberende pesticidene, diflubenzuron og teflubenzuron. Disse stoffene blir brukt til å behandle laks i oppdrettsanlegg som er smittet med lakselus. Det ble funnet påviselige konsentrasjoner av diflubenzuron og teflubenzuron i prøver av sediment, partikler i vann, blåskjell, tanglopper, taskekrabber og reker fra lokalitetene hvor det var behandlet med lakselusmidlene. Det ble ikke funnet diflubenzuron eller teflubenzuron i noen av torskeprøvene, og heller ikke i noen av prøvene fra referanseområdet.

Summary
This screening survey investigated the occurrence of two benzoylurea chitin synthesis inhibiting pesticides, diflubenzuron and teflubenzuron, used for the treatment of sea lice infestation in salomoid aquaculture. Detectable concentrations of diflubenzuron and teflubenzuron were measured in sediment, particulates, amphipods, brown crab, blue mussel and shrimp where sea lice treatment had occurred. Neither of the compounds were detected in any cod samples nor in any sample collected from the reference location.

4 emneord Akvakultur, teflubenzuron, diflubenzuron, Veterinærlegemidler	4 subject words Aquaculture, teflubenzuron, diflubenzuron, veterinary medicines

Preface

NIVA was commissioned by the Norwegian Climate and Pollution Agency (Klif) to establish the occurrence of selected aquaculture medicines in the marine environment. The results of the screening study are reported here.

The results show that the aquaculture industry contributes to the load of aquaculture medicines in the marine environment with compounds occurring in sediment, particulate matter, and biota. The results of a simple risk assessment using the limited toxicity and occurrence data available show that the concentrations released into the marine environment do at times exceed environmental quality standards set in the UK.

A gap in the data has been observed and it is recommended that marine sediment toxicity data are obtained for diflubenzuron so that an assessment of the risk to sediment dwelling organisms can be evaluated thoroughly.

Samples collection was coordinated by Sigurd Øxnevad and the sample analysis was coordinated by Katherine Langford. Merete Schøyen, Kate Hawley, Bjørnar Beylich, Lucy Brooks, Andreas Høgfeldt, Erling Bratsberg and Alfhild Kringstad all contributed to the sample collection or the sample analysis. We thank Einar Blikø, Svein Edvardsen, Hans Finnanger, Ove Morten Hagen, Rune Midtlien and Lars Moe for fishing crab, shrimp and cod for us. The risk assessment was completed by Kevin Thomas and quality assurance was the responsibility of Kevin Thomas and Kristin MacBeath. The project was lead by Katherine Langford and the report was written by Katherine Langford, Sigurd Øxnevad, Merete Schøyen and Kevin Thomas.

Bård Nordbø, the project coordinator at Klif, is also acknowledged for his support.

NIVA, Oslo, March 2011

Katherine Langford
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Contents

1. Abstract

The screening of new and emerging contaminants entering the environment is of increasing concern to environmental authorities. On behalf of the Norwegian Climate and Pollution Agency (Klif), the Norwegian Institute for Water Research (NIVA) investigated the occurrence of selected veterinary medicines used in the aquaculture industry during a screening program in 2010.

The screening survey investigated the occurrence of two benzoylurea chitin synthesis inhibiting pesticides, diflubenzuron and teflubenzuron, used for the treatment of sea lice infestation in salomoid aquaculture. Both pesticides are administered through feed, diflubenzuron is the active ingredient in Releeze vet and teflubenzuron is the active ingredient in Ektoban vet. Three salmon aquaculture locations were chosen in collaboration with Klif and the Norwegian Food Safety Authority (Mattilsynet) along with a reference location. The three fish farm locations all reported using Releeze and/or Ektoban, treatment prior to sample collection, resulting in the potential discharge of diflubenzuron and teflubenzuron to the marine environment. The reference location selected was the Oslofjord where there was no reported usage of either of the benzoylurea pesticides.

Diflubenzuron and teflubenzuron were measured in sediment, suspended particulates in water, amphipods, blue mussel, brown crab and shrimp, as well as cod filet, liver and skin, collected in close proximity (with 5 km) to all 3 fish farms and from the Oslofjord.

Detectable concentrations of diflubenzuron and teflubenzuron were measured in sediment, particulates, amphipods, brown crab, blue mussel and shrimp where sea lice treatment had occurred. Neither of the compounds were detected in any cod samples nor in any sample collected from the reference location.

An environmental quality standard (EQS) is the level used to assess the risk of chemical pollutant effects on water quality. With no Norwegian EQS available for either of the fish farm medicines selected for screening we compared the data with EQS values from the United Kingdom for sediment and water. Management practice in the UK dictates that there are EQSs for within and outside of an allowable zone of effects (AZE) which is defined as up to 25 m from the fish farm. All of the sediment samples collected outside of the 25 m limit at fish farms 1 and 2 contained levels of teflubenzuron greater than the UK EQS of 2 ng/g (dry weight). The other sediment samples collected from within 25 m of Fish farm 2 were below the teflubenzuron EQS for within the AZE of 10,000 ng/g (dry weight). The levels of teflubenzuron detected in sediments at certain locations are sufficiently high to be of risk to sediment dwelling organisms. No sediment EQS is available for diflubenzuron and a lack of relevant data make calculating a sediment predicted no-effect concentration (PNEC) difficult.

Water samples analysed from two of the farms contained dissolved levels of diflubenzuron above the UK EQS of 5 ng/L. Three water samples, collected between 300 and 900 m from Fish farm 2, contained levels of teflubenzuron above the EQS of 6 ng/L. The dissolved levels of diflubenzuron detected in water samples collected at the farms, and up to 1 km away, are sufficiently high to pose a risk to aquatic organisms. There is also some risk to aquatic organisms from dissolved concentrations of teflubenzuron.

It is not clear whether the concentrations detected in the biota samples collected will have effects on each individual species. It is however a concern that crab, shrimp and blue mussels are being exposed to both diflubenzuron and teflubenzuron at the sites selected. A crude

assessment of the levels detected in the shrimp collected from Farm 1 and the levels at which chronic effects are seen in shrimp (3 ng/L) would suggest that there is a potential risk to shrimp. It would also be reasonable to extrapolate this to any species that undergoes moulting in its life cycle.

2. Sammendrag

Det er økende oppmerksomhet rundt kartlegging av forekomst og tilførsel av nye miljøgifter til det marine miljø. I kartleggingsprogrammet for 2010 har Norsk institutt for vannforskning (NIVA) undersøkt forekomsten av utvalgte veterinærlegemidler brukt i akvakulturnæringen. Kartleggingen er gjennomført på oppdrag fra Klima- og forurensningsdirektoratet (Klif).

Kartleggingen undersøkte forekomsten av de to benzoylurea-kitinsynteseinhiberende pesticidene, diflubenzuron og teflubenzuron. Disse stoffene blir brukt til å behandle laks i oppdrettsanlegg som er smittet med lakselus. Begge pesticidene blir gitt via fiskefôret. Diflubenzuron er virkestoffet i medisinfôret Releeze vet og teflubenzuron er virkestoffet i medisinfôret Ektoban vet. Tre oppdrettsanlegg ble valgt ut av Klif og Mattilsynet til å være med i undersøkelsen. I tillegg ble det tatt prøver fra et referanseområde. De tre oppdrettsanleggene hadde brukt medisinfôrene Releeze eller Ektoban, eller en kombinasjon av begge behandlingene før prøveinnsamlingene. Dette kunne medføre tilførsel av diflubenzuron og teflubenzuron til det marine miljøet. Oslofjorden ble valgt som referanseområde, og har ingen rapportert bruk av benzoylurea pesticider.

De ble analysert for diflubenzuron og teflubenzuron i sediment, partikler i vann, blåskjell, tanglopper, taskekrabber, reker, torskefilét, torskelever og torskeskinn. Prøvene ble samlet inn i nærheten av oppdrettsanleggene og i Oslofjorden.

Det ble funnet påviselige konsentrasjoner av diflubenzuron og teflubenzuron i prøver av sediment, partikler i vann, blåskjell, tanglopper, taskekrabber og reker fra lokalitetene hvor det var behandlet med lakselusmidlene. Det ble ikke funnet diflubenzuron eller teflubenzuron i noen av torskeprøvene, og heller ikke i noen av prøvene fra referanseområdet.

En miljøkvalitetsstandard (EQS, Environmental Quality Standard) er nivået som brukes for å bedømme risiko for forurensningseffekter. Siden det ikke foreligger norske miljøkvalitetsstandarder EQS-verdier for oppdrettsmedisinene i denne undersøkelsen, har vi sammenlignet resultatene med EQS-verdier for sediment og vann fra Storbritannia. I Storbritannia bruker forvaltningen EQSer for innenfor, og utenfor sone for tillatte effekter (AZE, Allowable Zone of Effects), som er definert som opptil 25 meter fra oppdrettsanlegget. Alle sedimentprøvene som ble samlet inn utenfor 25 meters grensen for oppdrettsanlegg 1 og 2 hadde konsentrasjoner av teflubenzuron som oversteg EQS-verdien (for Storbritannia) på 2 ng/g (tørrvekt). De andre sedimentprøvene som ble samlet innenfor 25 meters grensen for oppdrettsanlegg 2 lå under EQS-verdien for teflubenzuron innenfor sonen for tillatte effekter på 10.000 ng/g (tørrvekt). De påviste konsentrasjonene av teflubenzuron i sedimentprøver fra enkelte lokaliteter er høye nok til å utgjøre en risiko for organismer som lever i sedimentene. Det finnes ingen EQS-verdi for diflubenzuron i sediment, og mangelen på relevante data gjør det vanskelig å beregne en grenseverdi for konsentrasjon som ikke gir toksiske effekter (PNEC, Predicted No-Effect Concentration).

Det ble påvist konsentrasjoner av diflubenzuron som ligger over EQS-verdien på 5 ng/L i vannprøver innsamlet ved to av oppdrettsanleggene. Tre vannprøver innsamlet mellom 300 og 900 meter fra oppdrettsanlegg 2 hadde konsentrasjoner av teflubenzuron over EQS-verdien på 6 ng/L. Påviste konsentrasjoner av diflubenzuron i vannprøver innsamlet ved oppdrettsanleggene og opp mot 1 km unna er tilstrekkelig høye til å utgjøre en risiko for akvatiske organismer. Konsentrasjoner av løst teflubenzuron utgjør også en potensiell risiko for akvatiske organismer.

Det er uklart om de påviste konsentrasjonene i biotaprøvene har effekter på de forskjellige artene. Det er imidlertid bekymringsfullt at krabber, reker og blåskjell blir eksponert for både diflubenzuron og teflubenzuron på de utvalgte lokalitetene, En grov vurdering av de påviste konsentrasjonene i reker samlet inn ved oppdrettsanlegg 1 og nivåene som gir kroniske effekter på reker (3 ng/L) tilsier at det er en potensiell risiko for effekter på reker. Det er også rimelig å videreføre dette til andre arter som gjennomgår skallskifte i løpet av livssyklusen.

3. Background and Introduction

3.1 General

Diflubenzuron and teflubenzuron are benzoylurea aquaculture medicines administered in fish food for the control of sea lice. Diflubenzuron is sold on the Norwegian market as Releeze vet and teflubenzuron is sold as Ektoban vet. Releeze is 0.6 g/kg diflubenzuron and Ektoban is 2 g/kg teflubenzuron and they are administered as feed additives.

Diflubenzuron and teflubenzuron have been previously used in Norway in the late 1990´s with a limited and temporary approval. A voluntary ban on their use began at the end of the 1990´s due to suspected adverse environmental effects. In recent years they have been in use again due to the resistance of sea lice to treatment with emamectin benzoate. The Norwegian Fish Health and Environment company (Fiskehelse og miljø AS) reported that 81 kg of emamectin benzoate was used in 2008 and this fell to 41 kg in 2009 with a corresponding increase of benzoylurea usage from 0 kg in 2008 to 1839 kg and 1080 kg of diflubenzuron and teflubenzuron respectively in 2010. In 2010, over 500 fish farms applied for a license to use benzoylurea pesticides.

Sea lice treatments are required in aquaculture because salmonoids are typically vulnerable to parasitic infections, in particular in fish farms where water quality can be poor, stress factors can be high and space can be a premium. Sea lice are amongst the most common parasitic crustacean in salmon fish farming and require treatment for the health of the fish and the economy of the fish farm as they browse on the skin of fish and cause lesions. The lesions cause additional stress to the fish and leave them vulnerable to further infection. Historically, organophosphate pesticides such as dichlorvos, trichlorphon and azamethiphos, avermectins such as emamectin benzoate, and pyrethroids have been used to control sea lice infections. However in recent years the use of these treatments has reduced due to difficult application processes or a build up of resistance by sea lice to these chemicals. This has resulted in a return to the use of benzoylureas such as diflubenzuron and teflubenzuron.

Diflubenzuron and teflubenzuron are chitin synthesis inhibitors. The exact biochemical mechanisms that affects chitin synthesis is still unclear but it is thought that benzoylureas act by inhibiting chitin synthetase during molting in immature stages of insect development (Verloop and Ferrell, 1977). Some studies have suggested that benzoylureas may not directly target chitin synthetase inhibitors, but they may be serine protease inhibitors that then block the conversion of chitin synthetase zymogen into the active enzyme (Leighton *et al.*, 1981). Whatever the mechanisms of action, they act by stopping the organism from casting its exuvium during the molting process resulting in death. Treatment is required in the early stages of sea lice infestation as the inhibition of chitin synthesis is more effective at the larval stage where molting is more frequent, and has less effect on adult lice that have already formed an exoskeleton. If adult sea lice are present during treatment, the adult lice will survive and breed meaning further treatment is required.

3.2 Benzoylurea pesticides as environmental contaminants

Due to their very specific mode of action, teflubenzuron and diflubenzuron are relatively non-toxic to fish and algae, but by their nature are likely to have adverse effects on many non-target insects and crustacean where chitin synthesis is an important part of their growth. Their hydrophobic nature means they are likely to bind to the suspended particulate matter or to the sediment phase. Nakagawa reported increasing toxicity to *Chilo suppressalis* larvae with

increasing Log K_{ow} and clear relationships with the molecular positions of the functional groups (Nakagawa *et al.*, 1991).
Transformation of diflubenzuron in water is pH and temperature dependant with higher rates observed at higher temperatures and pH (Ivie *et al.*, 1980; Marsella *et al.*, 2000), while photolysis is a more important mechanism of degradation than hydrolysis (Marsella *et al.*, 2000). Organic carbon content has also been shown to be of importance when considering the aquatic fate of benzoylurea pesticides (Carringer *et al.*, 1975).

It is estimated that approximately 10% of the dose of benzoylurea pesticides administered in feed is absorbed into the fish and that the remaining 90% is rapidly excreted in faeces. Uneaten food is another point source of the chemicals to the surrounding environment.

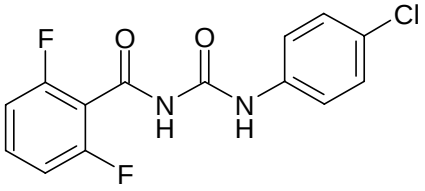
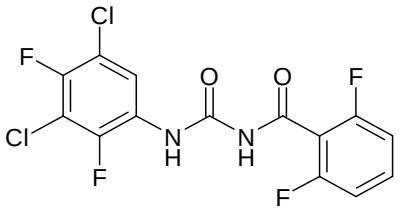
Much of the research reported to date is based on runoff to aquatic environments following spray applications on land to control pests such as mosquitoes and gypsy moth. This research has focused on freshwater and estuarine systems with little data available for the marine environment.

3.3 Physico-chemical properties of diflubenzuron and teflubenzuron

3.3.1 Diflubenzuron (CAS 35367-38-5)

Diflubenzuron is poorly water soluble (89 µg/L) and relatively hydrophobic with a Log K_{ow} 3.8 (Marsella *et al.*, 2000) (table 1). Diflubenzuron is persistent in the terrestrial environment and acts as a stomach poison as well as inhibiting chitin synthesis (Coppen and Jepson, 1996; Coppen and Jepson, 1996).

Table 1. Physico-chemical properties of diflubenzuron and teflubenzuron.

Active ingredient	**Diflubenzuron**	**Teflubenzuron**
Commercial formulation	Releeze vet	Ektoban vet
		
CAS number	35367-38-5	83121-18-0
Name	1-(chlorophenyl)-3-(2,6-difluorobenzoyl) urea	1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea
Formula	$C_{14}H_9ClF_2N_2O_2$	$C_{14}H_6C_{12}F_4N_2O_2$
RMM	310.7 g/mol	381.11 g/mol
Water solubility[1]	89 µg/L	9.4 µg/L
Log K_{ow} [1]	3.83	5.39
Half life [1] (pH9 deionised water)	8 (±2) days	7.6 (±0.9) days

[1] (Marsella *et al.*, 2000)

3.3.2 Teflubenzuron (CAS 83121-18-0)

Teflubenzuron is more potent and toxicologically active than diflubenzuron (Coppen and Jepson, 1996) and is less soluble in water (9.4 µg/L) and significantly more hydrophobic with a Log K_{ow} of 5.4 (Marsella *et al.*, 2000) (table 1). The reduced water solubility and increased hydrophobicity can be explained by the larger molecular size and presence of additional halogenated functional groups compared to diflubenzuron.

3.4 Environmental fate and effects

3.4.1 Diflubenzuron

3.4.1.1 Ecotoxicological effects

See table 2 for ecotoxicology data summary. The lethal dose for 50% of the exposed population (LD_{50}) for diflubenzuron and larvae of terrestrial insect *Chrysoperla carnea* was 2.26 ng/insect (Medina *et al.*, 2003) but was much higher for desert Locust (*Schistocerca gregaria*) nymph at 68.0 µg/insect (Coppen and Jepson, 1996). Most insects died after the first molt following treatment. In *haematobia irritans* fly larvae, the LC_{50} and LC_{90} were 25.5 µg/L and 34.6 µg/L respectively (Silva and Mendes, 2002) and the third molt stage was more sensitive than the previous two.

Diflubenzuron is metabolized faster, excreted faster and has a longer penetration time than teflubenzuron in the terrestrial insect larvae *Spodoptera littoralis* (El Saidy *et al.*, 1989). Diflubenzuron is metabolized to 4-chloroaniline which demonstrates a greater toxicity to fish than the parent compound (Fisher and Hall, 1992).

In a laboratory freshwater stream community, insect species were negatively affected at concentrations of diflubenzuron of 1 µg/L and greater (Hansen and Garton, 1982). Different insect species exhibited effects at different concentration. Algae and flora were also affected as a result of changing community structure although to a lesser degree and on a temporary basis. Bacteria were not affected.

A study of the acute effects of diflubenzuron on the freshwater fish, *Prochilodus lineatus*, showed adverse effects at 25 mg/L after 96 hours exposure (Maduenho and Martinez, 2008). Diflubenzuron exposure reduced the number of erythrocytes and hemoglobin content. Hepatic alterations were also induced which may impair normal liver function.
The 96 hr LC_{50} of diflubenzuron in the neotropical fish, *Prochilodus lineatus* was greater than 50 mg/L (Fischer and Hall, 1992). However, fish can accumulate diflubenzuron up to 160 times (Eisler, 1992) meaning the LC_{50} value may be significant during periods of treatment, however the study by Fischer and Hall (1992) demonstrated that there is a rapid elimination of diflubenzuron (7 days) after exposure had ceased.

Copepod (*Acartia tonsa*) hatching viability was <50% after 12 hr exposure to 1 µg/L of diflubenzuron and <5% after 24 hours exposure to 10 µg/L. Those that did hatch were abnormally shaped and failed to molt at the next stage of development (Tester and Costlow, 1981). Neither adult survival nor fecundity was affected at either concentration.

Table 2. Summary of ecotoxicology data.

Test	Results	Reference
Terrestrial insects		
Chrysoperla carnea larvae	LD_{50} 2.26 ng/insect	(Medina *et al.*, 2003)
Schistocerca gregaria nymph	LD_{50} 68.0 µg/insect	(Coppen and Jepson, 1996)
haematobia irritans larvae	LC_{50} 25.5 µg/L	(Silva and Mendes, 2002)
Cricotopus sp.	LC_{50} 1.6 µg/L	(Nebeker *et al.*, 1983)
Tanytarsus dissimilis	LC_{50} 1.02 µg/L	(Hansen and Garton, 1982)
	LC_{50} 4.9 µg/L	(Nebeker *et al.*, 1983)
Fish		
Cutthroat Trout	96 hr LC_{50} >50 mg/L	(Mayer and Ellerssieck, 1986)
Atlantic salmon fingerling	96 hr LC_{50} >50 mg/L	(Mayer and Ellerssieck, 1986)
Aquatic crustacea		
Acartia tonsa	Hatching viability <50% at 1 µg/L Hatching viability <5% at 10 µg/L	(Tester and Costlow, 1981)
Eurytemora affinis	48 hr LC_{50} 2.2 µg/L	(Savitz *et al.*, 1994)
Hyallela azteca	96 hr LC_{50} 1.84 µg/L	(Farlow *et al.*, 1978)
Daphnia magna	48 hr LC_{50} 4.42-6.89 µg/L	(Hansen and Garton, 1982)
Daphnia magna	48 hr LC_{50} 2 µg/L	(Nebeker *et al.*, 1983)
Daphnia magna	48 hr EC_{50} 0.5-1 µg/L	(Koyangi *et al.*, 1998)
Gammarus pseudolimnaes mature	24 hr LC_{50} 87 µg/L	(Mayer and Ellerssieck, 1986)
Clistoronia magnifica	30 day LC_{50} 0.1 µg/L	(Nebeker *et al.*, 1983)
Mysidopsis bahia juvenile	96 hr LC_{50} 2.06 µg/L	(Nimmo *et al.*, 1979)
Palaemonetes pugio larvae	96 hr LC_{50} 1.44 µg/L	(Wilson and Costlow, 1987)

The 48 hr LC_{50} for diflubenzuron and the estuarine copepod *Eurytemora affinis* was 2.2 µg/L. Short term effects were observed at the developmental stage (abnormal morphology) after 5 days exposure to 0.78 µg/L (Savitz *et al.*, 1994). In freshwater systems the amphipod *Hyallela azteca* had 96 hr LC_{50} of 1.84 µg/L (Farlow *et al.*, 1978) compared to the more resistant mature *Skwala sp*. with a 96 hr LC_{50} of more than 100 000 µg/L (Mayer and Ellerssieck, 1986).

Studies of crab larvae have shown significant adverse effects when exposed to diflubenzuron (Christiansen *et al.*, 1978; Christiansen and Costlow, 1982). Larval development of *Rhithropanpeus harrisii* and *Sesarma reticulatum* were affected at 1 µg/L. No effects were observed on the epicuticle development but negative effects were observed in the endo and exocuticle development indicating that chitin synthesis is inhibited in crab species in the same way as copepods as is supported in another study using horseshoe crabs which shows increased death rates after molting (Weis and Ma, 1987). Another study using fiddler crabs, *Uca pugilator*, also investigated effects on molting (Cunningham and Myers, 1987). The no-effect concentration for molting was 20 µg/L, the no-effect concentration for survival was 2 µg/L and the no-effect concentration for the ability to escape was 0.2 µg/L. Diflubenzuron has also been observed to reduce limb regeneration in fiddler crabs at concentrations as low as

5 µg/L (Weis *et al.*, 1987). Concentrations greater than 0.5 µg/L also affected the burrowing behaviour of fiddler crabs (Weis *et al.*, 1987) and less burrows containing fewer crabs were observed.

3.4.1.2 Environmental fate

Diflubenzuron has shown relatively slow degradation in brackish estuarine waters (Christiansen and Costlow, 1980). Water temperature and pH significantly affect the persistence of diflubenzuron. Degradation is more rapid at higher temperatures and neutral pH compared to lower temperature and a higher pH (Cunningham, 1986).

In marine waters, sediment acts as a sink and rapidly removes diflubenzuron from the water column (Cunningham and Myers, 1987) although it is likely that elevated sediment concentrations have a negative impact on sediment dwelling species. Maximum concentrations of 5.4 µg/g have been previously measured under a Norwegian fish farm and dispersed only 20 m from the fish cage. Less than 0.01 µg/g could be detected 15 months after treatment (Selvik *et al.*, 2002).

Diflubenzuron is highly toxic to target species but did not appear to bioaccumulate and was quickly eliminated from target insect species (Coppen and Jepson, 1996). A 67% elimination rate was observed after 12 hours in *Spodoptera exigua* and the remaining diflubenzuron was hydrolysed to 4-chlorophenylurea and 4-chloroaniline (Van Laecke and Degheele, 1991).

3.4.2 Teflubenzuron

3.4.2.1Ecotoxicological effects

There are very little data available on the ecotoxicological effects of teflubenzuron, particularly for aquatic species. LD_{50} for teflubenzuron and Desert Locust (*Schistocerca gregaria*) nymph was 0.71 µg/insect (Coppen and Jepson, 1996). Most insects died during the first molt. An LC_{50} of 56 µg/kg was observed for teflubenzuron in the *Callosobruchus maculates* terrestrial weevil (Abo-Elghar *et al.*, 2004) and of 7.3 mg/L in the fifth instar larvae *Spodoptera exigua,* another terrestrial species (Abo-Elghar *et al.*, 2004). A 48 hr EC_{50} of 1.2 µg/L for *Daphnia magna* immobilization was reported by (Koyangi *et al.*, 1998) and a NOEC concentration of 36 µg/L for 2 freshwater snails, *Juga plicifera* and *Physa sp*. In the UK, a sediment environmental quality standard (EQS) for teflubenzuron of 2 µg/kg has been set (Thomas, 2007).

3.4.2.1Environmental Fate

There are very little data available on the environmental fate of teflubenzuron but sediments are a likely sink due to the high Log K_{ow} value. Studies in Scottish lochs where teflubenzuron was in use measured it in sediment 1000 m from the fish cages but by 645 days after treatment, 98% had been degraded (SAMS, 2005). A half life of 115 days has been calculated (SEPA, 1999).

Teflubenzuron is highly toxic to target species but does not bioaccumulate and is quickly eliminated from target insect species (Coppen and Jepson, 1996). A 67% elimination rate was observed after 12 hours in *Spodoptera exigua* caused by mainly hydrolysis and conjugation processes (Van Laecke and Degheele, 1991).

The fate of teflubenzuron in the aquatic environment is largely dependant on the organic carbon and particulate load of the water phase as it remains bound to the organic carbon and particulates. If treatment occurs during an algal bloom, the biomass may be exposed to the

teflubenzuron and as the biomass sinks to the bottom after a bloom, the benthos dwelling organisms will be exposed to elevated concentrations (SAMS, 2005).

4. Materials and Methods

4.1 Description of sampling sites

Samples were collected from three fish farm locations and a reference location (figure 1) to address the potential release and accumulation of veterinary pharmaceuticals in the marine environment. The three fish farms included in the screening were selected by Klif in collaboration with Mattilsynet.

1. Fish farm 1 in Nord-Trøndelag county. This farm reported treating the fish with 40 tons of Ektoban (80 kg teflubenzuron) from 23rd to 25th of September and then treated with 220 tons of Releeze (132 kg diflubenzuron) from 25th of September to 6th of October 2010.

2. Fish farm 2 in Nord-Trøndelag county. There are two fish farms on this location. Both fish farms reported treating with Ektoban (teflubenzuron) in 2009 and one of the fish farms reported treating with 112.5 tons Ektoban (225 kg teflubenzuron) in spring 2010. Only one of the fish farms was in use in August 2010 at the time of sampling.

3. Fish farm 3 in Hordaland county. This fish farm reported using 15 tons of Releeze (9 kg diflubenzuron) from 27th of October to 10th of November 2010.

4. The Oslofjord. There are no fish farms in the Oslofjord, this fjord was therefore chosen as reference location.

4.1.1 Fish farm 1

Samples of sediment, blue mussel and amphipods were collected on 26th and 27th of October 2010. The water and particulate samples were collected on the 4th of October during treatment with Releeze. Shrimp were collected by trawling on the 26th of October. Brown crab and cod were caught between the 25th and 29th of October. Some cod were also caught on the 5th of January 2011.

The water and particulate samples were collected from approximately 10 meters depth at five stations. Soft sediment was only found in a shallow area south of the fish farm. It is very deep under the fish farm cages, and the bottom consisted of gravel and rocks. Blue mussels were collected from four buoys around the fish farm, and from a rope on a landing stage south of the fish farm. Amphipods were collected from three stations near the fish farm. Shrimp was collected by trawling, once from west to east and once from north to south in the fjord where the fish farm is located.

4.1.2 Fish farm 2

Samples of particulate, sediment, blue mussel and amphipods were collected on the 11th and 12th of August 2010. Cod and brown crab were caught between the 25th and 30th of September 2010. Shrimp was trawled on the 23rd of September 2010.

Particulate samples were collected at five stations and were taken near the sea bed. Sediment was collected in a transect near both fish farms at this location. Blue mussels were collected from five stations. Four of the blue mussel samples were collected from the fish net cages and

one sample was collected from a floating stage south of the fish farm. Amphipods were sampled from three stations south and south-east of the fish farm. Shrimp was collected by trawling in the fjord where the fish farm is located. Brown crab and cod were caught by using crab pots and gill nets near the fish farm.

4.1.3 Fish farm 3

Water and particulate samples were collected on the 9th of November, during treatment with Releeze. Samples of sediment, blue mussel and amphipods were collected on the 23rd of November. Shrimp were caught by trawling on 26th of November.

Water and particulate samples were collected from approximately 10 meters depth. Sediment samples were collected in a gradient from 40 to 100 meters depth. Blue mussel was sampled from three stations on the fish farm, and from two stations north of the fish farm. Amphipods were sampled from three locations near the fish farm. Shrimp was collected by trawling in the fjord where the fish farm is located. Brown crab and cod were caught by crab pots and gill nets near the fish farm.

4.1.4 The Oslofjord – reference area

Samples of particulate, sediment, blue mussel and amphipods were collected on the 20th of August 2010. Amphipods were also collected on the 11th of October. Shrimp were collected by trawling on the 20th of August, and cod were caught by trawling on the 20th of August and the 15th of November 2010. Brown crab were caught on the 28th of September 2011.

Samples of particulate, sediment, blue mussel and amphipods were collected from the mid part of Oslofjord. Shrimp were trawled in the mid part of Oslofjord. Cod were caught by trawling from the mid- and the Inner Oslofjord. Brown crab was caught by crab pots in Outer Oslofjord.

Figure 1. Map of the sampling sites. Map reference: Norwegian Mapping Authority (cc-by-sa-3.0).

At each location the following samples and species were collected (summarised in tables 3 and 4):

- Water for particulates
- Sediment
- Blue mussel (*Mytilus edulis*)
- Amphipods (*Gammarus locusta*)
- Shrimp (*Pandalus borealis*)
- Brown crab (*Cancer pagurus*)
- Cod (*Gadus morhua*)

Table 3. Overview of sample location, matrix and count (n).

Area	Matrix	Number of samples analysed	Sampling date
Fish farm 1	Water & particulates	5	4.10.2010
	Sediment	5	26.10.2010
	Blue mussel (5x30)	5	26.10.2010
	Amphipods	3	26.-27.10.2010
	Shrimp (2 kg)	3	26.10.2010
	Brown crab (n=20)	4	25.-29.10.2010
	Cod - filet (n=11)	11	25.-29.10.2010, 5.1.2011
	- liver (n=11)	11	
	- skin (n=11)	11	
Fish farm 2	Water for particulates	5	11.8.2010
	Sediment	5	11.8.2010
	Blue mussel (5x30)	5	11.8.2010
	Amphipods	3	11.-12.8.2010
	Shrimp (2 kg)	3	23.9.2010
	Brown crab (n=20)	4	25.-30.9.2010
	Cod - filet (n=11)	11	25.-30.9.2010
	- liver (n=11)	11	
	- skin (n=11)	11	
Fish farm 3	Water & particulates	5	9.11.2010
	Sediment	5	23.11.2010
	Blue mussel (5x30)	5	23.11.2010
	Amphipods	3	23.11.2010
	Shrimp (2 kg)	3	26.11.2010
	Brown crab (n=20)	4	22.-28.11.2010
	Cod - filet (n=5)	5	22.-28.11.2010
	- liver (n=5)	5	
	- skin (n=5)	5	
Oslofjord (reference area)	Water for particulates	5	20.8.2010
	Sediment	5	20.8.2010
	Blue mussel (5x30)	5	20.8.2010
	Amphipods	3	20.8.2010, 11.10.2010
	Shrimp (2 kg)	3	20.8.2010
	Brown crab (n=20)	4	28.9.2010
	Cod - filet (n=15)	15	20.8.2010, 15.11.2010
	- liver (n=15)	15	
	- skin (n=15)	15	

Table 4. Approximate distance between the fish farms and sampling stations

	Distance from Fish farm 1 (meters)	Distance from Fish farm 2 (meters)	Distance from Fish farm 3 (meters)
Water/Suspended Particulate 1	0	100	0
Water/Suspended Particulate 2	200	0	0
Water/Suspended Particulate 3	300	0	200
Water/Suspended Particulate 4	700	100	700
Water/Suspended Particulate 5	900	0	1000
Sediment sample 1	500	0	600
Sediment sample 2	200	0	300
Sediment sample 3	470	0	0
Sediment sample 4	450	0	300
Sediment sample 5	400	200	900
Blue mussel sample 1	0	380	0
Blue mussel sample 2	0	0	0
Blue mussel sample 3	0	0	0
Blue mussel sample 4	0	0	2300
Blue mussel sample 5	600	0	3000
Amphipod sample 1	780	100	2400
Amphipod sample 2	630	800	1300
Amphipod sample 3	660	900	1000
Brown crab	100-300	100-300	100-300
Cod	100-300	100-300	100-300
Shrimp	1000-3000	2000-5000	1000-5000

4.2 Sample collection

Samples of biota and sediment were put in clean, baked (500° C) glass jars. The water samples were put in clean, baked (500° C) bottles (2.5 liters).

4.2.1 Water and particulates

Water samples from location fish farm 1 and fish farm 3 were collected by the staff at the fish farms during treatment with Ektoban and Releeze for the analysis of water and particulates. The water samples from fish farm 2 and Oslofjord were collected by NIVA for the analysis of particulates. The samples were collected by using a Ruttner water sampler or a Nisikin water sampler (figure 2). The samples collected from fish farm 2 were collected from near the bottom, since it was several months since the treatment with Ektoban. The water samples at the other locations were taken from approximately 10 meters depth.

Figure 2. A Niskin water sampler was used to collect the water samples for particulate analysis (photo: Merete Schøyen).

4.2.2 Sediment

Sediment samples were collected from five stations at each location by using a van Veen grab (figure 3).The sediment sample was collected from the upper 2 cm of sediment of three parallel grabs.

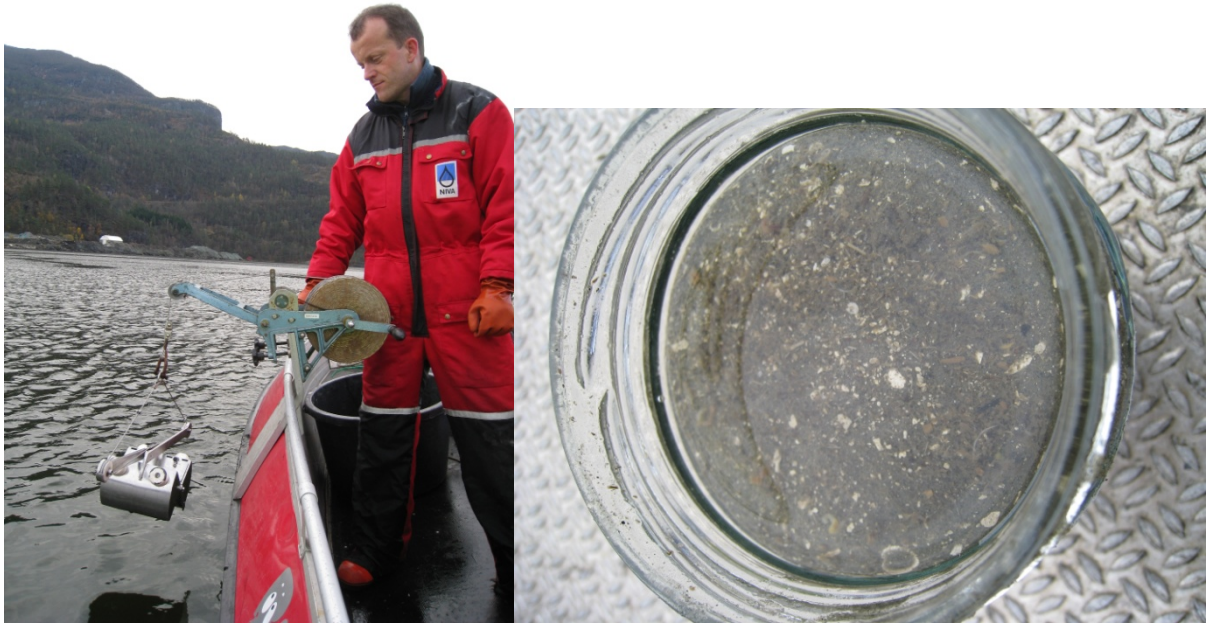


Figure 3. Sediment samples were collected by a van Veen grab (photos: Merete Schøyen).

4.2.3 Blue mussel

Blue mussels were collected from five stations at each location. At least 40 blue mussels (3-5 cm) were collected from each station (figure 4).

Figure 4. Sampling of blue mussels from location Fish farm 2 (photos: Merete Schøyen).

Blue mussels were frozen (-20° C) upon arrival at NIVA from the field. The soft tissue of 30 blue mussels were mixed into one bulk sample from each station (figure 5).

Figure 5. Thirty blue mussels were mixed into one sample (photos: Sigurd Øxnevad).

4.2.4 Amphipods

Amphipods were found under stones and gravel at low tide, and were picked by hand using a pair of tweezers. Approximately 400-500 amphipods were picket on each station. At a few stations only 50-100 individuals were found. The amphipods were put directly into the sample containers (figure 6).

Figure 6. Amphipods were sampled at low tide. The picture to the right shows one amphipod sample (photos: Sigurd Øxnevad).

4.2.5 Shrimp

Shrimp were caught by trawling by local fishermen, then frozen and sent to NIVA for analysis (figure 7). The shrimps were peeled and split into three parallel bulk samples from each location.

Figure 7. Shrimps ready for sampling (photo: Sigurd Øxnevad).

4.2.6 Cod

Cod were caught by trawling or using gill nets by local fishermen and then sent to NIVA for analysis (figure 8). Cod from the Inner Oslofjord, from the same station as for the Coordinated Environmental Monitoring Programme (CEMP station 30B, Green et al 2010) were collected by NIVA by trawling from the research vessel F/F Trygve Braarud (University of Oslo). The cod varied in size from 31 to 75 cm and 281 to 4200 grams (table 5). The cod

were sampled individually for fillet, liver and skin. Approximately 100 gram fillet, the whole liver and skin from both sides of the cod were sampled.

Table 5. Length and weight of cod from the four locations, with weight of samples.

Location	Length (cm)	Weight (g)	Fillet (g)	Liver (g)	Skin (g)
Fish farm 1	49 - 75	1322 - 4200	90 - 127	42.7 – 167.1	17.2 – 89.5
Fish farm 2	31 - 54	281 - 1394	71 - 128	2.2 – 25.9	12.4 – 38.0
Fish farm 3	49 - 60	1468 - 2250	100 - 118	90.2 – 138.9	32.4 – 86.7
Oslofjord	42 - 68	683 - 3294	82 - 111	5.7 – 85.5	17.2 – 68.6

Figure 8. Cod ready for dissection (photo: Sigurd Øxnevad).

4.2.7 Brown crab

Brown crab were caught by local fishermen in crab pots, frozen and sent to NIVA for analysis. Approximately 20 grams of soft tissue (white and brown tissue including hepatopancreas) of five crabs were mixed into one bulk sample (table 6 and figure 9). This resulted in four samples from each location.

Figure 9. Soft tissue of five brown crabs was mixed into one sample (photos: Sigurd Øxnevad).

Table 6. Size of the crabs from the four locations.

Location	Crab shell length (cm)
Fish farm 1	16.2 – 18.8
Fish farm 2	12.5 – 19.8
Fish farm 3	13.0 – 18.5
Oslofjord	13.7 – 19.7

4.3 Chemical analysis

Each sample matrix was extracted using a separate optimized method and the instrumental method of analysis was the same for all matrices.

4.3.1 Analyte determination

All samples were analysed by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) (UPLC-Quattro Premier XE, Micromass, Sweden).

Mass spectrometry parameters were optimsed by the standard procedure of direct injection of a 1 µg/ml standard solution made up in solvent. The mass transitions are shown in table 5. The source was operated at 100 ^{o}C, the desolvation temperature was 450 ^{o}C, the cone gas flow was 55 L/hr and the desolvation gas flow was 800 L/hr. Two mass transitions were used for diflubenzuron and diflubenzuron-d_4 internal standard, but only one transition was observable for teflubenzuron (table 7 and figure 10).

Table 7. Mass transitions and mass spectrometer conditions of targeted compounds.

Compound	Parent ion (m/z)	Daughter ion (m/z)	Cone voltage (V)	Collision energy (V)
Diflubenzuron d4	313.2	155.8	15	10
	313.2	293.1	15	10
Diflubenzuron	309.1	155.8	15	10
	309.1	289.1	15	10
Teflubenzuron	379.1	339.1	15	10

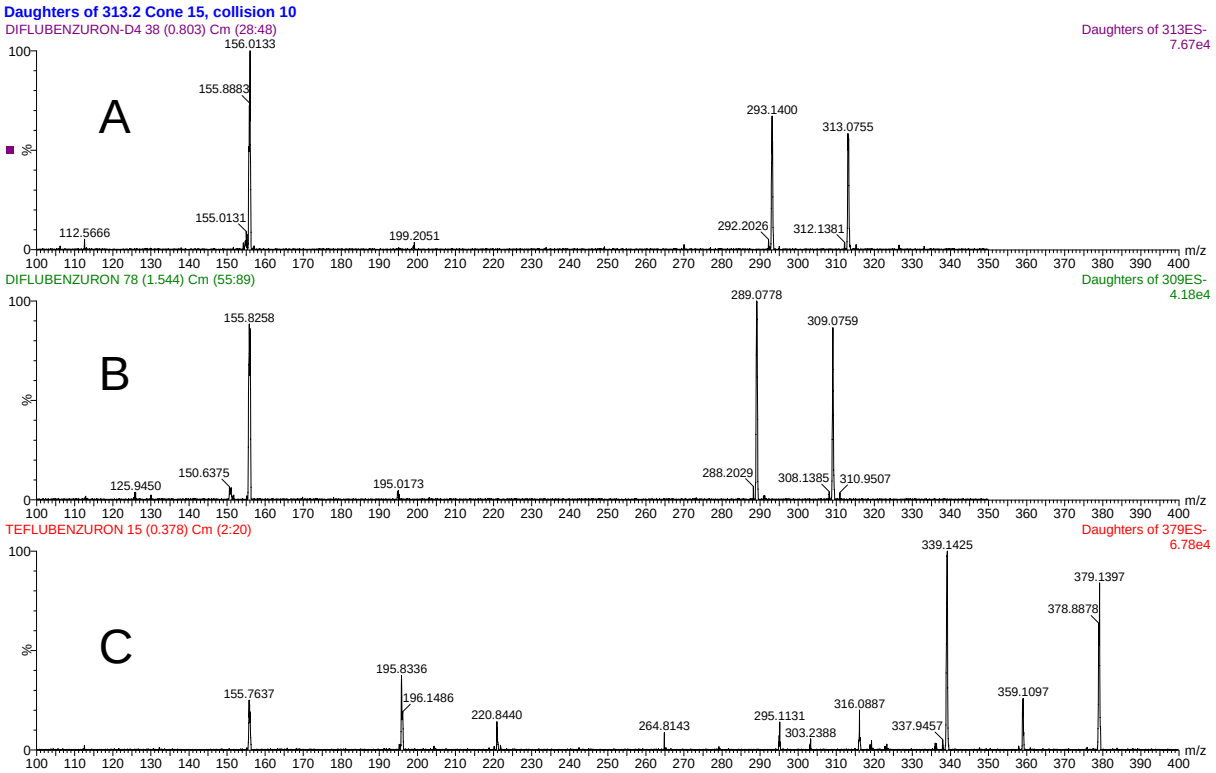


Figure 10. Mass spectrum of the MRM transitions of A, diflubenzuron-d_4; B, diflubenzuron and C, teflubenzuron.

Diflubenzuron, diflubenzuron-d_4 and teflubenzuron were separated using a C18 column (UPLC BEH 50 mm x 2.0 mm x 1.7 µm) (figure 11). The compounds were separated using a gradient elution program with acetonitrile and water as the mobile phases at a flow rate of 0.6 ml/min. The elution program is shown in table 8.

Table 8. HPLC gradient elution program.

Time (mins)	Water (%)	Acetonitrile (%)
Initial	65	35
0.2	65	35
2.0	30	70
2.1	1	99
3.1	1	99
3.1	65	35
4.2	65	35

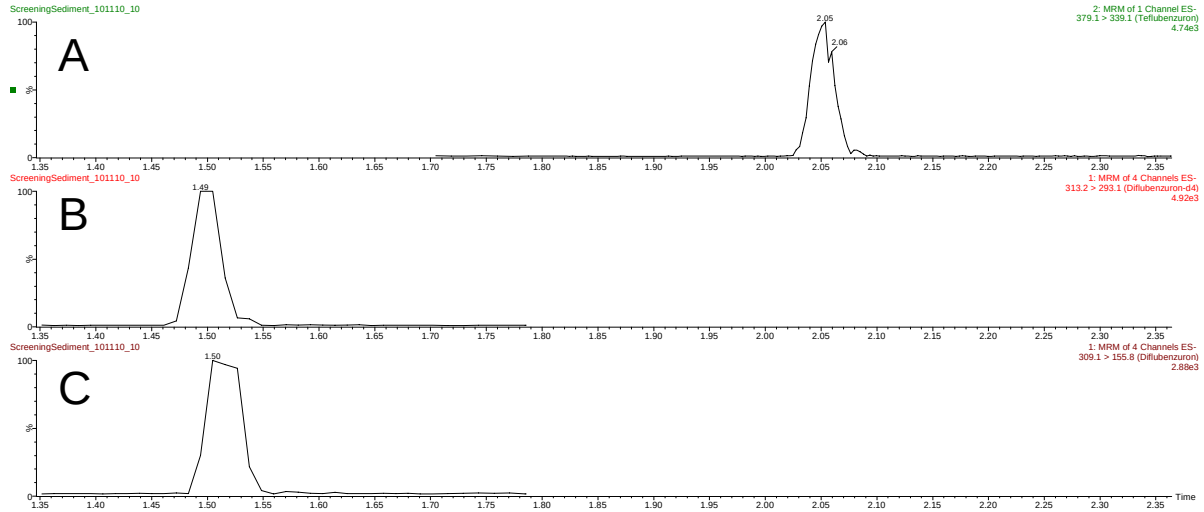


Figure 11. Chromatogram showing benzoylurea separation. A, teflubenzuron; B, diflubenzueron-d_4 and C, diflubenzuron.

4.3.2 Extraction Methods

4.3.2.1 Particulates

Water samples 1-2.5 L were filtered through dried pre-weighed GFC filters (Whatman). Filters were oven dried (60 ^{o}C) overnight and re-weighed. 100 ng diflubenzuron-d_4 was added as internal standard after transferring the filter papers to PET tubes.

5 ml acetone/methanol (50/50) was added and each sample was placed on a mechanical shaker for 30 minutes. The samples were centrifuged at 1400 g for 5 minutes and the extract removed by pipette into a clean tube. The extraction process was repeated and the solvent extracts combined. The solvent was evaporated under nitrogen to approximately 500 µl and 500 µl ultrapure water was added before transferring the extract to a 1 ml vial in preparation for analysis.

4.3.2.2 Water

Water samples 1-2.5 L were filtered through dried pre-weighed GFC filters (Whatman).

The samples were extracted by solid phase extraction (SPE) using Oasis HLB (Waters, Sweden). The SPE cartridges were conditioned with 6 ml methanol followed by 6 ml water. The samples were then applied to the SPE cartridge under vacuum at a rate of approximately 4 ml/min. After sample application, the cartridges were allowed to dry under vacuum for approximately 30 minutes to remove any excess water. Each cartridge was rinsed with 6 ml 2% methanol in ultrapure water and the eluant discarded. The benzoylureas were eluted with 12 ml methanol and this eluant was evaporated under nitrogen to approximately 500 µl and 500 µl ultrapure water was added before transferring the extract to a 1 ml vial in preparation for analysis.

4.3.2.3 Sediment

100 ng diflubenzuron-d_4 was added as internal standard to approximately 1 g freeze dried sediment sample in a PET tube. 5 ml acetone/methanol (50/50) was added and each sample was placed on a mechanical shaker for 30 minutes. The samples were centrifuged at 1400 g for 10 minutes and the extract removed by pipette into a clean tube. The extraction process

was repeated and the solvent extracts combined. The solvent was evaporated under nitrogen to approximately 1 ml and then diluted to approximately 50 ml with ultrapure water.

The extracts were cleaned up by solid phase extraction (SPE) using Oasis HLB (Waters, Sweden). The SPE cartridges were conditioned with 6 ml methanol followed by 6 ml water. The samples were then applied to the SPE cartridge under vacuum at a rate of approximately 4 ml/min. After sample application, the cartridges were allowed to dry under vacuum for approximately 30 minutes to remove any excess water. Each cartridge was rinsed with 6 ml 2% methanol in ultrapure water and the eluant discarded. The benzoylureas were eluted with 12 ml methanol and this eluant was evaporated under nitrogen to approximately 500 µl and 500 µl ultrapure water was added before transferring the extract to a 1 ml vial in preparation for analysis.

4.3.2.4 Biota

Cod, blue mussel, shrimp and crab samples were homogenized. 100 ng diflubenzuron-d_4 was added as internal standard to each sample aliquot (approximately 5 g blue mussel; 10 g shrimp, 10 g crab meat; 2 g cod liver; 5 g cod skin and 10 g cod filet). 10 ml acetone/methanol (90/10) was added and each sample was placed on a mechanical shaker for 30 minutes. The samples were centrifuged at 1400 g and the solvent extract removed by pipette into a clean tube. The extraction process was repeated and the solvent extracts combined. The extracts were evaporated to ensure all of the acetone was removed.

Fat was removed by the addition of 2 ml heptane. The extracts were briefly shaken and then centrifuged at 500 g for 5 minutes. The heptane layer was removed and discarded and the process was repeated. The remaining methanol layer was diluted into approximately 50 ml ultrapure water.

Cod filet, cod liver and shrimp extracts were cleaned up by SPE using Oasis HLB (Waters, Sweden). The SPE cartridges were conditioned with 6 ml methanol followed by 6 ml water. The samples were then applied to the SPE cartridge under vacuum at a rate of approximately 4 ml/min. After sample application, the cartridges were allowed to dry under vacuum for approximately 30 minutes to remove any excess water. Each cartridge was rinsed with 6 ml 2% methanol in ultrapure water and the eluant discarded. The benzoylureas were eluted with 12 ml methanol and this eluant was evaporated under nitrogen to approximately 500 µl and 500 µl ultrapure water was added before transferring the extract to a 1 ml vial in preparation for analysis.

Cod skin and crab extracts were cleaned up by SPE using Oasis MAX (Waters, Sweden). The SPE cartridges were conditioned with 6 ml methanol followed by 6 ml water. The samples were then applied to the SPE cartridge under vacuum at a rate of approximately 4 ml/min. After sample application, the cartridges were allowed to dry under vacuum for approximately 30 minutes to remove any excess water. The cartridges were rinsed with 6 ml 1% sodium acetate followed by 6 ml methanol and the benzoylureas were eluted with 12 ml 1% formic acid in methanol and this eluant was evaporated under nitrogen to approximately 500 µl and 500 µl ultrapure water was added before transferring the extract to a 1 ml vial in preparation for analysis.

4.3.2.5 Amphipod

100 ng diflubenzuron-d_4 was added as internal standard to approximately 1 g oven dried amphipod sample. 5 ml acetone/methanol (50/50) was added and each sample was placed on

a mechanical shaker for 30 minutes. The samples were centrifuged at 1400 g and the extract removed by pipette into a clean tube. The extraction process was repeated and the solvent extracts combined. The extracts were evaporated to ensure all of the acetone was removed.

Fat was removed by the addition of 2 ml heptane. The extracts were briefly shaken and then centrifuged at 500 g for 5 minutes. The heptane layer was removed and discarded and the process was repeated. The remaining methanol layer was diluted into approximately 50 ml ultrapure water.

The extracts were cleaned up by SPE using Oasis HLB. The SPE cartridges were conditioned with 6 ml methanol followed by 6 ml water. The samples were then applied to the SPE cartridge under vacuum at a rate of approximately 4 ml/min. After sample application, the cartridges were allowed to dry under vacuum for approximately 30 minutes to remove any excess water. Each cartridge was rinsed with 6 ml 2% methanol in ultrapure water and the eluant discarded. The benzoylureas were eluted with 12 ml methanol and this eluant was evaporated under nitrogen to approximately 500 µl and 500 µl ultrapure water was added before transferring the extract to a 1 ml vial in preparation for analysis.

4.3.2.6 Particle Size Analysis

Wet sediment is shaken by mechanical fractionater with < 63 µm sieves. Dry weight measurements are used for the particle size calculations.

4.3.2.7 Sediment TOC

Dried sediment sample aliquots (0.5-10 mg) are heated in a furnace at 1800 ^{o}C in the presence of oxygen free helium. The carbon dioxide gas produced is passed through a chromatography column and the total organic carbon is measured. The detection limit is 0.1%.

4.3.2.8 Cod liver lipid content

An aliquot of homogenised cod liver (approx 2 g) was weighed. 40 ml of cyclohexane/isopropanol (50/50) was added and the samples shaken for 2 hours. The samples were centrifuged at 2000 g for 10 minutes. The solvent phase was decanted into a clean tube and the extraction repeated with 30 ml of cyclohexane/isopropanol (50/50) and the extracts combined. 20 ml of 0.5% NaCl was added to the combined extracts and shaken before again centrifuging at 2000 g for 10 minutes. The cyclohexane layer was transferred to pre-weighed tubes and then evaporated under nitrogen. When the cyclohexane had been removed the tubes were heated at 60 ^{o}C to a constant weight (approx 24 hrs) and the lipid content calculated.

4.4 Method efficiencies and detection limits

The method efficiencies are shown in table 9. The method recovery data indicates the method efficiencies and the standard deviation in parentheses demonstrate the method uncertainty.

Table 9. Method efficiencies and detection limits.

Sample matrix	Method Recovery (%)		Detection Limits	
	Diflubenzuron	Teflubenzuron	Diflubenzuron	Teflubenzuron
Sediment	115±32	107±11	5 ng/g dw	1 ng/g dw
Particulate	77±18	65±15	1 ng/L	1 ng/L
Water	85±5	88±5	1 ng/L	1 ng/L
Amphipod	85±12	67±5	2 ng/g dw	0.5 ng/g dw
Brown Crab	102±15	58±11	1 ng/g dw	1 ng/g dw
Blue Mussel	80±9	93±7	7 ng/g ww	3 ng/g ww
Cod Liver	98±53	76±5	10 ng/g lipid	5 ng/g lipid
Cod Filet	117±11	105±40	5 ng/g ww	5 ng/g ww
Cod Skin	131±4	124±5	15 ng/g ww	20 ng/g ww

Where dw and ww represent dry weight and wet weight respectively.

5. Results

5.1 Diflubenzuron (table 10)

Particulate samples were collected at fish farm 1 during treatment with 132 kg diflubenzuron and the remaining samples were collected three weeks after diflubenzuron treatment finished. Fish farm 3 was treated with 9 kg diflubenzuron. Particulate samples were collected during treatment and all other sample matrices were collected two weeks after treatment.
Diflubenzuron was not detected in any of the cod samples collected (tables A8-A10). Diflubenzuron was measured in sediment and particulates at the 2 locations where salmon had been treated with Releeze, farm 1 and farm 3 (Figure 12 and 13; tables A1 and A3). The median particulate concentrations of 5.4 ng/L and 4.4 ng/L.

Diflubenzuron was also detected in shrimp, blue mussels and brown crab samples collected from fish farm 1 (figures 15, 16 and 17 respectively). 132 kg of diflubenzuron was administered here prior to sampling compared to only 9 kg at fish farm 3 where no diflubenzuron was measured in any samples. Fish farm 2 did not report using diflubenzuron and none was detected in the samples collected. Diflubenzuron was not detected at the reference location, which had no reported usage. 5.5 ng/g was measured in a single amphipod composite sample from farm 1 but it was not detected in any other amphipod samples from this location or the other three locations.

Table 10. Diflubenzuron occurrence summary.

Location	Farm 1		Farm 2		Farm 3		Reference	
Usage	**Diflubenzuron & teflubenzuron**		**Teflubenzuron**		**Diflubenzuron**		**No usage**	
	Range	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**
Sediment (ng/g) (dw)*	5.9-42.5	11.1	<5	-	0.7-136.6	0.7	<5	-
Particulate (ng/L)	1.1-15.2	5.5	<1	-	0.3-17.7	4.4	<1	-
Water (ng/L)	34.3-295.2	123.7	-	-	13.1-30.9	27.4	-	-
Shrimp (ng/g) (ww)*	<0.5-10	3.9	<0.5	-	<0.5	-	<0.5	-
Amphipod (ng/g) (dw)	<2-5.5	<2	<2	-	<2	-	<2	-
Crab (ng/g) (ww)	180.9-537.9	339.7	<1	-		-	<1	-
Blue mussel (ng/g) (ww)	2.7-22.0	8.5	<5	-	<7	-	<8	-
Cod filet (ng/g) (ww)	<5	-	<5	-	<5	-	<5	-
Cod liver (ng/g) (lipid wt)	<10	-	<10	-	<10	-	<10	-
Cod skin (ng/g) (ww)	<15	-	<15	-	<15	-	<15	-

*Where ww and dw represent wet weight and dry weight respectively. – represents not determined.

5.2 Teflubenzuron (table 11)

Fish farm 1 was treated with 80 kg of teflubenzuron 1 week prior to particulate sampling and 4 weeks prior to the sampling of all other matrices. Fish farm 2 was treated with 225 kg teflubenzuron and all samples were collected several months later.

Teflubenzuron was not detected in any cod samples collected from any of the 4 locations (tables A8-A10). It was detected in particulate samples (figure 13) at fish farm 2 with high usage (225 kg) and it was measured in sediment samples from this site with a median concentration of 65.2 ng/g (table 12, figure 12). It was also measured at fish farm 1 with a lower teflubenzuron application of only 80 kg. The median concentration here was 10.5 ng/g. Shrimp, blue mussel and brown crab samples from both locations where teflubenzuron was in use showed measurable concentrations (figures 15, 16 and 17 respectively). Median concentrations in shrimp were 9.6 ng/g and 0.4 ng/g for fish farms 1 and 2 respectively. Concentrations were higher in brown crab and 122.3 ng/g was the median concentration at fish farm 1 and 7.5 ng/g at fish farm 2. In blue mussels, the concentration range was from below the limit of detection for one sample point at each fish farm, up to 10.5 ng/g at fish farm 1 and 36 ng/g at fish farm 2. At fish farm 1, the sample point with <3 ng/g also showed the lowest concentration of diflubenzuron.

Sediment samples from the two locations chosen for the Klif screening in 2008 (prior to benzoylurea usage) showed no detectable concentrations of teflubenzuron (table A3).

Table 11. Teflubenzuron occurrence summary

Location	**Farm 1**		**Farm 2**		**Farm 3**		**Reference**	
Usage	**Diflubenzuron & teflubenzuron**		**Teflubenzuron**		**Diflubenzuron**		**No usage**	
	Range	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**
Sediment (ng/g) (dw)*	7.2-66.0	10.5	8.3-269.2	65.2	<1	-	<1	-
Particulate (ng/L)	<1	-	<1	-	<1	-	<1	-
Water (ng/L)	<1-12.9	6.0	-	-	<1	-	<1	-
Shrimp (ng/g) (ww)*	9.6-11-3	9.6	0.4	0.4	<0.2	-	<0.2	-
Amphipod (ng/g) (dw)	<0.5-3-5	<0.5	<0.5		<0.5	-	<0.5	-
Crab (ng/g) (ww)	43-185.7	122.3	2.7-20.9	7.5	<1	-	<1	-
Blue mussel (ng/g) (ww)	<3-10.5	3.4	<5-36	6.6	<5	-	<5	-
Cod filet (ng/g) (ww)	<5	-	<5	-	<5	-	<5	-
Cod liver (ng/g) (lipid wt)	<5	-	<5	-	<5	-	<5	-
Cod skin (ng/g) (ww)	<20	-	<20	-	<20	-	<20	-

*Where ww and dw represent wet weight and dry weight respectively. – represents not determined.

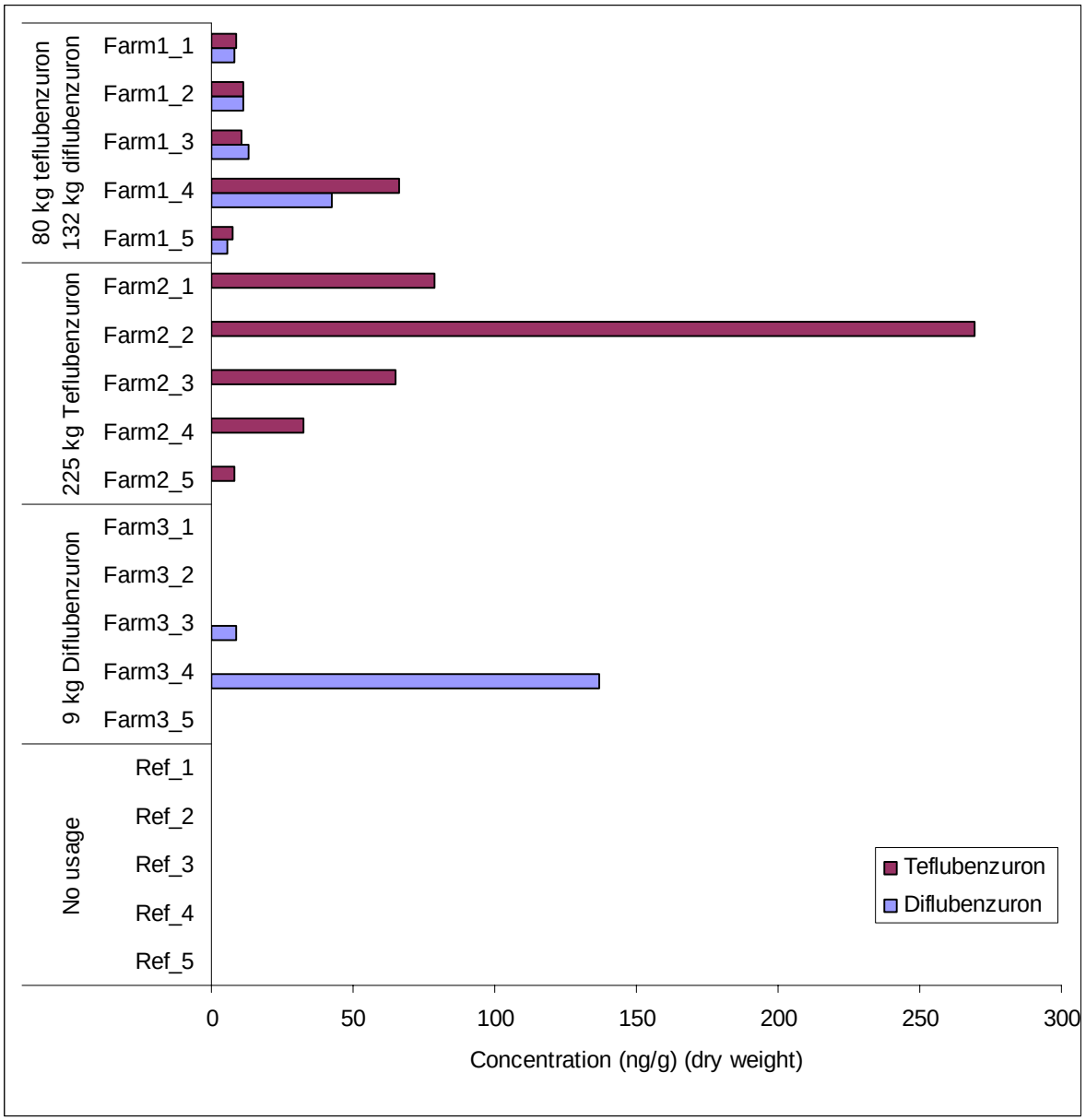


Figure 12. Diflubenzuron and teflubenzuron in sediment.

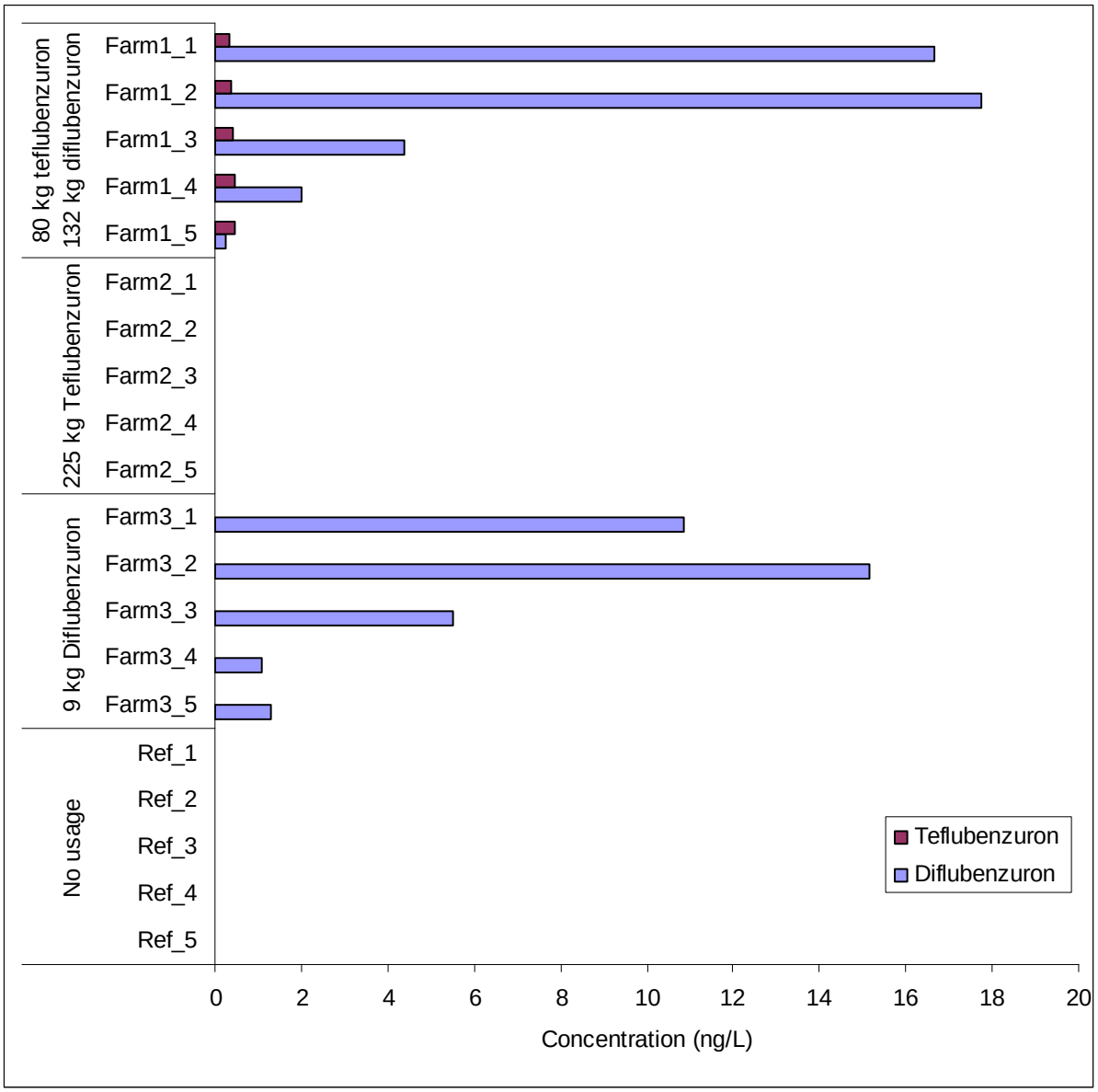


Figure 13. Diflubenzuron and teflubenzuron in particulates.

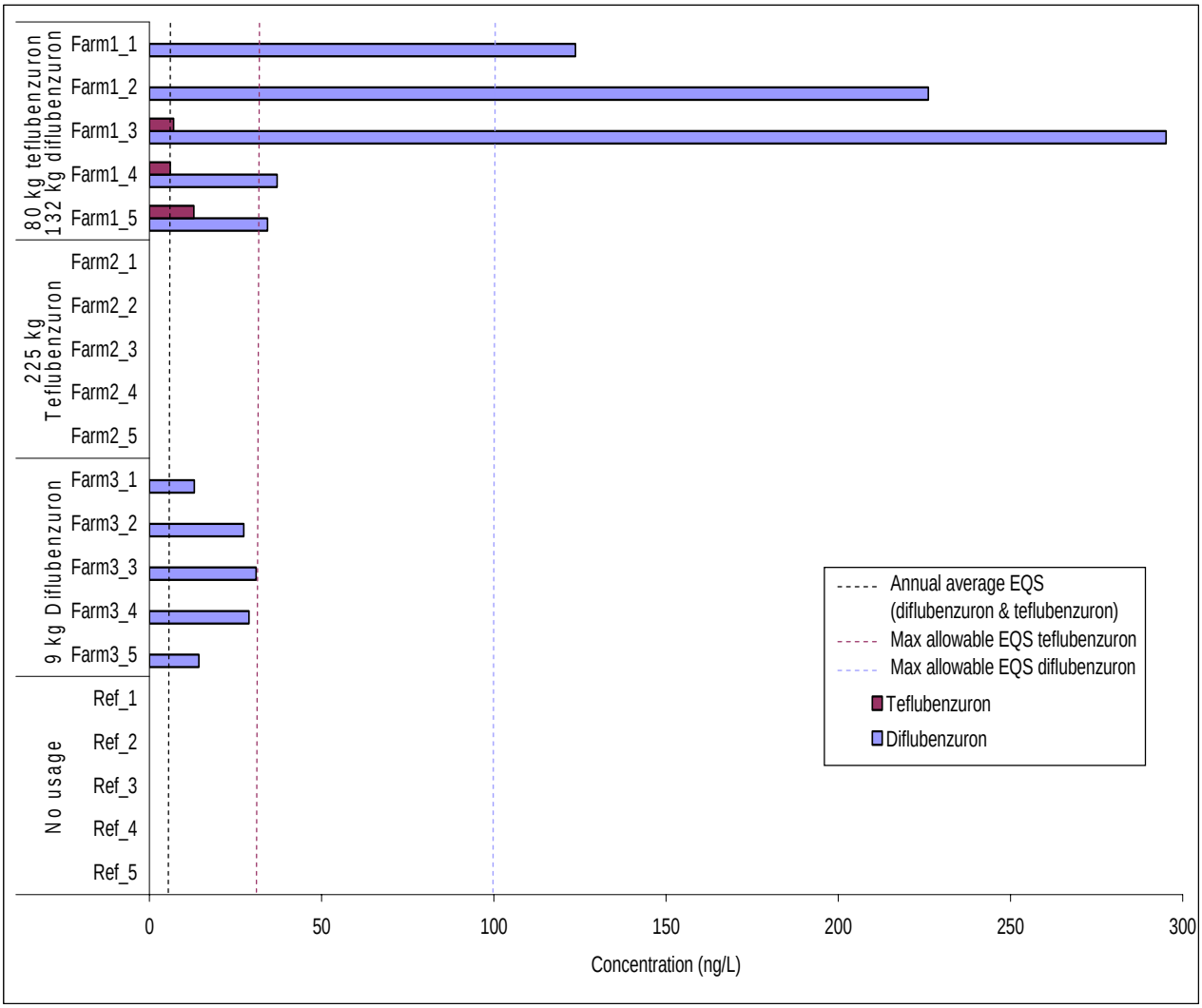


Figure 14. Diflubenzuron and teflubenzuron in water with the EQS (DoE, 1996; SEPA, 1998) values shown

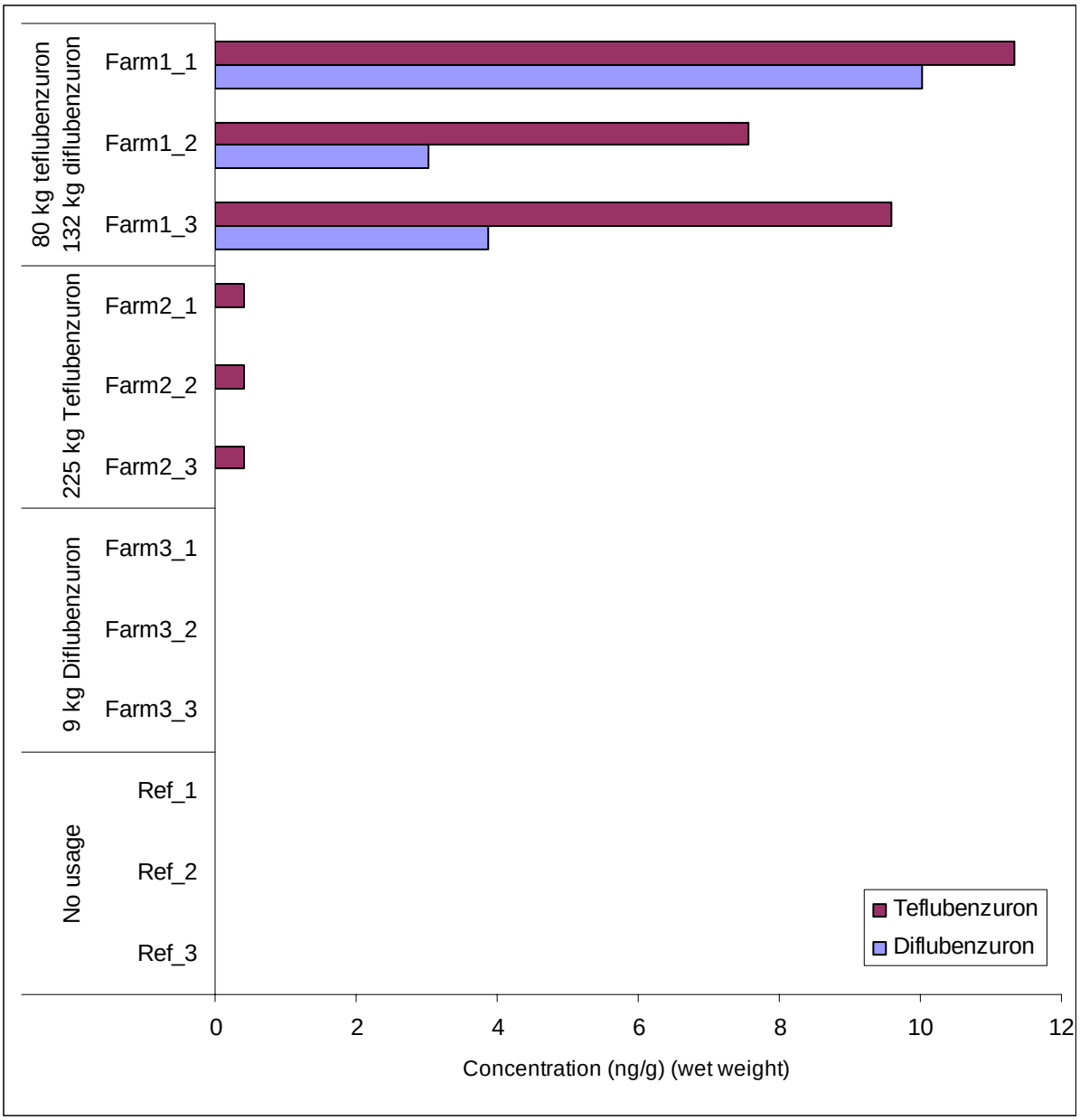


Figure 15. Diflubenzuron and teflubenzuron in shrimp.

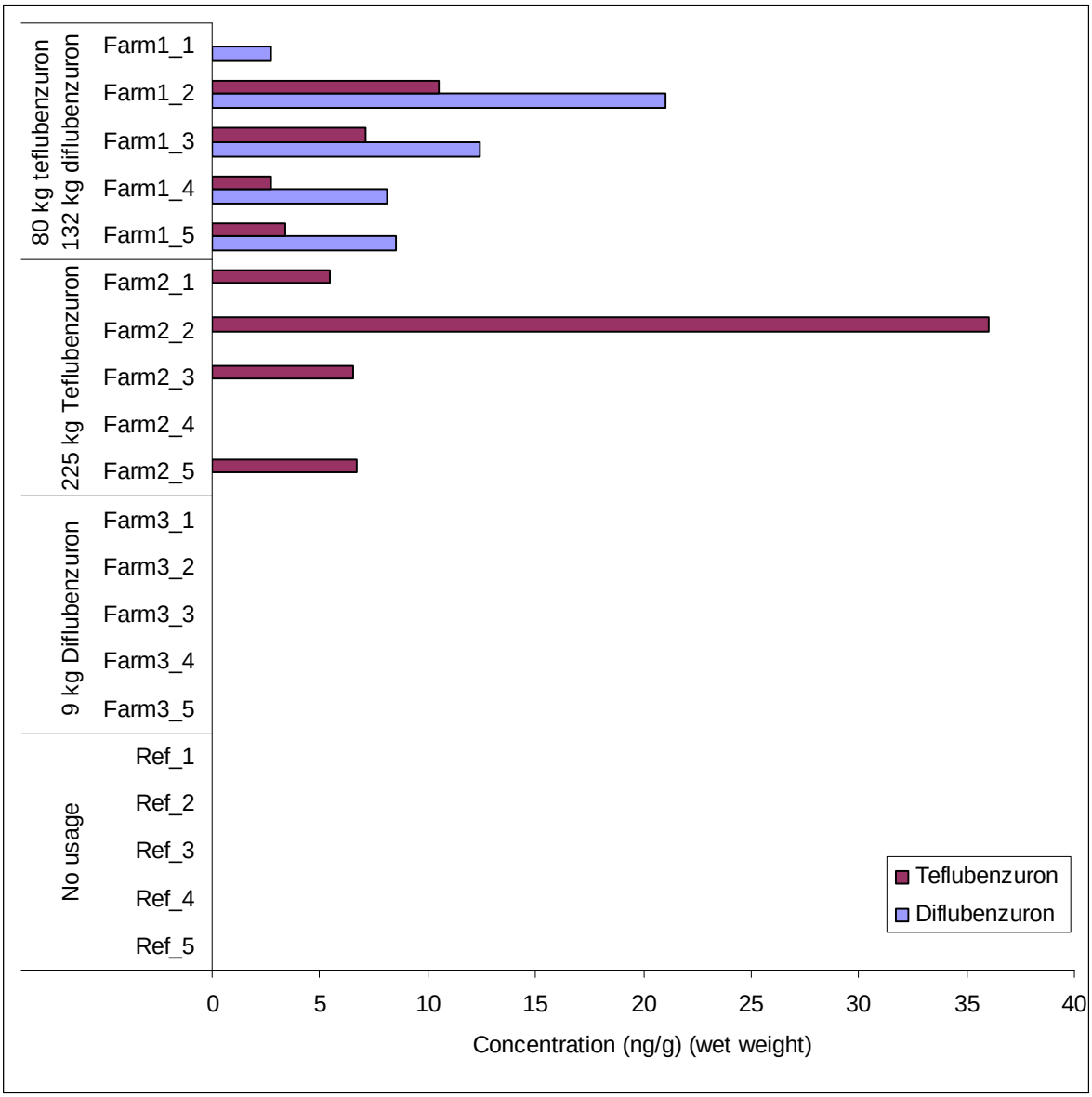


Figure 16. Diflubenzuron and teflubenzuron in blue mussel.

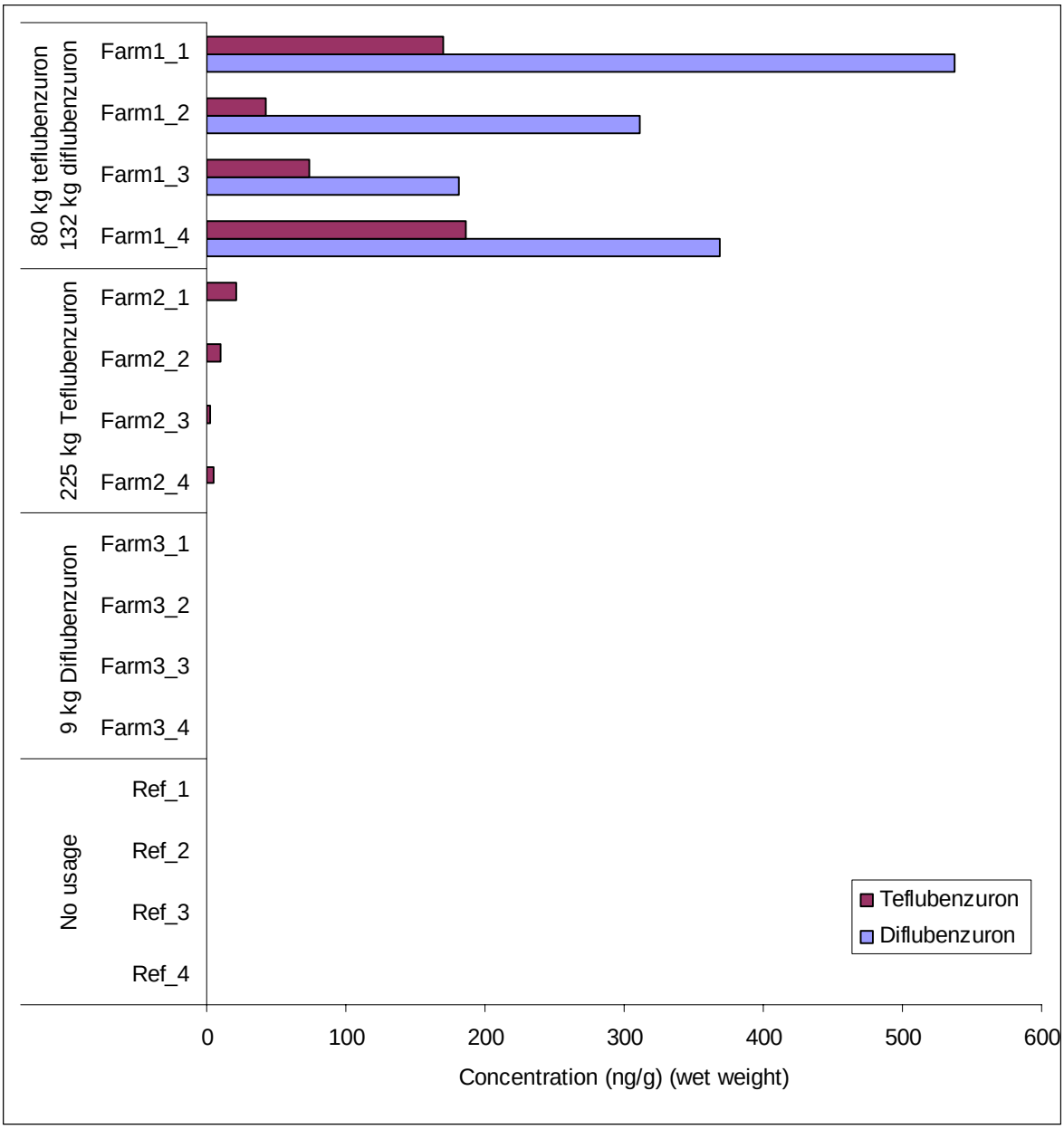


Figure 17. Diflubenzuron and teflubenzuron in brown crab.

6. Discussion

There are very few occurrence data reported for the marine environment for diflubenzuron or teflubenzuron. The majority of the published data relates to crop spraying. An earlier study in the Norwegian aquaculture industry measured diflubenzuron in sediment in the vicinity of a fish farm (Selvik *et al.*, 2002). Concentrations decreased rapidly with increasing distance from the fish farm with a maximum concentration of 5.4 µg/g (wet weight) directly under the fish cage and decreasing to below detection limits 20 m from the cage. In the present study, diflubenzuron was detected further from the fish cages than in the study by Selvik *et al*. (2002) although the measured concentrations were lower. This could be as a result of different usage patterns or due to different environmental conditions.

In the same study by Selvik *et al*. (2002), material was collected in sediment traps 2 m above the seabed and directly below the fish cage. These traps collected mainly feed and faeces and the diflubenzuron concentration range was 43-259 µg/g during 14 days of diflubenzuron treatment. These high concentrations confirm the pathway by which diflubenzuron enters the environment as via excess food and faeces.

Sediment screening surveys in the Scottish aquaculture industry in 2005 and 2006 did not detect diflubenzuron in any samples (Thomas, 2004; Thomas, 2005; Thomas, 2006; Thomas, 2007) and in 2003 and 2006 no teflubenzuron was detected in any sediment samples (Thomas, 2004; Thomas, 2007). Different locations were surveyed in 2004 and 2005. In 2004, teflubenzuron was detected in one sediment sample at 0.56 µg/kg (Thomas, 2005) and the following year it was detected in 25 of the 51 sample stations with a concentration range of 0.23-10.9 µg/kg (Thomas, 2006) which is comparable to the concentrations measured in this present Norwegian study.

With no Norwegian Environmental Quality Standard (EQS) available for either of the fish farm medicines selected for screening, we propose to compare the data with UK EQS for sediment and water (table 12). The EQS is a concentration which is set in order to protect the environment from a particular chemical. EQS values are derived from standard laboratory ecotoxicity tests using appropriate quality assurance and control that allow an assessment of the safe amount of a particular substance to be present in the environment. Therefore the EQS set for the UK are equally as applicable to the Norwegian environment since they are based on standardised test results and typically use safety assessment factors agreed upon by the international community. Management practice in the UK allows for the fish farms to impact an area of up to 25 m from the cages which is termed the allowable zone of effects (AZE). There are therefore different EQS for inside and outside the AZE (Table 12). We have used this UK classification system for comparing the data within this report.

Since all of the sediment samples were > 25 m from the cages at Fish farm 1, we have compared the data with the EQS for outside the AZE. All of the sediment samples collected from fish farm 1 contained levels greater than the UK sediment EQS for teflubenzuron of 2 ng/g. At Fish farm 2 only one sample was from outside the AZE (sample 5) and this too was above the UK EQS for outside the AZE. The other sediment samples collected from within 25 m of Fish farm 2 were below the teflubenzuron EQS for within the AZE of 10,000 ng/g. At fish farm 3 no teflubenzuron was detected. It appears that the sediment samples collected from outside the 25 m AZE at fish farms where teflubenzuron has been used were all above the EQS of 2 ng/g. No sediment EQS is available for diflubenzuron and a lack of relevant data make calculating a sediment predicted no-effect concentration (PNEC) difficult which is a concern since diflubenzuron is relatively stable in marine sediments (Selvik et al., 2002). It is

therefore recommended that marine sediment toxicity data are obtained for diflubenzuron so that an assessment of the risk to sediment dwelling organisms can be evaluated.

Table 12. Summary of UK environmental quality standard (EQS) data for diflubenzuron and teflubenzuron.

Chemical	EQS (ng/L)	Application	Source
Diflubenzuron	5	Annual average within a water body	(DoE, 1996)
	100	Maximum allowable concentration	(DoE, 1996)
Teflubenzuron	6	Annual average within a water body	(SEPA, 1998)
	30	Maximum allowable concentration	(SEPA, 1998)
	2 ng/g dry weight	Maximum allowable concentration outside 'zone of effects'	(SEPA, 1998)
	10,000 ng/g dry weight	Trigger level for further monitoring inside the 'zone of effects'	

Allowable zone of effects (AZE) is defined by the Scottish Environmental Protection Agency (SEPA) as an area of seabed under and close to the cages to a distance of 25 metres from beneath the cage edge.

Teflubenzuron and diflubenzuron have relatively low water solubility and a reported tendency to bind to sediment and organic materials. Water samples were only collected from Fish farms 1 and 3 with diflubenzuron present in all of the samples analysed. At Fish farm 1, the highest concentrations were observed near the farm with concentrations reducing further away from the farm. These data are similar to previously reported occurrence data for diflubenzuron in water from surveys performed in Norway and Ireland. Detectable concentrations of diflubenzuron were reported in 125 of 550 water samples collected from Norway at concentrations between 26 and 242 ng/L (Samulesen *et al.*, 2009). The levels of diflubenzuron detected at farms 1 and 3 were above the UK EQS of 5 ng/L with the samples collected within 300m of Fish farm 1 above the 'maximum allowable concentration' of 100 ng/L. Only the sample collected from 1 km away from Fish farm 2 contained levels of diflubenzuron greater than 100 ng/L. The concentration of teflubenzuron was highest 900 m from farm 1 with levels above the UK EQS of 6 ng/L detected between 700 and 900 m away from the farm. These data indicate that at the time of sampling the dissolved concentration of diflubenzuron in the water samples collected from Fish farms 1 and 3 were sufficiently high to pose a risk to aquatic organisms. The teflubenzuron in samples collected between 300 and 900 m of Fish farm 1 also contribute to this risk.

The association of diflubenzuron and teflubenzuron with particulate material is of particular concern for filter feeders such as blue mussels. In this study, diflubenzuron was detected in blue mussels at 2.7-21 ng/g at fish farm 1 where particulate concentrations were in the range 0.3-17.7 ng/L. Teflubenzuron was measured in blue mussels at both fish farms where it was in use (Farms 1 and 2) and it was also measured in all of the particulate phase samples collected at Fish farm 1 regardless of their distance from the farm. There is however evidence to suggest that mussels readily eliminate teflubenzuron (Burridge et al., 2010).

Crab and shrimp are bottom dwellers resulting in exposure from the sediment and also the water column. As shrimp are scavenger feeders, they will also be vulnerable to diflubenzuron and teflubenzuron associated with particulate matter. Both shrimp and crab will also accumulate diflubenzuron and teflubenzuron through the food that they eat. Concentrations measured in shrimp were in the low ng/g range which is lower than the concentrations measured in brown crab. Fish farm 1 had the highest usage of diflubenzuron and resulted in concentrations in brown crab of 181-538 ng/g. The concentrations of teflubenzuron were lower but due to the increased potency of teflubenzuron the concentrations may be equally significant. Low ng/g concentrations of teflubenzuron were also measured in crab close to fish farm 2 where it was is use.

Both chitin inhibitors were detected in a single sample of amphipod from fish farm 1. Amphipod samples were collected between 100 and 2400 m from the fish farms suggesting that the majority of the amphipods were not exposed. It is not possible to draw any conclusions from this data set.

There were no measurable concentrations of either compound in any of the cod samples. Cod are not confined to one location and were all fished some time after sea lice treatment and as a result they may not have been exposed to diflubenzuron or teflubenzuron. The hydrophobic nature of both compounds means sediment is the likely sink which will have less impact on cod than it does for the other species monitored. Bottom dwelling fish may be of more interest in future studies.

It is not clear whether the concentrations detected in the biota samples collected will have an effect on each individual species. It is however a concern that crab, shrimp and blue mussels are being exposed to both diflubenzuron and teflubenzuron at the sites selected. This suggests that diflubenzuron and teflubenzuron are present in a bioavailable form and supports the above call for dissolved measurements of the chemicals in future programmes. It is not surprising that the chemicals are bioaccumulating since this is as would be predicted from their Log K_{OW} (diflubenzuron= 3.8; teflubenzuron=5.4). What is unclear, due to the lack of published data, are what these levels mean to the organisms in question in terms of acute and chronic effects. The teflubenzuron EQS_{Water} presented above were derived from chronic life-cycle data as measured concentrations in a 27 day *Mysidopsis* shrimp test (Baird et al. 1997) and by applying a x2 (for the annual) and a x10 safety factor for the maxiumum allowable concentration (SEPA, 1999). A crude assessment of the levels detected in the shrimp collected from Farm 1 and the levels at which chronic effects are seen in shrimp would suggest that there is a potential risk to shrimp. It would also be reasonable to extrapolate this to any species that undergoes moulting in its life cycle (i.e. brown crab) (Burridge et al., 2010).

Outside of Norway, diflubenzuron is the most frequently used antiparasitic agent in the Brazilian freshwater aquaculture industry (Mabilia and de Souza, 2006), unlike in the UK and in Norway, the treatment method is an immersion bath rather than through feed (Mabilia *et al.*, 2008) which may result in a different loading compared to administering through feed, and potentially less stringent control may mean benzoylurea pesticide usage is also a concern outside of Norway.

6. Conclusions

Diflubenzuron and teflubenzuron were detected in particle, water, sediment and biota samples collected from around selected fish farms known to have used these chemicals for sea lice control.

The levels of teflubenzuron detected in sediments at certain locations are sufficiently high to exceed UK environmental quality standards and thus be of potential risk to sediment dwelling organisms. It was not possible to evaluate the risks associated with the levels of diflubenzuron detected due to a lack of pertinent ecotoxicity data.

The dissolved levels of diflubenzuron detected in water samples collected at the farms, and up to 1 km away, are also sufficiently high to exceed UK environmental quality standards and pose a risk to aquatic organisms. There is also some risk to aquatic organisms from dissolved concentrations of teflubenzuron.

The levels of both chitin inhibitors in shrimp and crab suggest that shrimp, crab and other moulting species are at potential risk at specific locations where the chemicals are being used.

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Appendix A

Table A1. Diflubenzuron and teflubenzuron concentration in particulate samples.

Sample Location	Diflubenzuron (ng/L)	Teflubenzuron (ng/L)	Diflubenzuron (ng/g) (dry weight)	Teflubenzuron (ng/g) (wet weight)
Reference 1	<1	<1	<100	<100
Reference 2	<1	<1	<100	<100
Reference 3	<1	<1	<100	<100
Reference 4	<1	<1	<100	<100
Reference 5	<1	<1	<100	<100
Fish farm 1 _ 1	16.7	0.5	3189.9	89.1
Fish farm 1 _ 2	17.7	0.5	17197.8	459.1
Fish farm 1 _ 3	4.4	0.4	1515.8	150.6
Fish farm 1 _ 4	2.0	0.4	1604.1	306.4
Fish farm 1 _ 5	0.3	0.3	87.3	106.7
Fish farm 2 _ 1	<1	<1	<100	<100
Fish farm 2 _ 2	<1	<1	<100	<100
Fish farm 2 _ 3	<1	<1	<100	<100
Fish farm 2 _ 4	<1	<1	<100	<100
Fish farm 2 _ 5	<1	<1	<100	<100
Fish farm 3 _ 1	10.9	<1	3072.2	<100
Fish farm 3 _ 2	15.2	<1	6683.8	<100
Fish farm 3 _ 3	5.5	<1	3327.3	<100
Fish farm 3 _ 4	1.1	<1	1030.2	<100
Fish farm 3 _ 5	1.3	<1	988.1	<100

Table A2. Diflubenzuron and teflubenzuron concentration in water samples.

Sample Location	Diflubenzuron (ng/L)	Teflubenzuron (ng/L)
Reference 1	-	-
Reference 2	-	-
Reference 3	-	-
Reference 4	-	-
Reference 5	-	-
Fish farm 1 _ 1	123.7	<1
Fish farm 1 _ 2	226.2	<1
Fish farm 1 _ 3	295.2	7.0
Fish farm 1 _ 4	37.0	6.0
Fish farm 1 _ 5	34.3	12.9
Fish farm 2 _ 1	-	-
Fish farm 2 _ 2	-	-
Fish farm 2 _ 3	-	-
Fish farm 2 _ 4	-	-
Fish farm 2 _ 5	-	-
Fish farm 3 _ 1	13.1	<1
Fish farm 3 _ 2	27.4	<1
Fish farm 3 _ 3	30.9	<1
Fish farm 3 _ 4	28.9	<1

Fish farm 3 _ 5	114.3	<1

- not determined

Table A3. Diflubenzuron and teflubenzuron concentration in sediment samples

Sample Location	Diflubenzuron (ng/g) (dry weight)	Teflubenzuron (ng/g) (dry weight)	Particle Size <63µm (% dry weight)	TOC/F (µg C/mg TS)
Reference 1	<5	<1	70	18.9
Reference 2	<5	<1	3	5.7
Reference 3	<5	<1	4	2.0
Reference 4	<5	<1	3	2.5
Reference 5 (n=2)	<5	<1	13	10.4
Fish farm 1 _ 1	8.2	8.7	18	4.7
Fish farm 1 _ 2	11.1	11.3	-	9.8
Fish farm 1 _ 3	12.9	10.5	6	2.6
Fish farm 1 _ 4	42.5	66.0	12	2.8
Fish farm 1 _ 5	5.9	7.2	13	4.3
Fish farm 2 _ 1	<5	78.8	4	1.9
Fish farm 2 _ 2	<5	269.2	3	6.4
Fish farm 2 _ 3	<5	65.2	4	2.1
Fish farm 2 _ 4	<5	32.8	3	1.2
Fish farm 2 _ 5	<5	8.3	3	1.6
Fish farm 3 _ 1	<5	<1	61	3.9
Fish farm 3 _ 2	0.7	<1	61	2.7
Fish farm 3 _ 3	9.0	<1	62	18.0
Fish farm 3 _ 4	136.6	<1	65	11.7
Fish farm 3 _ 5	0.6	<1	65	3.9
2008 screening 1	<5	<1	-	-
2008 screening 2	<5	<1	-	-
2008 screening 3	<5	<1	-	-
2008 screening 4	<5	<1	-	-
2008 screening 5	<5	<1	-	-
2008 screening 6	<5	<1	-	-

- not determined

Table A4. Diflubenzuron and teflubenzuron concentration in brown crab samples.

Sample Location	Diflubenzuron (ng/g) (dry weight)	Teflubenzuron (ng/g) (dry weight)
Reference 1	<1	<1
Reference 2	<1	<1
Reference 3	<1	<1
Reference 4	<1	<1
Fish farm 1 _ 1	537.9	170.3
Fish farm 1 _ 2	310.8	43.0
Fish farm 1 _ 3	180.9	74.3
Fish farm 1 _ 4	368.7	185.7
Fish farm 2 _ 1	<1	20.9
Fish farm 2 _ 2	<1	10.1

Fish farm 2 _ 3	<1	2.7
Fish farm 2 _ 4	<1	5.0
Fish farm 3 _ 1	<1	<1
Fish farm 3 _ 2	<1	<1
Fish farm 3 _ 3	<1	<1
Fish farm 3 _ 4	<1	<1

Table A5. Diflubenzuron and teflubenzuron concentration in shrimp samples.

Sample Location	Diflubenzuron (ng/g) (wet weight)	Teflubenzuron (ng/g) (wet weight)	Lipid (%)
Reference 1	<0.5	<0.2	-
Reference 2	<0.5	<0.2	-
Reference 3	<0.5	<0.2	-
Fish farm 1 _ 1	10.0	11.3	-
Fish farm 1 _ 2	3.0	7.6	-
Fish farm 1 _ 3	3.9	9.6	-
Fish farm 2 _ 1	<0.5	0.4	-
Fish farm 2 _ 2	<0.5	0.4	-
Fish farm 2 _ 3	<0.5	0.4	-
Fish farm 3 _ 1	<0.5	<0.2	1.3
Fish farm 3 _ 2	<0.5	<0.2	1.5
Fish farm 3 _ 3	<0.5	<0.2	1.4

- not determined

Table A6. Diflubenzuron and teflubenzuron concentration in Amphipod samples.

Sample Location	Diflubenzuron (ng/g) (dry weight)	Teflubenzuron (ng/g) (dry weight)
Reference 1	<2	<0.5
Reference 2	<2	<0.5
Reference 3	<2	<0.5
Fish farm 1 _ 1	5.5	3,5
Fish farm 1 _ 2	<2	<0.5
Fish farm 1 _ 3 (n=2)	<2	<0.5
Fish farm 2 _ 1	<2	<0.5
Fish farm 2 _ 2 (n=2)	<2	<0.5
Fish farm 2 _ 3	<2	<0.5
Fish farm 3 _ 1	<2	<0.5
Fish farm 3 _ 2	<2	<0.5
Fish farm 3 _ 3	<2	<0.5

Table A7. Diflubenzuron and teflubenzuron concentration in blue mussel samples.

Sample Location	Diflubenzuron (ng/g) (wet weight)	Teflubenzuron (ng/g) (wet weight)
Reference 1	<8	<3
Reference 2	<8	<3
Reference 3	<8	<3
Reference 4	<8	<3

Reference 5	<8	<3
Fish farm 1 _ 1	2.7	<3
Fish farm 1 _ 2	21.0	10.5
Fish farm 1 _ 3	12.4	7.1
Fish farm 1 _ 4	8.2	2.7
Fish farm 1 _ 5	8.5	3.4
Fish farm 2 _ 1 (n=2)	<7	5.5
Fish farm 2 _ 2	<7	36.0
Fish farm 2 _ 3	<7	6.6
Fish farm 2 _ 4	<7	<3
Fish farm 2 _ 5	<7	6.7
Fish farm 3 _ 1	<8	<3
Fish farm 3 _ 2	<8	<3
Fish farm 3 _ 3	<8	<3
Fish farm 3 _ 4	<8	<3
Fish farm 3 _ 5	<8	<3

Table A8. Diflubenzuron and teflubenzuron concentration in cod filet.

Sample Location	Diflubenzuron (ng/g) (wet weight)	Teflubenzuron (ng/g) (wet weight)
Reference 1	<5	<5
Reference 2	<5	<5
Reference 3	<5	<5
Reference 4	<5	<5
Reference 5	<5	<5
Reference 6	<5	<5
Reference 7	<5	<5
Reference 8	<5	<5
Reference 9	<5	<5
Reference 10	<5	<5
Reference 11	<5	<5
Reference 12	<5	<5
Reference 13	<5	<5
Reference 14	<5	<5
Reference 15	<5	<5
Fish farm 1 _ 1	<5	<5
Fish farm 1 _ 2	<5	<5
Fish farm 1 _ 3	<5	<5
Fish farm 1 _ 4	<5	<5
Fish farm 1 _ 5	<5	<5
Fish farm 1 _ 6	<5	<5
Fish farm 1 _ 7	<5	<5
Fish farm 1 _ 8	<5	<5
Fish farm 1 _ 9	<5	<5
Fish farm 1 _ 10	<5	<5
Fish farm 1 _ 11	<5	<5
Fish farm 2 _ 1	<5	<5

Fish farm 2 _ 2	<5	<5
Fish farm 2 _ 3	<5	<5
Fish farm 2 _ 4	<5	<5
Fish farm 2 _ 5	<5	<5
Fish farm 2 _ 6	<5	<5
Fish farm 2 _ 7	<5	<5
Fish farm 2 _ 8	<5	<5
Fish farm 2 _ 9	<5	<5
Fish farm 2 _ 10	<5	<5
Fish farm 2 _ 11 (n=2)	<5	<5
Fish farm 3 _ 1	<5	<5
Fish farm 3 _ 2	<5	<5
Fish farm 3 _ 3	<5	<5
Fish farm 3 _ 4	<5	<5
Fish farm 3 _ 5	<5	<5

Table A9. Diflubenzuron and teflubenzuron concentration in cod liver.

Sample Location	Diflubenzuron (ng/g) (lipid weight)	Teflubenzuron (ng/g) (lipid weight)	Lipid (%)
Reference 1	<10	<5	7.6
Reference 2	<10	<5	33.2
Reference 3	<10	<5	33.2
Reference 4	<10	<5	22.4
Reference 5	<10	<5	35.5
Reference 6	<10	<5	40.8
Reference 7	<10	<5	3.7
Reference 8	<10	<5	32.4
Reference 9	<10	<5	52.9
Reference 10	<10	<5	52.1
Reference 11	<10	<5	32.5
Reference 12	<10	<5	6.0
Reference 13	<10	<5	19.4
Reference 14	<10	<5	21.6
Reference 15	<10	<5	43.0
Fish farm 1 _ 1	<10	<5	63.5
Fish farm 1 _ 2	<10	<5	-
Fish farm 1 _ 3	<10	<5	-
Fish farm 1 _ 4	<10	<5	-
Fish farm 1 _ 5	<10	<5	-
Fish farm 1 _ 6	<10	<5	-
Fish farm 1 _ 7	<10	<5	-
Fish farm 1 _ 8	<10	<5	-
Fish farm 1 _ 9	<10	<5	-
Fish farm 1 _ 10	<10	<5	-
Fish farm 1 _ 11	<10	<5	-
Fish farm 2 _ 1	<10	<5	5.0
Fish farm 2 _ 2	<10	<5	3.5
Fish farm 2 _ 3	<10	<5	3.4

Fish farm 2 _ 4	<10	<5	1.8
Fish farm 2 _ 5	<10	<5	9.7
Fish farm 2 _ 6	<10	<5	28.3
Fish farm 2 _ 7 (n=2)	<10	<5	58.9
Fish farm 2 _ 8	<10	<5	2.9
Fish farm 2 _ 9	<10	<5	25.1
Fish farm 2 _ 10	<10	<5	2.8
Fish farm 2 _ 11	<10	<5	30.8
Fish farm 3 _ 1	<10	<5	72.5
Fish farm 3 _ 2	<10	<5	59.5
Fish farm 3 _ 3	<10	<5	65.2
Fish farm 3 _ 4	<10	<5	66.8
Fish farm 3 _ 5	<10	<5	60.6

- not determined

Table A10. Diflubenzuron and teflubenzuron concentration in cod skin.

Sample Location	Diflubenzuron (ng/g) (wet weight)	Teflubenzuron (ng/g) (wet weight)
Reference 1	<15	<20
Reference 2	<15	<20
Reference 3	<15	<20
Reference 4	<15	<20
Reference 5	<15	<20
Reference 6	<15	<20
Reference 7	<15	<20
Reference 8	<15	<20
Reference 9	<15	<20
Reference 10	<15	<20
Reference 11	<15	<20
Reference 12	<15	<20
Reference 13	<15	<20
Reference 14	<15	<20
Reference 15	<15	<20
Fish farm 1 _ 1	<15	<20
Fish farm 1 _ 2	<15	<20
Fish farm 1 _ 3	<15	<20
Fish farm 1 _ 4	<15	<20
Fish farm 1 _ 5	<15	<20
Fish farm 1 _ 6	<15	<20
Fish farm 1 _ 7	<15	<20
Fish farm 1 _ 8	<15	<20
Fish farm 1 _ 9	<15	<20
Fish farm 1 _ 10	<15	<20
Fish farm 1 _ 11	<15	<20
Fish farm 2 _ 1	<15	<20
Fish farm 2 _ 2	<15	<20
Fish farm 2 _ 3	<15	<20
Fish farm 2 _ 4	<15	<20

Fish farm 2 _ 5	<15	<20
Fish farm 2 _ 6	<15	<20
Fish farm 2 _ 7	<15	<20
Fish farm 2 _ 8	<15	<20
Fish farm 2 _ 9	<15	<20
Fish farm 2 _ 10	<15	<20
Fish farm 2 _ 11	<15	<20
Fish farm 3 _ 1	<15	<20
Fish farm 3 _ 2	<15	<20
Fish farm 3 _ 3	<15	<20
Fish farm 3 _ 4	<15	<20
Fish farm 3 _ 5	<15	<20

Statlig program for forurensningsovervåking

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Om Statlig program for forurensningsovervåking

Statlig program for forurensningsovervåking omfatter overvåking av forurensningsforholdene i luft og nedbør, skog, vassdrag, fjorder og havområder. Overvåkingsprogrammet dekker langsiktige undersøkelser av:

- overgjødsling
- forsuring (sur nedbør)
- ozon (ved bakken og i stratosfæren)
- klimagasser
- miljøgifter

Overvåkingsprogrammet skal gi informasjon om tilstanden og utviklingen av forurensningssituasjonen, og påvise eventuell uheldig utvikling på et tidlig tidspunkt. Programmet skal dekke myndighetenes informasjonsbehov om forurensningsforholdene, registrere virkningen av iverksatte tiltak for å redusere forurensningen, og danne grunnlag for vurdering av nye tiltak. Klima- og forurensningsdirektoratet er ansvarlig for gjennomføringen av overvåkingsprogrammet.

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