Screening of veterinary medicines used in aquaculture – diflubenzuron and teflubenzuron

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The study

- Investigated the occurrence of 2 chitin synthesis inhibitors
  - Diflubenzuron (Releeze)
  - Teflubenzuron (Ektoban)
- Used to treat sealice in salmon farms
- In use again after sealice became resistant to emamectin benzoate (Slice)

What are chitin synthesis inhibitors?

- Flubenzurons that stop chitin development and inhibit molting in early life stage development
- Both pesticides are administered in food
- Diflubenzuron is the active ingredient in Releeze vet
- Teflubenzuron is the active ingredient in Ektoban vet

Why are we screening?

- Approx 10% of the administered dose is absorbed by the fish
- Approx 90% is rapidly excreted through faeces
- Approx 5% of the feed pellets are not eaten

- Flubenzurons are not toxic to many marine species such as fish and algae, but by their nature are likely to have adverse affects on non-target species such as crab and shrimp that live in the area around the fishfarm

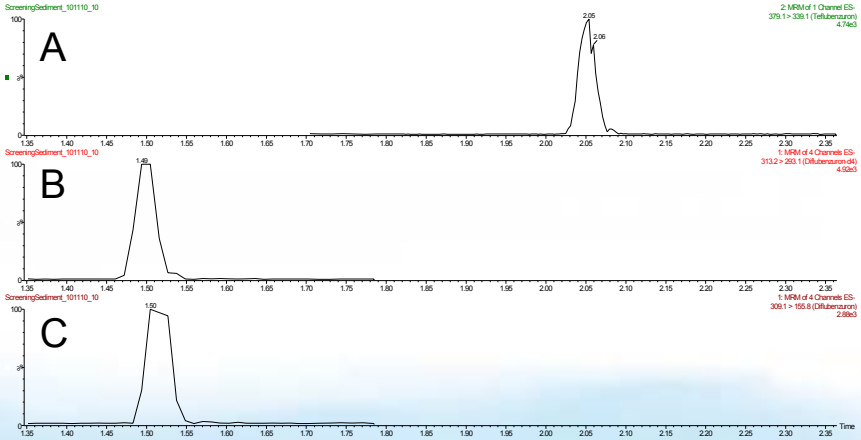
- **Farm1** – Nord-Trøndelag
  - 40 tonnes Ektoban (80 kg teflubenzuron)
  - 220 tonnes Releeze (132 kg diflubenzuron)
- **Farm2** – Nord-Trøndelag
  - 112 tonnes Ektoban (225 kg teflubenzuron)
- **Farm3** – Hordaland
  - 15 tonnes Releeze (9 kg diflubenzuron)
- **Reference** – Oslofjorden
  - No registered use of aquaculture medicines

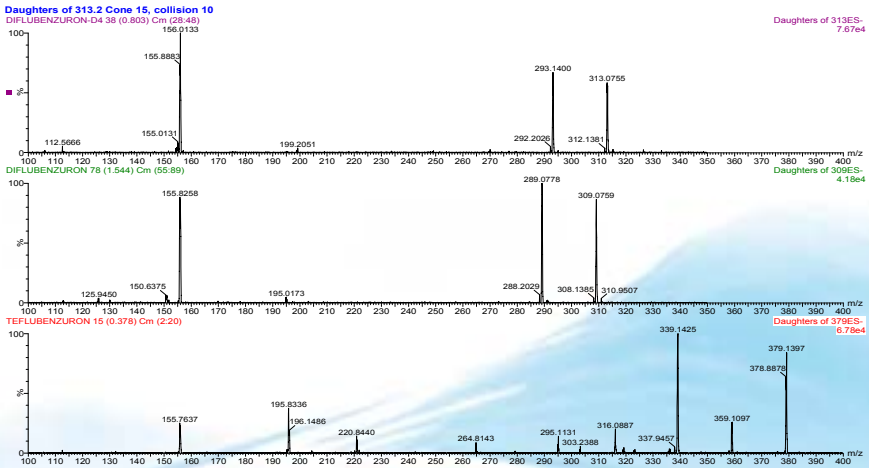
Samples collected

- Water and particulate
- Sediment
- Blue mussel
  (*Mytilus edulis*)
- Amphipod
  (*Gammarus locusta*)
- Shrimp
  (*Pandalus borealis*)
- Brown crab
  (*Cancer pagurus*)
- Cod (*Gadus morhua*)

Analysis

- Samples were solvent extracted
- Fat was removed from biota samples
- Analysed by LC/MS/MS



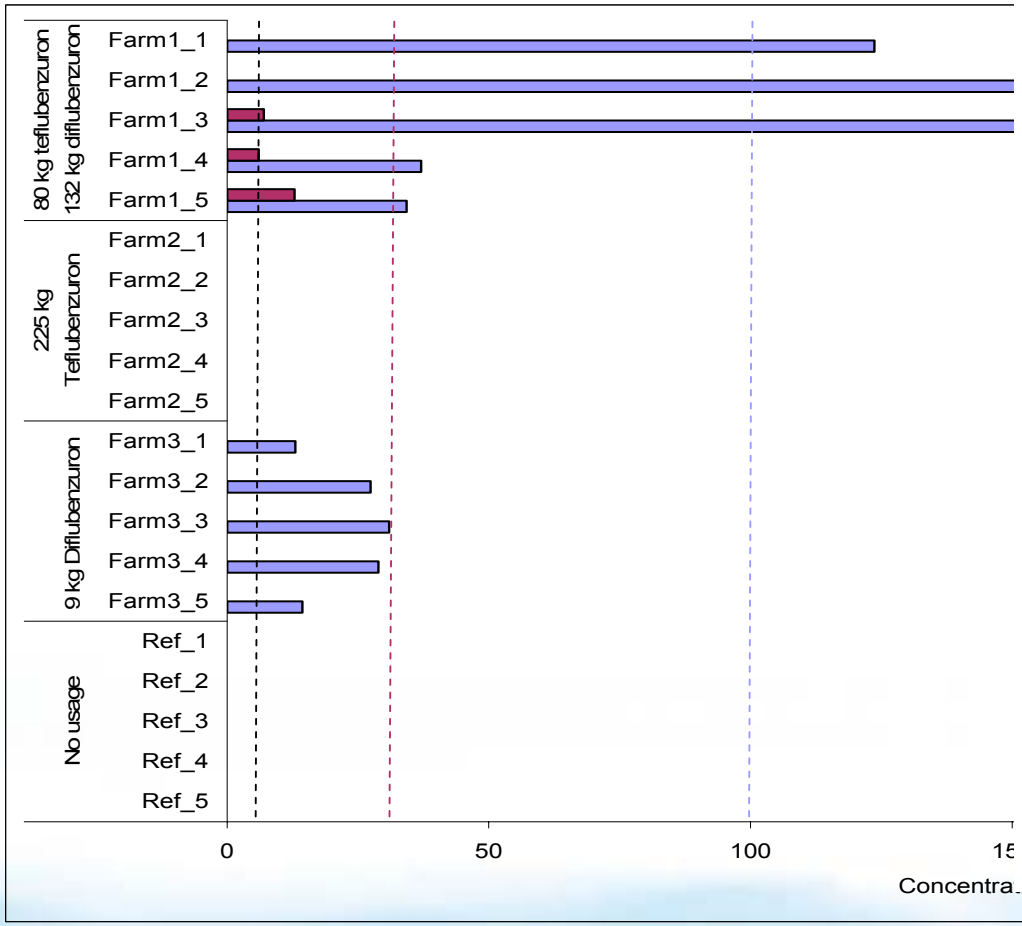


EQS og AZE

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

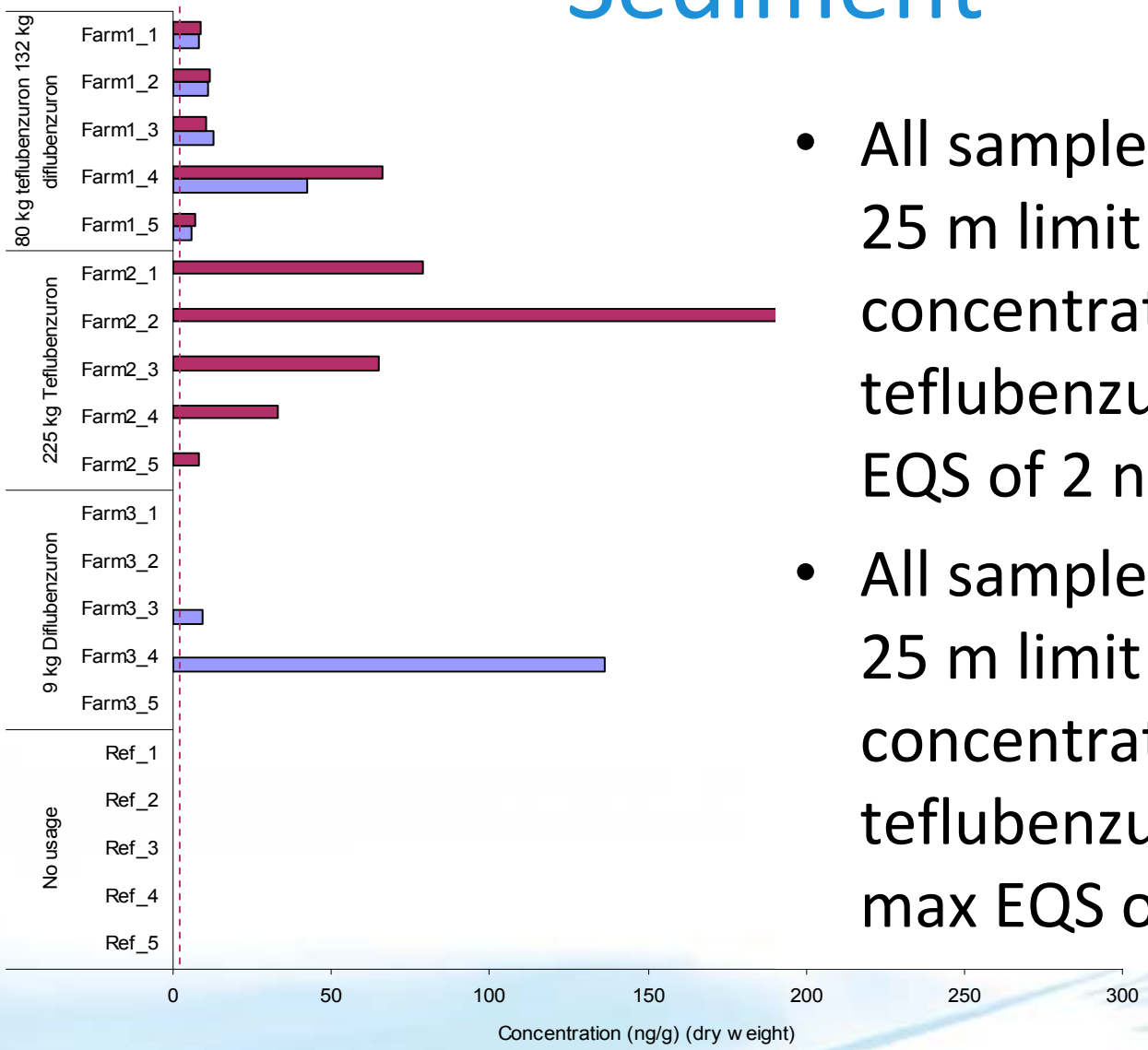
- EQS – Environmental Quality Standard
- AZE – Allowable Zone of Effects (up to 25 m from the fish farm)
- Used to evaluate the environmental risk of a compound
- No Norwegian EQS available so we used the British

Water



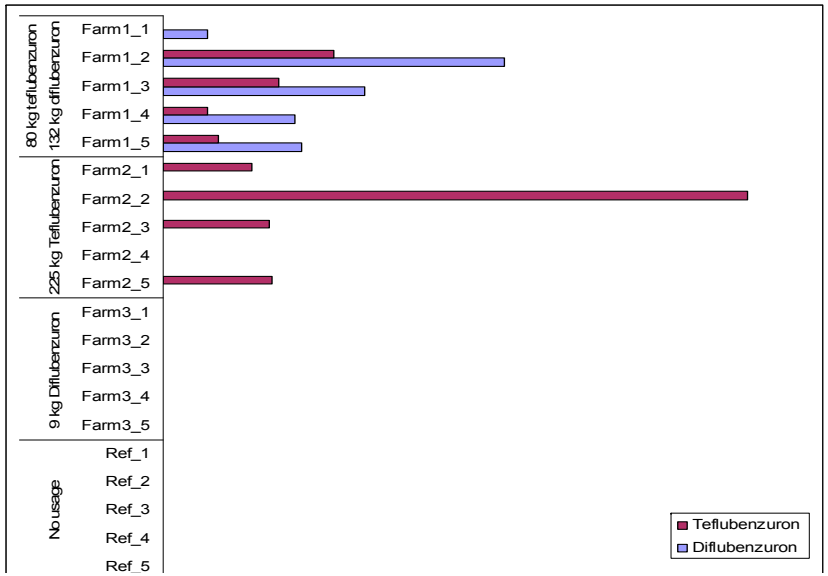
- Concentrations of diflubenzuron and teflubenzuron are above annual average EQS of 5 and 6 ng/L
- Concentration of diflubenzuron up to 1 km from the site over max allowable EQS
  - potentially high enough to pose a risk to aquatic organisms

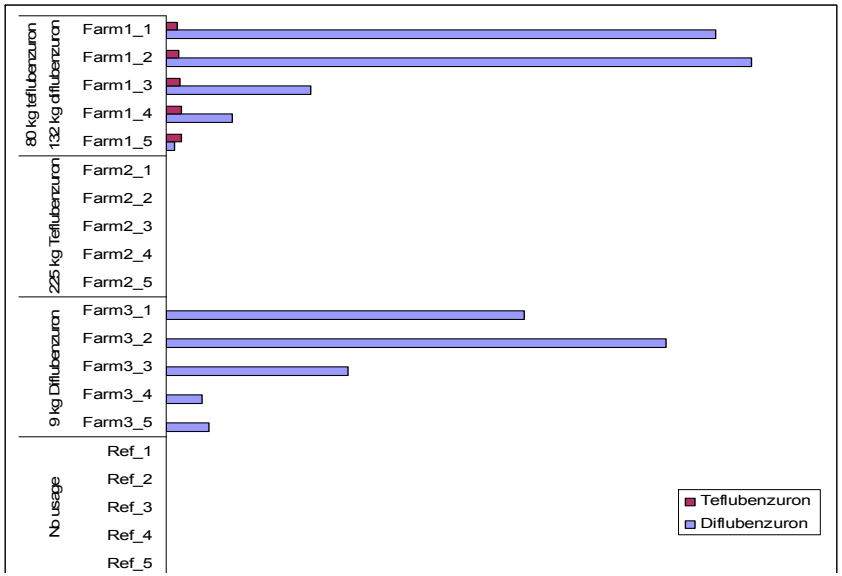
Sediment



- All samples outside the 25 m limit had concentrations of teflubenzuron over the EQS of 2 ng/g
- All samples within the 25 m limit had concentrations of teflubenzuron under the max EQS of 10 000 ng/g

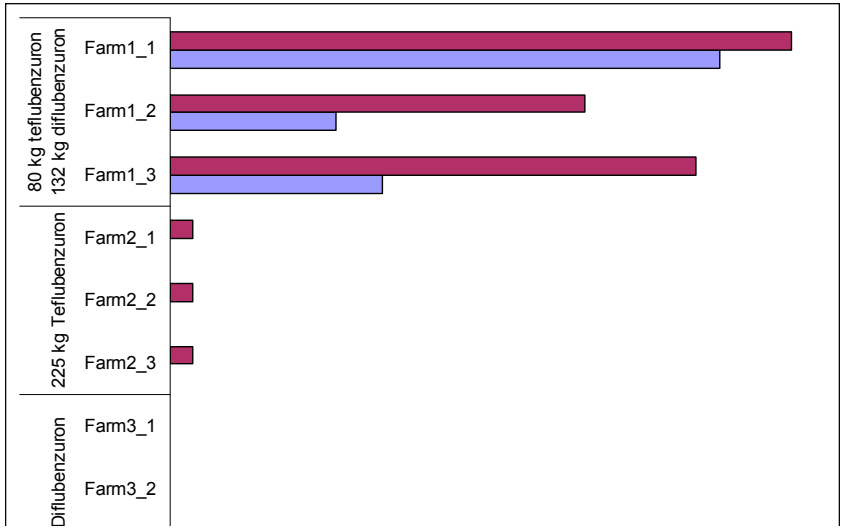
Filter feeders

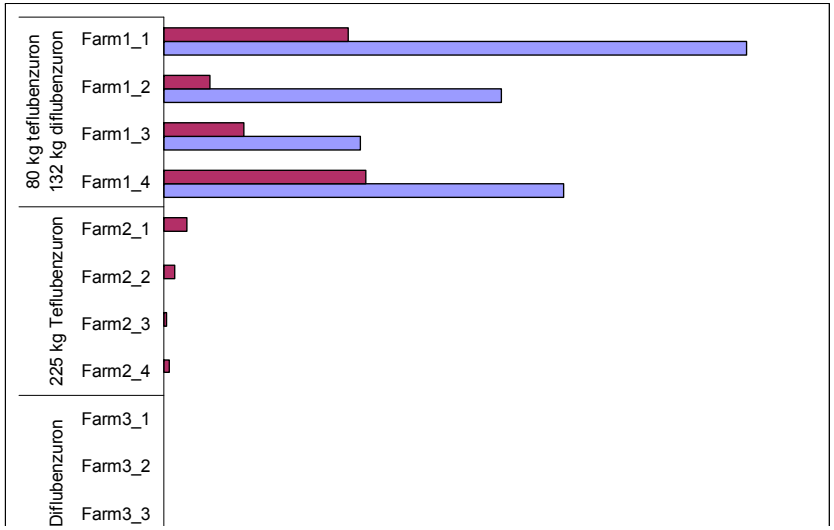




- Compounds bound to the particulate phase are of particular concern for filter feeders such as blue mussel
- High concentrations of diflubenzuron were detected in blue mussel where there were also high concentrations associated with particulates

Shrimp and Brown Crab

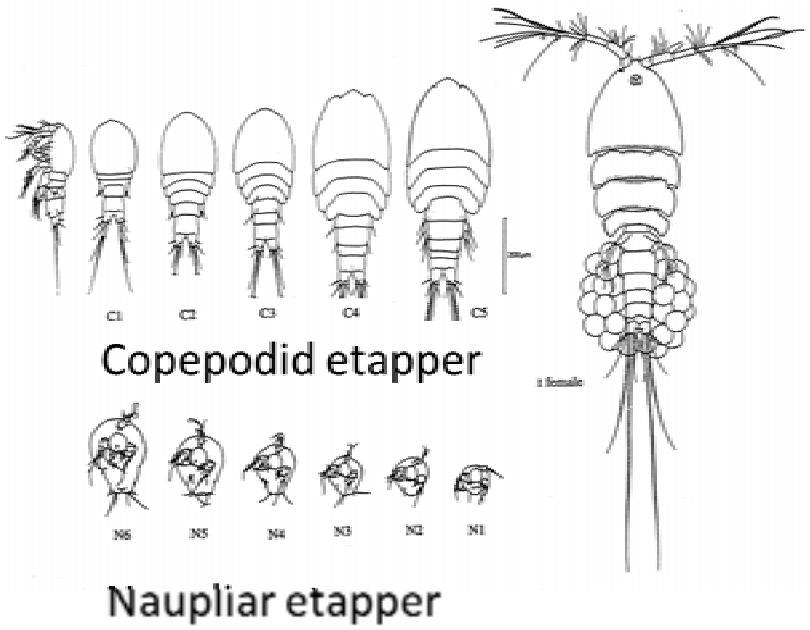




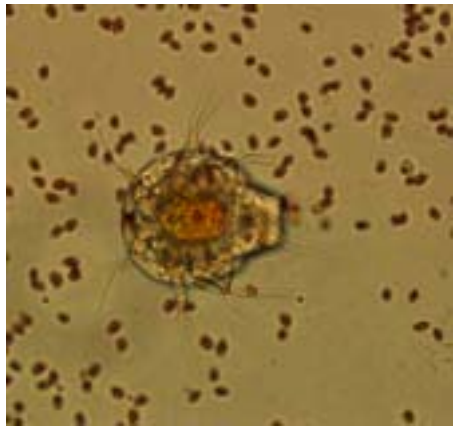
- Bottom dwelling organisms are exposed to sediment, water and particulate material
- Crustacea will then be subjected to the same toxic effects as sea lice
- A rough risk assessment of the concentrations measured in shrimp compared to toxic effects reported in the literature, indicate potential effects at these concentrations

Further evaluation of toxicity

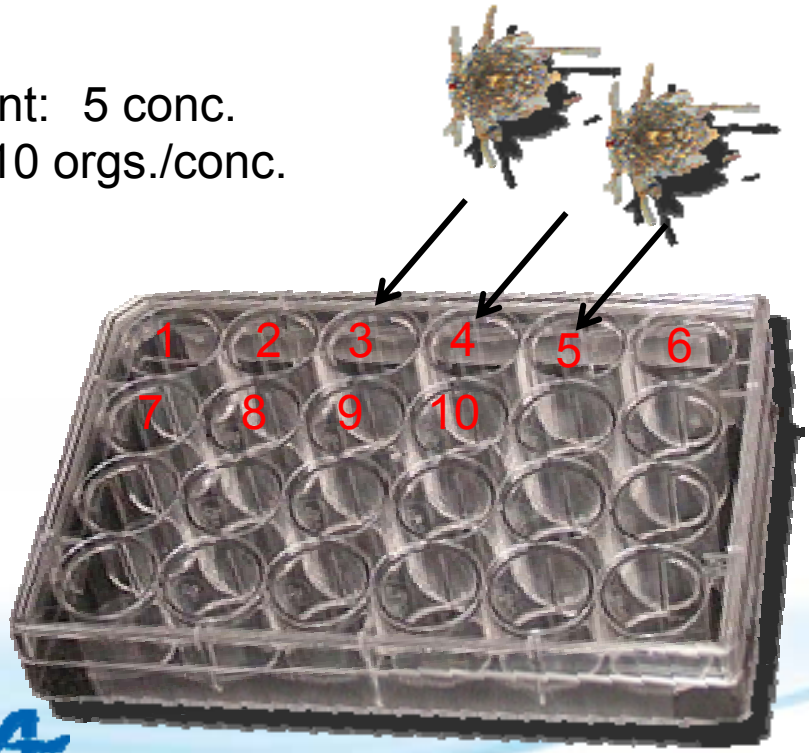
- Effects on crustacea
- Flubenzurons stop chitin development and inhibit molting
- *T. battagliai* development has six naupliar stages and five copepodid stages



Results



Experiment: 5 conc.
10 orgs./conc.



Lethality

•Diflubenzuron:

➢20% dødelighet på 1 mg/L

•Teflubenzuron:

➢LOEC = 1000 ng/L

➢NOEC = 100 ng/L

7 dag nauplier development

•Teflubenzuron:

➢LOEC = 0.10 ng/L

➢NOEC = 3.2 ng/L

Conclusion

- The concentrations of dissolved diflubenzuron and teflubenzuron in water are high enough to potentially pose a risk to aquatic organisms in specific areas
- The concentrations of diflubenzuron and teflubenzuron in some sediment samples were high enough to have potential risk to sediment dwelling organisms
- A rough risk assessment of the concentrations measured in shrimp compared to toxic effects reported in the literature, indicate potential effects at these concentrations
- It is safe to assume that these risks are also applicable to other species that rely on chitin synthesis and molting in their early lifestage development
- Significant development effects at 10 ng/L teflubenzuron
- Significant effects on death rates of day 3 nauplier at 320 ng/L teflubenzuron

kg aktive substance used

	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Azametifos							66	1884	3346	2437
Cypermethrin	62	59	55	45	49	30	32	88	107	48
Deltamethrin	23	16	17	16	23	29	39	62	61	54
Emamectin	20	23	32	39	60	73	81	**41**	**22**	**105**
Teflubenzuron								**2028**	**1080**	**26**
Diflubenzuron								**1413**	**1839**	**704**

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Thanks to ….

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- Bård Nordbø from Klif

Diflubenzuron

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	-

Teflubenzuron

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	-

DIFLUBENZURON	Results
Terrestrial insects	
Chrysoperla carnea larvae	LD_{50} 2.26 ng/insect
Schistocerca gregaria nymph	LD_{50} 68.0 µg/insect
haematobia irritans larvae	LC_{50} 25.5 µg/L
Cricotopus sp.	LC_{50} 1.6 µg/L
Tanytarsus dissimilis	LC_{50} 1.02 µg/L LC_{50} 4.9 µg/L
Fish	
Cutthroat Trout	96 hr LC_{50} >50 mg/L
Atlantic salmon fingerling	96 hr LC_{50} >50 mg/L
Aquatic crustacea	
Acartia tonsa	Hatching viability <50% at 1 µg/L Hatching viability <5% at 10 µg/L
Eurytemora affinis	48 hr LC_{50} 2.2 µg/L
Hyallela azteca	96 hr LC_{50} 1.84 µg/L
Daphnia magna	48 hr LC_{50} 4.42-6.89 µg/L
Daphnia magna	48 hr LC_{50} 2 µg/L
Daphnia magna	48 hr EC_{50} 0.5-1 µg/L
Gammarus pseudolimnaes mature	24 hr LC_{50} 87 µg/L
Clistoronia magnifica	30 day LC_{50} 0.1 µg/L
Mysidopsis bahia juvenile	96 hr LC_{50} 2.06 µg/L
Palaemonetes pugio larvae	96 hr LC_{50} 1.44 µg/L