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

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1. SUMMARY

This report summarises the results from the monitoring program for pharmaceuticals, illegal substances, and contaminants in Norwegian farmed fish according to directive 96/23/EC. In 2014, samples from 13 180 farmed fish were collected.

About 35% of the samples collected were analysed for substances with anabolic effects or unauthorized substances. These samples were collected by official inspectors at the farm, without prior notification to the farmers. The samples were collected at all stages of farming and are representative of farmed fish under production. Metronidazole was detected in fish from one fish farm. The findings were reported to the Norwegian Food Safety Authorities, which concluded that the samples had been contaminated. No other residues of unauthorized substances, including substances with anabolic effects were detected.

Samples tested for veterinary drugs were collected at processing plants, and are representative of Norwegian farmed fish ready for the market. Emamectin was detected in two out of 106 pooled samples, each pooled sample consisting of five fish. The highest concentration measured was 9.7 µg/kg, which is well below the current Maximum Residue Limit (MRL) for emamectin of 100 µg/kg. Cypermethrin was found in two out of 34 pooled samples. The highest level measured was 11 µg/kg, while the MRL for cypermethrin is 50 µg/kg. No other veterinary drug residues was detected in 2014. Other veterinary drugs, like antibiotics or drugs used against internal parasites, were not detected.

Samples analysed for contaminant were collected at processing plants, and are representative of Norwegian farmed fish ready for the market. 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. For contaminants, no samples exceeded the EUs maximum limits, where such limits have been established. The level of several of the contaminants have decreased over the last years.

2. INTRODUCTION

The aim of this program is to monitor the presence of illegal substances, pharmaceuticals and contaminants in Norwegian farmed fish. The residues or substance groups that are relevant to monitor for aquaculture animals are specified in Directive 96/23/EC:

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

In addition, BFR, PFC and PAH, which belongs to group B3f, others, are included.

2.1 Group A, Substances with anabolic effects and unauthorized substances

Group A includes growth promoters: steroids and stilbenes and substances for which no MRL can be established (EU 37/2010). Prohibited compounds considered relevant for aquaculture are chloramphenicol, nitrofurans, dyes and metronidazole. To ensure harmonized levels for the control of banned substances, analytical methods used should meet minimum required performance limits (MRPLs) set by the European reference laboratories (EU-RLs), National reference Laboratories (NRLs)

and member states of the European Union (2003/181/EC; 2004/25/EC; CRL 2007). Table. 8.5 gives an overview of MRPLs of relevant compounds.

Illegal substances are analysed in samples that are 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.

2.2 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. Consumption of food with drug residues below the MRL should, by a wide safety margin, not pose a health risk to the consumer. The MRLs for fish are set for muscle and skin in natural proportions.

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.

2.3 Group B, contaminants

Contrary to veterinary drugs, which are given to the fish intentionally, contaminants are unwanted substances that the fish receive primarily from the feed. The main contributor of organic contaminants like dioxin, dl-PCB and PCB-6 is the fish oil used in the feed, while the main contributor of mercury is the fishmeal. Maximum limits for some of the contaminants are set for fish, while for others, like the pesticides and BFR, maximum limits have not been established.

As for the veterinary drugs, these samples are collected from fish at processing plants, and are representative of fish ready for human consumption.

3. MATERIAL AND METHODS

3.1 Sampling

Samples are taken on fish farms in all fish-producing regions in Norway. The sampling plan was randomised with regards to season and region, and the sample identification was blinded for the analysts.

Fish species included in 2014 were Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), Turbot (*Scophthalmus maximus*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), Arctic char (*Salvelinus alpinus*) and Wolffish (*Anarhichas lupus*).

Samples consisted mainly of muscle tissue, however, for samples collected for microbiological screening, liver tissue was also included. The samples were transported to NIFES in a frozen state.

3.2 Pre-treatment

On arrival at NIFES, the Norwegian quality cut (NQC) was obtained from the fish (Johnsen 2011). Pooled samples of five fish from the same cage/farm were homogenised before analyses. Samples collected for analyses of substances with anabolic effects or unauthorized substances may include small fish from early life stages. In these cases, head, tail and gut were removed before the rest of the fish were analysed. A back-up sample is stored for all samples. For samples to be analysed for veterinary drugs with a MRL, skin is included in the back-up sample. If a veterinary drug is detected in an initially screening, the back-up sample will be analysed. Samples of liver were excised from the fish to be screened for residues of antimicrobial agents by the microbiological inhibition zone assay. Liver samples were examined individually, if residues is detected, the back-up sample of muscle will be analysed by chemical methods.

3.3 Analytical methods

The laboratory routines and most of the analytical methods are accredited in accordance with the standard ISO 17025 (Table 8.5). A summary of the analytical methods and their Limit of detection (LOD) and Limit of quantification (LOQ) are shown in table 8.5. 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, or by analysing certified reference material (CRM), where such exist.

3.3.1 Group A substances

A1 and A3, Stilbenes and steroids

Stilbenes and steroids were extracted by water and acetonitrile, and analysed by either GC-MS or LC-MS/MS.

A6, Illegal veterinary drugs

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

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 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 chemical analysis or a three plate microbiological assay, or by a combination of both.

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

Oxolinic acid and flumequine:

The analytes were extracted with acetonitrile, and analysis was performed by LC-MS/MS.

Oxytetracycline

The analyte was extracted with an EDTA-succinate aquatic buffer. Solid phase extraction was used for sample clean-up. The analyte was determined 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.

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 (Samuelsen et al. 2014).

Emamectin and ivermectin

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

Cypermethrin and deltamethrin

Cypermethrin and deltamethrin were extracted from the samples with acetone. 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. Diethyl ether and hexane were used for sample clean up. Praziquantel was determined by LC-UV.

B3a, Organochlorine compounds

PCDD/PCDF and dl-PCBs.

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).

PCB-6

PCB-6 were extracted by hexane. The extract was purified 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 using hexane. The sample extract was then divided in two. The extract was either acid treated and analysed on GC/MS in EI, or cleaned up through three columns, ChemElut, QuEChERS and C18, and subsequently detected on GC/MS in NCI (Berntssen, Julshamn et al. 2010). Some of the samples analysed for pesticides were analysed by a different method. Pesticides were extracted by organic solvent, and the extract were cleaned-up by column chromatography, before the pesticides were analysed by GC-HRMS.

B3b, Organophosphorus compounds

Azamethiphos and dichlorvos

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

B3c, elements

Lead, mercury, cadmium and arsenic

The sample was decomposed in acid, 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 separated from other arsenic compounds by anionic axchange 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

Ochratoxin A.

The sample material was weighed in together with Celite, before chloroform and phosphoric acid was added. The sample was further subjected to clean-up by an immunoaffinity column and quantification by HPLC with fluorescence detection.

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, and sulphuric acid were used for samples clean-up. The PBDEs were determined by GC-MS.

HBCD and TBBPA

The analytes were extracted from the sample by toluene. A liquid/liquid extraction were used for sample clean up before the analytes were detected and quantified by GC-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 organic solvent, the extract were purified by solid phase extraction. PAHs were analysed by GC/MS or GC-MS/MS

Table 3.1. Number of fish of each species and the number of parameters analysed

	Compounds	Fish	Atlantic salmon	Rainbow trout	Turbot	Atlantic halibut	Atlantic cod	Arctic char	Wolf-fish
A1 Stilbenes	Diethylstilboestrol Dienoestrol Hexoestrol	780	710	45		5	10	10	
	17beta-Estradiol alpha-Estradiol Estriol Estrone Ethinyl estradiol	290	265	15			5	5	
A3 Steroids	α- and β-Nandrolon α- and β-Trenbolon	480	440	25		5	5	5	
	16-Hydroxystanozolol 17alpha-Boldenone 17alpha-Trenbolone alpha-Nandrolone Boldenone Chlor-Testosterone Epitestosterone Methyl-Boldenone Methyltestosterone Nortestosterone/ Nandrolone Stanozolol Testosterone Testosterone propionate Trenbolone Trenbolone-acetate	295	265	20			5	5	
A6	Chloramphenicol	810	735	45		10	10	5	5

Illegal drugs	Metronidazole Metronidazole-OH	790	745	40				5	
	Nitrofuran metabolites (AOZ, AMOZ, AHD, SEM)	800	730	35		5	15	10	5
	Malachite green * Leucomalachite green Crystal violet Leucocrystal violet Brilliant green	750	695	45				10	
B1 **Chemical method in muscle**	Florfenicol	105	100				5		
	Oxytetracycline	90	90						
	Flumequine	100	95	5					
	Oxolinic acid	255	245	5			5		
B1 **Microbiological assay in liver**	Quinolones Tetracyclines Amphenicols Sulphonamides	1795	1615	120	5	15	15	25	
B2 **Other veterinary drugs**	Teflubenzuron	245	230	15					
	Diflubenzuron	260	245	15					
	Cypermethrin	170	145	25					
	Praziquantel	505	460	40			5		
	Fenbendazole	50	50						
	Emamectin	530	495	35					
	Ivermectin	75	75						
	Deltamethrin	160	135	25					
B3a **Organochlorine compounds**	DDT	840	745	65	5	5	20		
	Pesticides other than DDT	515	470	35			10		
	Dioxins and dl-PCBs	330	275	30	5	10	10		
	PCB-6	655	550	60	10	15	20		
B3b,	Azamethiphos	210	185	25					

Organophosphorus	Dichlorvos	50	50						
B3c Chemical elements	Lead Cadmium Mercury Arsenic	580	525	40		5	10		
	Inorganic Arsenic Methylmercury	105	105						
	Tributyltin	325	325						
B3d, Mycotoxins	Ochratoxin A	275	255	15			5		
B3e, Dyes	Malachite green * Leucomalachite green Crystal violet Leucocrystal violet Brilliant green	510	475	30				5	
B3f, Others	PBDE	360	350	10					
	TBBPA and HBCD	305	290	10			5		
	PAH	220	205	10			5		
	PFC	310	290	10			5	5	

*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 932 pooled fillet samples from 4 660 fish, were examined with respect to residues of illegal substances. For these substances, any presence of a compound, regardless of concentration, will lead to a non-compliant result.

4.1.1 Stilbenes

Stilbenes were examined in 156 pooled samples from a total of 780 fish. None of the substances was detected in the samples analysed.

4.1.2 Steroids

The presence of steroids was examined in 155 pooled samples from 775 fish. None of the substances was detected in the samples analysed.

4.1.3 Unauthorized veterinary drugs

A total of 630 pooled samples from 3 150 fish were analyzed for unauthorized veterinary drugs. No residues of chloramphenicol, nitrofurans or dyes were detected. Metronidazole was detected in three pooled samples. The samples were from the same fish farm, and the fish were 0.1 - 0.3 kg. The highest level measured were 1.8 ng/g.

4.2 Veterinary drugs or contaminants

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

4.2.1 Group B1, antibacterial agents

The antibacterial agents in class B1 was determined by a combination of chemical methods and the three plate bioassay. The broad groups a) quinolones, b) amphenicols and tetracyclines and c) sulphonamides, were measured in livers from 1 795 fish, giving a total of 5 385 bioassay determinations. The B1 antibacterial agents: florfenicol, oxytetracyclin, flumequin and oxolinic acid, were also analysed by chemical methods in 110 pooled fillet samples, representing 550 fish. The LODs/LOQs for each compound are listed in Table 8.5.

4.2.2 Group B2a anthelmintics

The levels of the anthelmintics; teflubenzuron, diflubenzuron, cypermethrin, deltamethrin, emamectin, ivermectin, praziquantel or fenbendazole were determined in 399 pooled muscle samples representing 1 995 fish. Emamectin was detected in two out of 106 pooled samples. According to the analytical protocol, any detection of drug residues would be followed by a re-analysis of the back up sample, consisting of muscle and skin in natural proportions, in duplicate. The highest concentration of emamectin measured in muscle and skin was 9.7 µg/kg. This concentration is well below the MRL of 100 µg/kg (EU 37/2010). Cypermetrin was detected in two out of 34 pooled samples. The highest concentration measured in muscle and skin was 11 µg/kg, which is well below the MRL of 50 µg/kg (EU 37/2010). Residues of other agents in this group were not detected in any of the samples. LODs/LOQs for the substances are specified in Table. 8.5.

4.2.3 Group B3b. Organophosphorous compounds

The levels of the B3b substances azamethiphos or dichlorvos were determined in 45 and 10 pooled fillet samples respectively. Residues of these agents were not detected in any of the examined samples.

4.2.4 Group B3a, Organochlorine compounds

The levels of organochlorine compounds were determined in 234 pooled samples of 1 170 fish. The results are summarised in Table 4.1 to 4.3.

4.2.5 Organochlorine pesticides

The sum of DDT is calculated as both lower bound (LB) and upper bound (UB). For LB calculation, analytes with levels below LOQ are calculated as zero. When using UB calculations, the numerical value of LOQ is used for analytes with levels below LOQ. UB represents a "worst case scenario". The UB-mean of sum DDT was 5.3 µg/kg w.w., and the highest concentration was 10 µg/kg w.w.

Table 4.1. DDT (µg/kg w.w.) in fillets of farmed fish

		Atlantic Salmon	Rainbow trout	Atlantic Cod	Atlantic Halibut	Turbot	All Groups
SUM DDT	N	149	13	4	1	1	168
	LB-Mean	5.0	4.8	0.08	10	2.0	4.9
	UB-Mean	5.4	5.4	0.18	10	2.0	5.3
	UB-Min	2.5	3.8	0.13	-	-	0.13
	UB-Max	8.6	8.1	0.31	10	2.0	10

The results for the other pesticides are summarised in Table 4.2. The highest level measured was 5 µg/kg w.w. of hexachlorobenzene (NIFES 2014).

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

Pesticide		Atlantic salmon	Rainbow Trout	Atlantic Cod	All Groups	LOQ
α- Hexachlorocyclo-hexane	N	94	7	2	103	
	#Values	64	4	0	68	
	UB-mean	0.12	0.13	-	0.12	
	Max	0.23	0.19	LOQ	0.23	0.05-0.2
β- Hexachlorocyclo-hexane	N	62	3	2	67	
	#Values	34	2	0	36	
	UB-mean	0.11	0.13	-	0.11	
	Max	0.20	0.19	LOQ	0.20	0.05-0.1
δ- Hexachlorocyclo-hexane	N	62	3	2	67	
	#Values	0	0	0	0	
	UB-mean	-	-	-	-	
	Max	LOQ	LOQ	LOQ	LOQ	0.05-0.2
γ-Hexachlorocyclo-hexane	N	94	7	2	103	
	#Values	28	3	0	31	
	UB-mean	-	-	-	-	
	Max	0.20	0.19	LOQ	0.20	0.02-0.08
Hexachlorobenzene	N	94	7	2	103	
	#Values	94	7	1	102	
	UB-mean	1.4	1.3	0.11	1.3	
	Max	5	1.7	0.11	5	0.03-0.1
Pentachlorobenzene	N	94	7	2	103	
	#Values	5	0	0	5	
	UB-mean	-	-	-	-	
	Max	0.3	LOQ	LOQ	0.3	0.05-0.4
Heptachlor	N	94	7	2	103	
	#Values	1	0	0	1	
	UB-mean	-	-	-	-	
	Max	0.05	LOQ	LOQ	0.05	0.02-0.07
Heptachlor A	N	94	7	2	103	
	#Values	10	0	0	10	
	UB-mean	-	-	-	0.11	
	Max	0.20	LOQ	LOQ	0.20	0.05-0.2
Cis-Heptachlor epoxide	N	62	3	2	67	
	#Values	62	3	0	65	
	UB-mean	0,3	0,3	-	0,3	
	Max	0,5	0,5	LOQ	0,5	0.03-0.04
Aldrin	N	94	7	2	103	
	#Values	0	0	0	0	
	UB-mean	-	-	-	-	
	Max	LOQ	LOQ	LOQ	LOQ	0.02-0.2
Dieldrin	N	94	7	2	103	

	#Values	94	7	0	101	
	UB-mean	1.3	1.3	-	1.3	
	Max	2.3	1.9	LOQ	2.3	0.01-0.04
Endrin	N	62	3	2	67	
	#Values	5	0	0	5	
	UB-mean	-	-	-	-	
	Max	0.3	LOQ	LOQ	0.3	0.06-0.2
Isoldrin	N	32	4	-	36	
	#Values	23	2		25	
	UB-mean	0.16	0.19		0.17	
	Max	0.5	0.4		0.5	0.03-0.08
α-endosulfan	N	94	7	2	103	
	#Values	0	0	0	0	
	UB-mean	-	-	-	-	
	Max	LOQ	LOQ	LOQ	LOQ	0.02-0.4
β-endosulfan	N	94	7	2	103	
	#Values	0	0	0	0	
	UB-mean	-	-	-	-	
	Max	LOQ	LOQ	LOQ	LOQ	0.02-0.3
Endosulfan sulphate	N	94	7	2	103	
	#Values	3	0	0	3	
	UB-mean	-	-	-	-	
	Max	0.25	LOQ	LOQ	0.25	0.02-0.3
***cis*-chlordane**	N	94	7	2	103	
	#Values	94	7	0	101	
	UB-mean	0.5	0.4	0.02	0.5	
	Max	1.0	0.6	LOQ	1.0	0.03-0.08
***oxy*-chlordane**	N	94	7	2	103	
	#Values	34	4	0	38	
	UB-mean	-	0.18	-	0.18	
	Max	0.3	0.4	LOQ	0.4	0.01-0.05
***trans*-chlordane**	N	94	7	2	103	
	#Values	93	7	0	100	
	UB-mean	0.09	0.08	-	0.09	
	Max	0.20	0.14	LOQ	0.20	0.01-0.04
***cis*-nonachlor**	N	32	4	-	36	
	#Values	32	4		36	
	UB-mean	0.3	0.24		0.3	
	Max	0.5	0.3		0.5	0.01-0.03
***trans*-nonachlor**	N	94	7	2	103	
	#Values	94	7	1	102	
	UB-mean	0.76	0.7	0.01	0.64	
	Max	1.4	1.3	0.02	1.4	0.02-0.05
TOX-26	N	94	7	2	103	
	#Values	94	7	0	101	
	UB-mean	0.5	0.5	0.11	0.5	
	Max	1.2	0.7	LOQ	1.2	0.04-0.1

TOX-32	N	32	4	-	36	
	#Values	0	0		0	
	UB-mean	-	-	-	-	
	Max	LOQ	LOQ		LOQ	0.1-0.3
TOX-40+41	N	32	4	-	36	
	#Values	32	4		36	
	UB-mean	0.24	0.16		0.23	
	Max	0.4	0.21		0.4	0.02-0.05
TOX-42a	N	32	4	-	36	
	#Values	32	3		35	
	UB-mean	0.13	0.10		0.12	
	Max	0.3	0.15		0.3	0.02-0.05
TOX-50	N	94	7	2	103	
	#Values	94	7	0	101	
	UB-mean	0.9	0.9	-	0.9	
	Max	2.2	1.8	LOQ	2.2	0.03-0.1
TOX-62	N	94	7	2	103	
	#Values	77	4	0	81	
	UB-mean	0.8	0.8	-	0.8	
	Max	3.6	2.1	LOQ	3.6	0.03-0.7
Mirex	N	94	7	2	103	
	#Values	28	1	0	29	
	UB-mean	-	-	-	-	
	Max	0.10	0.08	LOQ	0.10	0.02-0.08
Octachlorstyrol	N	62	3	2	67	
	#Values	62	3	0	65	
	UB-mean	0.3	0.24	-	0.3	
	Max	1.8	0.4	0.01	1.8	0.01
UB-mean: LOQ substituted for all values <LOQ in the calculation. No mean is given if more than 50% of the results are below LOQ.						

4.2.6 Dioxin, dl-PCBs and PCB-6

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

The level of dioxin is reported as ng toxic equivalents 2005 (TEQ05)/kg, and represents the sum of 17 different PCDD/F 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). Similar, the levels of dioxins and dl-PCBs are the sum of 17 PCDD/F and 12 dl-PCBs, each multiplied by their corresponding TEF value.

Sum dioxins ranged from 0.027 ng TEQ/kg to 0.43 ng TEQ/kg w.w., and the UB-mean sum was 0.20 ng TEQ/kg w.w. The maximum value of 0.43 ng TEQ/kg w.w. is below the EU maximum limit of 3.5 ng TEQ/kg w.w.

The sum of all 29 PCDD/F and dl-PCBs ranged from 0.034 to 1.4 ng TEQ/kg w.w. The UB-mean concentration was 0.53 ng TEQ/kg w.w. All values were below the EU maximum limit of 6.5 ng TEQ/kg w.w.

The concentrations of PCB-6 in farmed fish are shown in Table 4.3. In 2014, the data is mainly represented by Atlantic salmon (210 samples), but also samples from rainbow trout, Atlantic halibut, Atlantic Cod and turbot have been examined. The UB-mean of PCB-6 for all species was 5.0 µg/kg w.w. The congeners PCB-138 and PCB-153 have been the main contributors to the sum PCB-6. The EUs maximum limit for indicator PCBs in fish is 75 µg/kg w.w. The highest concentration of indicator PCBs measured in 2014 was 11 µg/kg w.w., which is well below the maximum limit.

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

		Atlantic Salmon	Rainbow trout	Atlantic halibut	Atlantic cod	Turbot	All Groups	Maximum limit
Sum dioxins (ng TEQ/kg w.w.)	Samples	55	6	2	2	1	66	
	Median	0.21	0.16	0.091	0.027	0.10	0.21	
	UB-Mean	0.21	0.18	0.091	0.027	0.10	0.20	
	Min	0.11	0.13	0.076	0.027		0.027	
	Max	0.43	0.24	0.11	0.028	0.10	0.43	3.5
Sum dioxin + dl-PCBs (ng TEQ/kg w.w.)	Samples	55	6	2	2	1	66	
	Median	0.56	0.39	0.25	0.037	0.33	0.56	
	UB-Mean	0.57	0.47	0.25	0.037	0.33	0.53	
	Min	0.35	0.37	0.16	0.034		0.034	
	Max	1.4	0.69	0.35	0.040	0.33	1.4	6.5
PCB-6 (µg/kg w.w.)	Samples	110	12	3	4	2	131	
	Median	4.9	4.6	3.8	0.12	2.7	4.8	
	UB-Mean	5.2	5.1	4.9	0.12	2.7	5.0	
	Min	2.0	2.8	1.2	0.08	1.8	0.08	
	Max	11	10	9.8	0.16	3.6	11	75

UB-mean: LOQ substituted for all values <LOQ in the calculation.

4.2.7 Group B3c, Chemical elements

In 2014, the highest concentration of total mercury in salmon was 0.059 mg/kg w.w. The highest level, 0.069 mg/kg w.w., was found in Atlantic halibut (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 well below the maximum limit. In addition to mercury, methylmercury was measured in 21 samples of salmon. The result showed that the levels of methylmercury (Table 8.1) were similar to the level of mercury, indicating that mercury in salmon is mainly present as methylmercury.

The concentrations of cadmium in most samples analysed since 2002 have been lower than the LOQ. In 2014, two of 116 samples were above 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 was 0.59 mg/kg w.w., and the highest concentration measured were 2.1 mg/kg w.w. (Table 4.4). None of the samples had concentrations of inorganic arsenic above the LOQ (4-6 µg/kg w.w.) (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.

Only 3% of the samples had detectable concentrations of lead (Table 4.4). The highest concentration was 0.026 mg/kg w.w. The EU maximum level for lead in muscle meat of fish is 0.30 mg/kg w.w. (EU 1881/2006). Thus, all samples were well below the limit.

Tributyltin was detected in two of the samples analysed. The highest level found was 0.60 µg/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 Cod	Atlantic halibut	All Groups	LOQ	EU-Limit
Arsenic (mg/kg w.w.)	N	105	8	2	1	116		
	#Values	104	8	2	1	115		
	Median	0.58	0.62	0.62		0.59		
	UB-Mean	0.66	0.67	0.62		0.67		
	Max	2.1	1.0	0.63	1.6	2.1	0.003	
Cadmium (mg/kg w.w.)	N	105	8	2	1	116		
	#Values	2	0	0	0	2		
	UB-Mean	-	-	-	-	-		
	Max	0.002	LOQ	LOQ	LOQ	0.002	0.001-0.003	0.050
Mercury (mg/kg w.w.)	N	105	8	2	1	116		
	#Values	103	8	2	1	114		
	Median	0.019	0.018	0.042		0.019		
	UB-Mean	0.021	0.020	0.042		0.022		
	Max	0.059	0.035	0.043	0.069	0.069	0.002	0.50
Lead (mg/kg w.w.)	N	105	8	2	1	116		
	#Values	4	0	0	0	4		
	UB-Mean	-	-	-		-		
	Max	0.026	LOQ	LOQ	LOQ	0.026	0.005-0.01	0.30
Tri butyltin (µg/kg w.w.)	N	59	4	2	0	65		
	#Values	2	0	0		2		
	UB-Mean	-	-	-		-		
	Max	0.60	LOQ	LOQ		0.60	0.3-0.5	

UB-mean: LOQ substituted for all values <LOQ in the calculation.
No mean is given if more than 50% of the results are below LOQ.

4.2.8 Group B3d, Mycotoxins

In 2014, 55 pooled samples were analysed for Ochratoxin-A, no residues were detected.

4.2.9 Group B3e, Dyes

A total of 102 pooled samples from 510 fish, sampled at processing plants, were examined 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.

4.2.10 Group B3f, others

PBDE, TBBPA and HBCD are compounds used as flame retardants, these are called brominated flame retardants (BFR). The summarised PBDE-7 (28, 47, 99, 100, 153, 154, 183) values are shown in Table 4.5. The levels in salmon ranged from 0.17 to 1.3 µg/kg w.w. with a mean value of 0.52 µg/kg w.w. The results of the other PBDEs are shown in table 8.2. All of the samples had TBBPA level below the LOQ. The highest concentration of sum HBCD were 0.69 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 cod	All Groups	LOQ
	Samples	70	2		72	
Sum PBDE 7	UB-Mean	0.52	0.47		0.52	
	Min	0.17	0.43		0.17	
	Max	1.3	0.51		1.3	
	Samples	58	2	1	61	
TBBPA	#Values	0	0	0	0	
	UB-Mean	-	-	-	-	
	Max	LOQ	LOQ	LOQ	LOQ	0.03-0.4
Sum HBCD (α,β,γ)	#Values	58	2	1	61	
	UB-Mean	0.21	0.20	-	0.20	
	Max	0.69	0.22	0.02	0.69	0.01

UB-mean: LOQ substituted for all values <LOQ in the calculation.
No mean is given if more than 50% of the results are below LOQ.

A total of 62 samples were analysed for the PFCs and all measurements were below the LOQ (Table 8.3).

Table 8.4 summarises the results for the PAH compounds analysed in farmed fish in 2014. PAH was analysed in 44 samples of salmon. Benzo(a)pyrene and Chrysene was detected in two samples each. 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 metronidazole were detected in three pooled samples. One sample was examined in the routine program, whereas the two other samples, from the same fish farm, were analyzed during the investigation. Metronidazole is a suspected carcinogen and therefore banned for use in animals used for food production. However, as metronidazole is allowed for pets and humans, the possibility that samples could have been contaminated after they were received at NIFES was investigated. It was concluded that this was unlikely. No Metronidazole was detected in the feed, and no residues of metronidazole was found in fish from the same farm three months after the first identification. The Norwegian Food Safety Authority concluded that the contamination occurred during sampling.

5.2 Veterinary drugs

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

Veterinary antihelmintics were detected in four of the samples. Two samples contained residues of emamectin, and two samples contained residues of cypermethrin. Residues of both compounds have also been found previously. No residues of antibiotics or endoparasitic agents have been detected the last decade in Norwegian farmed fish.

5.3 Contaminants

The monitoring of undesirables in Norwegian farmed fish has been executed at NIFES since the late 90s. The general trend for most contaminants analysed in this program, is that the levels in farmed fish

are significantly declining, mainly reflecting the shift from fish based to more vegetable based raw materials in the feed.

The mean levels of sum dioxins + dl-PCBs in farmed salmon have decreased from 1.4 ng TEQ/kg w.w. to 0.57 ng TEQ/kg w.w. from 2002 to 2014. The level of DDT in farmed salmon has declined from 11.8 µg/kg in 2002 to 5.4 µg/kg in 2014.

5.4 Food safety

The levels of contaminants in food are compared to maximum levels/regulatory limits for the specific commodity set by the EU or the WHO. However, EUs maximum limits for food are not toxicologically based but derived from the frequency distribution of occurrence 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 described in this report, tolerable intake values is 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 different governmental agencies, such as EFSA in Europe, EPA in the US, and WHO or JECFA on a worldly basis. The TWI level is based on an extensive evaluation of the available scientific literature.

The compound group most strongly restricting the advisable intake of all fish in this report is the dioxin and dl-PCB. However, according to a recent report by the Norwegian Scientific Committee for Food Safety (VKM 2014) more than 1 kg of farmed salmon can be eaten each week, in addition to other dietary sources containing these contaminants, without the TWI of dioxin and dl-PCB being exceeded.

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6. CONCLUSION

Norwegian farmed fish is safe food.

None of the substances with anabolic effect was detected in any of the samples analysed. The detection of Metronidazole was defined as a contamination by the Norwegian Food Safety Authority.

None of the veterinary drugs exceeded the MRL established for fish. Emamectin and cypermethrin were detected in two samples each; the levels measured were well below their respective MRLs.

Similarly to veterinary drugs, all the environmental contaminants analysed in farmed fish were found at levels below the EU maximum limits, for those compounds for which such limits have been established (sum dioxins, dl-PCBs, PCB-6, mercury, lead and cadmium).

The general trend for most contaminants analysed in this program shows that the level in farmed salmon is significantly declining, which mainly reflects the shift from fish based raw materials in the feed to more vegetable based.

7. RECOMMENDATIONS

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

8. TABLES

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

		Atlantic Salmon	LOQ
	N	21	
Inorganic arsenic (µg/kg w.w.)	#Values	0	
	UB-Mean	-	
	Max	LOQ	4-6
Methyl-mercury (mg/kg w.w.)	#Values	21	
	UB-Mean	0.023	
	Max	0.036	0.001

UB-mean: LOQ substituted for all values <LOQ in the calculation.
No mean is given if more than 50% of the results are below LOQ.

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

		Atlantic Salmon	Rainbow trout	All Groups	LOQ
	N	70	2	72	
PBDE 66	#Values	49	2	51	
	UB-Mean	0.01	0.01	0.01	
	Max	0.04	0.01	0.04	0.003-0.005
PBDE 119	#Values	11	0	11	
	UB-Mean	-	-	-	
	Max	0.01	LOQ	0.01	0.003-0.005
PBDE 138	#Values	0	0	0	
	UB-Mean	-	-	-	
	Max	LOQ	LOQ	LOQ	0.006-0.01

UB-mean: LOQ substituted for all values <LOQ in the calculation.
No mean is given if more than 50% of the results are below LOQ.

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

Compound	Atlantic Salmon	Rainbow trout	Arctic char	Atlantic cod	Total	Max value	LOQ
PFBA	58	2	1	1	62	<LOQ	1.0-2.1
PFBS							0.8-3
PFDA							0.5-2.1
PFDoDA							0.8-1.8
PFDS							1-1.8
PFHpA							0.7-2.4
PFHxA							0.9-1.8
PFHxDA							13-24
PFHxS							0.8-1.8
PFNA							0.9-1.8
PFOA							1.3-2.4
PFODA							7-24
PFOS							0.8-1.8
PFOSA							1.2-1.5
PFPeA							6-42
PFTeDA							1.1-2.4
PFTrDA							1.2-3.6
PFUdA							1-2.7

Table 8.4. PAH (µg/kg w.w.) in fillets of farmed fish

PAH congener	Atlantic salmon	Rainbow trout	Atlantic cod	Total	#values	Max	LOQ
5-Methylchrysene	41	2	1	44	0	<LOQ	0.1-1.0
Benzo(a)antracene					0	<LOQ	0.1-0.5
Benzo(a)pyrene					2	0.14	0.1-0.5
Benzo(b)fluoranthene					0	<LOQ	0.1-0.5
Benzo(ghi)perylene					0	<LOQ	0.1-0.5
Benzo(j)fluoranthene					0	<LOQ	0.1-0.5
Benzo(k)fluoranthene					0	<LOQ	0.1-0.5
Benzo(c)Fluorene					0	<LOQ	0.1-1.0
Chrysene					2	0.22	0.1-0.5
Cyclopenta(c,d)pyrene					0	<LOQ	0.1-1.0
Dibenzo(a,e)pyrene					0	<LOQ	0.5-1.0
Dibenzo(a,h)anthracene					0	<LOQ	0.1-0.5
Dibenzo(a,h)pyrene					0	<LOQ	0.5-1.0
Dibenzo(a,i)pyrene					0	<LOQ	0.5-1.0
Dibenzo(a,l)pyrene					0	<LOQ	0.5-1.0
Indeno(1,2,3-cd)pyrene					0	<LOQ	0.1-0.5

Table. 8.5. 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	GC-MS LC-MS/MS	0.4-1		Presence	OUH/ Eurofins
	Dienestrol		0.7-1			
	Hexestrol		0.6-1			
	B-Estradiol		1			
	α-Estradiol		1			
	Estriol		1			
	Estrone		1			
	Ethinyl estradiol		1			
A3 Steroids	α-nandrolon	GC-MS LC-MS/MS	0.6-1		Presence	OUH/ Eurofins
	β-nandrolon		0.6-1			
	α-trenbolon		0.6-1			
	β-trenbolon		0.6-1			
	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	0.25		Presence (MRPL = 0.3)	NIFES
	Metronidazole3	LC-MS/MS	0.3		Presence	
	Hydroxy-metronidazole3		2.0		Presence	
	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	NIFES
	Tetracyclines		200		100	
	Amphenicols		200		1000	
	Sulfonamides		400		100	
B1	Oxolinic acid	LC-MS/MS		50	100	Eurofins

Antibacterial substances Chemical method	Flumequine			50	600	
	Oxytetracycline	LC-MS/MS		50	100	
	Florfenicol	LC-MS/M	0.2	0.5	1000	NIFES
B2a Anthelmintics	Praziquantel	LC-UV	50	100	n.a.	NIFES
	Fenbendazole[3]	LC-MS/MS	0.3	1.0	n.a.	
	Emamectin	LC-MS	3	5.0	100	
	Ivermectin		25	50	n.a.	
	Diflubenzuron	LC-MS	10	20	1000	
	Teflubenzuron		5	15	500	
	Cypermethrin	GC-EC		10	50	Eurofins
	Deltamethrin			10	10	
B3a Organo-chlorine compounds	Dioxins and dlPCB	GC-HRMS		0.004-10 ng/kg	6.5 ng TEQ/kg	NIFES
	PCB-6	GC-MS		0.004 – 10	75	
	Pesticides	GC-MS		0.02-0.6	n.a.	NIFES/ Eurofins
B3b Organo-phosphorus compounds	Azametiphos	GC-FPD		20	n.a.	Eurofins
	Dichlorvos			10	n.a.	
B3c Chemical elements	Lead	ICP-MS		0.005-0.01 mg/kg	0.3 mg/kg	NIFES
	Cadmium			0.001-0.003 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		NIFES
	Methylmercury[3]	GC-ICP-MS		1.0		
	Tributyltin[3]	GC-ICP-MS		0.3-0.6		
B3d Mycotoxins	Ochratoxin A	HPLC-FLU	0.06		n.a.	NVI
B3e, dyes	Malachite green	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		0.003-0.01	n.a.	NIFES
	HBCD	GC-MS		0.01	n.a.	Eurofins
	TBBPA	GC-MS		0.03-0.4	n.a.	
	PAH	GC-MS		0.1-1.0	n.a.	NIFES/ Eurofins
	PFC	LC-MS/MS		0.5-42	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

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