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

ANNUAL REPORT FOR 2015

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1. SUMMARY

This report summarises the monitoring data collected in 2015 on the status of illegal substances, pharmaceuticals and contaminants in Norwegian farmed fish. A total of 12 285 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. All samples were collected by official inspectors.

Samples examined for illegal compounds were collected by official inspectors at the farm, without prior notification to the farmers. They 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. Crystal violet 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 prior to arrival at the laboratory. No other 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 the market. Residues of three anti sea lice agents; Emamektin, cypermethrin or diflubenzuron, were found in 6% of the samples analysed for anti sea lice agents. However, the concentration measured were below the Maximum Residue Limit (MRL) for all samples. Other veterinary drugs, like antibiotics or drugs used against internal parasites, were not detected.

Samples analysed for contaminants 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. All the environmental contaminants analysed in farmed fish were below the EU maximum limits, for those compounds for which such limits have been established. The level for several of the contaminants have decreased over the last years.

2. INTRODUCTION

2.1 Background

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

Group A Substances with anabolic effects and unauthorized substances:

A1: Stilbenes, derivatives and their salts and esters

A3: Steroids

A6: Prohibited substances

Group B Veterinary drugs and contaminants:

B1: Antibacterial agents

B2a: Anthelmintics

B3a: Organochlorine compounds

B3b: Organophosphorus compounds

B3c: Chemical elements

B3d: Mycotoxins

B3e: Dyes

B3f: Others

2.2 Group A, Substances with anabolic effects and unauthorized substances

Samples examined for illegal compounds 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, 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 Union (2002/657/EC), and European reference laboratories (EU-RLs), (CRL 2007). Table. 8.5 gives an overview of MRPLs of relevant compounds.

2.3 Group B, veterinary drugs

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

2.4 Group B, contaminants

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

3. MATERIAL AND METHODS

3.1 Sampling

Samples were taken on fish farms, by official inspectors, in all fish-producing regions in Norway. The sampling plan was randomised with regards to season and region. In 2015 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*) and Arctic char (*Salvelinus alpinus*).

One sample consists of five fish from the same cage/farm, these were collected and transported to NIFES in a frozen state. For most samples, the Norwegian quality cut (NQC) was obtained from the fish (Johnsen 2011). However, samples collected for analyses of substances with anabolic effects or unauthorized substances included small fish from early life stages, in these cases, head, tail and gut were removed. In addition, for most of the samples collected for analyses of antibiotics, liver were collected as well.

3.2 Pre-treatment

Upon arrival at NIFEs the sample identification were blinded for the analysts. Pooled samples of muscle from five fish from the same cage/farm were homogenised before analyses. 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) or 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, Stilbenes

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

A3, Steroids

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

A6, Illegal veterinary drugs

Chloramphenicol

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

Nitrofurans

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

Metronidazole

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

3.3.2 Group B substances

B1, Antibacterial agents (antibiotics)

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

Microbiological assay

For the three-plate microbiological inhibition method, 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 muscle and skin.

Oxolinic acid and flumequine

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

Oxytetracycline

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

Florfenicol

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

B2a, Anthelmintics

Diflubenzuron and teflubenzuron

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

Emamectin

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

Ivermectin

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 from the samples by soxhlet extraction. The extracts were purified by gel permeation chromatography. The samples were analysed and quantified 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 and quantified by LC-MS/MS.

Praziquantel

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

B3a, Organochlorine compounds

Dioxins, dl-PCBs and PCB-6.

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

PCB-6 and DDT

PCB-6 and DDTs 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 in addition to *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD and *o,p′*-DDD.

Chlorinated pesticides

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

B3b, Organophosphorus compounds

Azamethiphos and dichlorvos

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

Chlorpyriphos and Pirimiphos

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

B3c, elements

Lead, mercury, cadmium and arsenic

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

Inorganic Arsenic

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

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

Fumonisin

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

B3e, Dyes

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

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

B3f, Others

PBDE

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

HBCD and TBBPA

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

PFC

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

PAH

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

Table 3.1. Number of fish collected for each analysis.

	Compounds	Fish	Atlantic salmon	Rainbow trout	Turbot	Atlantic halibut	Arctic char
A1 Stilbenes	Zeranol 17alpha-Estradiol 17alpha-Ethinyl estradiol 17beta-Estradiol beta-Zearalanol Dienestrol Diethylstilbestrol Estriol Estrone Hexestrol	725	665	45		5	10
A3 Steroids	16-Hydroxystanozolol 17alpha-Boldenone 17alpha-Trenbolone alpha-Nandrolone Boldenone Chlor-Testosterone Epitestosterone Methyl-Boldenone Methyltestosterone Nortestosterone Stanozolol Testosterone Testosterone propionate Trenbolone Trenbolone-acetate	730	665	50		5	10
A6 Illegal drugs	Chloramphenicol	760	690	55		10	5
	Metronidazole Metronidazole-OH	730	670	50			10
	Nitrofuran metabolites (AOZ, AMOZ, AHD, SEM)	765	705	45		10	5
	Malachite green * Leuco malachite green Crystal violet Leuco crystal violet Brilliant green	705	650	45			10
B1 Chemical method in muscle	Florfenicol	90	90				
	Oxytetracycline	100	100				
	Flumequine	100	95	5			
	Oxolinic acid	240	235	5			
B1 Microbiological assay in liver	Quinolones Tetracyclines Amphenicols Sulphonamides	1605	1480	95	5	5	20
B2 Other veterinary drugs	Diflu- teflubenzuron	490	460	30			
	Cypermethrin	175	150	25			
	Praziquantel	430	390	40			
	Fenbendazole	55	55				

	Emamectin	460	425	35			
	Ivermectin	80	80				
	Deltamethrin	160	135	25			
B3a Organochlorine compounds	Pesticides	460	435	25			
	PCB-6, Dioxins and dl-	300	265	25	5	5	
	PCB-6 and DDT	300	265	25	5	5	
B3b, Organophosphorus compounds	Azamethiphos	225	200	25			
	Dichlorvos	55	55				
	Chlorpyriphos and Pirimiphos *	145	130	15			
B3c Chemical elements	Lead Cadmium Mercury Arsenic	345	330	10		5	
	Lead Cadmium Mercury Arsenic Inorganic Arsenic Methylmercury	100	95	5			
	Tributyltin	285	265	20			
B3d, Mycotoxins	Ochratoxin A	250	240	10			
B3e, Dyes	Malachite green ** Leuco malachite green Crystal violet Leuco crystal violet Brilliant green	455	425	25			5
B3f, Others	PBDE	310	305	5			
	TBBPA and HBCD	295	285	5			5
	PAH	210	205	5			
	PFC	295	280	10			5

Some of the samples collected have been analysed by two methods, meaning that the number of samples analysed could, for some substances, be more than that specified in this table.

*These samples consist of the same fish as the samples analysed for cypermethrin.

****** 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 883 pooled fillet samples from 4 415 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

The presence of stilbenes were examined in 145 pooled samples from a total of 725 fish. None of the substances was detected in the samples analysed.

4.1.2 Steroids

The presence of steroids was examined in 146 pooled samples from 730 fish. None of the substances was detected in the samples analysed.

4.1.3 Unauthorized veterinary drugs

A total of 592 pooled samples from 2 960 fish were analyzed for unauthorized veterinary drugs. No residues of chloramphenicol, nitrofurans or metronidazole were detected. Although malachite green, crystal violet and brilliant green belongs to the group B3e according to directive 96/23, these dyes are not allowed to be used for food producing animals, therefore 141 pooled samples were collected as group A samples at the fish farms, and analysed for dyes. Residues of crystal violet and leuco crystal violet were detected in one sample taken in the routine program. During the investigation, five samples, taken at the same time from the same fish farm were examined, residues of crystal violet were found in all five samples, while traces of leuco crystal violet was detected in one sample. The highest level measured for crystal violet was 15 ng/g, while the highest concentration detected for leuco crystal violet was 0.29 ng/g.

4.2 Veterinary drugs

Samples analysed for veterinary drugs were collected from fish at processing plants, and are representative of fish ready for human consumption. The maximum residue limit for veterinary drugs are determine 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.

4.2.1 Group B1, antibacterial agents

The antibacterial agents was 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 1 605 fish. Florfenicol, oxytetracyclin, flumequin and oxolinic acid, were also analysed by chemical methods in 106 pooled fillet samples, representing 530 fish. The LODs/LOQs for the compounds 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 426 pooled muscle samples representing 2 130 fish. Emamectin was detected in eight out of 133 pooled samples. The highest concentration of emamectin was 32 µg/kg. This concentration is below the MRL of 100 µg/kg (EU 37/2010). Cypermethrin was detected in seven out of 44 pooled samples. The highest concentration measured was 21 µg/kg, which is below the MRL of 50 µg/kg (EU 37/2010). Furthermore residues of diflubenzuron were detected in six of 128 pooled samples. The MRL of diflubenzuron is 1000 µg/kg, and the highest level measured were 14 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. 8.5.

4.2.3 Group B3b. Organophosphorous compounds

The levels of the B3b substances azamethiphos or dichlorvos were determined in 45 and 11 pooled fillet samples respectively. Residues of these agents were not detected in any of the examined samples.

4.2.4 Group B3e, Dyes

According to directive 96/23, malachite green, crystal violet and brilliant green 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 (A6, illegal drugs) and group B samples (B3e, dyes). A total of 102 pooled samples from 510 fish, were collected at processing plants, and analysed with respect to malachite green and its metabolite leuco malachite green, crystal violet and its metabolite leuco crystal violet, and brilliant green. No residues of these agents were detected in the samples collected at processing plants.

4.3 Contaminants

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

4.3.1 Group B3a, Organochlorine compounds

The levels of organochlorine compounds were determined in 212 pooled samples of 1 060 fish. The results are summarised in Table 4.1 to 4.3.

4.3.1.1 Organochlorine pesticides

The sum of DDT is calculated as upper bound (UB). 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-median of sum DDT was 5.1 µg/kg w.w., and the highest concentration was 13 µg/kg w.w.

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

		Atlantic Salmon	Rainbow trout	Atlantic Halibut	Turbot	All Groups
SUM DDT	N	140	10	1	1	152
	Median	5.1	5.2	10	5.4	5.1
	UB-Max	13	5.6	10	5.4	13

The results for the other pesticides are summarised in Table 4.2. The highest level measured was 4.3 µg/kg w.w. of hexachlorobenzene and toxaphene 50).

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

	Pesticide	Atlantic salmon	Rainbow Trout	All Groups	LOQ
	Samples	87	5	92	
α-Hexachlorocyclo-hexane	#Values	68	5	73	
	Median	0.13	0.10	0.13	
	Max	0.75	0.11	0.75	0.07-0.2
β-Hexachlorocyclo-hexane	#Values	62	5	67	
	Median	0.12	0.11	0.12	
	Max	0.46	0.14	0.46	0.07-0.2
δ-Hexachlorocyclo-hexane	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.04-0.2
γ-Heδxachlorocyclo-hexane	#Values	21	0	21	
	Median	LOQ	LOQ	LOQ	
	Max	0.50	LOQ	0.50	0.003-0.2
Hexachlorobenzene	#Values	87	5	92	
	Median	1.3	1.4	1.4	
	Max	4.3	1.5	4.3	0.03-0.1
Pentachlorobenzene	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.05-0.4
Heptachlor	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.02-0.08
Trans-Heptachlor epoxide	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.05-0.3
Cis-Heptachlor epoxide	#Values	87	5	92	
	Median	0.19	0.16	0.19	
	Max	0.69	0.19	0.69	0.03-0.04
Aldrin	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.01-0.08
Dieldrin	#Values	87	5	92	
	Median	1.0	0.81	1.0	
	Max	3.4	1.1	3.4	0.01-0.04
Endrin	#Values	10	1	11	
	Median	LOQ	LOQ	LOQ	
	Max	0.23	0.14	0.23	0.09-0.3
α-endosulfan	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.1-0.4
β-endosulfan	#Values	0	0	0	
	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.1-0.3
Endosulfan sulphate	#Values	0	0	0	

	Median	LOQ	LOQ	LOQ	
	Max	LOQ	LOQ	LOQ	0.1-0.3
cis-chlordane	#Values	87	5	92	
	Median	0.37	0.31	0.37	
	Max	1.2	0.37	1.2	0.1-0.3
oxy-chlordane	#Values	2	0	2	
	Median	LOQ	LOQ	LOQ	
	Max	0.18	LOQ	0.18	0.1-0.4
trans-chlordane	#Values	72	4	76	
	Median	0.07	0.06	0.07	
	Max	0.25	0.07	0.25	0.03-0.08
trans-nonachlor	#Values	87	5	92	
	Median	0.60	0.49	0.59	
	Max	2.1	0.63	2.1	0.02-0.05
TOX-26	#Values	85	5	90	
	Median	0.45	0.32	0.45	
	Max	1.4	0.54	1.4	0.04-0.1
TOX-50	#Values	86	5	91	
	Median	0.90	0.67	0.90	
	Max	4.3	1.2	4.3	0.03-0.1
TOX-62	#Values	31	2	33	
	Median	LOQ	LOQ	LOQ	
	Max	2.0	0.43	2.0	0.03-0.8
Mirex	#Values	37	2	39	
	Median	LOQ	LOQ	LOQ	
	Max	0.06	0.06	0.06	0.03-0.08
Octachlorstyrol	#Values	87	5	92	
	Median	0.31	0.33	0.33	
	Max	1.9	0.53	1.9	0.1-0.7

4.3.1.2 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 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).

The median of the sum of dioxins was 0.17 ng TEQ/kg w.w. The maximum value of 0.51 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 0.49 ng TEQ/kg w.w. The highest result 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 concentrations of PCB-6 in farmed fish are shown in Table 4.3. In 2015, the data is mainly represented by Atlantic salmon (105 samples), but also samples from rainbow trout, Atlantic halibut, and turbot have been examined. The median of PCB-6 for all species was 5.1 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. and the highest concentration of indicator PCBs measured in 2015 was 13 µg/kg w.w.

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

		Atlantic Salmon	Rainbow trout	Atlantic halibut	Turbot	All Groups	Maximum limit
Sum dioxins (ng TEQ/kg w.w.)	Samples	52	5	2	1	60	
	Median	0.17	0.19	0.25	0.13	0.25	
	UB-Mean	0.19	0.18	0.25	0.13	0.25	
	Max	0.51	0.22	0.40	0.13	0.51	3.5
Sum dioxin + dl-PCBs (ng TEQ/kg w.w.)	Samples	52	5	2	1	60	
	Median	0.49	0.48	0.71	0.40	0.71	
	UB-Mean	0.51	0.47	0.71	0.40	0.71	
	Max	1.3	0.59	1.2	0.40	1.3	6.5
PCB-6 (µg/kg w.w.)	Samples	105	10	3	2	120	
	Median	5.1	4.8	9.3	4.2	9.3	
	UB-Mean	5.4	4.8	8.3	4.2	8.3	
	Max	13	6.5	13	5.1	13	75

4.3.2 Group B3b. Organophosphorous compounds

Chlorpyriphos, chlorpyrifos-methyl, pirimiphos-methyl and pirimiphos-ethyl were examined in 29 samples, no residues were found.

4.3.3 Group B3c, Chemical elements

In 2015, the highest concentration of total mercury in salmon was 0.057 mg/kg w.w. The highest level, 0.061 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 20 samples. 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 2015, two of 91 samples were above LOQ. The highest concentration measured was 0.003 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 0.64 mg/kg w.w., and the highest concentration measured were 3.1 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 any of the 91 samples analysed. The LOQ varied from 0.006-0.03 mg/kg w.w., meaning that the level of lead were below 0.03 mg/kg w.w. in all samples. 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 one of the samples analysed. The highest level found was 0.61 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 halibut	All Groups	LOQ	EU-Limit
Arsenic (mg/kg w.w.)	N	87	3	1	91		
	#Values	87	3	1	91		
	Median	0.64	0.61	3.1	0.64		
	Max	2.0	0.88	3.1	3.1	0.003	n.a.
Cadmium (mg/kg w.w.)	N	87	3	1	91		
	#Values	2	0	0	2		
	Median	-	-	-	-		
	Max	0.0030	LOQ	LOQ	0.0030	0.001-0.002	0.050
Mercury (mg/kg w.w.)	N	87	3	1	91		
	#Values	87	3	1	91		
	Median	0.016	0.027	0.061	0.027		
	Max	0.057	0.027	0.061	0.061	0.002	0.50
Lead (mg/kg w.w.)	N	87	3	1	91		
	#Values	0	0	0	0		
	Median	-	-		-		
	Max	LOQ	LOQ	LOQ	LOQ	0.006-0.03	0.30
Tri butyltin (µg/kg w.w.)	N	55	3	0	58		
	#Values	0	1		1		
	Median	-	-		-		
	Max	LOQ	0.61	LOQ	0.61	0.3	

4.3.4 Group B3d, Mycotoxins

In 2015, 50 pooled samples were analysed for the mycotoxins ochratoxin-A and fumonisin, two of the samples were rainbow trout, and the rest of the samples were salmon. No residues were detected.

4.3.5 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 highest level measured was 1.2 µg/kg w.w. with a median value of 0.38 µg/kg w.w. The results of the other PBDEs are shown in table 8.2. TBBPA was below LOQ in all samples but one. HBCD was analysed in 59 samples, the highest concentration measured was 0.30 µg/kg w.w in arctic char. The median concentration of HBCD in salmon was 0.19 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	Arctic Char	All Groups	LOQ
	Samples	61	1	0	62	
Sum PBDE 7	Median	0.38	0.29	-	0.38	
	Max	1.2	0.29	-	1.2	
	Samples	57	1	1	59	
TBBPA	#Values	1	0	0	1	
	Median	-	-	-	-	
	Max	0.52	LOQ	LOQ	0.52	0.03-0.2
Sum HBCD (α,β,γ)	#Values	57	1	1	59	
	Median	0.19	0.15	0.30	0.30	
	Max	2.0	0.15	0.30	2.0	0.006-0.01

A total of 51 samples were analysed for the PFCs and all measurements were below the LOQ (Table 8.3). EU has no maximum level for PFC in food.

The results for PAH are summarised in table 8.4. PAH was analysed in 42 samples, all results were below the LOQ. There is no longer a maximum limit for PAH in fresh fish, since it has been concluded that PAH does not accumulate in muscle meat due to rapid metabolism (EU 835/2011).

5. DISCUSSION

5.1 Unauthorized substances

Crystal violet is a known fungicide, however, it is not authorized for use in food producing animals. Residues of crystal violet and leuco crystal violet were detected in one sample taken in the routine program. During the investigation, five samples, taken at the same time from the same fish farm were examined, residues of crystal violet were found in all five samples, while traces of leuco crystal violet was detected in one sample. Crystal violet in salmon is rapidly metabolized to leuco crystal violet (Chan 2012), the low levels of leuco crystal violet in two of the samples and the fact that it was not found in four samples, indicates a contamination of the samples rather than use of crystal violet for treatment. The use of crystal violet is allowed for pets and humans, therefore NIFES examined the possibility that the samples could have been contaminated after they were received at NIFES. It was concluded that this was unlikely. The Norwegian Food Safety Authority concluded that the contamination occurred during sampling prior to arrival at the laboratory.

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.

Similar as in 2014, residues of emamectin and cypermethrin were detected. In addition diflubenzuron were found as well. Although there was an increase in the percentage of samples where anti sea lice agents were detected, all the results were below the MRLs. 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 transition from marine ingredients, fishmeal and fish oil, to more vegetable ingredients in the feed.

The median level of sum dioxins + dl-PCBs in farmed salmon have decreased from 1.4 ng TEQ/kg w.w. to 0.49 ng TEQ/kg w.w. from 2002 to 2015. The median level of mercury in farmed salmon has declined from 0.029 mg/kg w.w. in 2002 to 0.016 mg/kg w.w.in 2015.

All the contaminants analysed in farmed fish were below the EU maximum limits. 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 international risk assessment bodies, such as EFSA in Europe, EPA in the US, and WHO or JECFA on a worldly basis.

The compound group most strongly restricting the advisable intake of all fish in this report are the dioxins and dl-PCBs. 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.

6. CONCLUSION

Norwegian farmed fish is safe food.

None of the substances with anabolic effect was detected in any of the samples analysed. Residues of crystal violet were detected in samples from one fish farm. The Norwegian Food Safety Authority concluded that the samples had been contamination during sampling.

None of the veterinary drugs exceeded the MRL established for fish. Emamectin, cypermethrin and diflubenzuron were detected in a total of 21 samples; the levels measured were well 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 shows that the level in farmed salmon is significantly declining, mainly reflecting the transition from marine ingredients, fishmeal and fish oil, to more vegetable ingredients in the feed.

7. RECOMMENDATIONS

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

8. TABLES

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

		Atlantic Salmon	Rainbow trout	All Groups	LOQ
	N	19	1	20	
Inorganic arsenic (µg/kg w.w.)	#Values	0	0	0	
	Median	-			
	Max	LOQ	LOQ	LOQ	4-6
Methyl-mercury (mg/kg w.w.)	#Values	19	1	20	
	Median	0.021	0.017	0.021	
	Max	0.038	0.017	0.038	0.001

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

		Atlantic Salmon	Rainbow trout	All Groups	LOQ
	N	61	1	62	
PBDE 66	#Values	45	1	46	
	Median	0.005	0.009	0.009	
	Max	0.03	0.009	0.03	0.004-0.005
PBDE 119	#Values	1	0	1	
	Median	-	-	-	
	Max	0.01	LOQ	0.01	0.003-0.005
PBDE 138	#Values	0	0	0	
	Median	-	-	-	
	Max	LOQ	LOQ	LOQ	0.006-0.01

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

Compound	Atlantic Salmon	Rainbow trout	Arctic char	Total	Max value	LOQ
PFBA	56	2	1	59	<LOQ	1.0
PFBS						0.8
PFDA						0.5
PFDoDA						0.8
PFDS						1
PFHpA						0.7
PFHxA						0.9
PFHxDA						13
PFHxS						0.8
PFNA						0.9
PFOA						1.3
PFODA						7
PFOS						0.8
PFOSA						1.2
PFPeA						6
PFTeDA						1.1
PFTrDA						1.2
PFUdA						1

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

PAH congener	Atlantic salmon	Rainbow trout	Total	Max	LOQ
5-Methylchrysene	41	1	42	<LOQ	1
Benzo(a)antracene					0.5
Benzo(a)pyrene					0.5
Benzo(b)fluoranthene					0.5
Benzo(ghi)perylene					0.5
Benzo(j)fluoranthene					0.5
Benzo(k)fluoranthene					0.5
Benzo(c)Fluorene					1
Chrysene					0.5
Cyclopenta(c,d)pyrene					1
Dibenzo(a,e)pyrene					1
Dibenzo(a,h)anthracene					0.5
Dibenzo(a,h)pyrene					1
Dibenzo(a,i)pyrene					1
Dibenzo(a,l)pyrene					1
Indeno(1,2,3-cd)pyrene					0.5

Table. 8.5. 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	1		Presence	Eurofins
	Dienestrol		1			
	Hexestrol		1			
	B-Estradiol		1			
	α-Estradiol		1			
	Estriol		1			
	Estrone		1			
	Ethinyl estradiol		1			
A3 Steroids	α-nandrolon	GC-MS LC-MS/MS	1		Presence	Eurofins
	β-nandrolon		1			
	α-trenbolon		1			
	β-trenbolon		1			
	Trenbolone-acetate		2			
	16-Hydroxy stanozolol		1			
	α -Boldenone		1			
	Boldenone		1			
	Chlor-Testoste rone (Clostebol)		1			
	Epitestosterone		1			
	Methyl-Boldenone (Dianabol)		1			
	Methyltestosterone		1			
	Nortestosterone/ Nandrolone		1			
	Stanozolol		1			
	Testosterone		1			
	Testosterone-propionate		2			
A6 Annex IV substances	Chloramphenicol	LC-MS/MS	0.25		Presence (MRPL = 0.3)	NIFES
	Metronidazole[3]	LC-MS/MS	0.3		Presence (MRPL = 3.0)	
	Hydroxy-metronidazole[3]		2.0			
	Nitrofuran AOZ	LC-MS/MS	0.5		Presence (MRPL =1.0)	
	Nitrofuran AHD		0.6		Presence (MRPL =1.0)	
	Nitrofuran AMOZ		0.4		Presence (MRPL =1.0)	
	Nitrofuran SEM		0.5		Presence (MRPL= 1.0)	
B1 Antibacterial Substances Micro-biological method	Quinolones	3-plate Screening Method[2]	200		100-600	NIFES
	Tetracyclines		200		100	
	Amphenicols		200		1000	
	Sulfonamides		400		100	
B1 Antibacterial substances	Oxolinic acid	LC-MS/MS		30-50	100	Eurofins
	Flumequine			30-50	600	
	Oxytetracycline	LC-MS/MS		30-50	100	

Chemical method	Florfenicol	LC-MS/MS	0.2	0.5	1000	NIFES
B2a Anthelmintics	Praziquantel	LC-MS/MS		1	n.a.	NIFES
	Fenbendazole[3]	LC-MS/MS		1	n.a.	
	Emamectin	LC-MS/MS		5-10	100	
	Diflubenzuron	LC-MS/MS		1-10	1000	
	Teflubenzuron			1-50	500	
	Ivermectin	LC-MS/MS		2	n.a.	Eurofins
	Cypermethrin	GC-MS/MS		5-10	50	
	Deltamethrin			10	10	
B3a Organo-chlorine compounds	Dioxins and dlPCB	HRGC-HRMS		0.0001-0.1 ng TEQ/kg	6.5 ng TEQ/kg	NIFES
	PCB-6	GC-MS GC-MS/MS		0.004 – 0.5	75	
	Pesticides	HRGC-HRMS		0.003-0.8	n.a.	Eurofins
B3b Organo-phosphorus compounds	Azametiphos	LC-MS/MS		10-20	n.a.	Eurofins
	Dichlorvos			10	n.a.	
	Chlorpyriphos Chlorpyrifos-methyl	GC-MS/MS		5	n.a.	
	Pirimiphos-methyl Pirimiphos-ethyl			10	n.a.	
B3c Chemical elements	Lead	ICP-MS		0.006-0.03 mg/kg	0.3 mg/kg	NIFES
	Cadmium			0.001-0.002 mg/kg	0.05 mg/kg.	
	Arsenic			0.003 mg/kg	n.a.	
	Mercury			0.002 mg/kg	0.5 mg/kg	
	Inorganic arsenic	LC-ICP-MS		4-6	n.a.	
	Methylmercury[3]	GC-ICP-MS		3	n.a.	
	Tributyltin[3]	GC-ICP-MS		0.3	n.a.	
B3d Mycotoxins	Ochratoxin A	HPLC-FLU		0.5	n.a.	Eurofins
	Fumonisin B1 Fumonisin B2	LC-MS/MS		20		
B3e, dyes	Malachite green[3]	LC-MS/MS	0.15		Presence (MRPL=2)	NIFES
	Leuco malachite green		0.15			
	Crystal violet		0.30		Presence	
	Leuco crystal violet		0.15		Presence	
	Brilliant green[3]		0.15		Presence	
B3f, others	PBDE	GC-MS		0.003-0.01	n.a.	NIFES
	HBCD	LC-MS/MS		0.006-0.01	n.a.	Eurofins
	TBBPA			0.03-0.2	n.a.	
	PAH	GC-MS		0.4-1.0	n.a.	Eurofins
	PFC	LC-MS/MS		0.5-13	n.a.	NIFES

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

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

[3] Not accredited

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