NIFES
NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

Report
2012

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 2011

Ole Jakob Nøstbakken, Helge Torbjørn Hove, Bjørn Tore Lunestad, Bjarte Holmelid and Bente E. Torstensen

Nasjonalt institutt for ernærings- og sjømatforskning (NIFES)

24.08.2012

ACKNOWLEDGEMENTS

Most of the analyses for the monitoring programme were conducted at NIFES. Annette Bjordal was in charge of the analytical work, while Anne Margrethe Aase and Elin Kronstad were responsible for the work related to sample storage, preparation and distribution within the institute. Manfred Torsvik and Vidar Fauskanger carried out the sample pre-treatment. Rita Hannisdal, Felicia D. Couillard, Eva Torgilstveit, Edel Erdal, Kari B Sele and Tore Tjensvoll were responsible for chemical analysis of residues of therapeutics. Karstein Heggstad, Tadesse T. Negash, Jannicke A. Berntsen, Dagmar Nordgård, Vivian Mui, Lene H. Johannessen, Britt Elin Øye, Pablo Cortez, Kari Breisten Sæle, Kjersti Pisani, Joseph Malaiamaan, Kjersti Kolås, Per Ola Rasmussen, Edel Erdal and Joar F. Breivik were responsible for analyses of organic contaminants. Jorun Haugsnes, Siri Bargård, Tonja Lill Eidsvik, Berit Solli, Edel Erdal and Laila Sedal carried out the analysis of the chemical elements. Anette Kausland, Annbjørg Bøkevoll and Anne Karin Syversen were responsible for communication with the Norwegian Food Safety Authority. Tone Galluzzi and Leikny Fjeldstad conducted the analyses of the antibacterial agents.

In 2011, NIFES used sub-contractors for analysis of some parameters: Oslo University Hospital for stilbenes and steroids, the Norwegian Veterinary Institute for mycotoxins and Eurofins for the analysis of selected therapeutic compounds (see Annex I for details).

TABLE OF CONTENTS

1. SUMMARY

The number of samples included in the 96/23 monitoring programme is dependent on the production volume, a minimum of 1 sample per 100 tonnes of annual production. This report for 2011 is based on 2 021 fish fillet samples (pooled samples of five fish) and 1 660 liver samples, a total of 11 765 farmed fish.

Group-A includes substances with anabolic effect and unauthorised substances, approximately 40% of the total samples were analysed for these substances. These samples were collected by official inspectors at the farm without prior notification. Samples were taken at all stages of farming and are representative of farmed fish under production. Group-B includes veterinary drugs and contaminants, approximately 60% of the total samples were analysed for these substances. The group B-samples were taken from fish at processing plants and are representative of commercial Norwegian farmed fish.

Of the therapeutic agents in group B, emamectin was detected in five of the 77 pooled samples of farmed fish analysed in 2011. Reanalysis of the positive samples showed mean concentrations ranging from 2.5 to 10.0 µg/kg, which are all below the current MRL for emamectin of 100 µg/kg. Residues of other substances in group B, or their metabolites, were not found in any of the farmed fish samples from 2011. The mycotoxin Ochratoxin A was not detected in any of the eight pooled salmon samples analysed for 2011.

The concentrations of dioxins (PCDDs and PCDFs), dioxin-like PCBs, PCB-7 and organochlorine pesticides in farmed salmon were similar to the results from this monitoring programme for the years 2003 to 2010, and also comparable with the concentrations of these compounds in farmed fish available in the online database: "Seafood data" (www.nifes.no). No samples exceeded the EU maximum limits, for the compounds where such limits have been established.

This year, only PBDE of the brominated flame retardants were analysed since no levels above LOQ were observed for HBCD and TBPPA in 2010. The maximum concentration of PBDE-7 was similar, albeit slightly lower with 1µg/kg in 2011, compared to 3.2µg/kg in 2010. There are currently now EU regulations regarding this compound.

Concentrations of lead, cadmium and mercury in farmed fish in 2011 were below the EU maximum limits for these elements in fish. There is currently no EU maximum limit for arsenic. Noteably, the concentration of arsenic in farmed fish is not a safety concern due to the low toxicity of the principal chemical compound (chemical species) in which this element is present in fish

2. TERMINOLOGY

For **"Limit of quantification"** and "**Limit of detection**" the internationally recognized abbreviations LOQ and LOD are used. LOQ is normally higher than LOD by a factor of 3.0 to 3.3. For compounds that are illegal in fish the LOD is most relevant, since detection of the compound (i.e. with > 95% probability) is important information. For other compounds quantification is required. The LOQ is the lower limit for a reliable quantitative measurement, and an experimental LOQ is provided for each method. However, the LOQ may vary somewhat according to differences in the matrix being measured. In this report both the "experimental" LOQ and the "variable" LOQ will be shown. Levels that cannot be quantified with acceptable reliability are reported as "less than LOQ", for example: < 2.0 µg/kg.

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 relevant "variable" 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 headings). 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. MRL is further discussed under the "regulations" chapter.

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

Congener: Congeners are very 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 dioxin-like PCBs in 1998 which were re-evaluated in 2005. For the year 2011 WHO 1998 TEFs values were still used. TEF values are applied to 17 PCDD/F congeners and 12 dioxin-like PCBs, and summed as toxic equivalents (TEQ) of dioxins (PCDD/F) and dioxin-like PCBs.

3. INTRODUCTION

3.1 Background

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

3.2 Regulations

The aim of this surveillance program is to monitor 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 pharmacological 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 and
iv) substances for which administration to food producing animals is prohibited. According to the current Commission Regulation (EU) No 37/2010, the MRLs 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 (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 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.

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

There will from 2012 be new regulations regarding dioxins, DLPCBs and indicator PCBs: A new limit will be assigned to the sum of six indicator PCBs (PCB6). For the dioxins (PCDDs and PCDFs) and the DLPCBs a new list of TEF values (TEF-2005) are to be used, also from 2012 (Commission regulation 1259/2011). However, these new regulations have not been implemented for 2011.

3.3 Compounds included in the program

Directive 96/23/EC lays down measures to monitor the substances and groups of residues which are listed in Annex I of the Directive, Group A and Group B. These two groups differ in their sampling intensity, sampling modus and selection of analytes. 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

Group A-substances are unauthorized substances, including compounds with anabolic effects. Fish at different stages of farming are sampled at fish farms with no prior notification by official inspectors from the Norwegian Food Safety Authority, 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 substances which are monitored vary to some extent from year to year according to priorities given by NFSA. The priorities are based on the monitoring of the prescription of pharmaceuticals in the 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 1 sample per 100 tonnes produced fish. In 2011 this applied to all farmed fish species. Roughly 40 % 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. The samples for most parameters were frozen muscle or liver tissue. As for other parameters, the sample material was whole gutted fish stored on ice. All samples were shipped frozen to NIFES. Most of the analyses were carried out on pooled fish samples, except for the liver samples which were all analysed individually. According to the directive, for B samples, fish muscle and skin shall be analysed in natural proportions. The difference between accumulation of residues in muscle and skin has in worst cases been reported to be up to 1:20 respectively. However, skin in natural proportions to muscle make up 10 % of total analysed tissue, so that in a worst case scenario, including skin may double the amount of residue, compared to analysis of muscle only. Due to homogenization difficulties, excluding the skin from the analyses ensure less variation in measurement. In addition, the LOD for all residues in this project is at least 10 times lower than MRL. Meaning that, the exclusion of skin from the screening analyses decrease variation, but still detects levels well below the MRL. Therefore it has been chosen to screen only muscle tissue in this program, but if anything is detected, skin is also analysed in a confirmatory method. This is also under the provisions of the Commission decision of 12 august 2002 (2002/657/EC) chapter 2. It can be mentioned that inclusion of skin in the different analyses is always under consideration, depending on methodology available and its detection limits and variation.

On arrival at NIFES fish muscle samples were pooled with equal amounts of sample contributing from each fish, 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.

Table 4.1 gives the number of fish analysed in the monitoring programme in 2011, whereas Table 4.2 gives the number of fish for each species. The total number of fish in 2011 was 11765 analysed in a total of 2021 fillet samples and 1 660 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	Diethylstilboestrol Dienoestrol Hexoestrol	290*	58	58 x 3 = 174	Accredited
	A3 Steroids	α-nandrolon β-nandrolon α-trenbolon β-trenbolon	255*	51	51 x 4 = 204	Accredited
	A6	Chloramphenicol	1150*	230	230	Accredited
	Illegal drugs: Annex IV to EEC 2377/90	Metronidazole Hydroxy-metronidazole	810*	162	162 x 2 = 324	-
		Nitrofuran metabolites 3-Amino-2-oxazolidone 1-Aminohydrantion 3-Amino-5-Morpholinomethyl-Semicarbazide	805*	161	161 x 4 = 644	-
	Sum of group A		3310*	662	1576	
Samples taken from processing plants	B1 Anti bacterial	Florfenicol	75	15	15	Accredited
		Oxytetracyclin	95	19	19	-
		Flumequin	85	17	17	-
		Oxolinic acid	75	15	15	-
	B2 Other veterinary	Teflubenzuron	230	46	46	Accredited
		Diflubenzuron	225	45	45	Accredited
		Cypermethrin	85	17	17	-
		Praziquantel	400	80	80	Accredited
		Fenbendazole	205	41	41	-
		Emamectin	385	77	77	Accredited
		Ivermectin	70	14	14	Accredited
		Deltamethrin	75	15	15	-
	B3a Organohlorine Compounds	α–HXH	275	55	55 x 26 = 1430	Accredited
		γ–HXH				
		HCB				
		Pentachlorobenzene				
		Heptachlor				
		Heptachlor epoxide				
		Heptachlor A				
		Aldrin				
		Dieldrin				
		Isodrin				
		Oxy-Chlordane				
		trans-Chlordane				
		cis-Chlordane				

		α–Endosulfan				
		β–Endosulfan				
		Endosulfan sulfate				
		cis-Nonachlor				
		trans-Nonachlor				
		Toxaphene 26				
		Toxaphene 32				
		Toxaphene 40+41				
		Toxaphene 42a				
		Toxaphene 50				
		Toxaphene 62				
		Mirex				
		DDT, DDE og DDD: orto-para and para-para congeners				
		Method 292: Dioxin, PBDE, PCB	145	29	29	Accredited
		PCB 7	235	47	47	Accredited
	B3b Organo-phosphorous Compounds	Azametiphos	220	44	44	-
		Dichlorvos	215	43	43	-
	B3c Chemical	Pb Cd Hg As	1640	328	328 x 4 = 1312	Accredited
	B3d Mycotoxins	Ochratoxin A	40	8	8	Accredited
	B3e, Dyes	Malachite green Leucomalachite green Chrystal violet Leuco Chrystal violet^	2020	404	4 x 404 = 1616	Accredited
	Sum B fillets, pooled		6795	1359	4922	
Liver	B1 Microbiological screening of liver	Quinolones	1660	1660	1660 x 3 = 4980	Accredited
		Tetracyclines and amphenicols				
		Sulphonamides				
Total sum B			8455	3019	9902	
Total sum fillet A+B			10105*	2021	6498	
Total sum, fillets, pooled and individual fish and liver			11765*	3681	11478	

Note: PCB-7, PBDE, and some dioxins, are analysed in the same samples affecting the sum.

***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.**

^Two samples are lacking due to experimental error.

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 (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 a standard addition procedure.

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.

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 standard addition 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.

B2a, Anthelmintics

Diflubenzuron and teflubenzuron

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

Analytical method:

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 standard addition method.

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 standard addition method.

Ivermectin and Emamectin

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

Analytical method:

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 standard addition method.

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 standard addition 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 internal standard.

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 a standard addition 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 (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants 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 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. Other congeners, with a higher toxicity (non-ortho PCBs and mono-ortho PCBs) are determined as part of the Dioxin method described in the next section. The PCB- list overlaps with the list of dioxin-like PCBs (described in the next section) in that PCB-118 is found in both lists. Thus it has been suggested that the PCB-7 sum should be replaced by a PCB-6 sum, "indicator PCBs" (the PCB-7 without PCB-118). Maximum limits for indicator PCBs are being discussed in the European Commission. 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.

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, however the EU legislation on maximum limits for dioxins and dioxin-like PCBs in feed and food was in 2011 still based on 1998 TEFs, and this is reflected in this report. 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, seven brominated flame retardant 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. The pesticides analysed include: Pentachlorobenzene, hexachlorobenzene, hexachlorocyclohexanes (α-, β- and γ), 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-50, TOX-62, TOX-42a, and the sum of TOX40 and TOX41.

Analytical method: Sample was extracted using hexane at 75^{o}C under 1500 psi pressure, and further clean up and detection were performed in two different manners. Either, the extract was acid treated and analysed on GC/MS in EI, or clean up through three columns, ChemElut, QuEChERS and C18 and detection on GC/MS in NCIQuantification was based on internal standard method.

B3b, Organophosphorous compounds

Azametiphos and Dichlorvos

Analytical method: The sample material was extracted with acetone. The extract was cleaned up by gel permeation chromatography and analysed by GC-FPD. The LOQ values are given in Annex I.

B3c, Chemical elements

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).

B3d, Mycotoxins

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. 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 a standard addition method (dose response method).

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*	270	5			15		
	A3 Steroids	α-nandrolon β-nandrolon α-Trenbolon β-trenbolon	255*	250					5	
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	1150*	1030	45		15	45	10	5
		Metronidazole Metronidazole-OH	810*	750	25			25	10	
		Nitrofuran metabolites 3-Amino-2-oxazolidone 1-Aminohydrantion 3-Amino-5-morpholinomethyl-2-oxazol Semicarbazide	805*	730	25			35	15	
Samples taken from processing plants	B1 Chemical method in muscle	Florfenicol	75	70	5					
		Oxytetracycline	95	95						
		Flumequine	85	80	5					
		Oxolinic acid	75	75						
	B1 Microbiological assay in liver	Quinolones Tetracyclines and amphenicols Sulphonamides	1660	1525	65	5		60	5	
	B2	Teflubenzuron	230	230						
		Diflubenzuron	225	220	5					

	Other veterinary drugs	Cypermethrin	85	85						
		Praziquantel	400	380	15			5		
		Fenbendazole	205	190	10			5		
		Emamectin	385	370	15					
		Ivermectin	70	65	5					
		Deltamethrin	75	70	5					
	B3a Organo-chlorine compound	α–HXH	275	245	15			15		
		β–HXH								
		γ–HXH								
		HCB								
		PeCB								
		Heptachlor								
		Heptachlor epoxide								
		Aldrin								
		Isodrin								
		Dieldrin								
		cis-Chlordane								
		trans-Chlordane								
		oxy-Chlordane								
		α–Endosulfan								
		β–Endosulfan								
		Endosulfan sulphate								
		cis-Nonachlor								
		trans-Nonachlor								
		Toxaphene 26								
		Toxaphene 32								
		Toxaphene 40+41								
		Toxaphene 50								
		Toxaphene 62								

		Mirex								
		DDT, DDE og DDD orto-para + para-para								
		Dioxins + Dioxin like PCBs	145	140				5		
		PCB-7	300	280	10			10		
	B3b Organophosphorous Compounds	Azametiphos	220	215	5					
		Dichlorvos	215	205	10					
	B3c Chemical elements	Pb Cd Hg As	1640	1505	75	5	5	50		
	B3d	Mycotoxins	40	40						
	B3e, Dyes	Malachite green Leucomalachite green	2020	1900	60			55	5	
		Crystal violet Leucocrystal violet^								

Note: PCB-7, PBDE, and some of the dioxins, are analysed in the same samples which affects the sum.

***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.**

^Two samples are lacking due to experimental error.

5. RESULTS AND DISCUSSION

5.1 Group A

A total of 662 pooled fillet samples from 3 310 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 diethylstilboestrol, dienoestrol 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 51 pooled samples from 255 fish from three 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 553 pooled samples from 2 765 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 1359 pooled fish samples of fillets from a total of 6775 fish, and additionally 1660 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 1660 fish representing a total of 4 980 analytical determinations. The B1 antibacterial agents: florfenicol, oxytetracyclin, flumequin and oxolinic acid, were also analysed chemical methods in a total of 66 pooled fillet samples, representing 330 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 2011.

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 335 pooled fillet samples representing 1 675 fish from three species. Emamectin was detected in five of the 77 pooled samples included in the monitoring programme this year. According to the analytical protocol, any detection of drug residues would be followed by a re-analysis of the same sample material in triplicate, and also analysis of a backup-sample when available. This was done for the positive samples, giving mean concentrations ranging from 2.5 to 10.0µg/kg. The current MRL for emamectin is 100µg/kg. 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 2011 there were 131 samples from 655 fish analysed for these compounds. The results are summarised in Tables 6.1 to 6.4.

Organochlorine pesticides

There were 49 salmon-, three trout-, and three cod sample analysed for organochlorine pesticides and the results are given in Tables 3 and 4. 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.

DDT and its metabolites

In 2011, the polychlorinated pesticides and DDT, and its metabolites, was measured using the same methodology. This method has experimental LOQ values for DDT and its metabolites, of 1.5 ng. When the different metabolites of DDT are summed up, several congeners display mean values below LOQ. If an upperbound calculation of sum DDT is performed using the methodological LOQ, the sum DDT will be artificially high. Therefore it was chosen not to include sum DDT in the report for 2011. Still, the results for each metabolite of DDT do not differ considerably from previous years, and levels are still considered to be low. These data are shown in table 6.1.

Hexachlorcyclohexane (α- and γ-HCH), penta-(PeCB), and hexachlorobenzene (HCB)

Alfa- and gamma HCH was quantified in salmon, trout and cod samples, salmon had a maximum level of 0.3 µg/kg w.w. α-HCH, and 2.2 µg/kg w.w. γ-HCH. In trout and cod, no values above LOQ was measured. In salmon a maximum value of 0.3 µg/kg w.w. pentachlorobenzene were found, while in trout and cod no quantities above the LOQ were measured. Hexachlorobenzene was quantified in salmon (2.9 µg/kg w.w.) and trout (1.4 µg/kg), while not measurable for cod. Data from these compounds are listed in table 6.2.

Table 5.1 DDT, DDD and DDE (µg/kg wet weight) in the fillets of pooled farmed fish samples

	op-DDT	**pp-DDT**	**op-DDD**	**pp-DDD**	**op-DDE**	**pp-DDE**
LOQ	0.01-0.5	0.05-1.0	0.01-0.5	0.05-0.1	0.05-0.6	0.1
Atlantic Salmon						
#values	18	45	12	49	11	49
UB-Mean	-	0.6	-	1.3	-	3.6
Min	LOQ	LOQ	LOQ	0.4	LOQ	1.4
Max	0.5	1.4	0.5	2.5	0.6	8.1
Rainbow trout						
#values	0	2	0	3	0	3
UB-Mean	-	LOQ	-	0.9	-	2.6
Min	LOQ	LOQ	LOQ	0.6	LOQ	1.6
Max	LOQ	0.5	LOQ	1	LOQ	3.2
Atlantic Cod						
#values	0	0	0	1	0	2
UB-Mean	-	-	-	-	-	-
Min	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Max	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
All groups						
#values	18	47	12	53	11	54
UB-mean	-	0.5	-	1.2	-	3.4
Min	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
Max	0.5	1.4	0.5	2.5	0.6	8.1

UB=”upper bound”, LOQ substituted for all values <LOQ in the calculation.

If more than 50% of results are below LOQ, no mean is given

Other organochlorine pesticides

The results for the other 22 pesticide compounds analysed are summarised in Table 4. The values ranged from <LOQ to 5.8 µg/kg w.w., the highest concentration in 2011 was for α-endosulfan. Most of these compounds 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. Low concentrations were found in cod fillets for all of these compounds, which is consistent with cod being a lean fish.

Table 5.2. Other pesticides (µg/kg wet weight) in fillets of pooled fish samples

Pesticide		Atlantic salmon	Rainbow Trout	Atlantic Cod	All Grps	LOQ Method	LOQ variable
	No. samples	49	3	3	55		
α-HCH	#Values	24	0	0	24	0.3	0.1-2.0
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.3	LOQ	LOQ	0.3		
γ-HCH	#Values	15	0	0	15	0.3	0.03-2.0
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	2.2	LOQ	LOQ	2.2		
HCB*	#Values	49	3	2	54	0.5	0.1
	Median	1	1	0.1	1		
	UB-mean	1.2	1.0	0.1	1.1		
	Min	0.4	0.6	LOQ	LOQ		
	Max	2.9	1.4	LOQ	3.0		
Pentachlorobenzene	#Values	34	1	0	35	0.5	0.1-0.2
	Median	0.1	-	-	0.1		
	UB-mean	0.1	-	-	0.1		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.3	LOQ	LOQ	0.3		
Heptachlor	#Values	0	0	0	0	0.2	0.02-0.1
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	LOQ	LOQ	LOQ	LOQ		
Heptachlor A	#Values	1	0	0	0	0.2	0.02-0.05
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.1	LOQ	LOQ	0.1		
Aldrin (lack one sample)	#Values	1	0	0	1	0.3	0.03-1,5
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.3	LOQ	LOQ	0.3		
Isodrin	#Values	3	0	0	3	0.3	0.03-6.0
	Median	-	-	-	-		

	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	1.6	LOQ	LOQ	1.6		
Dieldrin	#Values	49	3	3	55	0.1	-
	Median	1.5	1.7	0.1	1.5		
	UB-mean	1.64	1.51	0.08	1.55		
	Min	0.5	0.72	0.05	0.05		
	Max	3.9	2.1	0.1	3.9		
α-endosulfan	#Values	21	1	1	23	0.2	0.02-0.1
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	5.8	0.19	0.02	5.8		
β-endosulfan	#Values	21	-	-	21	0.2	0.03-1.0
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	1.2	LOQ	LOQ	1.2		
Endosulfan sulphate	#Values	33	1	0	34	0.2	0.02-0.2
	Median	0.1	-	-	0.1		
	UB-mean	0.2	-	-	0.1		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	1.3	0.2	LOQ	0.5		
***cis*-chlordane**	#Values	48	3	1	52	0.3	0.03-0.5
	Median	0.8	0.6	-	0.7		
	UB-mean	1.0	0.7	-	0.9		
	Min	LOQ	0.3	LOQ	LOQ		
	Max	2.8	1.2	LOQ	2.8		
***oxy*-chlordane**	#Values	46	2	0	48	0.2	0.02-0.4
	Median	0.2	0.3	-	-		
	UB-mean	0.2	0.3	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.7	0.4	LOQ	0.69		
***trans*-chlordane**	#Values	35	2	2	39	0.1	0.2-0.3
	Median	0.2	0.2	LOQ	0.2		
	UB-mean	0.2	0.2	0.1	0.2		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.3	0.2	0.2	0.3		
***cis*-nonachlor**	#Values	46	2	1	49	0.1	0.01-1.0
	Median	0.3	0.4	-	0.3		
	UB-mean	0.4	0.5	-	0.4		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	1	1	LOQ	1		
***trans*-nonachlor**	#Values	49	3	2	54	0.2	0.1
	Median	0.8	0.7	0.1	0.7		
	UB-mean	0.9	0.7	0.1	0.1		

	Min	0.2	0.4	LOQ	LOQ		
	Max	2.8	1	0.1	2.8		
Toxaphene-26 (11 samples could not be determined => n = 44)	#Values	35	2	0	37	0.4	0.04-0.8
	Median	0.5	0.8	-	0.5		
	UB-mean	0.6	0.8	-	0.6		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	3.1	0.9	0.1	3.1		
Toxaphene-32	#Values	1	0	0	1	1	0.1-1.0
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.4	LOQ	LOQ	0.4		
Toxaphene-40+41	#Values	36	3	0	39	0.2	0.02-0.5
	Median	0.5	0.6	-	0.5		
	UB-mean	0.6	0.5	-	0.6		
	Min	LOQ	0.3	LOQ	LOQ		
	Max	2	0.6	LOQ	2		
Toxaphene-42a	#Values	37	3	0	40	0.2	0.02-0.2
	Median	0.2	0.2	-	0.2		
	UB-mean	0.3	0.2	-	0.2		
	Min	LOQ	0.1	LOQ	LOQ		
	Max	0.7	0.3	LOQ	0.7		
Toxaphene-50	#Values	49	3	3	55	0.3	-
	Median	1.0	1.1	0.1	1.0		
	UB-mean	1.2	0.9	0.1	1.1		
	Min	0.4	0.4	0.1	0.1		
	Max	3.5	1.3	0.1	3.5		
Toxaphene-62	#Values	47	2	1	50	0.3	0.03-1.0
	Median	0.5	0.6	-	0.5		
	UB-mean	0.6	0.6	-	0.6		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	2	1	0.1	2		
Mirex	#Values	20	0	0	20	0.3	0.04-0.25
	Median	-	-	-	-		
	UB-mean	-	-	-	-		
	Min	LOQ	LOQ	LOQ	LOQ		
	Max	0.3	LOQ	LOQ	0.3		
#values means: Number of analytical measurements above the LOQ. *Mean and median is only calculated when >50% is over LOQ.*							

Polychlorinated biphenyls (PCB)

The concentrations of PCB-7 and indicator PCBs (PCB-6) in farmed fish are given in Table 6.3. For 2011, the data is mainly represented by Atlantic salmon (56 samples), but also two Rainbow trout and two Atlantic cod samples have been measured. The PCB-7, calculated as the "upper bound-LOQ" (UB) sum in the salmon samples ranged from 1.2 to 11.6 µg/kg w.w. In 2010 and 2009 the maximum values were 21.6 and 18 µg/kg w.w., respectively.

The maximum values are typically found in salmon samples, thus the maximum value is not influenced by the varying number of lean fish in each year's monitoring plan. The median sum PCB-7 in salmon was 4.5µg/kg in 2011 compared to 10.6 µg/kg and 7.8µg/kg w.w. the previous two years. Since 2003 the congeners PCB-138 and PCB-153 have been the main contributors to the sum concentration. There is no obvious trend in the data for the period since 2003 for sum PCB-7 concentrations. The EU has not yet established a maximum limit for these compounds in fish, however, draft EU legislation indicates that the maximum limit for indicator PCBs in fish may be set at 75µg/kg. The highest concentration of indicator PCBs measured in salmon in 2011 was 10.3µg/kg w.w., which is well below the proposed limit.

Table 5.3 PCB-7 and PCB-6 (µg/kg wet weight) in the fillets of pooled farmed fish samples

	PCB-28	**PCB-52**	**PCB-101**	**PCB-118**	**PCB-138**	**PCB-153**	**PCB-180**	**UB Sum PCB-7**	**UB Sum PCB-6**
LOQ	0.05	0.5	0.5	0.5	0.5	0.5	0.5		
LOQ variable	0.04-0.2	0.04-0.2	-	-	-	-	-		
Atlantic Salmon									
N	56	56	56	56	56	56	56	56	56
Median	0.2	0.5	0.9	0.6	1.1	1.3	0.3	4.8	4.2
UB-Mean	0.2	0.5	0.9	0.6	1.2	1.4	0.3	5.1	4.5
Min	LOQ	LOQ	0.2	0.2	0.3	0.3	0.1	1.2	1.01
Max	0.6	1.0	1.9	1.3	2.6	3.3	1.1	11.6	10.3
Rainbow trout									
N	2	2	2	2	2	2	2	2	2
Median	-	-	-	-	-	-	-	-	-
UB-Mean	-	-	-	-	-	-	-	-	-
Min	LOQ	0.2	0.5	0.4	0.8	0.9	0.2	3.1	2.8
Max	0.2	0.5	0.8	0.5	1.0	1.2	0.3	4.5	4.0
Atlantic Cod									
N	2	2	2	2	2	2	2	2	2
Median	-	-	-	-	-	-	-	-	-
UB-mean	-	-	-	-	-	-	-	-	-
Min	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	0.1	0.1
Max	LOQ	LOQ	0.1	LOQ	0.1	0.1	LOQ	0.3	0.3
All groups									
N	60	60	60	60	60	60	60	60	60
Median	0.2	0.5	0.9	0.6	1.1	1.3	0.3	4.7	4.2
UB-Mean	0.2	0.5	0.9	0.6	1.1	1.4	0.3	4.9	4.3
Min	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ	0.1	0.1
Max	0.6	1.0	1.9	1.3	2.6	3.3	1.1	11.6	10.3

UB=”upper bound”, LOQ substituted for all values <LOQ in the calculation.

Variable LOQ has been used since this gives higher resolution, if results are smaller than highest variable LOQ this will be signified by the letters LOQ.

Median and mean are not calculated for rainbow trout and cod, due to number of replicates being only two.

PBDE, dioxins, furans and dioxin like PCBs

Polybrominated diphenyl ethers (PBDE) are compounds used to prevent fire. They are parts of the larger compound group, brominated flame retardants. The sum $PBDE_7$ ranged from 0.6 to 1.0 µg/kg w.w. in salmon with a mean value of 0.8 µg/kg w.w. There is currently no EU maximum limit for brominated flame retardants in food.
A summary of the total WHO_{1998}TEQ values (ng/kg w.w.) for the 29 congeners is listed in Table 6.4. All figures in the table are calculated as the "upper bound-LOQ" sum (UB-sum). A total of 29 pooled and individual samples were analysed from 145 fish. The fish species analysed were Atlantic salmon and Atlantic cod.

For the 17 dioxin and furan compounds (PCDD + PCDF) the sum ranged from 0.2 ng TEQ/kg to 0.5 ng TEQ/kg w.w in salmon. The mean sum was 0.3 TEQ/kg w.w. for Atlantic salmon. No mean was calculated for cod since this was based on 1 sample. The maximum value of 0.5 ng TEQ/kg w.w is below the EU's maximum limit of 4.0 ng TEQ/kg w.w.

The sum of dioxins and dioxin like PCBs ranged from 0.4 to 1.2 ng TEQ/kg w.w. in salmon. The mean concentration was 0.8 ng TEQ/kg w.w. This is far below the EU maximum limit of 8.0 ng TEQ/kg w.w. Concentrations of dioxins and DLPCD in farmed salmon measured for this monitoring programme have always been found to be below the EU maximum limit by a fair margin. The values correspond well with the levels reported in NIFES online database "Seafood Data". Since 2004 the levels of DLPCB have been found to be higher than the PCDD/F levels.

Table 5.4 Brominated flame retardant (PBDE), dioxins (PCDD/F) and dioxins-like PCBs (ng WHO_{1998}TEQ/kg wet weight) in fillets of farmed fish

		Atlantic Salmon	**Atlantic Cod**	**All Groups**	**EU Limit**
Sum PDBE 7	Samples	6	1	7	
	Median	0.7	-	0.7	
	Mean	0.8	-	0.7	
	Min	0.6	-	LOQ	
	Max	1.0	LOQ	1.0	
Sum PCDD/F	Samples	28	1	29	
	Median	0.3	-	0.3	
	Mean	0.3	-	0.3	
	Min	0.2	-	0.1	
	Max	0.5	0.1	0.5	4.0
Sum DLPCB + PCDD/F	Samples	28	1	29	
	Median	0.7	-	0.7	
	Mean	0.8	-	0.8	
	Min	0.4	-	0.1	
	Max	1.2	0.1	1.2	8.0
When individual congeners are summed up, upper bound calculations are performed. LOQ: All LOQ values are related to the individual congeners					

5.2.4 Group B3b, Organophosphorous compounds

The levels of the B3b substances azametiphos and dichlorvos were determined in 44 and 43 pooled fillet samples respectively, representing 220 and 215 fish from two species. Residues of these two agents were not found in none of the examined samples.

5.2.5 Group B3c, Chemical elements

The concentrations of chemical elements were determined in 328 pooled fish samples from the fillets of 1640 fish (Table 6.5).

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 0.02 to 3.07 mg/kg w.w in 2011 (Table 6.5). The level of total arsenic in fillets measured in this project has been fairly constant in the period since 2004. The levels in the lean species cod does not differ from the others.

Cadmium

For the 2011 samples, less than 10 % of the values were above LOQ. The maximum concentration measured was 0.05 mg/kg w.w. The EU maximum limit for cadmium in most fish species is 0.05 mg/kg w.w.

Mercury

The concentration of total mercury in farmed fish ranged from LOQ to 0.13 mg/kg w.w in 2011 (Table7). This highest concentration was measured in a salmon sample. The EU maximum limit is 0.5 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.

Lead

Only 2 samples of farmed fish fillets out of 301 analysed had measurable concentrations of lead. The highest concentration was found in salmon, 0.04 mg/kg w.w. The EU maximum level for lead in muscle meat of fish is 0.3 mg/kg. Thus all samples are well below the limit.

Table 5.5. Chemical elements arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) (mg/kg wet weight) in the fillets of pooled farmed fish samples

Element		Salmon	Trout	Halibut	Turbot	Cod	All Grps	EU-Limit	LOQ	Var. LOQ
As	N	301	15	1*	1*	10	328			
	#Values	301	15	1	1	10	328			
	median	0.69	0.70	-	-	1.00	0.70			
	UB-Mean	0.74	0.80	-	-	0.95	0.75			
	Min	0.02	0.42	-	-	0.64	0.02			
	Max	3.07	1.67	1.91	1.19	1.21	3.07	-	0.01	-
Cd	#Values	29	1	0	1	2	33			
	median	-	-	-	-	-	-			
	UB-Mean	-	-	-	-	-	-			
	Min	LOQ	LOQ	-	-	LOQ	LOQ			
	Max	0.05	0.01	LOQ	0.01	0.01	0.05	0.05	0.005	0.001-0.002
Hg	#Values	300	15	1	1	10	327			
	median	0.02	0.02	-	-	0.06	0.02			
	UB-Mean	0.02	0.02	-	-	0.06	0.02			
	Min	LOQ	0.01	-	-	0.02	LOQ			
	Max	0.13	0.06	0.07	0.04	0.09	0.13	0.5	0.005	0.002
Pb	#Values	2	0	0	0	0	2			
	median	-	-	-	-	-	-			
	UB-Mean	-	-	-	-	-	-			
	Min	LOQ	LOQ	-	-	LOQ	LOQ			
	Max	0.04	LOQ	LOQ	LOQ	LOQ	0.04	0.3	0.03	0.01

If less than 50% of results are below LOQ, median will not be presented.

#values signifies number of results above LOQ.
**means only one variable, and hence no mean or median are calculated.*

Trends since 2002 for the heavy metals in farmed fish.

The concentrations of metals in farmed fish fillets have been fairly stable since 2002. A total of >1700 individual or pooled fish samples have been analysed for metals in this time period. There is currently no EU upper limit for either total arsenic or inorganic arsenic in fish fillets. Inorganic arsenic is toxic whereas arsenobetaine, the main chemical form of arsenic present in fish is considered non-toxic. The concentrations of cadmium in most samples analysed since 2002 have been less than or equal to 0.02 mg/kg w.w, which also applies to 2011 where 90% of the samples were at, or below, LOQ at 0.005 mg/kg w.w. However, a single measurement was observed at 0.05 mg/kg w.w, (artifact?) which when including the experimental variation, is just below the EU maximum limit for cadmium at 0.05 mg/kg w.w. All concentrations of mercury in farmed fish analysed since 2002 have been less or equal to 0.23 mg/kg w.w, below the EU maximum limit for mercury of 0.5 mg/kg w.w for most fish species. The levels of lead in all samples of farmed fish analysed since 2002 have been less than or equal to 0.1 mg/kg w.w., below the EU maximum limit for lead which is 0.3 mg/kg w.w. Thus, based on more than 1400 samples pooled from more than six thousand fish it is concluded that Norwegian farmed fish contain levels below the EUs maximum limits for heavy metals in fish.

5.2.6 Group B3d, Mycotoxins

The 8 pooled samples from 2011 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 404 pooled samples from 2 020 fish representing four species were examined with respect to malachite green and its metabolite leuco malachite green (LMG), crystal violet and its metabolite leuco crystal violet. The results for leucocrystal violet, for two samples, were excluded due to analytical issues. No residues of these agents were detected.

6. CONCLUSION

None of the substances with anabolic effect (group A1 and A3) were detected in any of the samples analysed in 2011. Nor were any residues found for the illegal compounds in group A6.

None of the group B compounds - veterinary drugs exceeded the maximum residue limits (MRL) established for fish, in the monitoring program in 2011. Emamectin was detected, however in concentrations well below the MRL values. Similarly to veterinary drugs, none of the environmental contaminants (organochlorine compounds and chemical elements) in the farmed fish analysed in 2011 were found at levels above or equal to the EU maximum limit for those compounds for which such limits have been established (BaP, dioxins, dioxin-like PCBs, mercury, lead and cadmium). The concentrations of dioxins (PCDDs and PCDFs), dioxin-like PCBs, PCB-7 and organic pesticides in farmed salmon in 2011 were comparable to those found in the monitoring programme for Directive 96/23/EC for the years 2003 to 2009. The maximum PBDE-7 concentration in farmed salmon in 2011 was 1.0 µg/kg.

7. ANNEX

Annex I Summary of analytical methods

Group of substances	Compounds	Matrix	Method	Screen-ing method LOD (w.w.) (µg/kg)	Analytical method LOD (w.w. in muscle) (µg/kg)	Analytical method LOQ (w.w.) (µg/kg)	Level of action	Laborato ry
A1 Stilbenes	Diethylstilboestrol	Muscle	GC-MS	n.a.	0.4	-	Presence	AUS
	Dienoestrol	Muscle	GC-MS	n.a.	0.7	-	Presence	AUS
	Hexoestrol	Muscle	GC-MS	n.a.	0.6	-	Presence	AUS
A3 Steroids	α-nandrolon	Muscle	GC-MS	n.a.	0.6	-	Presence	AUS
	β-nandrolon	Muscle	GC-MS	n.a.	0.6	-	Presence	AUS
	α-trenbolon	Muscle	GC-MS	n.a.	0.6	-	Presence	AUS
	β-trenbolon	Muscle	GC-MS	n.a.	0.6	-	Presence	AUS
A6 Annex IV substances	Chloramphenicol	Muscle	LC-MS	n.a.	0.25 (MRPL = 0.3)	1.0	presence	NIFES
	Metronidazole	Muscle	LC-MS/MS	n.a.	2.0	6.0	Presence	NIFES
	Hydroxy-metronidazole	Muscle	LC-MS/MS	n.a.	15	45	Presence	NIFES
	Nitrofuran 3-Amino-2-oxazolidone	Muscle	LC-MS/MS	n.a.	(MRPL =1.0)	0.5	Presence	Eurofins
	Nitrofuran 1-Aminohydrantoin	Muscle	LC-MS/MS	n.a.	(MRPL =1.0)	1	Presence	Eurofins
	Nitrofuran 3-Amino-5-morpholinomethyl-2-oxazol	Muscle	LC-MS/MS	n.a.	(MRPL =1.0)	0.5	Presence	Eurofins

	Nitrofuran Semicarbazide	Muscle	LC-MS/MS	n.a.	(MRPL = 1.0)	1	Presence	Eurofins
B1 Antibacterial substances Micro-biological method	Oxolinic acid	Liver	3-plate Screening method and HPLC-MS	200	10	30	100 µg/kg	NIFES
	Flumequine	Liver		200	10	20	600 µg/kg	NIFES
	Tetracyclines	Liver		200	2.0	5.0	100 µg/kg	NIFES
	Florfenicol	Liver		200	0.2	0.5	1000 µg/kg	NIFES
	Sulfonamides	Liver		400	n.a.	n.a.	100 µg/kg	NIFES
B1 Antibacterial substances Chemical method	Quinolones	Muscle	LC-MS/MS	n.a.	-	10	600	Eurofins
	Oxytetracycline	Muscle	LC-MS/MS	n.a.	-	50	100	Eurofins
	Chlorotetracycline	Muscle	LC-MS/MS	n.a.	-	50		Eurofins
	Tetracycline	Muscle	LC-MS/MS	n.a.	-	50		Eurofins
	Doxycycline	Muscle	LC-MS/MS	n.a.	-	50		Eurofins
	Florfenicol	Muscle	LC-MS	n.a.	0.2	0.5	1000µg/kg	NIFES
B2a Anthelmintics	Praziquantel	Muscle	LC-UV	n.a.	50	100	n.a.	NIFES
	Fenbendazole	Muscle	LC-MS/MS	n.a.	0.3	1.0	n.a.	NIFES
	Emamectin	Muscle	LC-MS	n.a.	2.5	5.0	100 µg/kg	NIFES
	Ivermectin	Muscle	LC-MS	n.a.	25.0	50	n.a.	NIFES
B2c Carbamates and pyrethroids	Cypermethrin	Muscle	GC-EC	n.a.	5	10	50 µg/kg	Eurofins
	Deltamethrin	Muscle	GC-EC	n.a.	10	20	10 µg/kg	Eurofins
B2f	Diflubenzuron	Muscle	LC-MS	n.a.	10	20	1000 µg/kg	NIFES
Other active substances	Teflubenzuron	Muscle	LC-MS	n.a.	5	15	500 µg/kg	NIFES
B3a Organo-chlorine compounds	PCDD, PCDF, and non orto-PCB	Muscle	GC-HRMS	n.a.	0.002-0.1 ng/kg	0.008-0.4 ng/kg	4 ng TEQ/ kg	NIFES
	PCB 28	Muscle	GC-MS	n.a.	0.006 - 0.016	0.02 - 0.05	n.a.	NIFES
	PCB 52	Muscle	GC-MS	n.a.	0.033 – 0.006	0.5	n.a.	NIFES
	PCB 101	Muscle	GC-MS	n.a.	0.033 – 0.006	0.5	n.a.	NIFES
	PCB 118	Muscle	GC-MS	n.a.	0.033 – 0.006	0.5	n.a.	NIFES
	PCB 138	Muscle	GC-MS	n.a.	0.033 – 0.006	0.5	n.a.	NIFES

PCB 153	Muscle	GC-MS	n.a.	0.033 – 0.006	0.5	n.a.	NIFES
PCB 180	Muscle	GC-MS	n.a.	0.015 – 0.003	0.5	n.a.	NIFES
α-HCH	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
γ-HCH	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
HCB	Muscle	GC-MS	n.a.	0.15	0.5	n.a.	NIFES
Pentachlorobenzene	Muscle	GC-MS	n.a.	0.15	0.5	n.a.	NIFES
Heptachlor	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
Heptachlor A	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
Aldrin	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
Isodrin	Muscle	GC-MS	n.a	0.1	0.3	n.a	NIFES
Dieldrin	Muscle	GC-MS	n.a	0.03	0.1	n.a	NIFES
Oxy-chlordane	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
trans-chlordane	Muscle	GC-MS	n.a.	0.03	0.1	n.a.	NIFES
cis-chlordane	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
α-endosulfan	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
β-endosulfan	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
Endosulfansulphate	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
cis-nonachlor	Muscle	GC-MS	n.a.	0.03	0.1	n.a.	NIFES
trans-nonachlor	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
Toxaphene 26	Muscle	GC-MS	n.a.	0.15	0.4	n.a.	NIFES
Toxaphene 32	Muscle	GC-MS	n.a.	0.3	1.0	n.a.	NIFES
Toxaphene 40+41	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
Toxaphene 42	Muscle	GC-MS	n.a.	0.05	0.2	n.a.	NIFES
Toxaphene 50	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
Sum Toxaphene 62	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
Mirex	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
DDT-op DDT-pp DDD-op	Muscle	GC-MS	n.a.	0.03	0.5	n.a.	NIFES

	DDD-pp DDE-op DDE-pp							
B3b Organo-phosphorous compounds	Azametiphos	Muscle	GC-FPD			20	Not applicable	Eurofins
	Dichlorvos	Muscle	GC-FPD			10	n.a.	Eurofins
B3c Chemical elements	Pb	Muscle	ICPMS	n.a.	0.07 µg/L	0.04 mg/kg	0.3 mg/kg	NIFES
	Cd	Muscle	ICPMS	n.a.	0.00 µg/L	0.01 mg/kg	0.05 mg/kg.	NIFES
	As	Muscle	ICPMS	n.a.	0.02 µg/L	0.03 mg/kg	n.a.	NIFES
	Hg	Muscle	ICPMS	n.a.	0.01 µg/L	0.03 mg/kg	0.5 mg/kg	NIFES
B3d Mycotoxins	Ochratoxin A	Muscle	Immuno affinity/ HPLC	n.a.	0.06 µg/kg		n.a.	NVI
B3e, dyes	Malachite green	Muscle	LC-MS/MS	n.a.	0.15 (MRPL=2.0) ∑ malachite green and leuco-malachite green= 2.0	0.5	Presence	NIFES
	Leuco malachite green	Muscle	LC-MS/MS	n.a.	0.15 ∑ malachite green and leuco-malachite green= 2.0	0.5	Presence	NIFES
	Crystal violet	Muscle	LC-MS/MS	n.a.	0.3	1.0	Presence	NIFES
	Leucocrystal violet	Muscle	LC-MS/MS	n.a.	0.15	0.5	Presence	NIFES