NIFES
NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Report
2017

Monitoring program for pharmaceuticals, illegal substances, and contaminants in farmed fish

ANNUAL REPORT FOR 2016

Rita Hannisdal, Ole Jakob Nøstbakken, Bjørn Tore Lunestad, Helge Torbjørn Hove, Livar Frøyland and Lise Madsen

National Institute of Nutrition and Seafood Research (NIFES)

23.06.2017

Commissioned by

the Norwegian Food Safety Authority

ISBN: 978-82-91065-48-9

ACKNOWLEDGEMENTS

Most of the analyses for the monitoring programme were conducted at NIFES. Bergitte Reiersen, Marita Kristoffersen and Anette Kausland were in charge of the analytical work, while Siren Hatland was responsible for the work related to sample storage, preparation and distribution within the institute. Anne Margrethe Aase, Manfred Torsvik, Vidar Fauskanger, Nawaraj Gautam, and Aina Bruvik carried out the sample pre-treatment. Annette Bjordal, Tore Tjensvoll and Felicia Dawn Couillard were responsible for chemical analysis of residues of therapeutics. Kjersti Kolås, Jannicke A. Bakkejord, Dagmar Nordgård, Lene H. Johannessen, Teclu Habtemariam Weldegebriel, Kari B. Sæle, Franziska Randers, Agnethe Hertzberg, Andreas L. Tomren, Annie Fürstenberg, Per-Ola Rasmussen, Ørjan Bjorøy and Amarjargal Sengee were responsible for analyses of organic contaminants. Tonja Lill Eidsvik, Berit Solli, Vivian Mui, Edel Erdal, Nina Margrethe Steinsvik, Snorri Gunnarsson and Laila Sedal carried out the analysis of the chemical elements. Tone Galluzzi and Leikny Fjeldstad conducted the analyses of the antibacterial agents by the bioassay method.

Eurofins were used as sub-contractors for analyses of some parameters (table 8.4).

TABLE OF CONTENTS

1. SUMMARY

This report summarises the results from the monitoring program for pharmaceuticals, illegal substances, and contaminants in Norwegian farmed fish. In 2016, a total of 13 415 fish were collected.

Samples examined for substances with anabolic effects or unauthorized substances were collected at fish farms at different stages. Crystal violet was detected in fish from one fish farm. The findings were reported to the Norwegian Food Safety Authority (NFSA), which concluded that the samples had been contaminated prior arrival at the laboratory. No other residues of substances with anabolic effects or unauthorized substances were detected.

Samples tested for approved veterinary drugs were collected at processing plants, and are representative of Norwegian farmed fish ready for human consumption. Residues of the anti-sea lice agents; emamectin, cypermethrin, diflubenzuron or teflubenzuron were found in 22 samples. However, the concentrations measured were all below the Maximum Residue Limit (MRL) for all samples. Other veterinary drugs, such as antibiotics or drugs used against internal parasites, were not found.

Samples analysed for contaminants were collected at processing plants, and are representative of Norwegian farmed fish ready for human consumption. The samples were analysed for dioxins (sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)), dioxin like PCBs (dl-PCBs), indicator PCB (PCB-6), pesticides, metals, PAH, PFC or/and BFR. No environmental contaminants were found above the EU maximum limits. The level for several of the contaminants have decreased over the last 15 years.

2. INTRODUCTION

2.1 Background

According to EU legislation (96/23/EC), all food producing animals should be monitored for certain substances and residues thereof. The following residues or substance groups are monitored in Norwegian farmed fish:

Group A Substances with anabolic effects and unauthorized substances:

A1: Stilbenes, derivatives and their salts and esters

A3: Steroids

A6: Prohibited substances

Group B Veterinary drugs and contaminants:

B1: Antibacterial agents

B2a: Anthelmintics

B3a: Organochlorine compounds

B3b: Organophosphorus compounds

B3c: Chemical elements

B3d: Mycotoxins

B3e: Dyes

B3f: Others

2.2 Group A, Substances with anabolic effects and unauthorized substances

Samples examined for illegal compounds is collected by official inspectors at the farm, without prior notification to the farmers. Fish are sampled at all stages of farming. Group A includes growth promoters like steroids and stilbenes, and unauthorized drugs. Unauthorized drugs considered most relevant for aquaculture are chloramphenicol, nitrofurans, dyes and metronidazole. To ensure harmonized levels for the control of unauthorized substances, analytical methods used should meet minimum required performance limits (MRPLs) set by the European Union (2002/657/EC), and European reference laboratories (EU-RLs), (CRL 2007). Table 8.4 gives an overview of MRPLs of relevant compounds.

2.3 Group B, veterinary drugs

Samples examined for veterinary drugs are collected from fish at processing plants and the samples are representative of fish ready to be placed on the market for human consumption. In order to use a veterinary drug for food producing animals, a maximum residue limit (MRL) has to be established (EU 37/2010). The MRL is the highest permitted residual concentration of legally applied pharmacologically active substances in animals or animal products intended for human consumption. Consumption of food with drug residues below the MRL should not pose a health risk to the consumer. The MRLs for fish are set for muscle and skin in natural proportions.

2.4 Group B, contaminants

Samples examined for contaminants are collected from fish at processing plants, and are representative of fish ready for human consumption. The EU (EU 1881/2006) has set a Maximum limit (ML) for some of the contaminants in fish, while for others, like the pesticides, PAH, PFC and BFR, maximum limits have not been established.

3. MATERIAL AND METHODS

3.1 Sampling

The samples were collected by official inspectors from the Norwegian Food Safety Authority. All fish-producing regions in Norway were included. The sampling was randomised with regards to season and region. The following fish species were included in the monitoring program: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*) Arctic char (*Salvelinus alpinus*) and spotted wolfish (*Anarchichas minor*).

Samples were transported to NIFES in a frozen state. For most samples, the Norwegian quality cut (NQC) was used for further analyses (Johnsen 2011). However, for most of the samples collected for analysis of antibiotics, individual livers were also collected. Samples to be used for analyses of substances with anabolic effects or unauthorized substances included small fish from early life stages and in these cases, the whole fish except head, tail and gut were homogenised. The samples were analysed as pooled samples, and one pooled sample consists of five fish from the same cage/farm

3.2 Pre-treatment

Upon arrival at NIFES the sample identification were anonymised for the analysts. A back-up sample was stored for all samples. Pooled samples of muscle from five fish from the same cage/farm were homogenised before analyses. Samples of liver were excised from the fish in samples to be screened for residues of antimicrobial agents by the microbiological inhibition zone assay. Liver samples were examined individually, if residues are detected, the back-up sample of muscle would be analysed by chemical methods. The maximum residue limit for veterinary drugs are set for muscle and skin in natural proportions (EU 37/2010). Therefore, according to the analytical protocol, any detection of drug residues in the muscle or liver would be followed by a re-analysis of the back up sample, consisting of muscle and skin in natural proportions, in duplicate.

3.3 Analytical methods

The laboratory routines and most of the analytical methods are accredited in accordance with the standard ISO 17025 (Table 8.4). A summary of the analytical methods and their limit of detection (LOD) or limit of quantification (LOQ) are shown in table 8.4. The LOD is the lowest level at which the method

is able to detect the substance, while the LOQ is the lowest level for a reliable quantitative measurement. For all methods, a quality control sample (QC) with a known composition and concentration of target analyte, is included in each series. The methods are regularly verified by participation in inter laboratory proficiency tests, and by analysing certified reference material (CRM), where such exist.

3.3.1 Group A substances

A1, Stilbenes

Stilbenes were extracted by water and acetonitrile, and analysed by LC-MS/MS.

A3, Steroids

Steroids were extracted by water and acetonitrile. Solid phase extraction was used for sample clean up, before the samples were analysed by LC-MS/MS.

A6, Illegal veterinary drugs

Chloramphenicol

Chloramphenicol was extracted with ethyl acetate. Liquid-liquid extraction was used to purify the extract. The samples were analysed by LC-MS/MS.

Nitrofurans

The nitrofuran metabolites were extracted with aqueous hydrochloric acid and derivatized with nitrobenzaldehyde. Solid phase extraction was used for sample clean up. The analytes were determined by LC-MS/MS.

Metronidazole

Metronidazole and its metabolite hydroxymetronidazole were extracted by ethyl acetate. Solid phase extraction was used for sample clean up. The analytes were determined by LC-MS/MS

3.3.2 Group B substances

B1, Antibacterial agents (antibiotics)

The presence of antibacterial agents was determined by a three plate microbiological assay or by chemical analysis.

Microbiological assay

For the three-plate microbiological inhibition method, small pieces of liver were placed on a plate containing growth agar and a specific and senitive bacterial strain, after that the plate was incubated. If the samples contained residues of antibacterial agents, the bacterial growth would be inhibited in a zone around each piece of liver tissue. Thus, a transparent zone with no bacterial growth surrounding the liver

sample would indicate a positive sample. Any positive detection has to be verified by chemical analysis of muscle and skin.

Quinolones

Oxolinic acid, flumequin, cinoxacin, ciprofloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, lomefloxacin, marbofloxacin, nalidixic acid, norfloxacin, ofloxacin and sarafloxacin were extracted with acetonitrile, and analysis was performed by LC-MS/MS.

Tetracyclines

Oxytetracycline, doxycycline, chlortetracycline and tetracycline were extracted with acetonitrile, and analysed by LC-MS/MS.

Florfenicol

The analyte was extracted with ethyl acetate. Liquid-liquid extraction was used to purify the extract. The samples were analysed by LC-MS/MS.

B2a, Anthelmintics

Diflubenzuron and teflubenzuron

The analytes were extracted with acetone. Solid phase extraction was used for sample clean up. The samples were analysed and quantified by LC-MS/MS (Samuelsen et al. 2014).

Emamectin

Emamectin was extracted with acetonitrile, and the extracts were purified by solid phase extraction. The samples were analysed by LC-MS/MS (Hamre et al. 2011).

Ivermectin, abamectin, doramectin, eprinomectin and moxidectin

The analytes were extracted with organic solvent, and the extract was purified by solid phase extraction. The samples were analysed by LC-MS/MS

Cypermethrin and deltamethrin

Cypermethrin and deltamethrin were extracted from the samples by soxhlet extraction and purified by gel permeation chromatography. The samples were analysed and quantified by GC-MS.

Fenbendazole

Fenbendazole was extracted using methanol and water. Sample clean up was performed by liquid-liquid extraction. The samples were analysed and quantified by LC-MS/MS.

Praziquantel

Praziquantel was extracted from the sample by acetone, and determined by LC-MS/MS.

B3a, Organochlorine compounds

Dioxins, dl-PCBs and PCB-6.

This is an adaptation to modern clean-up equipment of the US-EPAs (Environmental Protection Agency) methods No. 1613 and 1668. Separation and quantification were performed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The method determines all of the 29 compounds on the WHO list: 17 PCDD / PCDF congeners, four non-ortho substituted PCBs: PCB -77, 81, 126 and 169 and eight mono-ortho substituted PCBs: PCB-105, 114, 118, 123, 156, 157, 167 and 189 (Berntssen, Julshamn et al. 2010). The PCBs included in PCB-6, PCBs no. 28, 52, 101, 138, 153 and 180, are analysed by GC-MS/MS.

PCB-6

PCB-6 was extracted by hexane using an accelerated solvent extractor. The extract was purified by sulphuric acid before detection and quantification by GC-MS (Berntssen et al. 2011). The method quantifies the PCBs no. 28, 52, 101, 138, 153 and 180.

Chlorinated pesticides

Pesticides were extracted by organic solvent, and the extract were cleaned-up by column chromatography, before the pesticides were analysed by HRGC-HRMS.

B3b, Organophosphorus compounds

Azamethiphos and dichlorvos

The sample material was extracted with acetonitrile. The analytes were analysed by LC-MS/MS.

Chlorpyriphos and Pirimiphos

Chlorpyriphos, chlorpyrifos-methyl, pirimiphos-methyl and pirimiphos-ethyl were extracted from the samples by organic solvent. The samples were analysed and quantified by GC-MS.

B3c, elements

Lead, mercury, cadmium and arsenic

The sample was decomposed by acid treatment, assisted by heat and high pressure. The metals were detected and quantified by inductively coupled plasma mass spectrometer (ICP-MS) (Julshamn, Maage et al. 2007).

Inorganic Arsenic

Inorganic arsenic was extracted by hydrochloric acid in hydrogen peroxide at 90 °C. Inorganic arsenic includes As (III) and As (V). As (III) was oxidised to As (V) during the extraction. Inorganic arsenic was analysed by LC-ICP-MS.

Methylmercury

Methylmercury was extracted by Tetramethylammonium Hydroxide. The pH was adjusted before derivatization and extraction by hexane. The samples were analysed by GC-ICP-MS.

Tributyltin

Tributyltin was extracted by acetic acid/methanol. The pH was adjusted before derivatization and extraction by hexane. The samples were analysed by GC-ICP-MS.

B3d, Mycotoxins

Ochratoxin A.

Ochratoxin A. was extracted by sodium bicarbonate/methanol. The sample was subjected to clean-up by an immunoaffinity column and quantification by HPLC with fluorescence detection.

Fumonisin

Fumonisins were extracted by acetonitrile/water/methanol. The samples were analysed by LC-MS/MS.

B3e, Dyes

Malachite green (MG), crystal violet (CV), brilliant green (BG) and their metabolites.

The analytes were extracted with acetonitrile and dichloromethane. Samples clean-up were performed by solid phase extraction, and the analytes were determined by LC-MS/MS.

B3f, Others

PBDE

PBDEs were extracted by dichloromethane and hexane using an accelerated solvent extractor. Sulphuric acid were used for samples clean-up. The PBDEs were determined by GC-MS.

HBCD and TBBPA

The analytes were extracted by organic solvent. Column chromatography were used for sample clean up before the analytes were detected and quantified by LC-MS/MS.

PFC

PFCs were extracted by methanol, the extract were purified by solid phase extraction. PFCs were analysed by LC-MS/MS.

PAH

PAHs were extracted by KOH/methanol, the extract were purified by solid phase extraction. PAHs were analysed by GC/MS.

Table 3.1. Number of fish analysed for each substance.

	Compounds	Fish*	Atlantic salmon	Rainbow trout	Turbot	Atlantic halibut	Arctic char	Spotted wolfish
A1 Stilbenes	Zeranol 17alpha-Estradiol 17alpha-Ethinyl estradiol 17beta-Estradiol beta-Zearalanol Dienestrol Diethylstilbestrol Estriol Estrone Hexestrol	790	730	40		10	10	
A3 Steroids	16-Hydroxystanozolol 17alpha-Boldenone 17alpha-Trenbolone alpha-Nandrolone Boldenone Chlor-Testosterone Epitestosterone Methyl-Boldenone Methyltestosterone Nortestosterone Stanozolol Testosterone Testosterone propionate Trenbolone Trenbolone-acetate	775	725	30		10	10	
A6 Illegal drugs	Chloramphenicol	800	740	30		10	15	5
	Metronidazole Metronidazole-OH	760	715	35			10	
	Nitrofuran metabolites (AOZ, AMOZ, AHD, SEM)	815	745	40		10	15	5
	Malachite green Leuco malachite green Crystal violet Leuco crystal violet Brilliant green	760	720	25			15	
B1 Chemical Method (muscle)	Oxolinic acid, Flumequin, Cinoxacin, Ciprofloxacin, Danofloxacin, Difloxacin, Enoxacin, Enrofloxacin, Lomefloxacin, Marbofloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Sarafloxacin	355	340	5		5	5	

	Oxytetracycline, Doxycycline, Chlortetracycline, Tetracycline	95	95					
	Florfenicol	100	95	5				
B1 Micro-biological assay (liver)	Quinolones Tetracyclines Amphenicols Sulphonamides	1665	1540	100	5	5	15	
B2 Other veterinary drugs	Diflubenzuron and teflubenzuron	800	730	70				
	Praziquantel	500	460	40				
	Fenbendazole	55	50	5				
	Emamectin	910	830	80				
	Ivermectin, Abamectin, Doramectin, Eprinomectin, Moxidectin	75	75					
	Hexaflumeron and lufenuron	305	275	30				
	Deltamethrin and cypermethrin	455	410	45				
B3a Organo-chlorine compounds	Pesticides	495	470	25				
	PCB-6, Dioxins and dl-PCBs	300	255	30	5	10		
	PCB-6	305	265	30	5	5		
B3b, Organo-phosphorus compounds	Azamethiphos and dichlorvos	305	275	30				
	Chlorpyriphos, chlorpyriphos-methyl pirimiphos-methyl and pirimiphos-ethyl	455	410	45				
B3c Chemical elements	Lead Cadmium Mercury Arsenic	495	460	15		10	10	
	Inorganic Arsenic Methylmercury	100	90	10				
	Tributyltin	350	330	20				
B3d, Mycotoxins	Ochratoxin A, Fumonisin B1 and B2	255	235	20				
B3e, Dyes* *	Malachite green Leuco malachite green Crystal violet Leuco crystal violet Brilliant green	500	465	30			5	
B3f, Others	PBDE	355	345	10				
	TBBPA and HBCD	345	335	10				
	PAH	350	335	15				
	PFC	350	330	20				

*Some of the fish collected have been analysed for two groups of substances. Hence, the number of fish specified in this table will exceed the total number of fish collected.
**According to directive 96/23, malachite green, crystal violet and brilliant green belong to the group B3e. However, these dyes are not allowed to be used for food producing animals, therefore samples analysed for dyes have been collected as both group A6 samples (illegal drugs) and group B3e samples (dyes).

4. RESULTS

4.1 Substances with anabolic effects and unauthorized substances

In 2016 a total of 940 pooled samples, comprising 4 700 fish, were analysed for illegal substances. A detection of these substances, regardless of level, will be deemed a non-compliant sample.

4.1.1 Stilbenes

The presence of stilbenes were examined in 158 pooled samples, none of the stilbenes analysed were detected.

4.1.2 Steroids

The presence of steroids were examined in 155 pooled samples, none of the steroids analysed were detected.

4.1.3 Unauthorized veterinary drugs

Chloramphenicol, nitrofurans and metronidazole were not detected in any of the 475 samples analysed for these substances. Although malachite green, crystal violet and brilliant green belong to the group B3e according to directive 96/23, these dyes are not allowed to be used for food producing animals, therefore 152 pooled samples were collected at the fish farms. Residue of crystal violet was detected in one sample at a concentration of 0.39 ng/g. Due to this finding, three other pooled samples, taken at the same time from the same fish farm were examined, and residues of crystal violet were found in two of the samples. The highest level measured was 1.2 ng/g. Residue of the metabolite leuco crystal violet was not detected

4.2 Veterinary drugs

Samples analysed for veterinary drugs were collected from fish at processing plants, and are representative of fish ready for human consumption. The maximum residue limit for veterinary drugs are set for muscle and skin in natural proportions (EU 37/2010).

4.2.1 Group B1, antibacterial agents

No residues of antibacterial agents were found in any of the samples analysed for these substances.

4.2.2 Group B2a anthelmintics

Residues of emamectin was detected in 17 out of 182 pooled samples. The highest concentration of emamectin was 94 µg/kg. This concentration is below the MRL of 100 µg/kg (EU 37/2010). Residue

of cypermethrin was detected in one out of 91 pooled samples, at a concentration of 8 µg/kg, which is below the MRL of 50 µg/kg (EU 37/2010). Residues of diflubenzuron were detected in three of 160 pooled samples. The MRL of diflubenzuron is 1000 µg/kg, and the highest level measured was 4.1 µg/kg Furthermore residue of teflubenzuron were detected in one of 160 pooled samples, at a concentration of 13 µg/kg, the MRL of teflubenzuron is 500 µg/kg. Residues of other agents in this group were not detected in any of the samples. LOQs for the substances are specified in Table 8.4.

4.2.3 Group B3b. Organophosphorus compounds

The levels of the B3b substances azamethiphos and dichlorvos were determined in 61 pooled samples. Residues of these agents were not detected in any of the examined samples.

4.2.4 Group B3e, Dyes

According to directive 96/23, malachite green, crystal violet and brilliant green belong to the group B3e. However, these dyes are not allowed to be used for food producing animals (EU 37/2010), therefore samples analysed for dyes have been collected as both group A samples at fish farms (A6, illegal drugs) and group B samples at processing plants (B3e, dyes). A total of 100 pooled samples were collected at processing plants, and analysed with respect to malachite green and its metabolite leuco malachite green, crystal violet and its metabolite leuco crystal violet, and brilliant green. No residues of these agents were detected in the samples collected at processing plants.

4.3 Contaminants

Samples analysed for contaminants were collected from fish at processing plants. They are representative of fish ready for the marked.

4.3.1 Group B3a, Organochlorine compounds

The levels of organochlorine compounds were determined in 220 pooled samples. The results are summarised in Table 4.1 to 4.3.

4.3.1.1 Organochlorine pesticides

For a number of pesticides the amount present is calculated as a sum where metabolites or other transformation products are included (SANTE 2015). The results for these groups of pesticides are presented in table 4.1. To calculate the sum of the components, conversion factors (table 8.5) are used to adjust for different molecular weights (SANTE 2015). The sums in table 4.1. were calculated according to the upper bound (UB) formula. When using UB calculations, the numerical value of LOQ is substituted for analytes with levels below LOQ. UB represents a “worst case scenario”.

Table 4.1. The sum of groups of pesticides (µg/kg w.w.) in fillets of farmed fish

		Atlantic Salmon	Rainbow trout
Sum	N	94	5
DDT	Median (UB)	5.1	5.5
	Max (UB)	9.5	8.4
Endosulfan	Median (UB)	0.83	0.84
	Max (UB)	1.2	1.1
Aldrin and dieldrin	Median (UB)	1.0	1.1
	Max (UB)	1.9	1.4
Chlordane	Median (UB)	0.73	0.75
	Max (UB)	1.2	0.81
Heptachlor	Median (UB)	0.42	0.49
	Max (UB)	0.54	0.59
Toxaphene	Median (UB)	2.1	2.0
	Max (UB)	4.6	2.8

The results for other pesticides are summarised in Table 4.2. The highest level measured was 3.9 µg/kg w.w. of hexachlorobenzene. No MRLs have been established for pesticides in fish.

Table 4.2. Pesticides (µg/kg w.w.) in fillets of farmed fish.

Pesticide		Atlantic salmon	Rainbow Trout	LOQ
	Samples	94	5	
α-Hexachlorocyclo-hexane	#Values*	6	1	
	Median	-	-	
	Max	0.19	0.11	0.077-0.24
β-Hexachlorocyclo-hexane	#Values	11	1	
	Median	-	-	
	Max	0.29	0.15	0.077-0.24
δ-Hexachlorocyclo-hexane	#Values	0	0	
	Median	-	-	
	Max	LOQ	LOQ	0.077-0.24
γ-Hexachlorocyclo-hexane	#Values	2	1	
	Median	LOQ	LOQ	
	Max	0.11	0.13	0.077-0.24
Hexachlorobenzene	#Values	94	5	
	Median	1.1	1.1	
	Max	3.9	3.0	0.033-0.10
Pentachlorobenzene	#Values	0	0	
	Median	-	-	
	Max	LOQ	LOQ	0.15-0.48
Endrin	#Values	0	0	
	Median	-	-	
	Max	LOQ	LOQ	0.092-0.29
Trans-nonachlor	#Values	94	5	
	Median	0.52	0.67	
	Max	1.2	0.82	0.02-0.05
Mirex	#Values	4	0	
	Median	-	-	
	Max	0.064	LOQ	0.045-0.096
Octachlorstyrol	#Values	93	5	
	Median	0.15	0.13	
	Max	0.71	0.31	0.015-0.044
* Numbers of samples with level above the LOQ				

4.3.1.2 Dioxin, dl-PCBs and PCB-6

The sums of dioxins, dioxins + dl-PCBs and PCB-6 are calculated according to the upper bound procedure (EU 1259/2011). Accordingly, the numerical LOQ values were substituted for congeners with levels below LOQ.

The level of dioxins and dl-PCBs are reported as ng toxic equivalents 2005 (TEQ05)/kg, and represent the sum of 17 different PCDD/F and 12 dl-PCBs where each congener has been multiplied by a Toxic equivalency factor (TEF). TEF values are determined by WHO, and the toxicity of each congener has

been expressed relative to the most toxic form of dioxin, 2,3,7,8-TCDD which has a TEF value of 1 (EU 1259/2011).

The sums of dioxins, dioxins and dl-PCBs and PCB-6 in farmed fish are shown in Table 4.3. In 2016, the data is mainly represented by Atlantic salmon, but samples from rainbow trout, Atlantic halibut, and turbot have also been examined. The median of the sum of dioxins in salmon was 0.22 ng TEQ/kg w.w. The maximum value in salmon was of 0.47 ng TEQ/kg w.w. The highest measured level was in Atlantic halibut, 0.66 ng TEQ/kg w.w. This is well below the EU maximum limit of 3.5 ng TEQ/kg w.w.

The median of the sum of all 29 PCDD/F and dl-PCBs in salmon was 0.61 ng TEQ/kg w.w. The highest level was measured in an Atlantic halibut, 1.9 ng TEQ/kg w.w. All values were below the EU maximum limit of 6.5 ng TEQ/kg w.w. (table 4.3).

The median of PCB-6 in salmon was 5.3 µg/kg w.w. The EUs maximum limit for the PCB-6 in fish is 75 µg/kg w.w. and the highest concentration of PCB-6 measured in 2016 was 20 µg/kg w.w. in an Atlantic halibut (table 4.3).

Table 4.3 Dioxins, dl-PCBs and PCB-6 in fillets of farmed fish.

		Atlantic Salmon	Rainbow trout	Atlantic halibut	Turbot	Maximum limit
Sum dioxins (ng TEQ/kg w.w.)	Samples	50	6	2	1	
	Median (UB)	0.22	0.18	0.61	0.16	
	Mean (UB)	0.23	0.20	0.61	0.16	
	Max (UB)	0.47	0.29	0.66	0.16	3.5
Sum dioxin + dl-PCBs (ng TEQ/kg w.w.)	Samples	50	6	2	1	
	Median (UB)	0.61	0.56	1.9	-	
	Mean (UB)	0.60	0.59	1.9	-	
	Max (UB)	1.6	0.77	1.9	0.61	6.5
PCB-6 (µg/kg w.w.)	Samples	102	12	3	2	
	Median (UB)	5.3	5.9	17	5.2	
	Mean (UB)	5.3	6.1	16	5.2	
	Max (UB)	14	9.5	20	5.3	75

4.3.2 Group B3b. Organophosphorous compounds

Chlorpyriphos, chlorpyrifos-methyl, pirimiphos-methyl and pirimiphos-ethyl were examined in 91 samples, none of the substances were found.

4.3.3 Group B3c, Chemical elements

Mercury was measured in 99 samples. The median level in salmon was 0.015 mg/kg, while the highest level measured in salmon was 0.049 mg/kg. Atlantic halibut had the highest level of mercury, 0.076

mg/kg w.w. (table 4.4). The EU maximum limit is 0.50 mg/kg w.w. for mercury in the species analysed in this report (EU 1881/2006). Thus, the measured concentrations in all samples are well below the maximum limit. In addition to mercury, methylmercury was measured in 20 samples. The result showed that the levels of methylmercury (Table 8.1) were similar to the level of mercury in the same samples.

Arsenic is determined as the elemental concentration "total arsenic", comprising the sum of all arsenic species (table 4.4). The median level of total arsenic in salmon was 0.68 mg/kg w.w. The highest concentration, 5.3 mg/kg w.w., was found in Atlantic halibut. None of the samples had concentrations of inorganic arsenic above the LOQ (Table 8.1), indicating that arsenic in fish is present mainly as organo-arsenic compounds of low toxicity (Shiomi 1994). There is currently no EU maximum limit for neither total arsenic nor inorganic arsenic in fish fillets.

Cadmium or lead were not found in measurable quantities in any of the 99 samples analysed.

Tributyltin was detected in one of the 70 samples analysed, at a concentration of 0.61 µg Sn/kg w.w. There is currently no EU upper limit for tributyltin in fish fillets.

Table 4.4. Chemical elements in fillets of farmed fish

Element			Atlantic Salmon	Rainbow trout	Atlantic halibut	Arctic char	LOQ	EU-Limit
Arsenic (mg/kg w.w.)		N	92	3	2	2		
		#Values	92	3	2	2		
		Median	0.68	0.83	4.3	1.32		
		Max	2.5	1.3	5.3	1.6	0.003	n.a.
Cadmium (mg/kg w.w.)		N	92	3	2	2		
		#Values	0	0	0	0		
		Median	-	-	-	-		
		Max	LOQ	LOQ	LOQ	LOQ	0.001-0.002	0.050
Mercury (mg/kg w.w.)		N	92	3	2	2		
		#Values	92	3	2	2		
		Median	0.015	0.023	0.069	0.023		
		Max	0.049	0.025	0.076	0.028	0.002	0.50
Lead (mg/kg w.w.)		N	92	3	2	2		
		#Values	0	0	0	0		
		Median	-	-		-		
		Max	LOQ	LOQ	LOQ	LOQ	0.005-0.01	0.30
Tri butyltin (µg Sn/kg w.w.)		N	66	4	-	-		
		#Values	1	0				
		Median	-	-				
		Max	0.61	LOQ			0.3-0.5	n.a.

4.3.4 Group B3d, Mycotoxins

Ochratoxin-A and fumonisin were analysed in 51 pooled samples. Four of the samples were rainbow trout, and the rest were salmon. No mycotoxins were detected.

4.3.5 Group B3f, others

PBDE, TBBPA and HBCD are compounds in the group of brominated flame retardants (BFR). The results for the PBDEs are shown in table 4.5. PBDE-47 contributes most to the sum of PBDE in salmon and rainbow trout.

Table 4.5. PBDE (µg/kg w.w.) in fillets of farmed fish.

		Atlantic salmon	Rainbow trout	**LOQ**
	Samples	69	2	
PBDE 28	#Values	66	2	
	Median	0.01	0.02	
	Max	0.03	0.02	0.003-0.005
PBDE 47	#Values	69	2	
	Median	0.21	0.25	
	Max	0.76	0.27	0.006-0.009
PBDE 66	#Values	35	2	
	Median	0.005	0.01	
	Max	0.05	0.02	0.003-0.005
PBDE 99	#Values	69	2	
	Median	0.04	0.04	
	Max	0.11	0.05	0.006-0.009
PBDE 100	#Values	69	2	
	Median	0.05	0.07	
	Max	0.17	0.08	0.003-0.005
PBDE 119	#Values	7	0	
	Median	-	-	
	Max	0.02	LOQ	0.003-0.005
PBDE 138	#Values	0	0	
	Median	-	-	
	Max	LOQ	LOQ	0.006-0.009
PBDE 153	#Values	65	2	
	Median	0.01	0.01	
	Max	0.03	0.02	0.003-0.005
PBDE 154	#Values	69	2	
	Median	0.03	0.05	
	Max	0.13	0.06	0.003-0.005
PBDE 183	#Values	0	0	
	Median	-	-	
	Max	LOQ	LOQ	0.006-0.009

TBBPA was below LOQ in all, but one sample. Sum HBCD was analysed in 69 samples, the highest concentration measured was 0.93 µg/kg w.w in a salmon sample. The median concentration of sum HBCD in salmon was 0.18 µg/kg w.w. There is currently no EU maximum limit for BFR compounds in food.

Table 4.6 TBBPA and sum HBCD (µg/kg w.w.) in fillets of farmed fish.

		Atlantic Salmon	Rainbow trout	LOQ
	Samples	67	2	
TBBPA	#Values	1	0	
	Median	-	-	
	Max	0.27	LOQ	0.03-0.2
Sum HBCD (α,β,γ) (UB)	Median	0.18	0.21	
	Max	0.93	0.24	

A total of 70 samples, mostly salmon but also rainbow trout, were analysed for the PFCs. All measured values were below the LOQ (Table 8.2). EU has no maximum level for PFCs in food.

The results for PAH are summarised in table 8.3. PAH was analysed in 70 samples, all measurements were below the LOQ. There is no longer a maximum limit for PAH in fresh fish, since it has been concluded that PAH does not accumulate in muscle meat due to rapid metabolism (EU 835/2011).

5. DISCUSSION

5.1 Unauthorized substances

Residues of crystal violet were detected in fish from one fish farm. Crystal violet is a known fungicide, however, it is not authorized for use in food producing animals. Crystal violet in salmon is rapidly metabolized to leuco crystal violet (Chan 2012). Hence detection of crystal violet without a concomitant detection of leuco crystal violet could indicate a contamination of the sample. Further investigation performed by the NFSA and NIFES, revealed that during sampling the fish muscle had been put in a plastic bag and marked by a marker pen. For two of the samples, these bags were still available. To examine if the marker pen contained crystal violet, and whether this could have contaminated the samples, fish muscle were rubbed onto the writing on the plastic bag. The analysis of these fish samples showed levels of crystal violet of 150 ng/g. Therefore the use of the marker pen on the plastic bag that the samples were contained in is a likely source for crystal violet contamination. The NFSA concluded that the fish had been contaminated due to the use of a marker pen.

5.2 Veterinary drugs

The level of the measured anti-sea lice agents was below the MRL in all samples. However, one sample contained emamectin at a concentration of 94 ng/g, which is closer to the MRL (100 ng/g) compared to previous years. Residues of diflubenzuron were found in three samples. Although the levels were well below the MRL, there is a discussion about the use of diflubenzuron as the carcinogenic compound p-chloroaniline, is a possible metabolite in fish. Recently, JECFA concluded that no MRL could be set for diflubenzuron, due to lack of data in regards to p-chloroaniline (JECFA 2015). However, diflubenzuron is still allowed for use in the EU (EU 37/2010).

In 2016, no residues of antibiotics or endoparasitic agents were detected, this is in accordance with the results from the last decade.

Most samples reviewed in this report are from fillet of farmed fish. However, as the liver has a central function in the distribution and elimination of drugs, liver samples were analysed for certain antibiotics. Even though the bioassay used for the antibacterial agents is less sensitive than the chemical analytical methods, the higher concentrations of antibacterial agents in liver compared to fillet enhance the ability to detect any residues. Moreover, the ability of the bio-assay to detect a wider range of antibiotics than the more specific chemical methods, renders the method useful for screening purposes. Any positive

detection by the bioassay has to be verified by chemical analysis of the corresponding fillet sample sampled from the same fish.

5.3 Contaminants

No environmental contaminants were found above the EU maximum limits. However, EUs maximum limits for food are not toxicologically based but derived from the ALARA (as low as reasonably achievable) principle, with the aim to prevent those commodities with the highest contaminant levels to reach the market. In order to evaluate the toxicological relevancy of the different contaminant levels, tolerable intake values are implemented. Tolerable weekly/daily intake (TWI/TDI) is the weekly/daily intake of a chemical that can occur over a lifetime without appreciable health risk. The TWI is a threshold level set by international risk assessment bodies, such as EFSA in Europe, and WHO or JECFA on a worldly basis.

For dioxin and dl-PCBs a TWI of 14 pg WHO-TEQ/kg bw has been established (SCF, 2001). Using the median value from 2016, an intake of 200 g farmed salmon will contribute to 12% of TWI for a person of 70 kg, while the intake of 200 g of farmed Atlantic halibut will contribute to 39% of TWI for a person of 70 kg.

The general trend for most contaminants analysed in this program, is that the levels in farmed fish are significantly declining, The median level of sum dioxins + dl-PCBs in farmed salmon is higher in 2016 than 2015 (0.61 ng TEQ/kg w.w. compared to 0.49 ng TEQ/kg w.w.). However, there has been an overall decline in the median level of sum dioxins + dl-PCBs in farmed salmon from 2002 (1.4 ng TEQ/kg w.w.). The median level of mercury in farmed salmon has declined from 0.029 mg/kg w.w. in 2002 to 0.015 mg/kg w.w. in 2016. The decrease in several of the contaminants over the last decades mainly reflects the transition from the use of marine ingredients, fishmeal and fish oil, to more vegetable ingredients in the feed production.

6. CONCLUSION

Residues of crystal violet were detected in samples from one fish farm. The Norwegian Food Safety Authority concluded that the samples had been contaminated during sampling.

Anti sea lice agents; emamectin, cypermethrin diflubenzuron or teflubenzuron, were detected in a total of 22 samples. The levels measured were below their respective MRLs. No residues of other veterinary drugs, including antibiotics, were found.

For contaminants, no samples exceeded the EUs maximum limits, where such limits have been established.

7. RECOMMENDATIONS

Due to the present status of illegal and undesirable substances in farmed fish, there is no need for specific recommendations.

8. TABLES

Table 8.1. Inorganic arsenic and methylmercury in fillets of farmed fish

		Atlantic Salmon	Rainbow trout	LOQ
	N	18	2	
Inorganic arsenic (µg/kg w.w.)	#Values	0	0	
	Median	-		
	Max	LOQ	LOQ	4-6
Methyl-mercury (mg Hg/kg w.w.)	#Values	18	2	
	Median	0.019	0.024	
	Max	0.026	0.026	0.001

Table 8.2. PFCs (µg/kg w.w.) in fillets of farmed fish

Compound	Atlantic Salmon	Rainbow trout	Max value	LOQ
PFBA	66	4	<LOQ	1.0
PFBS				0.8
PFDA				0.5
PFDoDA				0.8
PFDS				1
PFHpA				0.7
PFHxA				0.9
PFHxDA	57			13
PFHxS	66			0.8
PFNA				0.9
PFOA				1.3
PFODA	57			7
PFOS	66			0.8
PFOSA				1.2
PFPeA				6
PFTeDA				1.1
PFTrDA				1.2
PFUdA				1

Table 8.3. PAH (µg/kg w.w.) in fillets of farmed fish

PAH congener	Atlantic salmon	Rainbow trout	Max	LOQ
5-Methylchrysene	67	3	<LOQ	1
Benzo(a)antracene				0.5
Benzo(a)pyrene				0.5
Benzo(b)fluoranthene				0.5
Benzo(ghi)perylene				0.5
Benzo(j)fluoranthene				0.5
Benzo(k)fluoranthene				0.5
Benzo(c)Fluorene				1
Chrysene				0.5
Cyclopenta(c,d)pyrene				1
Dibenzo(a,e)pyrene				1
Dibenzo(a,h)anthracene				0.5
Dibenzo(a,h)pyrene				1
Dibenzo(a,i)pyrene				1
Dibenzo(a,l)pyrene				1
Indeno(1,2,3-cd)pyrene				0.5

Table 8.4. Summary of analytical methods

Group of substances	Compounds[1]	Method	LOD (µg/kg w.w.)	LOQ (µg/kg w.w.)	Level of action (µg/kg w.w.)	Labora-tory
A1 Stilbenes	Diethylstilbestrol	LC-MS/MS	1		Presence	Eurofins
	Dienestrol		1			
	Hexestrol		1			
	B-Estradiol		1			
	α-Estradiol		1			
	Estriol		1			
	Estrone		1			
	Ethinyl estradiol		1			
A3 Steroids	α-nandrolon	LC-MS/MS	1		Presence	Eurofins
	β-nandrolon		1			
	α-trenbolon		1			
	β-trenbolon		1			
	Trenbolone-acetate		2			
	16-Hydroxy stanozolol		1			
	α -Boldenone		1			
	Boldenone		1			
	Chlor-Testoste rone (Clostebol)		1			
	Epitestosterone		1			
	Methyl-Boldenone (Dianabol)		1			
	Methyltestosterone		1			
	Nortestosterone/ Nandrolone		1			
	Stanozolol		1			
	Testosterone		1			
	Testosterone-propionate		2			
A6 Annex IV substances	Chloramphenicol	LC-MS/MS	0.25		Presence (MRPL = 0.3)	NIFES
	Metronidazole	LC-MS/MS	0.3		Presence (MRPL = 3.0)	
	Hydroxy-metronidazole[3]		2.0			
	Nitrofuran AOZ	LC-MS/MS	0.5		Presence (MRPL =1.0)	
	Nitrofuran AHD		0.6		Presence (MRPL =1.0)	
	Nitrofuran AMOZ		0.4		Presence (MRPL =1.0)	
	Nitrofuran SEM		0.5		Presence (MRPL= 1.0)	
B1 Antibacterial	Quinolones	3-plate Screening	200		100-600	NIFES
	Tetracyclines		200		100	

Substances Micro-biological method	Amphenicols	Method[2]	200		1000	
	Sulfonamides		400		100	
B1 Antibacterial substances Chemical method	Oxolinic acid	LC-MS/MS		30	100	Eurofins
	Flumequine			30	600	
	Cinoxacin			30	n.a.	
	Ciprofloxacin			30	100	
	Danofloxacin			30	100	
	Difloxacin			30	300	
	Enoxacin			30	n.a.	
	Enrofloxacin			30	100	
	Lomefloxacin			30	n.a.	
	Marbofloxacin			30	n.a.	
	Nalidixic acid			30	n.a.	
	Norfloxacin			30	n.a.	
	Ofloxacin			30	n.a.	
	Sarafloxacin			30	30	
	Oxytetracycline	LC-MS/MS		30	100	Eurofins
	Doxycycline,			30	100	
	Chlortetracycline,			30	100	
	Tetracycline			30	100	
	Florfenicol	LC-MS/MS		0.5	1000	NIFES
B2a Anthelmintics	Praziquantel	LC-MS/MS		1	n.a.	NIFES
	Fenbendazole[3]	LC-MS/MS		1	n.a.	
	Emamectin[4]	LC-MS/MS		2-10	100	NIFES/ Eurofins
	Diflubenzuron	LC-MS/M		1-10	1000	
	Teflubenzuron			1-50	500	
	Hexaflumeron			50	500	
	Lufenoron			50	1350	
	Ivermectin			2	n.a.	Eurofins
	Abamektin,			2	n.a.	
	Doramektin,			2	n.a.	
	Eprinomektin,			2	n.a.	
	Moxidectin			2	n.a.	
	Cypermethrin	GC-MS		5	50	
	Deltamethrin			10	10	
B3a Organo-chlorine compounds	Dioxins and dlPCB	HRGC-HRMS		0.0001-0.1 ng TEQ/kg	6.5 ng TEQ/kg	NIFES
	PCB-6	GC-MS GC-MS/MS		0.004 – 0.5	75	
	Pesticides	HRGC-HRMS		0.003-0.8	n.a.	Eurofins
B3b Organo-phosphorus compounds	Azametiphos	LC-MS/MS		10	n.a.	Eurofins
	Dichlorvos					
	Chlorpyriphos Chlorpyrifos-methyl	GC-MS		5	n.a.	
	Pirimiphos-methyl Pirimiphos-ethyl			10	n.a.	
B3c Chemical elements	Lead	ICP-MS		0.005-0.01 mg/kg	0.3 mg/kg	NIFES
	Cadmium			0.001-0.002 mg/kg	0.05 mg/kg.	
	Arsenic			0.003 mg/kg	n.a.	

	Mercury			0.002 mg/kg	0.5 mg/kg	
	Inorganic arsenic	LC-ICP-MS		4-6	n.a.	
	Methylmercury[3]	GC-ICP-MS		1	n.a.	
	Tributyltin	GC-ICP-MS		0.3-0.5	n.a.	
B3d Mycotoxins	Ochratoxin A	HPLC-FLU		0.5	n.a.	Eurofins
	Fumonisin B1 Fumonisin B2	LC-MS/MS		20		
B3e, dyes	Malachite green[3]	LC-MS/MS	0.15		Presence (MRPL=2)	NIFES
	Leuco malachite green		0.15			
	Crystal violet		0.30		Presence	
	Leuco crystal violet		0.15		Presence	
	Brilliant green[3]		0.15		Presence	
B3f, others	PBDE	GC-MS		0.003-0.009	n.a.	NIFES
	HBCD	LC-MS/MS		0.006-0.01	n.a.	Eurofins
	TBBPA			0.03-0.2	n.a.	
	PAH	GC-MS		0.5-1.0	n.a.	Eurofins
	PFC	LC-MS/MS		0.5-13	n.a.	NIFES

[1] All methods used muscle as sample matrix except for microbiological methods for antibacterial substances (B1), were liver was used

[2] Only screening method, positive results have to be confirmed by a chemical method.

[3] Not accredited

[4]The method used was unaccredited for a period.

Table 8.5. Calculation of sums for certain pesticides.

Sum	Substances included in the sum	Conversion factor
DDT (sum of p,p-DDT, o,p-DDT, p,p-DDD, o,p-DDD, p,p-DDE,and o,p-DDE expressed as DDT)	op-DDT	1
	pp-DDT	1
	op-DDD	1.108
	pp-DDD	1.108
	op-DDE	1.115
	pp-DDE	1.115
Endosulfan (sum of alpha- and beta-isomers and endosulfan-sulphate expressed as endosulfan)	alpha-endosulfan	1
	beta-endosulfan	1
	endosulfan sulphate	0.962
Aldrin and dieldrin (Aldrin and dieldrin combined expressed as dieldrin)	dieldrin	1
	aldrin	1.044
Chlordane (Sum of cis- and trans-isomers and oxychlordane expressed as chlordane)	trans-chlordane	1
	cis-chlordane	1
	oxychlordane	0.967
Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	heptachlor	1
	trans-heptachlor epoxide	0.959
	cis-heptachlor epoxide	0.959
Toxaphene (sum of toxaphene 26, toxaphene 50 and toxaphene 62)	Toxaphene 26	1
	Toxaphene 50	1
	Toxaphene 62	1

REFERENCES

Berntssen, M. H. G., Julshamn, K., Lundebye, A. K. (2010). Chemical contaminants in aquafeeds and Atlantic salmon (*Salmo salar*) following the use of traditional- versus alternative feed ingredients. Chemosphere 78: 637-646.

Berntssen, M. H. G., Maage A., Julshamn, K., Oeye, B. E., Lundebye, A. K. (2011). Carry-over of dietary organochlorine pesticides, PCDD/Fs, PCBs, and brominated flame retardants to Atlantic salmon (*Salmo salar*) fillets. Chemosphere 83: 95-103.

Chan, D., Tarbin, J. A., Stubbings, G., Kay, J., & Sharman, M. (2012). Analysis of incurred crystal violet in Atlantic salmon (*Salmo salar L.*): comparison between the analysis of crystal violet as an individual parent and leucocrystal violet and as total crystal violet after oxidation with 2, 3-dichloro-5, 6-dicyanobenzoquinone. Food Additives & Contaminants: Part A, 29, 66-72.

CRL (2007). CRL guidance paper (7 december 2007) CRLs view on state of the art analytical methods for national residue control plans.

EU (1996). Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products.

EU (2002). 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

EU (2006). Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

EU (2010). Commission Regulation (EU) No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.

EU (2011). Commission regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs.

EU (2011). Commission Regulation (EU) No. 1259/2011 amending Regulation (EC) No. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs.

Hamre, L. A., Lunestad, B. T., et al. (2011). An evaluation of the duration of efficacy of emamectin benzoate in the control of Caligus curtus Muller infestations in Atlantic cod (*Gadus morhua*). Journal of Fish Diseases 34: 453-457.

Johnsen, C. A., Hagen, Ø., Adler, M., Jönsson, E., Kling, P., Bickerdike, R., Solberg, C., Björnsson, B. T., Bendiksen, E.Å. (2011). "Effects of feed, feeding regime and growth rate on flesh quality, connective tissue and plasma hormones in farmed Atlantic salmon (*Salmo salar*). Aquaculture 318: 343-354.

Joint FAO/WHO Expert Committee on Food Additives (2015). Summary and Conclusions of the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA).81st meeting on Veterinary Drug Residues in Food.

Julshamn, K., Maage, A., Norli, H. S., Grobecker, K. H., Jorhem, L., Fecher, P. (2007). Determination of arsenic, cadmium, mercury, and lead by inductively coupled plasma/mass spectrometry in foods after pressure digestion: NMKL1 interlaboratory study. Journal of Aoac International 90: 844-856.

Samuelsen, O. B., Lunestad, B. T., Farestveit, E., Grefsrud, E. S., Hannisdal, R., Holmelid, B., Tjensvoll, T., Agnalt, A. L. (2014). Mortality and deformities in European lobster (*Homarus gammarus*) juveniles exposed to the anti-parasitic drug teflubenzuron. Aquatic Toxicology 149: 8-15.

Shiomi, K. (1994). Arsenic in marine organisms: chemical forms and toxicological aspects. Advances in environmental science and technology-New York: 261.

European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food (2001).Opinion of the SFC on the risk assessment of dioxins and dioxin-like PCBs in

food. [Update based on new scientific information available since the adoption of the the SCF opinion of 22nd November 2000. Adopted 30 May 2001.
European commission directorate-general for health and food safety (2015). Safety of the Food Chain Pesticides and biocides. SANTE/11945/2015.

ISBN: 978-82-91065-48-9