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

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Content

1 Summary

This report summarises the monitoring data collected in 2017 on the status of illegal substances, pharmaceuticals and contaminants in Norwegian farmed fish. A total of 13 640 fish were collected, of these almost 40% were analysed for illegal compounds, approximately 35% were analysed for veterinary drugs, and about 25% were examined for contaminants. Official inspectors from the Norwegian Food Safety Authority performed the sampling.

Samples examined for illegal compounds could be collected at all stages of farming and are representative of farmed fish under production. The samples were analysed for substances with anabolic effects or unauthorized substances. No residues of illegal compounds 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 three anti sea lice agents; Emamectin, cypermethrin or diflubenzuron, were found in 16 samples. The levels present were below the Maximum Residue Limit (MRL) for all samples. Other veterinary drugs, like 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 the human consumption. The samples were analysed for dioxins, 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 declining trend that previously has been observed for several of the contaminants seems to have stopped and today's level are similar to the results from the last years.

2 Introduction

1.1 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

B2d: Sedatives

B3a: Organochlorine compounds

B3b: Organophosphorus compounds

B3c: Chemical elements

B3d: Mycotoxins

B3e: Dyes

B3f: Others

1.2 Group A, Substances with anabolic effects and unauthorized substances

Samples examined for illegal compounds were collected by official inspectors at the farm, without prior notification to the farmers. Fish are sampled at all stages of farming and are representative of farmed fish during production. Group A includes growth promoters like steroids and stilbenes, and unauthorized drugs. Unauthorized drugs considered most relevant for aquaculture are chloramphenicol, nitrofurans, metronidazole and dyes. The dyes; malachite green, crystal violet and brilliant green are not allowed to use for food producing species (EU 2010/37), they are therefore considered an A substance and hence sampled throughout the production chain. However, according to directive 96/23 these dyes belong to the group B3e. Therefore some of the samples assigned to analysis of dyes were also collected at the slaughterhouse.

To ensure harmonized levels for the control of banned substances, the used analytical methods should meet minimum required performance limits (MRPLs) set by the European Union (2002/657/EC), and European reference laboratories (EU-RLs), (CRL 2007). Table. 8.3 gives an overview of MRPLs of relevant compounds.

1.3 Group B, veterinary drugs

Samples examined for veterinary drugs were 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 evaluated. 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.

1.4 Group B, contaminants

Samples examined for contaminants were 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.

2. Material and methods

3.1 Sampling

Samples were taken on fish farms or slaughterhouses, by official inspectors, in all fish-producing regions in Norway. The sampling plan was randomised with regards to season and region. In 2017 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 Atlantic cod *(Gadus morhua).*

Samples were transported to IMR 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 also 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 comprising five fish from the same cage/farm

3.2 Pre-treatment

Upon arrival at IMR 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 were 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 8.3). A summary of the analytical methods and their limit of detection (LOD) or limit of quantification (LOQ) are shown in table 8.3. 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 sample blank and 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, or by analysing certified reference material (CRM), where such exist.

3.3.1 Group A substances

A1, Stilbenes

Stilbenes were extracted by water and acetonitrile. Liquid-liquid extraction was used for sample clean up. The stilbenes were and analysed by LC-MS/MS.

A3, Steroids

Steroids were extracted by water and acetonitrile. Liquid-liquid extraction followed by 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

Malachite green (MG), crystal violet (CV), brilliant green (BG)

The analytes were extracted with acetonitrile and dichloromethane. Samples clean-up were performed by solid phase extraction. MG, CV, BG and the metabolites leuco malachite green (LMG) and leuco crystal violet (LCV), 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, a plate containing growth agar and a specific bacterial strain was added. Small pieces of liver were placed on the plates before incubation. 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 had to be verified by chemical analysis of muscle and skin.

Oxolinic acid and flumequine
The analytes were extracted with acetonitrile. Liquid-liquid extraction was used to purify the extract. The analysis was performed by LC-MS/MS.

Oxytetracyclin
The analyte was extracted with acetonitrile. Liquid-liquid extraction was used to purify the extract. Oxytetracyclin was 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 by LC-MS/MS (Samuelsen et al. 2014).

Emamectin
Emamectin was extracted with acetonitrile, and analysed by LC-MS/MS.

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

Cypermethrin and deltamethrin
Cypermethrin and deltamethrin were extracted by soxhlet extraction. The extracts were purified by gel permeation chromatography. The samples were analysed by GC-MS/MS.

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

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

B2d, Sedatives

Isoeugenol
Isoeugenol is analysed by GC coupled to a flame ionization detector (FID).

B3a, Organochlorine compounds

Dioxins, dl-PCBs, PCB-6 and PBDEs.
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. The PBDEs were analysed by GC/MS in a relevant solvent fraction from the EPA clean-up procedure (Pirard, De Pauw et al. 2003).

PCB-6

The six PCBs were 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 analytes were extracted with acetonitrile, and analysed by LC-MS/MS.

Chlorpyriphos and Pirimiphos

Chlorpyriphos, chlorpyrifos-methyl, pirimiphos-methyl and pirimiphos-ethyl were extracted by soxhlet extraction. The extracts were purified by gel permeation chromatography. The samples were analysed by GC-MS/MS.

B3c, elements

Lead, mercury, cadmium and arsenic
The sample was decomposed by acid treatment, assisted by heat and high pressure. The metals were analysed 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 separated from other arsenic compounds by anionic exchange HPLC, and detected by 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

Enniatin and beauvericin
The mycotoxins; beauvericin, enniatin A, enniatin A1, enniatin B and enniatin B1were extracted with acetonitrile and water. Solid phase extraction was used for sample clean up. The mycotoxins were analysed by LC-MS/MS.

B3f, Others

HBCD
HBCD was extracted by a soxhlet apparatus, using a mixture of acetone and hexane. Sulfuric acid was used for purification. The extract was further cleaned up by an alumina column. The HBCD isomers were analysed by LC-MS/MS.

TBBPA
TBBPA was extracted by a soxhlet apparatus using a mix of acetone and hexane. Sulfuric acid was used for purification. O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used for derivatization. The extract was purified using column chromatography. TBBPA was analyzed by GC-MS using Electron Ionization (EI).

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

PAH
PAHs were extracted by dichloromethane and cyclohexane by the use of an Accelerated Solvent Extractor (ASE). The extract was purified by solid phase extraction and analysed by GC-MS/MS.

Table 3.1. Number of fish analysed for each substance.

Compounds	Fish	Atlantic salmon	Rainbow trout	Turbot	Atlantic halibut	Arctic char	Atlantic Cod
A1 Stilbenes							
Zeranol 17alpha-Estradiol 17alpha-Ethinyl-estradiol 17beta-Estradiol beta-Zearalanol Dienestrol Diethylstilbestrol Estriol Estrone Hexestrol	805	765	25		5	5	5
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	810	780	25		5		
A6 Illegal drugs							
Chloramphenicol	815	775	25		10		5
Metronidazole	770	745	25				
Nitrofuran metabolites (AOZ, AMOZ, AHD, SEM)	805	765	30		5		5
Malachite green * Crystal violet Brilliant green	800	755	40			5	
B1 Antibiotics							
Florfenicol	100	95	5				
Oxytetracycline	100	95	5				
Flumequine Oxolinic acid	340	325	15				
Quinolones (liver) Tetracyclines (liver) Amphenicols (liver) Sulphonamides (liver)	1685	1560	105		5	10	5
B2 Other veterinary drugs							
Emamectin	910	840	65			5	

Cypermethrin Deltamethrin	560	510	50				
Diflubenzuron Teflubenzuron	845	775	70				
Hexaflumeron Lufenuron	455	425	30				
Ivermectin	75	75					
Praziquantel	490	450	40				
Fenbendazole	50	50					
Isoeugenol	155	140	10			5	
B3a Organochlorine compounds							
Pesticides	505	485	20				
Dioxins dl-PCBs	365	335	20	5	5		
PCB-6	690	630	45	5	10		
B3b, Organophosphorus compounds							
Azamethiphos Dichlorvos	305	285	20				
Chlorpyriphos Pirimiphos	560	510	50				
B3c Chemical elements							
Lead Cadmium Mercury Arsenic	355	335	10			5	5
Inorganic arsenic Methylmercury	100	95	5				
Tributyltin	360	330	30				
B3d, Mycotoxins							
Beauvericin Enniatin	500	470	30				
B3e, Dyes							
Malachite green Crystal violet Brilliant green *	500	475	25				
B3f, Others							
PBDE	370	345	20	5	5		
HBCD and TBBPA	350	340	10				
PAH	350	345	5				
PFC	350	340	10				

Some of the samples collected have been analysed by more than one method. Therefore, the total of fish in this table will be higher than the number of fish collected.

***** According to directive 96/23, malachite green, crystal violet and brilliant green belongs 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 A samples (illegal drugs) and group B samples (dyes).

4. Results

4.1 Substances with anabolic effects and unauthorized substances

Totally 1 056 pooled fillet samples from 5 280 fish, were examined with respect to residues of illegal substances. The samples analysed are mainly Atlantic salmon, but also samples from rainbow trout, Atlantic halibut, and turbot have been examined. For these substances, any presence of the compound, regardless of level, will lead to a non-compliant result.

4.1.1 Stilbenes

The presence of stilbenes were examined in 161 pooled samples. None of the substances was detected in the samples analysed.

4.1.2 Steroids

The presence of steroids were examined in 162 pooled samples. None of the substances was detected in the samples analysed.

4.1.3 Unauthorized veterinary drugs

A total of 735 pooled samples were analyzed for unauthorized veterinary drugs. No residues of malachite green, crystal violet, brilliant green, chloramphenicol, nitrofurans or metronidazol were 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 is defined 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 backup sample, consisting of muscle and skin in natural proportions, in duplicate.

4.2.1 Group B1, antibacterial agents

The antibacterial agents were determined by a combination of the three plate bioassay and chemical methods. The broad groups a) quinolones, b) amphenicols and tetracyclines and c) sulphonamides, were measured in livers from 1 685 fish. Florfenicol, oxytetracyclin, flumequin and oxolinic acid, were also analysed by chemical methods in 108 pooled fillet samples, representing 530 fish. No residues were detected in any of the samples analysed. The LODs/LOQs for the compounds are listed in Table 8.3.

4.2.2 Group B2a anthelmintics

The levels of the anthelmintics; teflubenzuron, diflubenzuron, cypermethrin, deltamethrin, emamectin, ivermectin, praziquantel or fenbendazole were determined in 507 pooled muscle samples representing 2 535 fish. Emamectin was detected in 14 out of 182 pooled samples. The highest concentration of emamectin was 35 µg/kg. This concentration is below the MRL of 100 µg/kg (EU 37/2010). Cypermethrin was detected in one out of 112 pooled samples. The level measured was 7 µg/kg, which is below the MRL of 50 µg/kg (EU 37/2010). Furthermore, residues of diflubenzuron were detected in one pooled sample, at a concentration of 1.2 µg/kg, which is below the MRL of 1000 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. 8.3.

4.2.3 Group B3b. Organophosphorous compounds

The levels of the anti sea lice agents azamethiphos or dichlorvos were determined in 61 pooled fillet samples. Residues of these agents were not detected in any of the examined samples.

4.2.4 Group B3d. Sedatives

There was not found any residues of isoeugenol in the 31 samples analysed for this sedative.

4.3 Contaminants

Samples analysed for contaminants were collected from fish at processing plants, and are representative of fish ready for human consumption.

4.3.1 Group B3a, Organochlorine compounds

The levels of organochlorine compounds were determined in 239 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 8.4) 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". As an example, all results of endosulfan are below LOQ, however a result is generated based on the LOQ.

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

		Atlantic Salmon	Rainbow trout
Sum	Samples	97	4
DDT	Median (UB)	5.8	5.8
	Max (UB)	11	6.5
Endosulfan	Median (UB)	0.85	0.84
	Max (UB)	1.4	0.87
Aldrin and dieldrin	Median (UB)	1.2	1.1
	Max (UB)	2.3	1.4
Chlordane	Median (UB)	0.81	0.72
	Max (UB)	1.7	0.85
Heptachlor	Median (UB)	0.45	0.43
	Max (UB)	0.64	0.51
Toxaphene	Median (UB)	2.2	1.9
	Max (UB)	6.8	2.5

The results for the other pesticides are summarised in Table 4.2. The highest level measured was 2.0 µg/kg w.w. of trans-nonachlor and 1.8 µg/kg w.w. hexachlorobenzene.

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

Pesticide		Atlantic salmon	Rainbow Trout	LOQ
	Samples	97	4	
α-Hexachlorocyclo-hexane	#Values	4	0	
	Median	LOQ	LOQ	
	Max	0.21	LOQ	0.06-0.3
β-Hexachlorocyclo-hexane	#Values	3	0	
	Median	LOQ	LOQ	
	Max	0.19	LOQ	0.06-0.3
δ-Hexachlorocyclo-hexane	#Values	0	0	
	Median	LOQ	LOQ	
	Max	LOQ	LOQ	0.06-0.3
γ-Heδxachlorocyclo-hexane	#Values	1	0	
	Median	LOQ	LOQ	
	Max	0.060	LOQ	0.06-0.3
Hexachlorobenzene	#Values	96	4	
	Median	0.94	1.1	
	Max	1.8	1.3	0.06-0.3
Pentachlorobenzene	#Values	0	0	
	Median	LOQ	LOQ	
	Max	LOQ	LOQ	0.1-0.5
Trans-Nonachlor	#Values	97	4	
	Median	0.63	0.57	
	Max	2.0	0.74	0.02-0.1
Endrin	#Values	1	0	
	Median	LOQ	LOQ	
	Max	0.23	LOQ	0.06-0.3
Mirex	#Values	3	0	
	Median	LOQ	LOQ	
	Max	0.11	LOQ	0.02-0.1
Octachlorstyrol	#Values	95	4	
	Median	0.10	0.10	
	Max	0.26	0.20	0.01-0.5

4.3.1.2 Dioxin, dl-PCBs and PCB-6

The concentrations of dioxin, dl-PCBs and PCB-6 in farmed fish are shown in Table 4.3. The data is mainly represented by Atlantic salmon, but also samples from rainbow trout, Atlantic halibut, and turbot have been examined.

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

The level of dioxins and dl-PCBs are reported as ng toxic equivalents 2005 (TEQ05)/kg, and represents 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).

For salmon, the median of the sum of dioxins was 0.24 ng TEQ/kg w.w. The maximum value of 0.57 ng TEQ/kg w.w. is 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 was 0.57 ng TEQ/kg w.w for salmon. The highest result for salmon was 1.1 ng TEQ/kg w.w. All values were below the EU maximum limit of 6.5 ng TEQ/kg w.w.

The median of PCB-6 for salmon was 5.1 µg/kg w.w. The EUs maximum limit for PCB-6 in fish is 75 µg/kg w.w. and the highest concentration of PCB-6 measured in 2017 was 22 µg/kg w.w. in an Atlantic halibut sample.

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

		Atlantic Salmon	Rainbow trout	Atlantic halibut	Turbot	Maximum limit
	Samples	67	4	1	1	
Sum dioxins (ng TEQ/kg w.w.)	Median	0.24	0.25	0.38	0.28	
	Max	0.57	0.35	0.38	0.28	3.5
Sum dioxin + dl-PCBs (ng TEQ/kg w.w.)	Median	0.57	0.68	1.4	0.94	
	Max	1.1	0.83	1.4	0.94	6.5
	Samples	126	9	2	1	
PCB-6 (µg/kg w.w.)	Median	5.1	6.2	19	7.5	
	Max	9.8	8.6	22	7.5	75

4.3.2 Group B3b. Organophosphorous compounds

The pesticides chlorpyriphos, chlorpyrifos-methyl, pirimiphos-methyl and pirimiphos-ethyl were analysed in 114 pooled samples, 104 of the samples were salmon and 10 of the samples were rainbow trout, no residues were found.

4.3.3 Group B3c, Chemical elements

In 2017, the highest concentration of total mercury in salmon was 0.040 mg/kg w.w. The highest level, 0.14 mg/kg w.w., was found in Atlantic cod (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 concentrations measured in all samples are 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.

The concentrations of cadmium in most samples analysed since 2002 have been lower than the LOQ. In 2017, two out of 71 samples were above LOQ. The highest concentration measured was 0.0025 mg/kg w.w. which is well below EUs maximum limit of 0.05 mg/kg w.w. (EU 1881/2006).

Arsenic is determined as "total arsenic", comprising the sum of all arsenic species. The median level of total arsenic in Atlantic salmon was 0.87 mg/kg w.w., and the highest concentration measured was 1.8 mg/kg w.w. (Table 4.4). 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 upper limit for neither total arsenic nor inorganic arsenic in fish fillets.

Lead were not detected in one of the 71 samples analysed. The EU maximum level for lead in muscle meat of fish is 0.30 mg/kg w.w. (EU 1881/2006). The highest concentration measured was 0.049 mg/kg w.w. Thus, all samples were well below the limit.

Tributyltin was detected in one of the samples analysed. The highest level found was 7.4 µg/kg w.w. This is higher than previously found. There is currently no EU upper limit for tributyltin in fish fillet.

Table 4.4. Chemical elements in fillets of farmed fish

Element		Atlantic Salmon	Rainbow trout	Arctic Char	Cod	LOQ	EU-Limit
	N	67	2	1	1		
Mercury (mg/kg w.w.)	#Values	65	2	1	1		
	Median	0.017	0.025	-	-		
	Max	0.040	0.036	0.022	0.14	0.002	0.50
Arsenic (mg/kg w.w.)	#Values	67	2	1	1		
	Median	0.78	1.7	-	-		
	Max	1.8	2.5	1.2	0.88	0.003	n.a.
Cadmium (mg/kg w.w.)	#Values	1	1	0	0		
	Median	-	-	-	-		
	Max	0.0025	0.0013	LOQ	LOQ	0.009-0.002	0.050
Lead (mg/kg w.w.)	#Values	1	0	0	0		
	Median	-	LOQ	LOQ	LOQ		
	Max	0.049	LOQ	LOQ	LOQ	0.005-0.01	0.30
	N	66	6				
Tributyltin (µg Sn/kg w.w.)	#Values	24	6				
	Median	-	0.48				
	Max	0.23	7.4			0.06-0.09	n.a.

4.3.4 Group B3d, Mycotoxins

In 2017, 94 pooled samples of salmon and six pooled samples of rainbow trout were analysed for enniatin A, enniatin A1, enniatin B, enniatin B1 and beauvericin. No residues of these mycotoxins were detected.

4.3.5 Group B3f, others

The group B3f, others is a group not required for finfish products by the directive 96/23EC, but are deemed relevant for analyses in Norwegian aquaculture by the NSFA and IMR. This group currently consist of brominated flame retardants (BFR), perfluorinated compounds (PFC) and polyaromatic hydrocarbons (PAHs). These are undesirable compounds present in the environment and may affect food safety.

4.3.5.1 Brominated flame retardants

PBDE, TBBPA and HBCD are compounds used as flame retardants. The summarised PBDE-7 (28, 47, 99, 100, 153, 154, 183) and PBDE 66, 119 and 138 are shown in Table 4.5. The highest level of PBDE-7 was 0.80 µg/kg w.w. with a median value of 0.49 µg/kg w.w for salmon. TBBPA was below LOQ in all samples. HBCD was analysed in 70 samples, the highest level was 0.38 µg/kg w.w. The median concentration of HBCD in salmon was 0.16 µg/kg w.w.. There is currently no EU maximum limit for BFRs in food.

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

		Atlantic Salmon	Rainbow trout	Atlantic halibut	Turbot	LOQ
	Samples	66	4	1	1	
Sum PBDE 7	Median	0.49	0.66	-	-	
	Max	0.80	0.87	1.18	0.53	
PBDE 66	#Values	65	4	1	1	
	Median	0.010	0.011	-	-	
	Max	0.024	0.013	0.018	0.011	0.002-0.01
PBDE 119	#Values	14	2	0	1	
	Median	-	-	-	-	
	Max	0.0060	0.0051	LOQ	0.0030	0.002-0.01
PBDE 138	#Values	2	0	0	0	
	Median	-	LOQ	--	-	
	Max	0.0078	LOQ	LOQ	LOQ	0.003-0.02
	Samples	68	2	0	0	
TBBPA	#Values	0	0			
	Median	-	-			
	Max	LOQ	LOQ			0.03-0.14
	Samples	68	2	0	0	
UB-Sum HBCD(α,β,γ)	Median	0.16	0.19			
	Max	0.38	0.22			

4.3.5.2 Perfluorinated compounds

A total of 70 samples were analysed for the PFCs. All results were below the LOQ (Table 8.3). EU has no maximum level for PFC in food.

4.3.5.3 Polycyclic aromatic hydrocarbons

The results for PAH are summarised in table 4.6. PAH was analysed in 70 samples, from which 69 samples were salmon and one was rainbow trout. There is no maximum limit for PAH in fresh fish (EU 835/2011).

Table 4.6 PAH (µg/kg w.w.) in fillets of farmed fish.

PAH		Atlantic salmon	Rainbow Trout	LOQ
	Samples	69	1	
5-methylchrysene	#Values	1	0	0.074 - 0.14
	Max	0.13	LOQ	
Benz(a)anthracene	#Values	2	0	0.074 - 0.14
	Max	1.3	LOQ	
Benzo(a)pyrene	#Values	0	0	0.074 - 0.14
	Max	LOQ	LOQ	
Benzo(b)fluoranthene	#Values	0	0	0.074 - 0.14
	Max	LOQ	LOQ	
Benzo(c)fluorene	#Values	1	0	0.074 - 0.14
	Max	0.79	LOQ	
Benzo(ghi)perylene	#Values	1	0	0.074 - 0.14
	Max	0.10	LOQ	
Benzo(j)fluoranthene	#Values	0	0	0.074 - 0.14
	Max	LOQ	LOQ	
Benzo(k)fluoranthene	#Values	0	0	0.074 - 0.14
	Max	LOQ	LOQ	
Chrysene	#Values	4	0	0.074 - 0.14
	Max	2.4	LOQ	
Cyclopenta(cd)pyrene	#Values	12	0	0.087 – 0.14
	Max	0.28	LOQ	
Dibenz(ah)anthracene	#Values	1	0	0.074 - 0.14
	Max	0.11	LOQ	
Dibenzo(a,e)pyrene	#Values	0	0	0.37 – 0.68
	Max	LOQ	LOQ	
Dibenzo(a,h)pyrene	#Values	0	0	0.37 – 0.68
	Max	LOQ	LOQ	
Dibenzo(a,i)pyrene	#Values	0	0	0.37 – 0.68
	Max	LOQ	LOQ	
Dibenzo(a,l)pyrene	#Values	0	0	0.37 – 0.68
	Max	LOQ	LOQ	
Indeno(1,2,3,-cd)pyrene	#Values	8	0	0.074 - 0.14
	Max	0.16	LOQ	

5. Discussion

5.1 Unauthorized substances

No residues of unauthorized substances were detected in any of the samples analysed.

5.2 Veterinary drugs

Most samples reviewed in this report are from fillets of farmed fish. However, as the liver has a central function in the distribution and elimination of veterinary drugs, liver samples were analysed for 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 bioassay 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 inhibition assay has to be verified by chemical analysis of the corresponding fillet sample sampled from the same fish. No residues of antibiotics or endoparasitic agents were detected which is in accordance with previous results from the last years.

Similar as in 2016, residues of emamectin, cypermethrin and diflubenzuron were detected. The percentage of positive samples for anti sea lice agents were similar to 2016. However, all the results were well below the MRLs. For diflubenzuron, the European Medicines Agency (EMA) has recommended to lower the MRL from 1000 µg/kg to 10 µg/kg (EMA 2018). The level of diflubenzuron found in one pooled sample was below the recommended new MRL.

5.3 Contaminants

No environmental contaminants were found above the EU maximum limits, for the contaminants where such have been implemented. However, the 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 intake (TWI) is the weekly 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. The compound group with the highest influence on restricting the recommended intake of fish in this report is the dioxins and dl-PCBs, for which a TWI of 14 pg WHO-TEQ/kg bw has been established (SCF, 2001). Using the median value from 2017, an intake of 200 g farmed salmon will contribute to about 12% of TWI for a person of 70 kg, while the intake of 200 g of farmed Rainbow trout will contribute to 14% of TWI for a person of 70 kg. While using the single sample of Atlantic halibut would amount to 29% of the TWI. Although the level of dioxin and dlPCB decreased from 2006 until 2012 reflecting the increased inclusion of vegetable ingredients in the feed, the level now seem to have stabilized at around 0.5 ngTEQ/kg w.w. in farmed Atlantic salmon. This level has been stable from 2012 up to current date.

Unexpectedly high levels of TBT was found in rainbow trout from one fish farm. The reason for the high level in the fish has not been established. However, according to the tolerable daily intake (TDI) set by EFSA (EFSA 2004), an intake of 200 g rainbow trout containing of 7.4 µg Sn/kg w.w. amount to 21% of the TDI for a person of 70 kg, meaning that the food safety is attended. Except for this sample, all other measurements were low, comparable to previous years.

One sample contained unexpected high levels of some of the PAHs (table 4.6). There are currently no ML or TWI for PAH in muscle meat of fresh fish, however, compared to the ML for muscle meat of smoked fish, the levels we found were below.

6. Conclusion

None of the substances with anabolic effect was detected in any of the samples analysed.

None of the veterinary drugs exceeded the MRL established for fish. Emamectin, cypermethrin and diflubenzuron were detected in a total of 16 samples; the levels measured were below their respective MRLs.

For contaminants, no samples exceeded the EUs maximum limits, where such limits have been established (sum dioxins, sum dioxins and dl-PCBs, PCB-6, mercury, lead and cadmium).

The general trend for most contaminants analysed in this program, reveals that the level of contaminants in farmed salmon has remained stable for the last 5 years.

7. Recommendations

Due to the present situation regarding illegal and undesirable substances in Norwegian 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	19	1	
Inorganic arsenic (µg/kg w.w.)	#Values	0	0	
	Median	-		
	Max	LOQ	LOQ	4-5
Methyl-mercury (mg Hg/kg w.w.)	#Values	19	1	
	Median	0.017	0.027	
	Max	0.044	0.027	0.001

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

Compound	Atlantic Salmon	Rainbow trout	Max value	LOQ
PFBA	68	2	<LOQ	1.0
PFBS				0.8
PFDA				0.5
PFDoDA				0.8
PFDS				1
PFHpA				0.7
PFHxA				0.9
PFHxS				0.8
PFNA				0.9
PFOA				1.3
PFOS				0.8
PFOSA				1.2
PFTeDA				1.1
PFTrDA				1.2
PFUdA				1

Table 8.3. 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)	IMR
	Metronidazole	LC-MS/MS	0.3		Presence (MRPL = 3.0)	
	Hydroxy-metronidazole		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 Substances Micro-biological method	Quinolones	3-plate Screening Method[2]	200		100-600	IMR
	Tetracyclines		200		100	
	Amphenicols		200		1000	
	Sulfonamides		400		100	
B1 Antibacterial substances Chemical method	Oxolinic acid	LC-MS/MS		30	100	Eurofins
	Flumequine			30	600	
	Oxytetracycline	LC-MS/MS		30	100	Eurofins
	Florfenicol	LC-MS/MS		0.5	1000	IMR

B2a Anthelmintics	Praziquantel	LC-MS/MS		1	n.a.	IMR/ Eurofins
	Fenbendazole[3]	LC-MS/MS		1	n.a.	
	Emamectin	LC-MS/MS		2-10	100	
	Diflubenzuron	LC-MS/M		1-10	1000	
	Teflubenzuron			1-50	500	
	Hexaflumeron			1-50	500	
	Lufenoron			1-50	1350	
	Ivermectin	LC-MS/M		2	n.a.	Eurofins
	Cypermethrin	GC-MS		5	50	
	Deltamethrin			10	10	
	Isoeugenol[3]	GC-FID		50	6000	
B3a Organo-chlorine compounds	Dioxins and dlPCB	HRGC-HRMS		0.0001-0.1 ng TEQ/kg	6.5 ng TEQ/kg	IMR
	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	IMR
	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	GC-ICP-MS		1	n.a.	
	Tributyltin	GC-ICP-MS		0.3-0.5	n.a.	
B3d Mycotoxins	Beauvericin, Enniatin A, A1, B and B1	LC-MS/MS		10	n.a.	Eurofins
B3e, dyes	Malachite green[3]	LC-MS/MS	0.15		Presence (MRPL=2)	IMR
	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.	IMR
	HBCD	LC-MS/MS		0.006-0.01	n.a.	Eurofins
	TBBPA	GC-MS		0.03-0.2	n.a.	
	PAH	GC-MS/MS		0.5-1.0	n.a.	Eurofins
	PFC	LC-MS/MS		0.5-13	n.a.	IMR

[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

Table 8.4. 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

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