MONITORING PROGRAM FOR PHARMACEUTICALS, ILLEGAL SUBSTANCES, AND CONTAMINANTS IN FARMED FISH

– Annual report for 2018

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Summary (English):

This report summarises the monitoring data collected in 2018 on the status of illegal substances, pharmaceuticals and contaminants in Norwegian farmed fish. A total of 13 920 fish were collected. 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 and contaminants were collected at processing plants, and are representative of Norwegian farmed fish ready for human consumption. Residues of anti sea lice agents were found in four 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. No environmental contaminants were found above the EU maximum limits.

Summary (Norwegian):

Denne rapporten oppsummerer overvåkingsresultatene fra 2018 for ulovlige stoffer, legemidler og miljøgifter i norsk oppdrettsfisk. Totalt ble det samlet 13 920 fisk. Prøver som analyseres for ulovlige forbindelser, som stoffer med anabole effekter eller uautoriserte legemidler, kan bli tatt ut under alle livsstadier, og er representative for oppdrettsfisk under produksjon. Ingen rester av ulovlige forbindelser ble detektert. Prøver som testes for godkjente veterinære legemidler og miljøgifter ble samlet inn på slakterier, og er representativt for norsk oppdrettsfisk som er klar for markedet. Rester av lusemidler ble funnet i fire prøver, nivåene var under grenseverdien (MRL) for alle prøvene. Andre veterinære legemidler, som antibiotika eller legemidler brukt mot interne parasitter ble ikke funnet. Ingen miljøgifter ble funnet over EUs maksimumsgrenser.

Content

1 - 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

Fish tested for illegal compounds were collected at the farm by official inspectors from the Norwegian Food Safety Authorities, without prior notification to the farmers. Sampling can be done 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 unauthorized substances, the analytical methods should meet a minimum required performance limits (MRPLs) set by the European Union (2002/657/EC), and European reference laboratories (EU-RLs), (CRL 2007). Table. 7.3 gives an overview of MRPLs of relevant compounds.

1.3 - Group B, veterinary drugs

In order to protect public health, Maximum Residue Limits (MRLs) have been established. According to current EU legislation (EU 37/2010) each substance is assigned a MRL, which is the highest permitted residual concentration of legally applied pharmacologically active substances in products intended for human consumption. The MRLs for fish are set for muscle and skin in natural proportions. 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.

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.

1.5 - Ethoxyquin

Ethoxyquin (EQ) is a synthetic antioxidant, widely used as an additive (E324) in components for animal feed for pets and livestock to preserve product quality and increase shelf live. Because of its high efficacy, EQ has also been widely used by the global fishmeal industry both as a nutritional preservative, but also to avoid oxidation and self-ignition under long-distance transport.

2 - Material and methods

2.1 - Sampling

Samples were taken on fish farms or slaughterhouses, by official inspectors, in all fish-producing regions in Norway. The sampling plan was randomised according to season and region. In 2018, 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.

2.2 - 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 limits 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.

2.3 - Analytical methods

The laboratory routines and most of the analytical methods are accredited in accordance with the standard ISO 17025 (Table 7.3). A summary of the analytical methods and their limit of detection (LOD) or limit of quantification (LOQ) is shown in table 7.3. 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, are 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.

2.3.1 - 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.

2.3.2 - 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 specific bacterial strain was added to a plate containing growth agar and. 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, flumequine, enrofloxacin, ciprofloxacin and trimethoprim

The analytes were extracted with acetonitrile and water. 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, teflubenzuron, lufenuron and hexaflumuron

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 measures 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, were 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 using an Accelerated Solvent Extractor (ASE). The extract was purified by solid phase extraction and analysed by GC-MS/MS.

Ethoxyquin

EQ and EQDM were extracted with hexane from pooled muscle samples, after saponification in a mixture of ethanol, NaCl and NaOH. EQ and EQDM were quantified by reversed-phase high-performance liquid chromatography with fluorescence detection, using an external standard curve, as previously described by Bohne et al. (2007), with modifications described by Ørnsrud et al. (2011).

Table 2.1. Number of fish analysed for each substance.

Compounds	Fish	Atlantic salmon	Rainbow trout	Atlantic halibut	Arctic char	Turbot
A1 Stilbenes						
Zeranol, 17alpha-Estradiol, 17alpha-Ethinyl-estradiol, 17beta-Estradiol, beta-Zearalanol, Dienestrol, Diethylstilbestrol, Estriol, Estrone, Hexestrol	815	760	50		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	815	765	50			
A6 Illegal drugs						
Chloramphenicol	820	770	50			
Metronidazole	815	770	35	10		
Nitrofuran metabolites (AOZ, AMOZ, AHD, SEM)	825	770	45	10		
Malachite green, Crystal violet, Brilliant green*	835	775	45	10	5	
B1 Antibiotics						
Florfenicol	260	250	10			
Oxytetracycline	100	100				
Flumequine, Oxolinic acid, Enrofloxacin, Ciprofloxacin, Trimethoprim	360	345	15			
Quinolones (liver), Tetracyclines (liver), Amphenicols (liver), Sulphonamides (liver)	1500	1365	115	5	10	5
B2 Other veterinary drugs						
Emamectin	845	810	40		5	
Cypermethrin, Deltamethrin	600	575	25			
Diflubenzuron, Teflubenzuron, Hexaflumeron, Lufenuron	800	740	55		5	
Ivermectin	75	60	10		5	

Praziquantel	495	455	40			
Fenbendazole	50	45	5			
Isoeugenol	140	140				
B3a Organochlorine compounds						
Pesticides	485	445	40			
Dioxin and dl-PCBs	350	340	5			5
PCB-6	680	650	20	5 10		5
B3b, Organophosphorus compounds						
Azamethiphos, Dichlorvos	250	245	5			
Chlorpyriphos, Pirimiphos	600	575	25			
B3c Chemical elements						
Lead Cadmium Mercury Arsenic	435	410	25			
Inorganic arsenic Methylmercury	100	95	5			
Tributyltin	260	250	10			
B3d, Mycotoxins						
Beauvericin, Enniatin	495	460	30		5	
B3e, Dyes						
Malachite green, Crystal violet, Brilliant green *	460	455	5			
B3f, Others						
PBDE	350	340	5			5
HBCD, TBBPA	340	320	20			
PAH	355	325	25		5	
PFC	365	350	15			
Ethoxyquin	375	335	35		5	

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).

3 - Results

3.1 – Substances with anabolic effects and unauthorized substances

A total of 1 085 pooled fillet samples from 5 425 fish, were examined for of illegal substances. The analyzed samples were mainly from Atlantic salmon, but also samples from rainbow trout, and Arctic char were examined. For illegal substances, any presence of the compound will lead to a non-compliant result, regardless of the level.

3.1.1 - Stilbenes

The presence of stilbenes was examined in 163 pooled samples. None of the included stilbenes was detected in the samples analysed.

3.1.2 - Steroids

The presence of steroids was examined in 163 pooled samples. None of the substances was detected in the samples analysed.

3.1.3 - Unauthorized veterinary drugs

Totally 751 pooled samples were analyzed for unauthorized veterinary drugs. No residues of malachite green, crystal violet, brilliant green, chloramphenicol, nitrofurans or metronidazole were detected.

3.2 – 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.

3.2.1 - 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 1 500 fish. Florfenicol, (52 pooled samples), oxytetracyclin (20 pooled samples) and flumequin, oxolinic acid, enrofloxacin, ciprofloxacin and trimethoprim (72 pooled samples) were also analysed by chemical methods. No residues were detected in any of the analysed samples. The LOQs of the respective compounds are listed in Table 7.3.

3.2.2 - Group B2a anthelmintics

The levels of the anthelmintics; teflubenzuron, diflubenzuron, cypermethrin, deltamethrin, emamectin, ivermectin, praziquantel or fenbendazole were determined in 510 pooled muscle samples representing 2 550 fish. Emamectin was detected in 3 out of 162 pooled samples of Atlantic salmon. The highest concentration of emamectin was found at 5.2 µg/kg. This concentration is below the MRL of 100 µg/kg (EU 37/2010). Residues of the anti sea lice agent lufenuron was found in one sample at a concentration of 12 µg/kg. The MRL for lufenuron is 1350 µg/kg (EU 37/2010). Residues

of other agents in this group were not detected in any of the samples. LOQs for the substances are specified in Table. 7.3.

3.2.3 - Group B3b. Organophosphorous compounds

No residues of azamethiphos or dichlorvos were detected in the 50 samples analysed for these analytes.

3.2.4 - Group B3d. Sedatives

Residue of isoeugenol was not detected in any of the 28 samples analysed for this sedative.

3.3 - Contaminants

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

3.3.1 - Group B3a, Organochlorine compounds

The levels of organochlorine compounds were determined in 239 pooled samples. The results are summarised in Table 3.1 to 3.3.

3.3.2 - Organochlorine pesticides

For a number of the pesticides, the amount present is calculated as a sum including metabolites or transformation products (SANTE 2015). The results for these groups of pesticides are presented in table 3.1.

Table 3.1. The sum of groups of pesticides (µg/kg w.w.) in fillets of farmed fish

		Atlantic Salmon	Rainbow trout
Sum	Samples	89	8
DDT	Median (UB)	6.5	8.8
	Max (UB)	13	12
Endosulfan	Median (UB)	0.85	0.86
	Max (UB)	3.1	0.89
Aldrin and dieldrin	Median (UB)	1.3	1.6
	Max (UB)	3.6	2.2
Chlordane	Median (UB)	1.0	1.1
	Max (UB)	2.5	1.7
Heptachlor	Median (UB)	0.47	0.50
	Max (UB)	1.3	0.67
Toxaphene	Median (UB)	2.6	2.5
	Max (UB)	9.1	5.5

To calculate the sum of the components, conversion factors (table 7.4) are used to adjust for different molecular weights (SANTE 2015). The sums in table 3.1. 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 measurements of endosulfan are below LOQ, however, a sum is generated based on the LOQ-values.

The results for the other pesticides are summarised in Table 3.2. The highest level measured was 2.3 µg/kg w.w. of trans-nonachlor and 4.1 µg/kg w.w. hexachlorobenzene.

Table 3.2. Pesticides (µg/kg w.w.) in fillets of farmed fish.

Pesticide		Atlantic salmon	Rainbow Trout	LOQ
	Samples	89	8	
α-Hexachlorocyclo- hexane	#Values	12	0	
	Median	LOQ	LOQ	
	Max	0.24	LOQ	0.13-0.60
β-Hexachlorocyclo- hexane	#Values	3	2	
	Median	LOQ	LOQ	
	Max	0.19	0.26	0.13-0.60
δ-Hexachlorocyclo- hexane	#Values	0	0	
	Median	LOQ	LOQ	
	Max	LOQ	LOQ	0.13-0.60
γ-Hexachlorocyclo- hexane	#Values	0	0	
	Median	LOQ	LOQ	
	Max	LOQ	LOQ	0.13-0.60
Hexachlorobenzene	#Values	88	8	
	Median	1.4	1.5	
	Max	4.1	2.7	0.06-1.0
Pentachlorobenzene	#Values	3	0	
	Median	LOQ	LOQ	
	Max	0.37	LOQ	0.30-1.2
Trans-Nonachlor	#Values	89	8	
	Median	0.74	1.0	
	Max	2.3	1.9	0.13-0.60
Endrin	#Values	4	0	
	Median	LOQ	LOQ	
	Max	0.49	LOQ	0.15-0.71
Mirex	#Values	21	4	
	Median	LOQ	LOQ	
	Max	0.12	0.11	0.05-0.24
Octachlorstyrol	#Values	82	7	
	Median	0.09	0.10	
	Max	0.18	0.20	0.03-0.12

3.3.3 - Dioxin, dl-PCBs and PCB-6

The levels of dioxin, dl-PCBs and PCB-6 in farmed fish are shown in Table 3.3. 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 was 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 0.23 ng TEQ/kg w.w. The maximum value of 0.47 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 0.58 ng TEQ/kg w.w for salmon. The highest result for salmon was 1.3 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 4.7 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 2018 was 12 µg/kg w.w. in an Atlantic halibut sample.

Table 3.3 Dioxins, dl-PCBs and PCB-6 in fillets of farmed fish.

		Atlantic Salmon	Rainbow trout	Atlantic halibut	Turbot	Maximum limit
	Samples	68	1		1	
Sum dioxins (ng TEQ/kg w.w.)	Median	0.23	-		-	
	Max	0.47	0.24		0.17	3.5
Sum dioxin + dl-PCBs (ng TEQ/kg w.w.)	Median	0.58	-		-	
	Max	1.3	0.60		0.65	6.5
	Samples	130	4	1	1	
PCB-6 (µg/kg w.w.)	Median	4.7	6.0	-	-	
	Max	11	8.9	12	5.5	75

3.3.4 - Group B3b. Organophosphorous compounds

The pesticides chlorpyriphos, chlorpyrifos-methyl, pirimiphos-methyl and pirimiphos-ethyl were analysed in totally 120 pooled samples, 115 of the samples were salmon and 5 of the samples were rainbow trout, no residues were found.

3.3.5 - Group B3c, Chemical elements

In 2018, the highest measured concentration of total mercury were 0.062 mg/kg w.w. in a salmon sample and 0.027 mg/kg w.w. in a rainbow trout sample (Table 3.4). 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 7.1) 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 2018, one sample had levels above the LOQ. The highest concentration measured was 0.002 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 0.75 mg/kg w.w., and the highest concentration measured was 2.2 mg/kg w.w. (Table 3.4). None of the samples had concentrations of inorganic arsenic above the LOQ (Table 7.1), 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.

A quantifiable concentration of lead was detected in one of the 87 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 0.011 mg/kg w.w. Thus, all samples were well below the limit.

Monobutyltin was found in concentrations above the LOQ in 12 out of 50 samples analyzed, the maximum level was 2 µg/kg w.w.. All samples analysed for dibutyltin were below the LOQ. Tributyltin was detected in 17 of the analysed samples. The highest measured level was 0.3 µg/kg w.w.. There is currently no EU upper limit for tin in fish fillet.

Table 3.4. Chemical elements in fillets of farmed fish

Element		Atlantic Salmon	Rainbow trout	Arctic Char	Cod	LOQ	EU- Limit
	N	82	5				
Mercury (mg/kg w.w.)	#Values	80	5				
	Median	0.020	0.022				
	Max	0.062	0.027			0.002	0.50
Arsenic (mg/kg w.w.)	#Values	82	5				
	Median	0.75	0.79				
	Max	2.2	1.1			0.003	n.a.
Cadmium (mg/kg w.w.)	#Values	1	0				
	Median	-	-				
	Max	0.002	LOQ			0.009-0.002	0.050
Lead (mg/kg w.w.)	#Values	1	0				
	Median	-	-				
	Max	0.011	LOQ			0.005-0.01	0.30
	N	50	2				
Monobutyltin (µg Sn/kg w.w.)	#Values	11	1				
	Median	0.5	-				
	Max	2	0.40			0.4-1	n.a.
Dibutyltin (µg Sn/kg w.w.)	#Values	0	0				
	Median	-	-				
	Max	LOQ	LOQ			0.2-0.5	n.a.
Tributyltin (µg Sn/kg w.w.)	#Values	15	2				
	Median	0.08	0.3				
	Max	0.2	0.3			0.06-0.09	n.a.

3.3.6 - Group B3d, Mycotoxins

In 2018, 93 pooled samples were analysed for enniatin A, enniatin A1, enniatin B, enniatin B1 and beauvericin. No residues of these mycotoxins were detected.

3.3.7 - 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. In addition, in 2018, levels of the technological feed additive ethoxyquin (EQ) and its main transformation product ethoxyquin dimer (EQDM) were examined.

3.3.8 - 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 3.5. The highest level of PBDE-7 was 0.96 µg/kg w.w. with a median value of 0.46 µg/kg w.w for salmon. Out of 64 pooled samples of Atlantic salmon and 4 pooled samples of rainbow trout, TBBPA was found at a quantifyable level in one sample of salmon (0.05 µg/kg w.w.). HBCD was analysed in 68 samples, the highest level was 1.2 µg/kg w.w. The median concentration of HBCD in salmon was 0.11 µg/kg w.w.. There is currently no EU maximum limit for BFRs in food.

Table 3.5 BFR (µg/kg w.w.) in fillets of farmed fish.

		Atlantic Salmon	Rainbow trout	Turbot	LOQ
	Samples	68	1	1	
UB-Sum PBDE 7	Median	0.46	-	-	
	Max	0.96	0.61	0.40	
PBDE 66	#Values	68	1	1	
	Median	0.011	-	-	
	Max	0.035	0.010	0.011	0.002-0.01
PBDE 119	#Values	41	1	1	
	Median	0.0030	-	-	
	Max	0.014	0.0027	0.0027	0.002-0.01
PBDE 138	#Values	0	0	0	
	Median	-	-	-	
	Max	LOQ	LOQ	LOQ	0.003-0.02
	Samples	64	4	0	
TBBPA	#Values	1	0		
	Median	-	-		
	Max	0.05	-		0.03-0.14
	Samples	64	4	0	
UB-Sum HBCD(α,β,γ)	Median	0.12	0.11		
	Max	1.2	0.27		

3.3.9 - Perfluorinated compounds

A total of 73 samples were analysed for the PFCs. All results were below the LOQ (Table 7.3). EU has no maximum level for PFC in food.

3.3.10 - Polycyclic aromatic hydrocarbons

The results for PAH are summarised in table 3.6. PAH was analysed in 71 samples, of which 65 samples were from salmon, five from rainbow trout and one was Arctic char. There is no maximum limit for PAH in fresh fish (EU 835/2011).

Table 3.6 PAH (µg/kg w.w.) in fillets of farmed fish.

PAH		Atlantic salmon	Rainbow trout	Arctic char	LOQ
	Samples	65	5	1	
5-methylchrysene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Benz(a)anthracene	#Values	5	0	0	0.09 - 0.13
	Max	0.2	LOQ	LOQ	
Benzo(a)pyrene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Benzo(b)fluoranthene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Benzo(c)fluorine	#Values	1	0	0	0.09 - 0.13
	Max	0.2	LOQ	LOQ	
Benzo(ghi)perylene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Benzo(j)fluoranthene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Benzo(k)fluoranthene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Chrysene	#Values	8	0	0	0.09 - 0.13
	Max	0.3	LOQ	LOQ	
Cyclopenta(cd)pyrene	#Values	2	0	0	0.09 - 0.13
	Max	0.1	LOQ	LOQ	
Dibenz(ah)anthracene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	
Dibenzo(a,e)pyrene	#Values	0	0	0	0.44 – 0.66
	Max	LOQ	LOQ	LOQ	
Dibenzo(a,h)pyrene	#Values	0	0	0	0.44 – 0.66
	Max	LOQ	LOQ	LOQ	
Dibenzo(a,i)pyrene	#Values	0	0	0	0.44 – 0.66
	Max	LOQ	LOQ	LOQ	
Dibenzo(a,l)pyrene	#Values	0	0	0	0.44 – 0.66
	Max	LOQ	LOQ	LOQ	
Indeno(1,2,3,-cd)pyrene	#Values	0	0	0	0.09 - 0.13
	Max	LOQ	LOQ	LOQ	

3.3.11 - Ethoxyquin

EQ and EQDM levels were measured in 74 pooled fillet samples. The samples were mostly taken from Atlantic salmon, but also samples from rainbow trout and Atlantic char were included (Table 3.7 of EQ and EQDM was calculated as upper bound, using the numerical LOQ values for measurements below LOQ.

For salmon samples, the median level of the sum of EQ&EQDM was 0.11 mg/ kg ww. Rainbow trout and trout contained EQ&EQDM at a median concentration of 0.09 mg/kg ww. One sample of Atlantic char was analysed and this sample contained 0.04 mg EQ&EQDM/ kg ww .

The maximum values of EQ and EQDM were 0.01 and 0.34 mg/kg ww, respectively, and were found in salmon.

Table 3.7 Ethoxyquin (mg/kg w.w.) in fillets of farmed fish.

		Atlanti salmon	Rainbow trout	Atlantic char	LOQ
	Samples	67	7	1	
EQ (mg/kg ww)	#Values	41	7	-	
	Median	0.002	0.001	LOQ	
	Max	0.01	0.009	LOQ	0.001
EQDM (mg/kg ww)	#Values	66	7	1	
	Median	0.11	0.08	-	
	Max	0.34	0.15	0.04	0.005
Sum EQ&EQDM (mg/kg ww) UB					
	Median	0.11	0.09	-	
	Max	0.34	0.15	0.04	

4 - Discussion

4.1 – Unauthorized substances

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

4.2 – 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 is verified by chemical analysis of the corresponding fillet sampled from the same fish. In accordance with previous results from the last years, no residues of antibiotics or endoparasitic agents were detected.

Residues of the anti sea lice agents emamectin and lufenuron were found in three and one sample, respectively. The percentage of positive samples for anti sea lice agents were lower than in 2017. Residues of emamectin have also been detected previously; however, this is the first time residues of lufenuron has been detected in this surveillance. All samples had levels below the MRL.

4.3 – Contaminants

Although the level of dioxins and dl-PCBs decreased from 2006 until 2012, reflecting the increased inclusion of vegetable ingredients in the feed, the level appears to have stabilized at approximately 0.5 ng TEQ/kg w.w. in farmed Atlantic salmon. This level has been stable from 2012 up to, and including, 2018.

No environmental contaminants were found above the EU maximum limits (ML) in 2018, for the contaminants where MLs have been implemented. However, the EUs MLs 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.

To evaluate the toxicological relevancy of the different contaminant levels, tolerable intake values are implemented. The Tolerable weekly intake (TWI) estimates the amount per kg body weight (bw) of a potentially harmful chemical that can be ingested per week over a lifetime without appreciable health risk. The TWI is a threshold level set by international risk assessment bodies, such as WHO and JECFA, or EFSA in Europe. The compound group with the strongest impact on restricting the recommended intake of fish is the dioxins and dl-PCBs. The TWI for dioxins and dl-PCBs was re-evaluated by EFSA in 2018 (EFSA 2018), and a new TWI of 2 pg WHO-TEQ/kg bw was established. This TWI is 7-fold lower than the previous TWI set in 2001 by the Scientific Committee of Food (SCF, 2001). Importantly, the new TWI ascertains the risk of dioxins and dl-PCBs, not the combined risk and benefit of fish consumption. Therefore, the NFSA has commissioned a new report from the Norwegian Scientific Committee for Food and Environment (VKM) on the risk and benefit of seafood consumption

4.4 - Ethoxyquin

Due to limited data on toxicity of EQ and its metabolites, a precautional maximum residue level (MRL) at the limit of analytical quantification (0.05 mg/kg), is currently applied in the EU (EFSA 2005). The list of products where a MRL has been established includes products of animal origin, but no MRL has yet been defined for fish. Measurements of fillet from Atlantic salmon, rainbow trout and Atlantic char do not show an exceedance of this level for EQ alone. However, EQ is metabolized quickly in salmon and accumulates mainly as EQDM in the edible parts of the fish. The median concentration of the sum EQ&EQDM exceeds the currently set precautional MRL value. Yet, with the measured level, a daily intake of one kg of salmon is still considered acceptable without appreciable health risks based on the currently applied ADI established by JECFA in 2005. A risk assessment of ethoxyquin by EFSA is currently in progress.

5 - Conclusion

No substances with anabolic effect were detected in any of the samples analysed.

None of the veterinary drugs were detected at levels exceeding the MRL established for fish. Emamectin or lufenuron were detected in a total of four samples; but the measured levels 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).

6 - Advice

The new TWI for dioxins and dl-PCBs of 2 pg WHO-TEQ/kg bw ascertains risk only, and a holistic risk-benefit perspective is not considered. We therefore support the decision by the NFSA to commission a new report from VKM concerning the risk and benefit of fish consumption.

7 - Tables

Table 7.1. 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	2-3
Methyl-mercury (mg Hg/kg w.w.)	#Values	19	1	
	Median	0.020	-	
	Max	0.060	0.019	0.001

Table 7.2. PFCs (µg/kg w.w.) in fillets of farmed fish

Compound	Atlantic Salmon	Rainbow trout	Max value	LOQ
PFBA	70	3		1.0
PFBS				1.0
PFDA				0.2
PFDoDA				0.2
PFDS				0.2
PFHpA				0.2
PFHxA				0.5
PFHxS				1.0
PFNA				0.2
PFOA				0.4
PFOS				0.2
PFOSA				0.5
PFTeDA				0.2
PFTrDA				0.2
PFUdA				0.2

Table 7.3. Summery of analytical methods

Group of substances	Analyte1	Method	LOD (µg/kg w.w.)	LOQ (µg/kg w.w.)	Level of action (µg/kg w.w.)	Laboratory
A1 Stilbenes	Diethylstilbestrol	LC-MS/MS	1		Presence	Eurofins
	Dienestrol		1			
	Hexestrol		1			
	β-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-Testosterone (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 Method2	200		100-600	IMR
	Tetracyclines		200		100	
	Amphenicols		200		1000	
	Sulfonamides		400		100	
B1 Antibacterial substances Chemical method	Oxolinic acid	LC-MS/MS		40	100	IMR
	Flumequine			40	600	
	Enrofloxacin			10	100	
	Ciprofloxacin			10	100	
	Trimethoprim			2	50	
	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	LC-MS/MS		1	n.a.	
	Emamectin	LC-MS/MS		2-10	100	
	Diflubenzuron	LC-MS/M		1-10	1000	
	Teflubenzuron			1-50	500	
	Hexaflumuron			1-50	500	
	Lufenuron			1-50	1350	
	Ivermectin	LC-MS/M		2	n.a.	Eurofins
	Cypermethrin	GC-MS		5	50	
	Deltamethrin			10	10	
	Isoeugenol3	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	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		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.	Eurofins
	PAH	GC-MS/MS		0.5-1.0	n.a.	IMR
	PFC	LC-MS/MS		0.5-13	n.a.	IMR
	Ethoxyquin	HPLC-FLD		0.001	n.a.	IMR
	Ethoxyquin dimer			0.005	n.a.	

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.

Table 7.4. 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

8 - Referances

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