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

(Conducted to fulfil Norwegian obligations as laid down in Council Directive 96/23/EC)

ANNUAL REPORT FOR 2012

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In 2012, NIFES used sub-contractors for analysis of some parameters: Oslo University Hospital (OUH) for stilbenes and steroids, the Norwegian Veterinary Institute (NVI) for mycotoxins and Eurofins for the analysis of selected therapeutic compounds (see Annex I for details).

INNHOLD

1. SUMMARY

This report summarises the results of the analysis performed on Norwegian farmed fish according to directive 96/23/EC. The Directive lays down specific sampling levels and frequencies, as well as the groups of substances to be monitored for each commodity. The number of samples to be collected from finfish farming products must be at least 1 per 100 tonnes of annual production. This report for 2012 is based on 1 999 fish fillet samples (pooled samples of five fish) and 1 590 liver samples, originating from a total of 11 585 farmed fish.

As defined in the 96/23 monitoring programme, group-A includes substances with anabolic effect and unauthorised substances, approximately 30% of the total samples were analysed for these substances. These samples were collected by official inspectors at the farm without prior notification to the farmers. Samples were taken at all stages of farming and are representative of farmed fish under production. Group-B includes veterinary drugs and contaminants, and approximately 70% of the total samples were analysed for these substances. The group B-samples were taken from fish at processing plants and are representative of Norwegian farmed fish ready to be put on the market.

There was not detected any residues of banned substances (Group A) in any of the samples included in the program for 2012.

For the therapeutic agents in group B, emamectin was detected in two of the 68 pooled samples of farmed fish analysed in 2012. The highest concentration measured was 18 µg/kg, which is far below the current Maximum Residue Limit (MRL) for emamectin of 100 µg/kg. In addition, cypermethrin was detected in one of the 16 pooled samples examined; the measured concentration was 15 µg/kg, which is below the MRL for cypermethrin of 50 µg/kg. Residues of other substances in group B, or their metabolites, were not found in any of the farmed fish samples from 2012.

The mycotoxin Ochratoxin A was not detected in any of the eight pooled salmon samples analysed for 2012.

The concentrations of dioxins (PCDDs and PCDFs) and the organochlorine pesticides other than DDT, in farmed salmon, were comparable to the results from this monitoring programme for the years 2003 to 2011. For dioxin like PCBs (dlPCBs), PCB-7 and DDT the level has decreased over the last ten years. No samples exceeded the EUs maximum limits, where such limits have been established.

The concentrations of mercury, cadmium and lead in farmed fish were below the EU maximum limits for these elements in fish. There is currently no EU maximum limit for arsenic.

Of the brominated flame retardants, only polybrominated diphenyl ethers (PBDEs) were analysed in 2012. The Upper Bound (UB)-mean concentration of PBDE-7 was 0.7 µg/kg in 2012, which is the same as found in 2011. There are currently no EU regulations regarding this compound. Polycyclic aromatic hydrocarbons (PAHs) and perfluorinated compounds (PFCs) were not detected above their limit of quantification (LOQ). There are no maximum limits in the EU for these compounds in fresh fish.

2. TERMINOLOGY

Limit of detection (LOD) and Limit of quantification (LOQ): The terms refer to the methodology applied for detection and quantification, respectively. For compounds that are illegal in fish the LOD is most relevant, since detection of the compound (i.e. with > 99% probability) is important information. For other compounds quantification is required. LOQ is normally higher than LOD by a factor of 3.0 to 3.3. The LOQ is the lower limit for a reliable quantitative measurement; the LOQ of a method may vary somewhat according to differences in the matrix being measured.

Upper bound (UB) calculation: or "upper bound LOQ" calculation is required to be used for certain contaminants according to EU legislation. In UB calculation, all values below the LOQ are replaced with their LOQ value. UB calculation is intended to prevent any methodological limitations from giving artificially low concentrations. In this report UB calculations are used for several contaminants (specified in the table). When relative large parts of the data are below the LOQ, the mean will be artificially high when using UB calculations. Therefore, the median are also presented in several of the cases in order to get a better representation of the "true" average value. In cases where the number of values below the LOQ exceeds 50% of the sample material for a certain parameter, no mean or median is calculated for the parameter. Only the maximum value, the number of values above LOQ, and the LOQ, are reported in these cases.

Maximum residue limit (MRL): is the highest permitted concentration of legally applied agents in products from food animals intended for human consumption.

Minimum required performance limit (MRPL): Is the minimum required performance limit for methods used to determine residues of illegal agents or their metabolites in food. The MRPL is established in accordance with the EU Commission Decision 2002/657/EC

Congener: Congeners are closely related chemicals, analogous compounds within the classes PCB, PBDE, dioxins, furans and toxaphenes. A congener is generally assigned an identification number e.g PCB-153 or PBDE-47.

TEF and TEQ: The WHO established toxic equivalency factors (TEF) for dioxins and dlPCBs in 1998 which were re-evaluated in 2005. For the year 2012, WHO 2005 TEFs values were used. However, the results using WHO 1998 TEFs values are also included so that the results can be compared to previous years. TEF values are applied to 17 PCDD/F congeners and 12 dlPCBs, and summed as toxic equivalents (TEQ) of dioxins and dlPCBs.

3. INTRODUCTION

3.1 Background

Norway is obliged by international agreements and EU legislations to have a monitoring program for pharmaceuticals, illegal substances, and contaminants in food-producing animals both of terrestrial and marine origin. The Norwegian Food Safety Authority (NFSA) is responsible for the enforcement, planning, and sampling related to EU legislation in Norway. On behalf of the NFSA, the National Institute of Nutrition and Seafood Research (NIFES) is responsible for the analyses and reporting in the monitoring program for Norwegian farmed fish.

3.2 Regulations

The aim of this monitoring program is to ensure that the food on the market is safe for human consumption by monitoring residues of pharmaceuticals, illegal substances, and contaminants in the Norwegian farmed fish in accordance with Directive 96/23/EC "On measures to monitor certain substances and residues thereof in live animal and animal products" and specified in Directive 2002/657/EC on the implementation of the above mentioned directive.

In contrast to contaminants, pharmaceuticals are purposely administered in order to provide a desired therapeutic effect. Since low concentrations of drug residues may be found in treated animals for an extended time post therapy, is it necessary to establish acceptable legal residue concentrations in food producing animals. According to current EU legislation each substance is assigned a Maximum Residue Limit (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 are given for each substance either for all food producing species or for groups of related species, such as salmonid fish.

Until 2009, the MRLs were established in accordance with Council Regulation (EEC) No 2377/90. After 2009, Regulation (EC) No 470/2009 set the community procedures for the establishment of residue limits. According to the regulations, substances are to be classified into:

i) those where an MRL is established,
ii) those with a provisional MRLs,
iii) compounds where the establishment of an MRL is not considered necessary
iv) substances for which administration to food producing animals is prohibited.

According to the current Commission Regulation (EU) No 37/2010, the MRLs for fish are set for muscle and skin in natural proportions.

For prohibited pharmacologically active substances, a Minimum Required Performance Level (MRPL) applies to the analytical methods used for monitoring purposes. The MRPL defines a minimum detection limit for the methods, and is set in accordance with the EU Commission Decision 2002/657/EC. This commission decision also dictates analytical performance criterias, and interpretation of results.

Several EU directives exist for environmental pollutants in fish and fishery products for human consumption:

- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
- Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs
- Commission Regulation (EC) No 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs.
- Commission Regulation (EC) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs an non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006.
- Commission Recommendation 2006/88/EC of 6 February 2006 on the reduction of the presence of dioxins, furans and PCBs in feedingstuffs and foodstuffs.
- Commission Recommendation 2006/794/EC of 16 November 2006 on the monitoring of background levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs.
- Commission directive 2002/70/EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs.
- Commission Recommendation 2002/201/EC of 4 march 2002 on the reduction of the presence of dioxins, furans and PCBs in feedingstuffs and foodstuffs.
- Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs
- Commission Directive 2006/77/EC of 29 September 2006 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for organochlorine compounds in animal feed.
- Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs

Regarding the pesticides analysed in this project, there are no maximum limits for fish and seafood, but there are for feedingstuffs including both fish feed and feed ingredients:

- Regulation (EC) No 396/2005 Of The European Parliament and of The Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

Commission Regulation (EU) No 1259/2011 applies from 1 January 2012. A maximum level is assigned to the sum of six indicator PCBs (PCB6). For the dioxins (PCDDs and PCDFs) and the DLPCBs new maximum levels are given and a new list of TEF values (TEF-2005) is to be used.

3.3 Compounds included in the program

Directive 96/23/EC lays down specific sampling levels and frequencies, the groups of substances to be monitored for each food commodity is listed in Annex I of the Directive, The residue or substance group which are monitored for aquaculture animals are:

Group A Substances with anabolic effects and unauthorized substances:

A1: Stilbenes, derivatives and their salts and esters.

A3: Steroids

A6: Prohibited substances according to Regulation (EC) 470/2009.

Group B Veterinary drugs and contaminants:

B1: Antibacterial agents

B2a: Anthelmintics

B2c: Carbamates and pyrethroids

B2f: Other pharmacologically active substances

B3a: Organochlorine compounds

B3b: Organophosphorous compounds

B3c: Chemical elements

B3d: Mycotoxins

B3e: Dyes

B3f: Others, including PBDE, PAH and PFCs

Group A-substances are unauthorized substances, including compounds with anabolic effects. Fish at different stages of farming are sampled by official inspectors from the Norwegian Food Safety Authority (NFSA) at farms with no prior notification, and subsequently analysed for group A-substances. Sampling is performed in such a manner that the samples are representative of farmed fish in all production stages. Group B includes veterinary drugs and contaminants. The group B-samples are taken from fish at processing plants and the samples are representative of fish ready to be placed on the market for human consumption. The number of analysis in each group varies to some extent each year because of targeting of the sampling. This means that the plans made by the NFSA targets the groups to find the combinations where the probability of finding residues is the highest. The knowledge is based on the monitoring of the prescription of pharmaceuticals in the aquaculture industry and on the requirement for an up to date documentation of the levels of pollutants and other contaminants in the products.

4. MATERIALS AND METHODS

According to Directive 96/23/EC the minimum number of samples to be taken each year is one sample per 100 tonnes produced fish. In 2012 this applied to all farmed fish species. Roughly 30 % of the samples were analysed for group A substances, and the remainder of the samples were analysed for group B compounds. Farm sites from all regions with aquaculture activity, and at least 10% of the total number of sites were included in the sampling plan. The sampling plan was randomised with regards to season and region, and the sample identification was blinded for the analysts. Samples consisted of muscle or liver tissue and muscle were shipped to NIFES in a frozen state. Most of the analyses were carried out on pooled fish samples, except for the liver samples which were all analysed individually.

On arrival at NIFES, muscle from five fish were pooled in equal amounts, and then homogenised. Farmed fish are kept in net pens, containing large numbers of fish. The fish from the same cage is therefore exposed to the same feed and environmental influences which may affect contaminant content of fillet. All samples pooled originate from the same cage/farm, and are therefore representative of the cage/farm.

According to Commission regulation (EU) No 37/2010 of 22 December 2009, the MRLs for fish apply for muscle and skin in natural proportions. Due to homogenization difficulties, excluding the skin from the analyses ensure less variation in measurement. Therefore muscle tissue is initially screened for pharmaceutical residues. If any residues are detected, skin is included in an extended confirmatory examination. This is also in the agreement with the provisions of the Commission decision of 12 august 2002 (2002/657/EC) chapter 2. Inclusion of skin in the different analyses is always under consideration, depending on methodology available and its detection limits and variation.

Table 4.1 gives the number of fish analysed in the monitoring programme in 2012, whereas Table 4.2 gives the number of fish for each species. The total number of fish in 2012 was 11 585, consisting of 1 999 fillet samples and 1 590 liver samples. A plan for the sample collection, and analysis was prepared by the NFSA made to ensure statistically independent and representative samples. Since some samples were analysed for more than one parameter, the total sum of the samples was less than the sum of the samples analysed for each parameter. However, as a general rule each sample was only analysed for one parameter.

4.1 Sample data

Substance group		Compound	Fish	Samples	Determinations	Accredited
Samples taken from the farms with no pre- notice	A1 Stilbenes	Diethylstilbestrol Dienestrol Hexoestrol	290[1]	58	58 x 3 = 174	Accredited
	A3 Steroids	α-nandrolon β-nandrolon α-trenbolon β-trenbolon	250[1]	50	50 x 4 = 200	Accredited
	A6	Chloramphenicol	1170[1]	234	234	Accredited
	Illegal drugs: Annex IV To EEC 2377/90	Metronidazole Hydroxy-metronidazole	800[1]	160	160 x 2 = 320	-
		Nitrofuran metabolites 3-Amino-2-oxazolidone 1-Aminohydrantion 3-Amino-5-Morpholinomethyl-Semicarbazide	845[1]	169	169 x 4 = 676	Accredited
	Sum of group A		3355[1]	671	1604	
Samples taken from processing plants	B1 Anti bacterial agents	Florfenicol	85	17	17	Accredited
		Oxytetracyclin	85	17	17	Accredited
		Flumequin	85	17	17	Accredited
		Oxolinic acid	95	19	19	Accredited
	B2 Other veterinary	Teflubenzuron	235	47	47	Accredited
		Diflubenzuron	235	47	47	Accredited
		Cypermethrin	80	16	16	Accredited
		Praziquantel	415	83	83	Accredited
		Fenbendazole	205	41	41	-
		Emamectin	340	68	68	Accredited
		Ivermectin	70	14	14	Accredited
		Deltamethrin	80	16	16	Accredited
	B3a Organohlorine Compounds	DDT, DDE and DDD	260	52	52 * 6 = 312	Accredited
		Pesticides other than DDT, DDE and DDD			1229	
		Dioxin and dlPCB	160	32	32 x 29 = 928	Accredited
		PCB 6	400	80	80 x 6 = 480	Accredited
	B3b Organophosphorous Compounds	Azametiphos	210	42	42	Accredited
		Dichlorvos	205	41	41	Accredited
	B3c	Lead Cadmium	1650	330	330 x 4 = 1320	Accredited

	Chemical elements	Mercury Arsenic				
		Inorganic Arsenic	105	21	21	Accredited
		Methylmercury	110	22	22	Accredited
		Tributyltin	100	20	20	-
	B3d Mycotoxins	Ochratoxin A	40	8	8	Accredited
	B3e, Dyes	Malachite green Leucomalachite green Chrystal violet Leuco Chrystal violet	1995	399	4 x 399 = 1596	Accredited
		Brilliant green	1325	265	1 x 265 = 265	-
	B3f others	PBDE	160	32	32 x 7 = 224	Accredited
		PAH	105	21	21 x 16 = 336	Accredited
		PFC	105	21	21 x 18 = 378	Accredited
	Sum B fillets, pooled		6640[2]	1328[2]	7624	
Liver	B1 Microbiological screening of Liver	Quinolones	1590	1590	1590 x 3 = 4770	Accredited
		Tetracyclines and amphenicols				
		Sulphonamides				
Total sum B			8230[2]	2918[2]	12394	
Total sum fillet A+B			9995[1,2]	1999[2]	9228	
Total sum, fillets, pooled and individual fish and liver			11585[1,2]	3589[2]	13998	

[1]For the A samples, some fish may be so small that more than 5 fish is needed to get enough sample material. This means that the number of fish may be slightly higher than reported.
[2] Some analytes (i.e inorganic arsenic, methylmercury, tributyltin, PBDE, PAH and PFC) have been measured in samples where other substances have been measured as well, in the sum these samples have been counted only once.

4.1 Analytical methods

The analytical methods and the laboratory routines are accredited in accordance with the standard ISO 17025. A few non-accredited methods are still used. These methods are quality assured by the same protocol as the accredited methods, though usually with fewer validation experiments. An overview of accredited methods is shown in table 4.1. Accreditation of these methods is an on-going process, priority is given to group A parameters and to the methods with the highest number of samples to be analysed. The LOD, LOQ and MRPL values for the various analytical methods are given in Annex I.

4.1.1 Quality assurance

For all methods, except for the dioxin method, a quality control sample (QCS) with a known composition and concentration of target analyte, is included in each analytical series. A series is equivalent to the analytical capacity for one day. The dioxin method quantification principle is based on the isotope dilution method which integrates a higher level of quality assurance in the method. Thus the frequency of the QCS analysis is reduced to allow a higher analytical capacity for the dioxins method.

For all methods the QCS results are checked to be within pre-defined limits before the results from a series are approved. With a certain frequency also a "blank analysis" routine is

performed in which a full analysis is carried out without a sample. If a positive value is found for this "sample" this reflects a contamination of reagents or equipment that could affect the results of the actual samples. All methods are regularly verified by participation in inter laboratory proficiency tests, and by analysing certified reference material of relevant test materials (CRM). The results for the verification should be within pre-defined limits before the method is approved for continued use.

The fillet samples are pooled samples of five fish with the exception of the microbiological assay for antibiotics, where liver samples are tested individually. Group A samples may include small fish from early life stages. In this case the pooled sample may be prepared from the whole gutted fishes, or the whole round fishes in order to get enough sample material for the analysis. When the fish was very small, more than 5 fish may be required in the pooling of the sample to obtain enough sample material for performing the analyses. A summary of the analytical methods used is shown in Annex I.

4.1.2 Group A substances

The group-A samples were analysed for hormone-like substances in the group of stilbenes (A1), steroids (A3), and for illegal drugs (A6).

Group A1 and A3

The stilbenes (A1) diethylstilbestrol, dienesterol, hexosterol and steroid compounds (A3) compounds α-nandrolon, β-nandrolon, α-trenbolon and β-trenbolon, were analysed by GC/ MS. If positive findings should occur they would be verified by confirmatory methods, including an additional clean-up step by HPLC before a new derivatization step followed by a final analytical determination by GC/MS.

Group A6, Annex IV substances to council regulation EEC 2377/90

Chloramphenicol
Chloramphenicol is an antibiotic with activity against a broad spectrum of microorganisms. It has been used in human and veterinary medicine since 1949, but due to a rare but serious dose-independent adverse effect (*e.g.* aplastic anaemia); this agent is no longer authorized in the treatment of food-producing animals.

Analytical method: An internal standard (chloramphenicol-*d*5) was added to the sample before extraction with ethyl acetate. The sample was analysed by LC-MS, with a reversed phase C18 column for separation. The sample was ionized by ESI and detected as negative ions using the SIM mode. Quantification was based on the internal standard method.

Nitrofurans
This group of synthetic antibacterial agents are derivatives of nitrofurane. These pharmaceuticals have previously been widely used in veterinary medicine. In the fish tissue these agents are rapidly metabolized. Thus the metabolites 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semicarbazide (SEM) have been included in the assay.

Analytical method: The analytes 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 in the positive ionisation mode.

Metronidazole and its metabolite hydroxymetronidazole
Metronidazole is a synthetic antimicrobial compound that is used against infections caused by anaerobic bacteria and certain parasites. Metronidazole is not authorized in the treatment of food-producing animals.

Analytical method: Internal standard (dimetronidazole-*d*3) was added to the homogenized sample. The analytes were extracted by ethyl acetate and the extract was analysed by LC-MS/MS. A reversed phase C18 column was used for the chromatographic separation, and the components were ionized by ESI and the fragments detected as positive ions using the MRM mode. Quantification was based on the internal standard method.

4.1.3 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

Analytical method: In this assay for antibacterial agents, a three-plate microbiological inhibition method was applied. Each plate contained growth agar and a specific bacterial strain particularly sensitive to these analytes was added. The applied combination of agars and strains were *Escherichia coli* CCUG 2468 (syn. ATCC 11303) on Test Agar pH 7.2 for the quinolones, *Bacillus cereus* var. *mycoides* ATCC 11778 on Antibiotic Agar pH 5.85 for the tetracyclines and amphenicols, and *Kocuria rhizophila* CCUG 42340 (syn. *Micrococcus luteus* ATCC 9341) on Mueller Hinton Agar pH 7.3 for the sulphonamide group. Finally, small pieces of liver were placed on the plates. 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.

Oxolinic acid and flumequine: Oxolinic acid and flumequine belong to a family of synthetic antibacterial agents termed quinolones. These agents have been, and are presently applied in the treatment of bacterial infections in fish.

Analytical method: The analytes were extracted with acetonitrile, and analysis was performed by LC-MS/MS in the positive mode.

Oxytetracycline
Oxytetracycline belongs to the tetracycline antibiotics. It is a broad spectrum antibiotic that is active against a wide range of bacteria.

Analytical method: The analyte was extracted with an EDTA-succinate aquatic buffer. Solid phase extraction was used for sample clean-up. The analyte was then determined by LC-MS/MS in the positive ionisation mode.

Florfenicol
Florfenicol belongs to a group of antibiotics termed amphenicols. The compound has found wide application in treatment of bacterial diseases in fish.

Analytical method: An internal standard (chloramphenicol-*d*5) was added to the sample, and the analyte were extracted with ethyl acetate. The extract was analysed by LC-MS and detected as negative ions in the SIM mode. Quantification was based on the internal standard method.

B2a, Anthelmintics

Diflubenzuron and teflubenzuron
Diflubenzuron and teflubenzuron are both chitin synthesis inhibitors used in treatment against sea lice.

Analytical method: For the analyses of diflubenzuron, internal standard (teflubenzuron) was added to the sample, and the analytes were extracted with acetone. The samples were analysed by LC-MS and detected as negative ions using the SIM mode. Quantification was based on the internal standard method.

Analytical method: For the analyses of teflubenzuron, internal standard (diflubenzuron) was added to the sample, and the analytes were extracted with acetone. The samples were analysed by LC-MS and detected as negative ions using the SIM mode. Quantification was based on the internal standard method.

Ivermectin and Emamectin
Ivermectin and emamectin belong to the class of avermectins. Emamectin is used against external parasites on fish.

Analytical method: For the analysis of emamectin, internal standard (ivermectin) was added to the sample and the sample was analysed by LC-MS (APCI) and detected as positive ions using the SIM mode. Quantification was based on the internal standard method.

Analytical method: For the analysis of ivermectin, internal standard (emamectin) was added to the sample and the sample was analysed by LC-MS (APCI) and detected as positive ions using the SIM mode. Quantification was based on the internal standard method.

Cypermethrin and deltamethrin
Cypermethrin and deltamethrin are synthetic pyrethroids used in bath treatment against sea lice.

Analytical method: Cypermethrin and deltamethrin were extracted from the samples with acetone. The samples were analysed and quantified by gas chromatography-electron capture detector (GC-ECD). The quantification is based on the internal standard method.

Fenbendazole
Fenbendazole is a broad spectrum benzimidazole used against intestinal parasites in fish.

Analytical method: The samples are extracted using methanol and water, before the fat is removed using petroleum ether. Sodium dihydrogen phosphate and a mixture of diethyl ether/ethyl acetate were then added to the polar extract before shaking and centrifugation, and the upper layer was collected and vaporized. The extracted sample was dissolved in a solution of acetonitrile and water prior to analysis on LC-MS/MS and detected as positive ions in the MRM mode. Oxibendazol was used as an internal standard and quantification was based on the internal standard method.

Praziquantel
Praziquantel is an isoquinolin agent used against intestinal parasites in fish.

Analytical method: Praziquantel is extracted from the homogenized sample by acetone. Diethyl ether and hexane are used for further extraction before the sample is solubilised in mobile phase and analysed using LC-UV. Quantification is based on external calibration curve.

B3a, Organochlorine compounds

This is a heterogeneous group of lipophilic compounds such as PCBs and dioxins that exhibit a range of chemical and pharmacological properties. They are persistent in the environment and accumulate in the food chain. For this reason they are of environmental concern, and a food safety issue.

Polychlorinated biphenyls (PCB)
Commercial PCB mixtures were previously produced on a large scale for a variety of industrial applications. There are 209 theoretical PCB congeners and technical mixtures used to contain a varying amount of a high number of congeners. The use of PCB was banned in Norway in 1980. European PCB levels in food and feed are monitored and regulated according to a number of EU regulations:

- Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs
- Commission Regulation (EC) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs an non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006.
- Commission Recommendation 2006/88/EC of 6 February 2006 concerning the reduction of the presence of dioxins, furans and PCBs in feedingstuffs and foodstuffs.
- Commission Recommendation 2006/794/EC of 16 November 2006 on the monitoring of background levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs

The International Council for the Exploration of the Sea (ICES) selected seven congeners for monitoring PCB contamination in the marine environment. This list is known as PCB-7 or ICES-7 and consists of these PCB compounds: PCB-28, -52, -101, -118, -138, -153 and -180. The PCB- list overlaps with the list of dlPCBs (described in the next section) in that PCB-118

is found in both lists. Thus the new maximum level for non-dlPCBs are the sum of 6 PCBs (PCB-6) This report provides data for both the sum PCB-6 and the sum PCB-7.

Analytical method: Sample were extracted using hexane, while fat is broken down on-line with sulphuric acid impregnated silica gel in the cells. The extract is further purified of fat using sulphuric acid. The extracted sample was analysed on GC/MS in SIM mode with electron impact ionization. Quantification was based on the internal standards method. The method quantified the PCBs no. 28, 52, 101, 118, 138, 153 and 180. The LOQ values for the compounds are listed in Table 5.3.

Dioxins, furans, and the non-orto and mono-orto DLPCBs.
Dioxins (PCDD and PCDF) are unwanted by-products in various industrial processes, and from the combustion in waste incineration plants. A selection of seventeen dioxin/ furane congeners and twelve PCB congeners were assigned toxic equivalency factors (TEFs) in 1998 by the WHO. The TEF values are relative to the most toxic dioxin: 2,3,7,8 TCDD. In 2005 the TEF list was revised by the WHO. Concentrations are expressed in toxic equivalency units (TEQ) which are the analytical concentrations multiplied by the corresponding TEF value.

Analytical method: This is an adaptation to modern clean-up equipment of the US-EPAs (Environmental Protection Agency) methods No. 1613 and 1668. 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. Also, 10 BFR congeners in the PBDE class are analysed in this method. Recovery data is calculated for each sample based on the recoveries of the internal standards relative to the two labelled recovery standards. There are individual LOQ values assigned to each congener.

Polychlorinated pesticides, including DDT and its metabolites
This group of compounds include a wide range of complex molecular structures and physical and chemical properties. The method determines a selection of compounds that are persistent in the environment and accumulate in food chains. All of these compounds are potentially of food safety concern. In 2012, some samples have been analysed for pesticides at NIFES while some samples have been analysed by a sub-contractor. There are a few variation in what pesticides the methods measures. The method at NIFES include: Pentachlorobenzene, hexachlorobenzene, α-HCH, γ-HCH, DDT and its metabolites (pp-DDT, op-DDT, pp-DDD, op-DDD, pp-DDE and op-DDE), heptachlor, heptachlor epoxide, aldrin, dieldrin, isodrin, mirex, oxy-chlordane, trans-chlordane, cis-chlordane, α-endosulfan, β-endosulfan, endosulfan-sulphate, trans-nonachlor, cis-nonachlor and the toxaphene congeners TOX-26, TOX-32, TOX-40+41, TOX-50 and TOX-62. Most of these pesticides are included in the method performed at the sub-contractor as well, however, Isodrin, Cis-nonachlor, TOX 32, TOX-40+41 and TOX-42a, is not determined. In addition the sub-contractor determines β-HCH, δ-HCH, endrin, octachlorstyrol and cis-heptachlor epoxide.

Analytical method: Method at NIFES: Pesticides were extracted using hexane at 75^{o}C under 1500 psi pressure. The sampleextract was then divided in twos. Either, the extract was acid treated and analysed on GC/MS in EI, or cleaned up through three columns, ChemElut, QuEChERS and C18 and detection on GC/MS in NCI. Quantification was based on the internal standard method.

Method at subcontractor: Pesticides were extracted from the sample, and cleaned up by column crohatograpgy. The solution was analysed by HRGC/HRMS. Quantification was based on the internal standard method.

B3b, Organophosphorous compounds

Azametiphos and dichlorvos
These are both agents that have found applications in bath treatments against sea lice on fish.

Analytical method: The sample material was extracted with acetone. The extract was cleaned up by gel permeation chromatography and analysed by GC-FPD.

B3c, Chemical elements

Lead, mercury, cadmium and tin
Chemical elements such as cadmium and mercury occur both naturally in the environment and also as a result of anthropogenic activity. From a food safety perspective, contamination of the feed during feed production, storage or transportation is potentially the most serious source of contamination in farmed fish. In Norway there has been one incident where a mineral mixture added to fish- and other animal feed was contaminated by cadmium, the source was identified and the feed was collected and destroyed.

Analytical method: The sample was decomposed in acid, assisted by heat and high pressure. The analytes in solution were then simultaneously measured quantitatively by inductively coupled plasma mass spectrometer (ICPMS). The elements measured were: arsenic, cadmium, mercury and lead. Rhodium was used as an internal standard and gold was added to stabilize mercury. As part of the quality control, two certified reference materials (CRM) were analysed in each analytical series: Tort-2 (lobster hepatopancreas) and Dorm-2 (dogfish muscle).

Inorganic Arsenic, methylmercury and tributyltin
In addition to the chemical elements, inorganic arsenic, methylmercury and tributyltin was determined as well.

Analytical methods: Inorganic arsenic is extracted by hydrochloric acid in hydrogen peroxide at 90 °C. Inorganic arsenic includes As (III) and As (V). As (III) is oxidised to As (V) during the extraction. Inorganic arsenic is separated from other arsenic compounds by anionic axchange HPLC, and detected by ICP-MS. Quantification is based on external calibration curve.
Methylmercury is extracted by Tetramethylammonium Hydroxide. The pH is adjusted before derivatization and extraction by hexane. The samples were analysed by GC-ICP-MS.
Tributyltin is extracted by acetic acid/methanol. The pH is adjusted before derivatization and extraction by hexane. The samples were analysed by GC-ICP-MS.

B3d, Mycotoxins

Ochratoxin A.
Feed and food can be infected by moulds if stored under inappropriate conditions. Some moulds produce toxic secondary metabolites, collectively known as mycotoxins. One mycotoxin of specific relevance for farmed fish feed is Ochratoxin A.

Analytical method: The sample material is weighed in together with Celite, before chloroform and phosphoric acid is added. This is 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.
These dyes are triphenylmethane compounds. Historically some of these compounds were used to treat fish and fish eggs against fungal infections in the fresh water phase, MG was formerly used for this purpose in Norway. However, all three compounds are considered toxic, and their uses in food-producing animals are now forbidden. MG and CV are quickly metabolized in fish tissue, and are normally detected as their "leuco" derivative (LMG and LCV). If only MG or CV is found, without a simultaneous presence of LMG and LCV it may indicate that the fish have been contaminated *post mortem*.

Analytical method: The samples were extracted with acetonitrile and dichloromethane and analysed by LC-MS/MS. A reversed phase C18 column was used for separation and the components is ionized by ESI and detected as positive ions using the MRM mode. Quantification was based on the internal standard method.

B3f, Others

These compounds are not included in Directive 96/23/EC, but were included in the monitoring programme for 2012 on request of the Norwegian Food Safety Authority. They include PBDE, PFC and PAH.

PBDE
PBDEs are organobromine compounds that are used as flame retardant. The family of PBDEs consists of 209 possible congeners. PBDEs are structurally very similar to PCBs, except the former contain bromine instead of chlorine. The most common PBDE congeners in the environment and in food are at present: PBDE-47, PBDE-99 and PBDE-100. NIFES measures the congeners included in PBDE-7: 28, 47, 99, 100, 153, 154 and 183. PBDE 66, 119 and 138 are also measured.

Analytical method: PBDE are measured together with the dioxins, furans, and the non-orto and mono-orto DLPCBs (B3a, Organochlorine compounds).

PFC
PFCs have unique properties which make materials stain, oil, and water resistant, and are these compounds are widely used in diverse applications. PFCs persist in the environment as persistent organic pollutants; hence they were included as analytes in this monitoring programme.

Analytical method: mass-labelled internal standards were added to the sample prior to extraction and sample clean up. PFCs (18 different forms including PFOS and PFOA) were analysed by LC/MS/MS and quantified using internal standards and calibration curves.

PAH

PAHs are formed by incomplete combustion or heat-induced decomposition of organic matter. Several are genotoxic and carcinogenic. Food is a major route of exposure which can be contaminated with PAHs from environmental sources, industrial food processing and from certain home cooking practices.

Analytical method: Deuterated Benzo(a)pyrene was added to the samples as an internal standard. Cyclohexane was added to extract the PAHs from the sample which was analysed by GC/MS in single ion mode.

Table 4.2 Number of fish of each species and the number of parameters analysed

Class of compounds		Compounds	Fish	Atlantic Salmon	Rainbow trout	Turbot	Atlantic Halibut	Atlantic Cod	Arctic char	Wolf-fish
Samples taken from the farms with no pre-notice	A1 Stillebenes	Diethylstilboestrol Dienoestrol Hexoestrol	290*	280	5			5		
	A3 Steroids	α-nandrolon β-nandrolon α-Trenbolon β-trenbolon	255*	240	10					
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	1170*	1060	50	5	10	35	10	
		Metronidazole Metronidazole-OH	800*	735	40			20	5	
		Nitrofuran metabolites (AOZ, AMOZ, AHD, SEM)	845*	730	45	5	5	40	15	5
Samples taken from processing plants	B1 Chemical method in muscle	Florfenicol	85	80	5					
		Oxytetracycline	85	80	5					
		Flumequine	85	80	10					
		Oxolinic acid	95	90	5					
	B1 Microbiological assay in liver	Quinolones Tetracyclines and Amphenicols Sulphonamides	1590	1435	110	5		30	10	
	B2 Other veterinary drugs	Teflubenzuron	235	225	10					
		Diflubenzuron	225	220	15					
		Cypermethrin	80	75	5					
		Praziquantel	415	385	25			5		
		Fenbendazole	205	190	10					
		Emamectin	340	310	30					
		Ivermectin	70	65	5					

		Deltamethrin	80	75	5					
	B3a Organochlorine compound	DDT, DDE og DDD	260	245	15					
		Pesticides other than DDT, DDE and DDD								
		Dioxins and dlPDBs	160	155	5					
		PCB-7	400	375	15					
	B3b Organophosphorous Compounds	Azametiphos	210	200	10					
		Dichlorvos	205	195	10					
	B3c Chemical elements	Lead Cadmium Mercury Arsenic	1650	1525	10			5		
		Inorganic Arsenic	105	105	105					
		Methylmercury	110	110	110					
		Tributyltin	100	100	100					
	B3d	Mycotoxins	35	35						
	B3e, Dyes	Malachite green Leucomalachite green	1995	1855	120			15	5	
		Crystal violet Leucocrystal violet								
		Brilliant green	1325	1250	70			5		
	B3f, Others	PBDE	160	155	5					
		PAH	105	105						
		PFC	105	100	5					

5. RESULTS AND DISCUSSION

5.1 Group A

A total of 671 pooled fillet samples from 3 355 fish, were examined with respect to residues of pharmacologically active substances in group A. The samples were collected at the fish farm by inspectors from the Norwegian Food Safety Authority with no prior notice. The samples in this group are collected from different growth phases, not only from market-sized fish. This sometimes means that more than five fish are needed to obtain enough sample material, meaning that number of fish analysed may be slightly higher than reported.

5.1.1 Group A1

The levels of the group A1 substances diethylstilbestrol, dienestrol and hexoesterol were examined in 58 pooled samples from a total of 290 fish from three species. The detection limits (LOD) are listed in Annex I and the number of fish from each species is listed in Table 4.2. None of the substances were detected in any of the samples analysed.

5.1.2 Group A3

The levels of group A3 substances nortestosterone (α and β nandrolon) and α and β trenbolon, were analysed in 50 pooled samples from 250 fish from two species. The detection limits (LOD) are listed in Annex I, the number of fish from each species is listed in Table 4.2. None of the substances were detected.

5.1.3 Group A6 (annex IV to EEC 2377/90)

A total of 563 pooled samples from 2 815 fish were analysed in this group. The detection limits (LOD) are listed in Annex I, and the number of fish analysed of each species is listed in Table 4.2. No residues were detected in this group.

5.2 Group B

There were 1 328 pooled fish samples of fillets from a total of 6 640 fish, and additionally 1 590 individual fish liver samples for the inhibition test. Samples were taken at processing plants of fish that were market-size.

5.2.1 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 1 590 fish representing a total of 4 770 analytical determinations. The B1 antibacterial agents: florfenicol, oxytetracyclin, flumequin and oxolinic acid, were also analysed by chemical methods in a total of 70 pooled fillet samples, representing 350 fish. The LODs for each compound are listed in Annex I.

The liver has a central function in the distribution and elimination of drugs from fish as for other vertebrates. Higher concentrations of these compounds are thus generally found in the liver compared to muscle. 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 antibiotics. Moreover, the bio-assay is able to detect a wider range of antibiotics than the more specific chemical methods. This makes it 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. However, no positive samples were found in the B1 group in farmed fish livers in 2012.

5.2.2 Group B2a, anthelmintics, B2c, carbamates and pyrethroids and B2f, others.

The levels of the B2 substances teflubenzuron (B2f), diflubenzuron (B2f), cypermethrin (B2c), praziquantel (B2a), fenbendazole (B2a), emamectin (B2a), ivermectin (B2a) and deltamethrin (B2c) were determined in 332 pooled fillet samples representing 1 660 fish from four species. Emamectin was detected in two out of 68 pooled samples and cypermethrin was detected in one out of 16 pooled samples. According to the analytical protocol, any detection of drug residues would be followed by a re-analysis of muscle and skin in natural proportions in duplicate, and also analysis of a backup-sample when available. Analysis of muscle and skin gave concentrations ranging from 2.5 to 18 µg/kg for emamectin. The current MRL for emamectin is 100 µg/kg. The concentration of cypermethrin was 15 µg/kg, while the MRL is 50 µg/kg. This means that all three samples were far below their MRL. Residues of other agents in this group, or their metabolites were not found in any of the samples. Detection limits (LOD) for the substances are specified in Annex I.

5.2.3 Group B3a, Organochlorine compounds

These compounds have traditionally received much focus from a food safety perspective. In 2012 there were 164 samples from 820 fish analysed for these compounds. The results are summarised in Tables 5.1 to 5.4.

Organochlorine pesticides

There were 52 samples analysed for organochlorine pesticides, the results are given in Tables 5.1 and 5.2. For several of the parameters there are no measurable values since levels were below the LOQ. Data from previous years suggest that there is a significant variation in levels among fish species, and the levels reflect the variation in their fat content. This is consistent with the lipophilic nature of these compounds. This year only samples from salmon and rainbow trout were measured.

The UB-mean of sum DDT was 7 µg/kg w.w. and the highest concentration was 20 µg/kg w.w. The concentration of DDT has decreased over the last ten years.

Table 5.1 DDT, DDD and DDE (µg/kg w.w.) in fillets of farmed fish

	op-DDT	pp-DDT	op-DDD	pp-DDD	op-DDE	pp-DDE	UB-Sum DDT
LOQ min		0.4	0.4				
LOQ max	0.4	0.6	0.5	0.4	0.4	0.4	
Atlantic Salmon							
Number	49	49	49	49	49	49	49
#values	16	29	16	48	16	49	
UB-Mean	-	0.7	-	1	-	4	7
Min	LOQ	LOQ	LOQ	LOQ	LOQ	1.0	3
Max	0.2	2	0.6	5	0.3	12	20
Rainbow trout							
Number	3	3	3	3	3	3	3
#values	1	1	1	3	1	3	
UB-Mean	-	-	-	1	-	3	5
Min	LOQ	LOQ	LOQ	0.5	LOQ	1	4
Max	0.1	0.6	0.3	2	0.1	5	8
All groups							
Number	52	52	52	52	52	52	52
#values	17	30	17	51	17	52	
UB-mean	-	0.7	-	1	-	3	7
Min	LOQ	LOQ	LOQ	LOQ	LOQ	1.0	3
Max	0.5	2	0.6	5	0.3	12	20

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

The results for the other 29 pesticide compounds analysed are summarised in Table 5.2. The values ranged from <LOQ to 5 µg/kg wet weight, the highest concentration in 2012 was for dieldrin and TOX-50. Most of the pesticides were present at concentrations below their respective LOQ values hence it was not possible to calculate a representative mean or median, value. These low levels are consistent with the findings from previous years.

Table 5.2. Pesticides (µg/kg w.w.) in fillets of farmed fish

Pesticide		Atlantic salmon	Rainbow Trout	All Groups	LOQ
α-HCH	No. samples	49	3	52	
	#Values	33	2	35	
	UB-mean	0.2	0.2	0.2	
	Min	0.1	LOQ	LOQ	0.2
	Max	0.5	0.3	0.5	0.3
γ-HCH	No. samples	31	2	33	
	#Values	29	2	31	
	UB-mean	0.3	0.4	0.4	
	Min	LOQ	0.1	LOQ	0.3
	Max	0.4	0.7	0.7	4
HCB	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	1.7	1.1	1.6	
	Min	0.3	1	0.3	
	Max	4	1.9	4	0.2
Pentachlorobenzene	No. samples	49	3	52	
	#Values	19	1	20	
	UB-mean				
	Min	0.2	LOQ	LOQ	0.1
	Max	0.4	0.4	0.4	0.2
Heptachlor	No. samples	49	3	52	
	#Values	2	0	2	
	UB-mean				
	Min	0.1		LOQ	0.1
	Max	0.1	LOQ	0.1	0.3
Heptachlor A	No. samples	49	3	52	
	#Values	0	0	0	
	UB-mean				
	Min				0.1
	Max	LOQ	LOQ	LOQ	0.3
Aldrin	No. samples	49	3	52	
	#Values	0	0	0	
	UB-mean				
	Min				0.1
	Max	LOQ	LOQ	LOQ	0.3
Isodrin	No. samples	33	2	34	
	#Values	0	0	0	
	UB-mean				
	Min				0.1
	Max	LOQ	LOQ	LOQ	1.7
Dieldrin	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	2.1	1.2	2.0	

	Min	0.8	1	1	
	Max	5	2.1	5	0.1
α-endosulfan	No. samples	49	3	52	
	#Values	12	0	12	
	UB-mean				
	Min	0.1		LOQ	0.1
	Max	0.5	LOQ	0.5	0.3
β-endosulfan	No. samples	49	3	52	
	#Values	4	0	4	
	UB-mean				
	Min	0.1		LOQ	0.1
	Max	0.3	LOQ	0.3	0.5
Endosulfan sulphate	No. samples	49	3	52	
	#Values	24	1	25	
	UB-mean				
	Min	0.1	LOQ	LOQ	0.1
	Max	0.6	0.2	0.6	6.0
***cis*-chlordane**	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	0.8	0.4	0.8	
	Min	0.3	0.2	0.2	
	Max	1.8	0.6	1.8	0.2
***oxy*-chlordane**	No. samples	49	3	52	
	#Values	47	2	49	
	UB-mean	0.2	0.1	0.2	
	Min	LOQ	LOQ	LOQ	
	Max	0.7	0.2	0.7	0.1
***trans*-chlordane**	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	0.2	0.1	0.2	
	Min	0.1	0.1		
	Max	0.5	0.1	0.5	0.1
***cis*-nonachlor**	No. samples	33	2	35	
	#Values	33	2	35	
	UB-mean	0.3	0.1	0.3	
	Min	0.1	0.1	0.1	
	Max	0.5	0.2	0.5	0.1
***trans*-nonachlor**	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	1.0	0.5	0.9	
	Min	0.3	0.2	0.2	
	Max	2.5	0.8	2.5	0.2
TOX-26	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	0.9	0.5	0.9	
	Min	0.2	0.2	0.2	

	Max	2.4	0.9	2.4	0.2
TOX-32	No. samples	33	2	35	
	#Values	0	0	0	
	UB-mean				
	Min				
	Max	LOQ	LOQ	LOQ	0.3
TOX-40+41	No. samples	33	2	35	
	#Values	33	2	35	
	UB-mean	0.3	0.1	0.3	
	Min	0.1	0.1	0.1	
	Max	1.1	0.1	1.1	0.1
TOX-42a	No. samples	33	2	35	
	#Values	31	2	33	
	UB-mean	0.2	0.1	0.2	
	Min	LOQ	0.1	LOQ	
	Max	0.5	0.1	0.5	0.1
TOX-50	No. samples	49	3	52	
	#Values	49	3	52	
	UB-mean	1.5	0.7	1.4	
	Min	0.4	0.4	0.4	
	Max	5	1.3	5	0.3
TOX-62	No. samples	36	2	38	
	#Values	34	1	35	
	UB-mean	1.0		0.9	
	Min	0.2	0.2	0.2	0.4
	Max	2.5	0.2	2.5	1.5
Mirex	No. samples	49	3	52	
	#Values	20	1	21	
	UB-mean				
	Min	LOQ	LOQ	LOQ	
	Max	0.2	0.1	0.2	0.1
δ-HCH	No. samples	16	1	17	
	#Values	1	0	1	
	UB-mean				
	Min	LOQ		LOQ	
	Max	0.1	LOQ	0.1	0.1
Endrin	No. samples	16	1	17	
	#Values	14	1	15	
	UB-mean	0.3		0.3	
	Min	LOQ		LOQ	0.1
	Max	0.5	0.1	0.5	0.2
Octachlorstyrol	No. samples	16	1	17	
	#Values	16	1	17	
	UB-mean	0.1		0.1	
	Min	0.1		0.1	
	Max	0.2	0.1	0.2	

β-HCH	No. samples	16	1	17	
	#Values	16	1	17	
	UB-mean	0.4		0.3	
	Min	0.2		0.2	
	Max	0.7	0.3	0.7	
Cis-Heptachlor epoxide	No. samples	16	1	17	
	#Values	16	1	17	
	UB-mean	0.6		0.5	
	Min	0.3		0.3	
	Max	0.9	0.3	0.9	

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

Polychlorinated biphenyls (PCBs)

The concentrations of PCB-7 and indicator PCBs (PCB-6) in farmed fish are given in Table 5.3. For 2012, the data is mainly represented by Atlantic salmon (77 samples), but also three rainbow trout samples have been measured. The PCB-7, calculated as the "upper bound-LOQ" (UB) sum in the salmon samples ranged from 2.2 to 10.5 µg/kg w.w.. The UB-mean sum PCB-6 in salmon was 4.0 µg/kg in 2012 compared to 4,5 µg/kg in 2011. For PCB-7 the UB-mean level has been halved the last ten years. Since 2003 the congeners PCB-138 and PCB-153 have been the main contributors to the sum PCB-7. The EUs maximum limit for indicator PCBs in fish is 75 µg/kg w.w.. The highest concentration of indicator PCBs measured in salmon in 2012 was 9.4 µg/kg w.w., which is well below the maximum limit.

Table 5.3 PCB-7 and PCB-6 (µg/kg w.w.) in fillets of farmed fish

	PCB -28	PCB -52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	UB Sum PCB-7	UB Sum PCB-6
LOQ min	0.03		0.03	0.01	0.03	0.03	0.03		
LOQ max	0.10	0.10	0.10	0.10	0.10	0.10	0.05		
Atlantic Salmon									
N	77	77	77	77	77	77	77	77	77
# Values	73	74	77	77	77	77	77	77	77
Median	0.2	0.4	0.7	0.5	1.0	1.1	0.3	4.2	3.8
UB-Mean	0.2	0.4	0.7	0.5	1.1	1.2	0.3	4.5	4.0
Min	0.1	0.1	0.3	0.2	0.5	0.6	0.2	2.2	2.0
Max	0.6	0.7	1.6	1.1	2.5	3.1	0.8	10.5	9.4
Rainbow trout									
N	3	3	3	3	3	3	3	3	3
# Values	1	3	3	3	3	3	3	3	3
Median	-	0.4	0.6	0.4	0.9	1.0	0.3	4.3	3.3
UB-Mean	-	0.3	0.6	0.4	0.9	0.9	0.3	3.6	3.2
Min	-	0.2	0.4	0.3	0.7	0.8	0.2	4.3	2.5
Max	0.3	0.4	0.8	0.5	1.0	1.0	0.4	4.3	3.8
All groups									
N	80	80	80	80	80	80	80	80	80
# Values	74	77	80	80	80	80	80	80	80
Median	0.2	0.4	0.7	0.5	1.0	1.1	0.3	4.2	3.7
UB-Mean	0.2	0.4	0.7	0.5	1.1	1.2	0.3	4.5	4.0
Min	LOQ	LOQ	0.3	0.2	0.5	0.6	0.2	2.2	2.0
Max	0.6	0.7	1.6	1.1	2.5	3.1	0.8	10.5	9.4

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

Dioxins, furans and dioxin like PCBs

A summary of the total WHO_{2005}TEQ values (ng TEQ/kg w.w.) for the 29 congeners is listed in Table 5.4. All figures in the table are calculated as the "upper bound-LOQ". A total of 32 pooled and individual samples were analysed from 160 fish. The fish species analysed were Atlantic salmon and rainbow trout.

For the 17 dioxin and furan compounds (PCDD + PCDF) the sum ranged from 0.1 ng TEQ/kg to 0.6 ng TEQ/kg w.w. in salmon. The mean sum was 0.2 ng TEQ/kg w.w. for Atlantic salmon. No mean was calculated for rainbow trout since this was based on 1 sample. The maximum value of 0.6 ng TEQ/kg w.w. is below the EU's maximum limit of 3.5 ng TEQ/kg w.w.

The sum of dioxins and dioxin like PCBs ranged from 0.2 to 1.1 ng TEQ/kg w.w. in salmon. The mean concentration was 0.5 ng TEQ/kg w.w. All values were below the EU maximum limit of 6.5 ng TEQ/kg w.w.

Since calculations of dioxins, furans and dlPCBs previously has been done by WHO_{1998}TEF values, these results are included in the table to be able to compare this year's data with previous results. The UB-mean results for PCDD/F in 2012 calculated with WHO_{1998}TEF values was 0,3 ng TEQ/kg w.w. which is the same as in 2011. For PCDD/F and DLPCB the UB-mean was 0,6 ng TEQ/kg w.w. in 2012 which is slightly lower than in 2011 where the UB-mean were 0,8 ng TEQ/kg w.w. Over the last ten years the concentrations of dioxin have been fairly stable, while the concentration of dlPCBs has decreased.

Concentrations of dioxins and dlPCBs in farmed salmon measured for this monitoring programme have always been found to be below the EU maximum limit by a fair margin.

Table 5.4 Dioxins (PCDD/F) and dlPCBs (ng TEQ/kg w.w.) in fillets of farmed fish

		Atlantic Salmon	Rainbow trout	All Groups	EU Limit
Sum PCDD/F$_{2005}$	Samples	31	1	32	
	Median	0.2	-	0.2	
	UB-Mean	0.2	-	0.2	
	Min	0.1	-	0.1	
	Max	0.6	0.2	0.6	3.5
Sum PCDD/F$_{2005}$ + dlPCB	Samples	31	1	32	
	Median	0.5	-	0.5	
	UB-Mean	0.5	-	0.5	
	Min	0.2	-	0.2	
	Max	1.1	0.5	1.1	6.5
Sum PCDD/F$_{1998}$	Samples	31	1	32	
	Median	0.2	-	0.2	
	UB-Mean	0.3	-	0.3	
	Min	0.1	-	0.1	
	Max	0.6	0.3	0.6	4.0
Sum PCDD/F$_{1998}$ + dlPCB	Samples	31	1	32	
	Median	0.6		0.6	
	UB-Mean	0.6		0.6	
	Min	0.1		0.1	
	Max	1.3	0.5	1.3	8.0

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

5.2.4 Group B3b, Organophosphorous compounds

The levels of the B3b substances azametiphos and dichlorvos were determined in 42 and 41 pooled fillet samples respectively, representing 210 and 205 fish from two species. Residues of these two agents were not found in any of the examined samples.

5.2.5 Group B3c, Chemical elements

The concentrations of chemical elements were determined in 330 pooled fish samples from the fillets of 1650 fish (Table 5.5).

Arsenic

Arsenic in fish is present mainly as organo-arsenic compounds of low toxicity. The measured values are "total arsenic", the sum of arsenic from all arsenic containing molecular species in the sample. The arsenic levels in the fillets of farmed fish ranged from 0.16 to 1.2 mg/kg w.w. (Table 5.5). The level of total arsenic, in fillets of farmed fish, measured in this project has gradually decreased over the last 10 years from 2.2 mg/kg w.w. in 2003 to 0.56 mg/kg w.w. in 2012. There is currently no EU upper limit for either total arsenic or inorganic arsenic in fish fillets.

Cadmium

The concentrations of cadmium in most samples analysed since 2002 have been less than the LOQ, which also applies to 2012 where only 10 were above LOQ. However, there have been some measurements where the cadmium level has been close to the EUs maximum limit. For 2012, the maximum concentration measured was 0.042 mg/kg w.w.

Mercury

The concentration of total mercury in farmed fish ranged from 0,007 to 0.045 mg/kg w.w. in 2012 (Table 5.5). UB-mean was calculated to 0,015 mg/kg w.w. The EU maximum limit is 0.50 mg/kg w.w. for mercury in most species of fish with the exception of predatory fish such as halibut and tuna, which have a maximum limit of 1 mg/kg. Thus all samples are well below the maximum limit. The concentration of mercury in Norwegian farmed fish fillets has been fairly stable over the last ten years.

Lead

Only seven samples of farmed fish fillets out of 330 analysed had measurable concentrations of lead (5.5). 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. Thus all samples are well below the limit.

Table 5.5. Chemical elements (mg/kg w.w.) in fillets of farmed fish

Element		Salmon	Reinbow trout	Cod	All Groups	EU-Limit	LOQ
	N	305	24	1	330		
Arsenic	#Values	305	24	1	330		
	Median	0.52	0,58	-	0.53		
	UB-Mean	0.55	0.63	-	0.56		
	Min	0.16	0.46	-	0.16		
	Max	1.2	1.1	0.65	1.2		0.01
Cadmium	#Values	31	0	0	31		
	Median	-	-	-	-		
	UB-Mean	-	-	-	-		
	Min	LOQ	-	-	-		0.001
	Max	0.042	LOQ	LOQ	0.042	0.050	0.002
Mercury	#Values	305	4	1	330		
	Median	0.013	0.015	-	0.013		
	UB-Mean	0.014	0.016	-	0.015		
	Min	0.007	0.011	-	0.007		0.007
	Max	0.045	0.028	0.017	0.045	0.50	0.010
Lead	#Values	7	0	0	7		
	Median	-	-	-			
	UB-Mean	-	-	-			
	Min	LOQ	-	-	0.001		0.001
	Max	0.026	LOQ	LOQ	0.026	0.30	0.010

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

Although arsenic is present above the LOQ in all samples, none of the samples had concentrations of inorganic arsenic above the LOQ (table 5.6).

Methylmercury had a UB-mean of 0.018 mg/kg w.w. (table 5.6), calculation of UB-mean for mercury for the same 22 samples also gave a UB-mean of 0.018 mg/kg w.w., showing that mercury in salmon and rainbow trout are present as methylmercury.

Tributyltin was detected in all 20 samples. The UB-mean was 0.35 µg/kg w.w, and the highest level found was 1.8 µg/kg w.w (table 5.6).

Table 5.6. Inorganic arsenic, methylmercury and tributyltin in fillets of farmed fish

Compound		Salmon	Reinbow trout	All Groups	LOQ
Inorganic arsenic (mg/kg w.w.)	N	19	2	21	
	#Values	0	0	0	
	UB-Mean	-	-	-	
	Min	-	-	-	0.004
	Max	LOQ	LOQ	LOQ	0.005
Methyl-mercury (mg/kg w.w.)	N	20	2	22	
	#Values	18	2	20	
	UB-Mean	0.018	0.016	0.018	
	Min	LOQ	0.020	LOQ	
	Max	0.37	0.024	0.037	0.001
Tributyltin (µg/kg w.w.)	N	18	2	20	
	#Values	18	2	20	
	UB-Mean	0.28	0.77	0.35	
	Min	0.06	0.24	0.06	0,05
	Max	1.8	1.7	1.8	0.3

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

5.2.6 Group B3d, Mycotoxins

The eight pooled samples from 2012 were analysed for Ochratoxin-A, by an analytical method adapted for marine samples. All samples were from salmon, and consisted of muscle material from five fish each. Ochratoxin-A was not detected in any of the samples.

5.2.7 Group B3e, Dyes

A total of 399 pooled samples from 1 995 fish representing five species were examined with respect to malachite green and its metabolite leuco malachite green (LMG), crystal violet and its metabolite leuco crystal violet. Brilliant green were examined in 265 pooled samples. No residues of these agents were detected.

5.2.8 Group B3f, others

PBDE are compounds used to prevent fire. They are parts of the larger compound group, brominated flame retardants. The sum PBDE-7 in salmon ranged from 0.2 to 4.1 µg/kg w.w. with a mean value of 0.7 µg/kg w.w. (Table 5.7), this is slightly lower than in 2011 were the mean value were 0,8 µg/kg w.w. There is currently no EU maximum limit for brominated flame retardants in food.

Table 5.7 PBDE (µg/kg w.w.) in fillets of farmed fish.

		Atlantic Salmon	Rainbow trout	All Groups
Sum PDBE-7	Samples	31	1	32
	Median	0.5	-	0.5
	UB-Mean	0.7	-	0.7
	Min	0.2		0.2
	Max	4.1	0,5	4.1

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

Table 5.8 summarises the results for the PAH compounds analysed in farmed fish in 2012. PAH was measured in 21 salmon samples, and none of the samples had levels above LOQ. In 2010, fluorene and phenantrene were measurable in more than 50% of the farmed fish samples, while anthracene and fluoranthene were detected in some samples. There was previously a maximum level for PAH in fresh fish, however it has been shown that PAH are quickly metabolised in fresh fish and do not accumulate in the muscle meat. Therefore, maintaining a maximum level was no longer appropriate (Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs).

Table 5.8. PAH (µg/kg w.w.) in fillets of farmed fish

PAH compound	N	#values	Max	LOQ
5-Methylchrysene	21	0	<LOQ	1.0
Benzo(a)antracene	21	0	<LOQ	0.5
Benzo(a)pyrene	21	0	<LOQ	0.5
Benzo(b)fluoranthene	21	0	<LOQ	0.5
Benzo(ghi)perylene	21	0	<LOQ	0.5
Benzo(j)fluoranthene	21	0	<LOQ	0.5
Benzo(k)fluoranthene	21	0	<LOQ	0.5
Benzo(c)Fluorene	21	0	<LOQ	1.0
Chrysene/Triphenylene	21	0	<LOQ	0.5
Cyclopenta(c,d)pyrene	21	0	<LOQ	1.0
Dibenzo(a,e)pyrene	21	0	<LOQ	1.0
Dibenzo(a,h)anthracene	21	0	<LOQ	0.5
Dibenzo(a,h)pyrene	21	0	<LOQ	1.0
Dibenzo(a,i)pyrene	21	0	<LOQ	1.0
Dibenzo(a,l)pyrene	21	0	<LOQ	1.0
Indeno(1,2,3-cd)pyrene	21	0	<LOQ	0.5

A total of 21 samples were analysed for the PFCs, of which one sample was trout and the rest were salmon, results are given in Table 5.9. All the results were below the LOQ. PFCs were measured in this program in 2010 as well, then five samples had PFHxA values above LOQ, while the 17 other PFCs compounds had levels below the LOQ in all samples.

Table 5.9. PFCs (µg/kg w.w.) in fillets of farmed fish

Compound	N	#Values	Max value	LOQ
PFBA	21	0	<LOQ	1.5
PFBS	21	0	<LOQ	1.5
PFDA	21	0	<LOQ	0.3
PFDoDA	21	0	<LOQ	0.3
PFDS	21	0	<LOQ	0.3
PFHpA	21	0	<LOQ	0.3
PFHxA	21	0	<LOQ	0.3
PFHxDA	21	0	<LOQ	24
PFHxS	21	0	<LOQ	0.3
PFNA	21	0	<LOQ	0.3
PFOA	21	0	<LOQ	0.3
PFODA	21	0	<LOQ	24
PFOS	21	0	<LOQ	0.3
PFOSA	21	0	<LOQ	0.9
PFPeA	21	0	<LOQ	0.3
PFTeDA	21	0	<LOQ	0.3
PFTrDA	21	0	<LOQ	0.3
PFUdA	21	0	<LOQ	0.3

6. CONCLUSION

None of the substances with anabolic effect (group A1 and A3) were detected in any of the samples analysed in 2012. Nor were any residues found for the illegal compounds in group A6.

None of the veterinary drugs exceeded the MRL established for fish, in the monitoring program in 2012. Emamectin and cypermethrin were detected, however in concentrations well below the MRL.

Similarly to veterinary drugs, all the environmental contaminants (organochlorine compounds and chemical elements) in the farmed fish analysed in 2012 were found at levels below the EU maximum limit for those compounds for which such limits have been established (dioxins, dlPCBs, PCBs, mercury, lead and cadmium).

The concentrations of dioxins (PCDDs and PCDFs), organic pesticides other than DDT and PBDE in farmed fish in 2012 were comparable to those found in the monitoring programme for Directive 96/23/EC for the years 2003 to 2011. The level of dlPCBs, PCB-7 and DDT has decreased over the last decade.

The level of mercury, cadmium and lead were similar to previous years, while the level of arsenic has decreased over the last ten years.

7. ANNEX

Annex I 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	0.4	-	Presence	OUH
	Dienestrol		0.7	-	Presence	
	Hexoestrol		0.6	-	Presence	
A3 Steroids	α-nandrolon	GC-MS	0.6	-	Presence	
	β-nandrolon		0.6	-	Presence	
	α-trenbolon		0.6	-	Presence	
	β-trenbolon		0.6	-	Presence	
A6 Annex IV substances	Chloramphenicol	LC-MS	0.25	1.0	Presence (MRPL = 0.3)	NIFES
	Metronidazole	LC-MS/MS	2.0	6.0	Presence	NIFES
	Hydroxy-metronidazole		15	45	Presence	
	Nitrofuran AOZ	LC-MS/MS	0.2	0.5	Presence (MRPL =1.0)	Eurofins
	Nitrofuran AHD		0.3	1	Presence (MRPL =1.0)	
	Nitrofuran AMOZ		0.2	0.5	Presence (MRPL =1.0)	
	Nitrofuran SEM		0.3	1	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 Chemical method	Oxolinic acid	LC-MS/MS	10	30	100	Eurofins
	Flumequine		10	20	600	
	Oxytetracycline	LC-MS/MS	2.0	5.0	100	Eurofins
	Florfenicol	LC-MS/M	0.2	0.5	1000	NIFES
B2a Anthelmintics	Praziquantel	LC-UV	50	100	n.a.	NIFES
	Fenbendazole	LC-MS/MS	0.3	1.0	n.a.	NIFES
	Emamectin	LC-MS	2.5	5.0	100	NIFES
	Ivermectin		25	50	n.a.	
B2c Carbamates and pyrethroids	Cypermethrin	GC-EC	5	10	50	Eurofins
	Deltamethrin		10	20	10	
B2f Other active substances	Diflubenzuron	LC-MS	10	20	1000	NIFES
	Teflubenzuron	LC-MS	5	15	500	
B3a Organo-chlorine compounds	Dioxins and dlPCB	GC-HRMS	0.002-0.1 ng/kg	0.008-0.4 ng/kg	3.5 ng TEQ/kg	NIFES
	PCB 28	GC-MS		0.03 – 0.010	n.a.	NIFES
	PCB 52			0.010	n.a.	
	PCB 101			0.03 – 0.010	n.a.	

	PCB 118			0.03 – 0.010	n.a.	
	PCB 138			0.03 – 0.010	n.a.	
	PCB 153			0.03 – 0.010	n.a.	
	PCB 180			0.03 – 0.010	n.a.	
	α-HCH	GC-MS or HRGC/HRMS	0.1	0.3	n.a.	NIFES/ Eurofins
	γ-HCH		0.1	0.3		
	HCB		0.15	0.5		
	Pentachlorobenzene		0.15	0.5		
	Heptachlor		0.05	0.2		
	Heptachlor A		0.05	0.2		
	Aldrin		0.1	0.3		
	Isodrin		0.1	0.3		
	Dieldrin		0.03	0.1		
	Oxy-chlordane		0.05	0.2		
	trans-chlordane		0.03	0.1		
	cis-chlordane		0.1	0.3		
	α-endosulfan		0.05	0.2		
	β-endosulfan		0.05	0.2		
	Endosulfansulphate		0.05	0.2		
	cis-nonachlor		0.03	0.1		
	trans-nonachlor		0.05	0.2		
	Toxaphene 26		0.15	0.4		
	Toxaphene 32		0.3	1.0		
	Toxaphene 40+41		0.05	0.2		
	Toxaphene 42		0.05	0.2		
	Toxaphene 50		0.1	0.3		
	Toxaphene 62		0.1	0.3		
	Mirex		0.1	0.3		
	DDT-op DDT-pp DDD-op DDD-pp DDE-op DDE-pp	GC-MS or HRGC/HRMS		0.4-0.6	n.a.	NIFES/ Eurofins
B3b Organo-phosphorous compounds	Azametiphos	GC-FPD	1.5	4.0	n.a.	Eurofins
	Dichlorvos		5.0	10	n.a.	
B3c Chemical elements	Lead	ICP-MS		0.01 mg/kg	0.3 mg/kg	NIFES
	Cadmium			0.01 mg/kg	0.05 mg/kg.	
	Arsenic			0.01 mg/kg	n.a.	
	Mercury			0.01 mg/kg	0.5 mg/kg	
	Inorganic arsenic	LC-ICP-MS		4-5		NIFES
	Methylmercury	GC-ICP-MS		1		
	Tributyltin	GC-ICP-MS		0.3		

B3d Mycotoxins	Ochratoxin A	HPLC-FLU	0.06		n.a.	NVI
B3e, dyes	Malachite green	LC-MS/MS	0.15	0.5	Presence (MRPL=2.0, ∑ malachite green and leuco-malachite green)	NIFES
	Leuco-malachite green		0.15	0.5		
	Crystal violet		0.30	1.0	Presence	
	Leuco-crystal violet		0.15	0.5	Presence	
	Brilliant green		0.15	0.5	Presence	
B3f, others	PBDE	GC-MS		0.002-0.1	n.a.	NIFES
	PAH	GC-MS		0.5-1.0	n.a.	Eurofins
	PFC	LC-MS/MS		0.3-24	n.a.	NIFES

[1] All methods used muscle as sample matrix except for micro-biological methods for antibacterial substances (B1), were liver was used.
[2] Only screening method, positive results have to be confirmed by a chemical method.