MONITORING PROGRAMME FOR RESIDUES OF THERAPEUTIC AGENTS, ILLEGAL SUBSTANCES AND OTHER UNDESIRABLE SUBSTANCES IN FARMED FISH

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

ANNUAL REPORT FOR 2010

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In 2010, 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 PAH analyses and the analysis of selected therapeutic compounds (see Annex I for details).

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1. SUMMARY

The number of samples included in the 96/23 monitoring programme is dependent on the production volume, a minimum of one sample per 100 tonnes of annual production. This report for 2010 is based on 1608 fish fillet samples (including 53 individual samples and the remaining were pooled samples of five fish) and 1585 liver samples, a total of 9261 farmed fish.

Group-A includes substances with anabolic effect and unauthorised substances, one third of the total samples were analysed for these substances. These samples were collected by official inspectors at the fish 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, two thirds 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.

Chloramphenicol was detected in one of the 194 pooled samples analysed at a concentration between the Limit of Detection (0.25 ng/g) and the Limit of Quantification (LOQ = 1.0 ng/g). Additional analysis of the salmon sample confirmed this finding and the Norwegian Food Safety Authority was informed, the source remains unclear.

Of the therapeutic agents in group B, emamectin benzoate was detected in eight of the 188 pooled samples of farmed fish analysed in 2010. Re-analysis of the positive samples showed mean concentrations ranging from 2.5 to 24.9 µg/kg, below the current MRL for emamectin benzoate 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 2010. The mycotoxin Ochratoxin A was not detected in any of the nine pooled salmon samples analysed for 2010.

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 2009, 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.

Concentrations of lead, cadmium and mercury in farmed fish in 2010 were below the EU maximum limits for these elements in fish. There is currently no EU maximum limit for arsenic, however the concentration in farmed fish is not a safety concern due to the chemical form present in fish, arsenobetaine which is considered harmless.

Brominated flame retardants (PBDE, HBCD and TBBPA) and perfluorinated compounds were included in the programme for 2010. The maximum PBDE-7 concentration in farmed salmon in 2010 was 3.2 µg/kg. The concentrations of the other brominated flameretardants (α-, β- and γ-hexabromocyclododecane (HBCD) and Tetrabromobisphenol A (TBBPA)) in farmed fish were all below their LOQ value of 1.0 µg/kg wet weight. The only PFC level in farmed fish above the LOQ was PFHxA with a maximum concentration of 1.4 µg/kg. The concentrations of the remaining 17 PFCs analysed in farmed fish were below their LOQ. There are no maximum limits in the EU for these compounds in food.

Benzo[a]pyrene (BaP) is currently the only PAH with an established maximum limit in food. The concentration of BaP in all of the samples of farmed fish analysed since 2007 has been below the LOQ value.

The synthetic antioxidants ethoxyquin, BHT and BHA are authorised feed additives in the EU. The results show that there is carry-over of synthetic antioxidants from the feed to the fish fillets, in particular for BHT. Levels in farmed fish in 2010 were lower than those found in previous years, and all of the samples were compliant with the Japanese maximum residue limits. There is currently no EU maximum residue limit for these compounds in food of animal origin as a result of their use as feed additives.

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. Levels that cannot be quantified with acceptable reliability are reported as "less than LOQ", for example: <2.0 µg/kg. “Detected” for illegal compounds is used to indicate that the concentration is in the range from LOD to LOQ.

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 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). In cases where the number of values below the LOQ exceeds 2/3 of the samples for a certain parameter no mean is calculated for the parameter and only the maximum value and the LOQ is reported.

Maximum residue limit (MRL): is the highest permitted concentration of legally applied agents in products from food animals intended for human consumption. Until 2009, the MRLs were established in accordance with the Council Regulation (EEC) 2377/90. After 2009 Regulation (EC) 470/2009 set the community methods for the establishment of residue limits of pharmacologically active substances in foodstuff of animal origin. According to the regulations, substances are to be classifies into i) those where an MRL is established, ii) those with a provisional MRL, iii) compounds where the establishment of an MRL is not considered necessary and iv) substances which are prohibited to administer to food producing animals. According to the Commission Regulation 37/2010, MRLs are set for muscle and skin in natural proportions for all agents where an MRL has been established.

Minimum required performance level (MRPL): This is the minimum required detection 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 related chemicals, in this context “congeners” refers to 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. Current legislation applies to 1998 TEFs. TEFs 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

The aim of this programme is to monitor residues of therapeutic agents, illegal substances and other substances in 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. The Norwegian Food Safety Authority (NFSA) is responsible for the enforcement, planning and sampling following up this directive 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 results for aquacultured animals.

3.2 Scope of the monitoring programme

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 and analytes and also depend on the type of farm animal, feedingstuff or animal product. 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
- B3d: Chemical elements
- B3d: Mycotoxins
- B3e: Dyes
- B3f: Others

Group A-substances are compounds with anabolic effects and unauthorized substances. Fish at different stages of farming are sampled at fish farms without prior notification by official inspectors from the Norwegian Food Safety Authority and analysed for group A-substances. Sampling is designed to ensure that samples are representative of farmed fish in 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 consumption. The substances included in "Others" are not defined in Directive 96/23/EC, but are included in the monitoring programme on request by the Norwegian Food Safety Authority. The substances which are monitored vary from year to year depending on priority.

4. PARAMETERS AND METHODS

According to Directive 96/23 the minimum number of samples to be taken each year is 1 per 100 tonnes produced fish. In 2010 this applied to salmon and trout farming, whereas as a national measure the sample frequency for other farmed species was increased to one sample per 25 tonnes fish produced. One-third of the total number of samples were analysed for group A substances, and two-thirds 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 analysts were blinded to sample identification The samples for most parameters were frozen muscle (either fillets- or cutlets with skin) or liver tissue, as for other parameters gutted fish stored on ice were the sample material. Samples were shipped frozen to NIFES, except the samples of individual fish for analyses of synthetic antioxidants which were performed on fresh fillets. For practical reasons some individual fish samples were also analysed for other chemical parameters. The total number of fish analysed includes both individual and pooled fish samples. In 2010 there were 33 individual fish samples. The liver samples were all analysed individually.

On arrival at NIFES fish muscle samples were pooled with equal contribution from each fish, then homogenised. The advantage of pooled samples is that a large number of fish can be included in the surveillance. The standard deviations reflect the variability among the farms.

Table 1 gives the number of fish analysed in the monitoring programme in 2010, whereas Table 2 gives the number of fish for each species that was the basis for the samples examined in this programme. The total number of fish in 2010 was 9261 analysed in a total of 1608 fillet samples and 1 585 liver samples. Sample collection, and number of samples for each analyte were done according to the plan drawn up by the NFSA 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 number of the samples analysed for each parameter. However, as a general rule each sample was only analysed for one parameter.

Table 1. Sampling data

Substance		Parameter	Number of fish	Number of samples	Number of determinations
Samples taken from the farms with no pre- notice	A1 Stilbenes	Diethylstilboestrol	270	54	54
		Dienoestrol			54
		Hexoestrol			54
	A3 Steroids	α-nandrolon	285	57	57
		β-nandrolon			57
		α-trenbolon			57
		β-trenbolon			57
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	970	194	194
		Metronidazole	760	152	152
		Hydroxy metronidazole			152
		Nitrofuran metabolites	555	111	
		3-Amino-2-oxazolidone			111
		1-Aminohydrantion			111
		3-Amino-5-Morpholinomethyl-2-oxazol			111
		Semicarbazide			111
	Sum of group A		2 840	568	1 332
Samples taken from processing plants	B1 Anti bacterial agents	Flumequine	95	19	19x14=266
		Oxolinic acid			
		Cinoxacin			
		Ciprofloxacin			
		Danofloxacin			
		Difloxacin			
		Enoxacin			
		Enrofloxacin			
		Lomefloxacin			
		Marbofloxacin			
		Nalidixic acid			
		Norfloxacin			
		Ofloxacin			
		Sarafloxacin			
		Florfenicol	45	9	9
		Oxytetracycline Tetracycline Chlorotetracycline Doxycycline	50	10	10x4=40
	B2 Other veterinary drugs	Teflubenzuron	65	13	13
		Diflubenzuron	70	14	14
		Cypermethrin	105	21	21
		Praziquantel	395	79	79
		Fenbendazole	220	44	44
		Emamectin	940	188	188
		Ivermectin	45	9	9
		Deltamethrin	105	21	21
	B3a Organohlorine Compounds	α-HCH	165	33	33x33=1089
		β-HCH			
		γ-HCH			
		HCB			
		Pentachlorobenzene			
		Heptachlor			
		Heptachlor epoxide			
		Aldrin			
		Dieldrin			
		Isodrin			
		Oxy-Chlordane			
		trans-Chlordane			
		cis-Chlordane			
		α-Endosulfan			
		β-Endosulfan			

Table 1. Sampling data

Substance		Parameter	Number of fish	Number of samples	Number of determinations
		Endosulfan sulfate			
		cis-Nonachlor			
		trans-Nonachlor			
		Toxaphene 26			
		Toxaphene 32			
		Toxaphene 40+41			
		Toxaphene 42			
		Toxaphene 50			
		Toxaphene 62			
		Mirex			
		DDT, DDE og DDD : orto-para and para-para congeners			
		Dioxins and Dioxin-like PCBs	203	67	67x29=1943
		PCB-7	33	33	33x7=231
		PBDE-10			33x10=660
		α, β + γ-HBCD			31x4=124
		TBBPA			31
	B3b Organo-phosphorous Compounds	Azametiphos	140	28	28
		Dichlorvos			28
	B3c Chemical elements	Pb Cd Hg As	890	178	178x4=836
	B3d Mycotoxins	Ochratoxin A	45	9	9
	B3e, Dyes	Malachite green Leucomalachite green	825	165	2x165 = 330
		Chrystal violet Leuco Chrystal violet			
		Brilliant green	380	76	76
	B3f Others	BHT BHA	33	33	32x2=64
		Ethoxyquin + dimer			2x2=4
		PFC (10)			33x18=594
		PAH (13)			33x13=429
	Sum B fillets, pooled and from individual fish, chemical method + 20 for B1		4836	1036	7180
B (liver)	B1 Microbiological screening of liver	Quinolones	1585	1585	1605x3=4815
		Tetracyclines and amphenicols			
		Sulphonamides			
Total sum B			6421	2621	11995
Total sum fillet A+B			7676	1608	8512
Total sum, fillets, pooled and individual fish and liver			9261	3193	13327
Note: PCB-7, BHT, BHA, PAH, PFC, all the brominated flame retardants, synthetic antioxidants (BHT, BHA, and ethoxyquin) and some of the dioxins, are analysed in the same samples which affects the sum.					

4.1 Analytical methods

The analytical methods and the laboratory routines are accredited in accordance with the standard ISO 17025, unless otherwise specified. 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. 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 analyzed. The LOD and LOQ for the various analytical methods are given in Annex I.

4.1.1 Quality assurance

For all methods a quality control sample (QCS) with known composition is included in each analytical series. A series is equivalent to the analytical capacity for one day except for the dioxin method. This method is based on the isotope dilution principle which integrates quality assurance thus the frequency of the QCS analysis is reduced to allow a higher analytical capacity.

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. If a positive value is found for this “sample” this reflects 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 analyzing 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 33 individual fish samples. In the microbiological assay for antibiotics, liver samples are tested individually. The assay is a qualitative method and the results are therefore "detected" or "not detected". A summary of the analytical methods used is shown in Annex I.

4.1.2 Group A substances

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

4.1.2.1 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 analyzed by GC/ MS. If positive findings were found they would be verified by confirmatory methods, including an additional clean-up by HPLC before a new derivatization step followed by a final analytical determination by GC/MS.

4.1.2.2 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-d5) was added to the sample before extraction with ethyl acetate. The sample was analyzed by LC-MS, with a reversed phase C18 column for separation. The components were ionized by ESI and detected as negative ions using the SIM mode. Quantification was based on a standard addition method.

Nitrofurans
This group of synthetic antibacterial agents are derivatives of nitrofuran. The compounds have previously been widely used in veterinary medicine. These agents are rapidly metabolized in the tissue, thus in this surveillance programme the metabolites 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semicarbazide (SEM) have been included.

Analytical method: The analytes were extracted with aqueous hydrochloric acid and derivatized with nitrobenzaldehyde. Solid phase extraction was applied for sample clean up. The analytes were determined by LC-MS/MS in the positive 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-d3) was added to the homogenized sample. The analytes were extracted by ethyl acetate and analysed by LC-MS/MS. A reversed phase C18 column was used for separation, and the components ionized by ESI and fragments detected as positive ions using the MRM mode. Quantification was based on the standard addition method. This method was not accredited during 2010.

4.1.3 Group B substances

4.1.3.1 B1, Antibacterial agents (antibiotics)

The antibacterial agents in class B1 was determined by a combination of chemical methods and the three plate bioassay.

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. Pieces of liver were placed on the plates. If the samples had contained residues of antibacterial agents, the bacterial growth would be inhibited in a zone around each piece of liver tissue. 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 EDTA-succinate buffer. Solid phase extraction was used for sample preparation. The analyte was determined by LC-MS/MS in the positive 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: Internal standard was added to the sample, and the analytes were extracted with ethyl acetate. The samples were analyzed by LC-MS and detected as negative ions using the SIM mode. Quantification was based on a standard addition method.

4.1.3.2 B2a, Anthelmintics

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

Analytical method: Internal standard was added to the sample, and the analytes were extracted with acetone. The samples were analyzed 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: Internal standard was added to the sample and the sample was analyzed by LC-MS 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 analyzed by gas chromatography-electron capture (GC-EC).

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

Analytical method: 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. 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 were added to the extract and praziquantel was detected by LC-UV. Quantification was based on a standard addition method.

4.1.3.3 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 and accumulate in the food chain. For this reason they are of environmental concern, and a threat to human health

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 congeners. The use of PCB was banned in Norway in 1980. Europe 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 on 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 beind discussed in the European Commission. This report provides data for both PCB-6 and PCB-7.

Analytical method: The extracted sample was analysed on GC/MS in SIM mode with electron impact ionization. Quantification was based on an 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. The method was not accredited in 2010.

Dioxins, furans, and the non-orto and mono-orto PCBs.
Dioxins (PCDD and PCDF) are unwanted by-products in various industrial processes, and by waste incineration plants. Seventeen of the 210 dioxin congeners and 12 (dioxin-like) PCB congeners were assigned toxic equivalency factors (TEFs) in 1998 by the WHO relative to the most toxic dioxin: 2,3,7,8 TCDD. In 2005 the TEF list was revised by the WHO, however EU legislation on maximum limits for dioxins and dioxin-like PCBs in feed and food are still based on 1998 TEFs. Concentrations are expressed in toxic equivalency units (TEQ) which are the concentrations multiplied by the corresponding TEF value. Thus all 29 congeners are reported as the "sum of TEQ" for dioxins, and dioxin-like PCBs.

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-orto substituted PCBs: PCB -77, 81, 126 and 169 and eight mono-orto substituted PCBs: PCB-105, 114, 118, 123, 156, 157, 167 and 189. 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 for each congener.

Polychlorinated pesticides, including DDT and its metabolites
This group of compounds include a wide range of complex molecular structures, the pesticides of most concern are the chlorinated pesticides due to their persistence in the environment, and their high toxicity compared with more water soluble pesticides. 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: Analysis of the extracted sample was carried out by GC/MS in negative chemical ionization and SIM mode. Quantification was based on an internal standard method with isotope labelled internal standards. The individual LOQ values for each of the compounds are listed in Table 6.

4.1.3.4 B3b, Organophosphorous compounds

Azametiphos and Dichlorvos

The sample material was extracted with acetone. A measured aliquot of the organic phase was dried with sodium sulfate and then reduced in volume. Equal volumes of ethyl acetate and cyclohexane were added in succession to the residue. The extract was cleaned up by gel permeation chromatography and analysed by gas chromatography with a Flame Photometric detector (GC-FPD).

4.1.3.5 B3c, Chemical elements

Chemical elements such as cadmium and mercury occur both naturally in the environment and as a result of anthropogenic activity From a food safety perspective contamination during

feed production is potentially the most serious threat to farmed fish. In Norway there has been one incident where the 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 analytes in solution of digested samples were measured quantitatively by inductively coupled plasma mass spectrometer (ICPMS). These 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) from were analyzed in each analytical series: Tort-2 (lobster hepatopancreas) and Dorm-2 (dogfish muscle).

4.1.3.6 B3d, Mycotoxins

Mycotoxins can be formed in foods, raw materials for food production, or in animal feeds. Feed and food can be contaminated 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 is Ochratoxin A. The method for detection of this mycotoxin in muscle tissue, included extraction from the matrix, clean-up by an immunoaffinity column and quantification by HPLC with fluorescence detection.

4.1.3.7 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 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 analyzed 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).

4.1.3.8 B3f, Others

These compounds are not included in Directive 96/23/EC, but were included in the monitoring programme for 2010 on request of the Norwegian Food Safety Authority. They include the synthetic antioxidants BHA, BHT and ethoxyquin, perfluorinated organic compounds (PFC) and polycyclic aromatic hydrocarbons (PAH).

Brominated flame retardants (BFRs)
There are four main classes of BFR: Polybrominated diphenyl ethers (PBDE), Tetrabromobisphenol-A (TBBPA), Hexabromocyclododecane (HBCD) and Polybrominated biphenyls (PBB). The molecular structures of PBBs and PBDEs are very similar 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 also measures the congeners no. 28, 153, 154 and 183 (66, 119, 138). In addition to the individual levels of these compounds NIFES report their results as upper bound sum: PBDE-

7. Both lists, PCB-7 and PBDE-7, are based on their observed concentrations in marine samples rather than on their toxicity.

HBCD exists as three isomers: α-, β- and γ-HBCD. Tetrabromobisphenol-A (TBBPA) is the most widely used BFR and it is more common in Asia than in Europe and America. Due to increasing trade and atmospheric transport levels in the environment are expected to increase also in Europe.

Analytical method for PBDE: PBDE-139 was added as the internal standard to the extracted sample which was analysed by GC / MS in SIM mode with negative chemical ionizing. Quantification was according to an internal standard method based on a five point linear dose-response curve.

Analytical method for HBCD and TBBPA: A ^{13}C-labelled "recovery standard" was added to the solution prior to the instrumental analysis. The α-, β- and γ-HBCD and TBBPA were determined on LC / MS / MS with electro spray (ES) in the negative ionization mode with Multiple Reaction Monitoring (MRM) mode. Quantification was based on the "isotope dilution" method using ^{13}C-labeled internal standards.

Perfluorinated compounds
Perfluorinated compounds (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. Perfluorinated compounds (18 different forms of PFCs including PFOS and PFOA) were analysed by LC/MS/MS and quantified using internal standards and calibration curves.

PAH
Polycyclic aromatic hydrocarbons (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. One of the most toxic forms, Benzo (a) pyrene (BaP), is used as an indicator of PAH contamination, and the EU has an upper limit for BaP in fish of 2 μg/kg. In 2008 the European Food Safety Authority (EFSA) concluded that BaP, the only PAH presently regulated in food, is not a suitable "indicator" for the occurrence of PAHs in food and proposed a sum of either 4 or 8 PAH, as more suitable "indicators" for protecting consumer health. The European Commission is considering amending the current regulation for PAH in food.

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 GC7MS in single ion mode.

Synthetic antioxidants
The synthetic antioxidants ethoxyquin, BHT (4-methyl-2,6-ditert-butyl-phenol) and BHA (4-amino-2-hydroxy-benzoic acid) and ethoxyquin are authorised for use in animal feed. Antioxidants are necessary in feed ingredients to prevent self ignition during bulk transport

and because oxidized fats in feed may have adverse nutritional effects. The parent compound ethoxyquin and its dimer were analysed in the programme, but only the parent compounds of BHT and BHA were analysed.

Analytical method for ethoxyquin: Pyrogallol, ascorbic acid and EDTA were added to prevent oxidation and the samples were extracted with acetonitrile mixed with ascorbic acid. Ethoxyquin and its dimer were quantified by reverse phase HPLC with fluorescence detection. Quantification was based on an external standard method. This method was not accredited in 2010.

Analytical method for BHT: Pyrogallol, ascorbic acid and EDTA were added to prevent oxidation and the analyte was extracted with acetonitrile containing 0.1% ascorbic acid. After centrifugation the extract was filtered before quantification using reverse phase HPLC and fluorescence detection. Quantification was based on an external standard method.

Analytical method for BHA: BHA was extracted with acetonitrile with 0.1% ascorbic acid. The BHA concentration in the extract was measured using reverse phase HPLC with acetonitrile as the mobile phase and fluorescence detection. Quantification was based on an external standard method.

Table 2. Number of fish of each species and the number of parameters analysed

Class of compounds			# fish	Salmon	Rainbow trout	Turbot	Halibut	Cod	Arctic char	Saithe
Samples taken from the farms with no pre-notice	A1 Stillebenes	Diethylstilboestrol	270	245	5			20		
		Dienoestrol								
		Hexoestrol								
	A3 Steroids	α-nandrolon	285	255	10			20		
		β-nandrolon								
		α-Trenbolon								
		β-trenbolon								
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	970	750	70	5	20	105	20	
		Metronidazole Metronidazole-OH	760	710	40			10		
		Nitrofuran metabolites 3-Amino-2-oxazolidone	555	455	60			35	5	
		1-Aminohydrantion								
		3-Amino-5-morpholinomethyl-2-oxazol								
		Semicarbazide								
Samples taken from processing plants	B1 Chemical method in muscle	Flumequine								
		Oxolinic acid								
		Cinoxacin								
		Ciprofloxacin								
		Danofloxacin								

		Difloxacin	95	30				60	5	
		Enoxacin								
		Enrofloxacin								
		Lomefloxacin								
		Marbofloxacin								
		Nalidixic acid								
		Norfloxacin								
		Ofloxacin								
		Sarafloxacin								
		Florfenicol	45	15				30		
		Oxytetracycline Tetracycline Chlorotetacycline Doxycyclne	50	20				25	5	
	B1 Micro-biological assay in liver	Quinolones	1605	1520	85					
		Tetracyclines and amphenicols								
		Sulphonamides								
	B2 Other veterinary drugs	Teflubenzuron	65	65						
		Diflubenzuron	70	70						
		Cypermethrin	105	95	5			5		
		Praziquantel	395	380	10	5				
		Fenbendazole	220	205	5			10		
		Emamectin	940	845	95					
		Ivermectin	45	40	5					
		Deltamethrin	105	95	5			5		
	B3a Organo-chlorine compound	α-HCH	165	120	20		5	20		
		β-HCH								
		γ-HCH								
		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	203	183	20					
		PCB-7	33	32	1					
		PBDE-10						20		
		α , β + γ-HBCD								
		TBBPA								
	B3b Organo-phosphorous Compounds	Azametiphos	140	135	5					
		Dichlorvos	140	135	5					
	B3c Chemical elements	Pb								
		Cd								
		Hg								
		As	890	615	155			95	25	
	B3d	Mycotoxins	45	45						
	B3e, Dyes	Malachite green Leucomalachite green	825	795	30					
		Crystal violet Leucocrystal violet								
		Brilliant green	380	360	20					
	B3f Others	BHT								
		BHA	33	32	1					
		Ethoxyquin+ dimer								
		PAH								
		PFC								
Note: PCB-7, BHT, BHA, PAH, PFC, all the brominated flame retardants, synthetic antioxidants (BHT, BHA, and ethoxyquin) and some of the dioxins, are analysed in the same samples.										

6. RESULTS AND DISCUSSION

6.1 Group A

A total of 568 pooled fillet samples from 2 840 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 without prior notice. The samples in this group are from all growth phases, not only from market-sized fish.

6.1.1 Group A1

The levels of the group A1 substances diethylstilboestrol, dienoestrol and hexoesterol were examined in 54 pooled samples from a total of 270 fish from three species. The detection limits (LOD) are listed in Annex I and the number of fish of each species is listed in Table 2. None of the substances were detected in any of the samples analysed.

6.1.2 Group A3

The levels of group A3 substances nortestosterone (α and β nandrolon) and α and β trenbolon, were analyzed in 57 pooled samples from 285 fish from three species. The detection limits (LOD) are listed in Annex I, the number of fish of each species is listed in Table 2. None of the substances were detected.

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

A total of 457 pooled samples from 2 285 fish were analysed in this group. The detection limits (LOD) are listed in Annex I, the number of fish of each species is listed in Table 2. Chloramphenicol was detected in one out of 194 pooled sample at a concentration between the LOD (0.25 ng/g) and the LOQ (1.0 ng/g). The positive sample was of salmon, and this sample was re-analysed together with the back-up sample using a method which had an improved detection limit. This re-analysis confirmed the initial positive finding.

6.2 Group B

There were 1036 pooled and single fish samples of fillets from a total of 4835 fish, and additionally 1585 individual fish liver samples for the inhibition test. Samples were taken at processing plants of fish that were market-size.

6.2.1 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 1585 fish and muscle from 20 fish (where the liver was not supplied in the sample), representing a total of 1605 fish and 4 815 analytical determinations. The results from the bioassay on muscle are reported together with the liver samples. The B1 antibacterial agents were also analyzed by three chemical methods in 38 pooled fillet samples, representing 190 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 detection of antibiotics. Moreover, the method is able to detect a wider range of antibiotics than the more specific chemical methods which makes the bioassay a useful screening method. Positive samples detected by the inhibition assay are verified by chemical analysis of the corresponding fillet sample received together with the liver sample. However, no positive samples were found in the B1 group in salmon livers in 2010.

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

The levels of the B2 substances teflubenzuron (B2f), diflubenzuron (B2f), cypermethrin (B2c), praziquantel, fenbendazole (B2a), emamectin benzoate (B2a), ivermectin (B2a) and deltamethrin (B2c) were determined in 389 pooled fillet samples representing 1 945 fish from four species. Emamectin benzoate was detected in eight of the 188 pooled samples included in the monitoring programme of 2010. 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 24.9 µg/kg. The current MRL for emamectin benzoate is 100 µg/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.

6.2.3 Group B3a, Organochlorine compounds

These compounds receive much focus from a food safety perspective. There were 100 samples from 368 fish that were analysed for these compounds. The results are summarised in Tables 3 to 6.

6.2.3.1 Organochlorine pesticides

There were 24 salmon-, four trout-, four cod and one Atlantic halibut 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 this reflects the variation in their fat content. This is consistent with the lipophilic nature of these compounds.

DDT and its metabolites

UB-sum of DDT and its metabolites in 2010 varied between 3.3 µg/kg to 13.6 µg/kg wet weight. The highest level was found in the single halibut sample analysed, followed by salmon, trout and cod with UB-sum mean values of 8.7, 8.3 and 1.35 µg/kg wet weight respectively. In the last two years the levels in salmon were respectively 10.2 and 8.8 µg/kg wet weight. The use of UB calculation in the reports since 2007 must be taken into account when comparing results with years prior to 2007. Correcting for this there is no obvious trend in DDT concentrations in farmed fish since 2003. Generally, in this period the para-para compounds have been present at higher concentration levels than the orto-para compounds.

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

Alfa- and gamma HCH was quantified in salmon and trout samples, both species had a maximum level of 0.2 µg/kg wet weight. Beta HCH was not found in measurable quantities in any of the samples regardless of fish species, nor was pentachlorobenzene. Hexachlorobenzene though had measurable levels for in four species: Halibut contained 0.9 µg/kg wet weight, salmon 1.1 µg/kg wet weight, trout 1.2 µg/kg wet weight and cod 0.2 µg/kg wet weight. The difference in concentrations between the species reflects their varying lipid content as expected considering the lipophilic nature of these compounds.

Table 3. DDT, DDD and DDE (µg/kg wet weight) in fillets of pooled farmed fish samples

	op-DDT	pp-DDT	op-DDD	pp-DDD	op-DDE	pp-DDE	UB-sum DDT
LOQ	0.9	0.9	0.9	0.9	0.9	0.9	-
Salmon	N=24						
UB-Mean	-	0.8	-	1.8	-	5.3	8.5
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.3	4.1
Max	<LOQ	1.1	<LOQ	2.6	<LOQ	7.6	11.6
Rainbow trout	N=4						
UB-Mean	-	0.9	-	1.8	-	5.0	8.3
Min	<LOQ	<LOQ	<LOQ	1.0	<LOQ	2.8	5.3
Max	<LOQ	1.2	<LOQ	2.8	<LOQ	7.1	11.6
Cod	N=4						
UB-Mean	-	-	-	-	-	-	-
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-
Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	-
Halibut	N=1						
Value	<LOQ	1.2	<LOQ	2.9	<LOQ	9.1	14.1
All groups	N=33						
UB-Mean	-	0.8	-	1.8	-	5.4	7.8
Min	<LOQ	<LOQ	<LOQ	0.8	<LOQ	<LOQ	-
Max	<LOQ	1.2	<LOQ	2.9	<LOQ	9.1	14.1

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

Other organochlorine pesticides

The results for the other 22 pesticide compounds analysed are summarised in Table 4. The values ranged from <LOQ to 3.6 µg/kg w.w., the highest concentration in 2010 was for dieldrin, as was the case in 2009. Most of these compounds were present at concentrations below their respective LOQ values hence it was not possible to calculate a representative mean value. These low levels are consistent with the findings from previous years. No measurable concentrations weres found in cod fillets for any of these compounds. This is consistent with cod being a lean fish.

Table 4. Other pesticides (µg/kg wet weight) in fillets of pooled farmed fish samples

Pesticide		Atlantic Halibut	Atlantic salmon	Rainbow Trout	Cod	All Grps	LOQ
	No. samples	1	24	4	4	33	
α-HCH	#Values	0	11	2	0	13	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	0.2	0.2	<LOQ	0.2	0.3
β-HCH	#Values	0	0	0	0	0	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	1.2-
	Max	<LOQ	<LOQ	<LOQ	<LOQ	3.0	3.0
γ-HCH	#Values	0	9	2	0	11	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	0.2	0.3	<LOQ	0.2	0.3
HCB	UBMean	0.9	1.1	1.2	-	1.0	0.3
	Min		0.4	0.7	<LOQ	0.3	
	Max	0.9	1.8	2.0	<LOQ	2.0	
Pentachlorobenzene	#Values	0	0	0	0	0	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.3
Heptachlor	#Values	0	0	0	0	0	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.3
Heptachlor epoxide	#Values	0	0	0	0	0	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.3-
	Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.5
Aldrin	#Values	0	0	1	0	1	
	Min	-	-	<LOQ	-	<LOQ	0.9-
	Max	<LOQ	-	3.40	-	3.40	1.5
Isodrin	#Values	0	3	0	0	3	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	0.2	<LOQ	<LOQ	0.2	0.9
Dieldrin	UBMean	2.7	2.1	2.0	-	1.8	
	Min		0.7	1.0	<LOQ	<LOQ	
	Max	2.7	3.2	3.6	<LOQ	3.6	0.15
α-endosulfan	#Values	0	8	1	0	9	
	Min		<LOQ	<LOQ	-	<LOQ	
	Max	<LOQ	0.08	0.03	-	0.08	0.15
β-endosulfan	#Values	0	3	0	0	3	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	0.06	<LOQ	<LOQ	0.06	0.15
Endosulfan sulphate	#Values	0	4	2	0	6	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	0.2	0.3	<LOQ	0.3	0.15

cis-chlordane	UBMean	1.9	1.0	1.5	-	1.0	
	Min	-	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	1.9	1.9	3.6	<LOQ	3.6	0.9
oxy-chlordane	UBMean	0.4	0.3	0.2	-	0.2	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	0.4	0.5	0.4	<LOQ	0.5	0.3
trans-chlordane	UBMean	0.2	0.1	0.1	-	0.1	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	0.1-
	Max	0.2	0.2	0.2	<LOQ	0.2	0.3
cis-nonachlor	UBMean	1.3	0.5	0.4	-	0.4	
	Min		0.2	0.2	<LOQ	<LOQ	
	Max	1.3	1.0	0.6	<LOQ	1.3	0.15
trans-nonachlor	UBMean	2.6	1.1	0.9	-	1.0	
	Min	-	0.4	0.5	<LOQ	<LOQ	
	Max	2.6	2.2	1.5	<LOQ	2.6	0.15
Toxaphene-26	UBMean	1.6	0.9	0.6	-	0.8	
	Min		<LOQ	<LOQ	<LOQ	<LOQ	
	Max	1.6	1.8	0.8	<LOQ	1.8	0.6
Toxaphene-32	#Values	0	0	0	0	0	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.5
Toxaphene-40+41	UBMean	1.5	0.8	0.5	-	0.7	
	Min	-	0.2	0.2	<LOQ	<LOQ	
	Max	1.5	1.7	0.8	<LOQ	1.7	0.1-0.6
Toxaphene-42	#Values	0	11	1	0	12	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.3-
	Max	<LOQ	0.7	0.3	<LOQ	0.7	0.6
Toxaphene-50	UBMean	3.0	1.5	0.9	-	1.3	
	Min	-	0.8	<LOQ	<LOQ	<LOQ	
	Max	3.0	3.0	1.7	<LOQ	3.0	0.6
Toxaphene-62	#Values	0	8	1	0	9	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	1.3	1.1	<LOQ	1.3	0.6
Mirex	#Values	1	4	0	0	5	
	Min	-	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	0.2	0.30	<LOQ	<LOQ	0.30	0.3

#values means: Number of analytical measurements above the LOQ.

6.2.3.2 Polychlorinated biphenyls (PCB)

The concentrations of PCB-7 and indicator PCBs (PCB-6) in farmed fish are given in Table 5. This years the data are mainly for salmon (32 samples), and a single trout sample. The PCB-7, calculated as the "upper bound-LOQ" (UB) sum in the salmon samples ranged from 5.9 to 21.6 µg/kg wet weight. In 2009 and 2008 the maximum values were 18 µg/kg and 15.5 µg/kg wet weight, 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 mean sum PCB-7 value in salmon was 10.6 µg/kg in 2010 compared to 7.8 µg/kg and 8.8 µg/kg wet weight 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 75 µg/kg. The highest concentration of indicator PCBs measured in salmon in 2010 was 18.7 µg/kg wet weight which is well below the proposed limit

Table 5. PCB-7 and PCB-6 (µg/kg wet weight) in 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	0.06	0.09	0.09	0.09	0.12	0.09	0.15	7.9	7.0
Salmon									
N	32	32	32	32	32	32	32	32	32
UB-Mean	0.3	0.8	1.6	1.2	2.8	3	0.8	10.6	9.4
Min	0.1	0.4	0.9	0.7	1.6	1.7	0.4	5.9	5.2
Max	0.4	1.2	2.8	2.4	6.0	6.1	2.4	21.1	18.7
Rainbow trout									
N	1	1	1	1	1	1	1	1	1
UB-Mean	-	-	-	-	-	-	-	-	-
Min	-	-	-	-	-	-	-	-	-
Max	<0.3	0.5	1.1	0.9	2.2	2.3	0.6	7.9	7.0
All groups									
N	33	33	33	33	33	33	33	33	33
UB-Mean	0.3	0.8	1.6	1.2	2.8	3.0	0.8	10.5	9.3
Min	0.1	0.4	0.9	0.7	1.6	1.7	0.4	5.9	5.2
Max	0.4	1.2	2.8	2.4	6.0	6.1	2.4	21.1	18.7
UB="upper bound", LOQ substituted for all values <LOQ in the calculation.									

6.2.3.3 Dioxins, furans and dioxin like PCBs

A summary of the total WHO_{1998}TEQ values (ng/kg w.w.) for the 29 congeners is listed in Table 6. All figures in the table are calculated as the "upper bound-LOQ" sum (UB-sum). A total of 67 pooled and individual samples were analysed from 203 fish. The species analysed were Atlantic salmon and rainbow trout.

For the 17 dioxin and furan compounds (PCDD + PCDF) the sum ranged from 0.2 ng TEQ/kg to 1.1 ng TEQ/kg w.w. The mean sum was 0.5 TEQ/kg w.w. for both species, though the trout data were only based on four samples. Both the means and the range are consistent with concentrations measured annually since 2004, thus there seems to be no trend in this period. The maximum value of 1.1 ng TEQ/kg w.w is below the EU's maximum limit of 4.0 ng TEQ/kg w.w.

The dioxin-like PCBs (DLPCB) are PCB congeners with non- and mono-ortho molecular structure. The sum of the DLPCB levels range from 0.4 to 1.2 ng TEQ/kg wet weight, and the mean concentration was 0.7 ng TEQ. Like PCDD and PCDF values, both mean and range are consistent with the levels found annually since 2004. Thus there is no obvious trend in this period.

The sum of dioxins and DLPCBs ranged from 0.7 to 2.1 ng TEQ/kg w.w. The mean concentration was 1.2 ng TEQ/kg w.w, with no apparent difference between the levels in Atlantic salmon and rainbow trout. This is well below the EU maximum limit of 8.0 ng TEQ/kg w.w. Concentration of dioxins and DLPCD in farmed salmon or rainbow trout measured for this monitoring programme have alway been considerably below the EU maximum limit. The results correspond to 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 6. Dioxins (PCDD/F) and dioxins-like PCBs (ng WHO_{1998}TEQ/kg wet weight) in fillets of farmed fish samples

		Atlantic Salmon	Rainbow Trout	All Groups	EU Limit
Parameter	N	63	4	67	
Sum DLPCB	Mean	0.7	0.6	0.7	
	Min	0.4	0.5	0.4	
	Max	1.2	0.6	1.2	
SUM PCDD/F	Mean	0.5	0.5	0.5	
	Min	0.2	0.3	0.2	
	Max	1.1	0.7	1.1	4.0
SUM DLPCB + PCDD/F	Mean	1.2	1.1	1.2	
	Min	0.7	0.9	0.7	
	Max	2.1	1.4	2.1	8.0

UB: All sums and averages are”upper bound” calculations.
LOQ: All LOQ values are related to the individual congeners

6.2.4 Group B3b, Organophosphorous compounds

The levels of the B3b substances azametiphos and dichlorvos were determined in 28 pooled fillet samples representing 140 fish from two species. Residues of these two agents were not found in any of the examined samples.

6.2.5 Group B3c, Chemical elements

The concentrations of chemical elements were determined in 178 pooled fish samples from the fillets of 890 fish (Table 7).

6.2.5.1 Arsenic

The arsenic levels in the fillets of farmed fish ranged from 0.39 to 2.7 mg/kg w.w in 2010 (Table 7). The level of arsenic in fillets has been fairly constant in the period since 2004. The levels in the lean species cod are not significantly different from the others.

6.2.5.2 Cadmium

For the 2010 samples a total of 20 samples out of 209 had values above LOQ. The maximum concentration measured in farmed fish was 0.02 mg/kg w.w, comparable with the maximum levels found in 2009. The EU maximum limit for cadmium in most fish species is 0.05 mg/kg w.w.

6.2.5.3 Mercury

The concentration of total mercury in farmed fish ranged from 0.01 to 0.27 mg/kg w.w in 2010 (Table7). The highest concentration measured in 2010 was in farmed salmon, whereas the highest level in 2009 was found in cod. 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.

6.2.5.4 Lead

Only six samples of farmed fish fillets out of 209 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.

Table 7. Chemical elements arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) (mg/kg wet weight) in fillets of pooled farmed fish samples								
Element		**Salmon**	**Trout**	**Cod**	**Arctic Char**	**All Grps**	**EU-Limit**	**LOQ**
As	UB-Mean	0.84	0.93	0.98	1.77	0.89		
	N	123	31	19	5	178		
	Min	0.39	0.59	0.54	0.88	0.39		
	Max	2.40	1.40	1.60	2.70	2.70	-	0.03
Cd	UB-Mean	-	-	-	-	-		
	N	123	31	19	5	178		
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		
	Max	0.02	0.02	<LOQ	<LOQ	0.02	0.05	0.01
Hg	UB-Mean	0.03	0.02	0.08	0.03	0.03		
	N	123	31	19	5	178		
	Min	0.01	0.02	0.04	0.02	0.01		
	Max	0.27	0.04	0.14	0.04	0.27	0.5	0.03
Pb	UB-Mean	-	-	-	-	-		
	N	154	31	19	5	178		
	Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ		
	Max	0.04	<LOQ	<LOQ	<LOQ	0.04	0.3	0.01

Trends since 2002

The concentrations of metals in farmed fish fillets have been fairly stable since 2002. A total of >1400 individual or pooled fish samples have been analysed for metals in this time period. The maximum concentration of total arsenic was 6.3 mg/kg w.w. 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 all samples analysed since 2002 have been less than or equal to 0.02 mg/kg w.w., except for one single sample in 2008 which was 0.05 mg/kg w.w, the EU maximum limit for cadmium in most fish species. 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 levels for heavy metals in fish.

6.2.6 Group B3d, Mycotoxins

The 9 pooled samples from 2010 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 examined.

6.2.7 Group B3e, Dyes

The components in the B3e group were collected and examined both as A and B samples. A total of 241 pooled samples from 1 205 fish representing three species were examined with respect to malachite green and its leuco form, crystal violet and its leuco form or brilliant green. No residues of any of these agents were detected.

6.2.8 Group B3f, Others

6.2.8.1 Brominated flame retardants (BFR)

The concentrations of brominated flame retardants found in farmed fish in 2010 are summarised in Table 8. The sum $PBDE_7$ ranged from 0.5 to 3.2 µg/kg wet weight in salmon with a mean value of 1.1 µg/kg w.w. The single value in 2009 was 2.9 µg/kg wet weight. In 2008 the range was from 0.8 to 2.0, with a mean of 1.3 µg/kg wet weight. The concentrations of α-, β- and γ-hexabromocyclododecane (HBCD) and Tetrabromobisphenol A (TBBPA) in farmed fish were all below their LOQ value of 1.0 µg/kg wet weight. There are currently no EU maximum limits for brominated flame retardants in food.

Table 8. Brominated flame retardants, PBDE, HBCD and TBBPA (µg/kg wet weight) in fillets of pooled and individual farmed fish samples					
Congener	N	Salmon	Trout	All	
		32	1	33	LOQ
PBDE-28	Mean	0.04	-	0.04	0.006
	Min	0.01	-	0.01	
	Max	0.12	0.03	0.12	
PBDE-47	Mean	0.8	-	0.8	0.006
	Min	0.3	-	0.3	
	Max	2.4	0.38	2.4	
PBDE-99	Mean	0.1	-	0.1	0.006
	Min	0.05	-	0.05	
	Max	0.2	0.1	0.2	
PBDE-100	Mean	0.1	-	0.1	
	Min	0.06	-	0.06	
	Max	0.4	0.1	0.4	
PBDE-153	Mean	0.02	-	0.02	0.006
	Min	0.01	-	0.01	
	Max	0.04	0.03	0.04	
PBDE-154	Mean	0.1	-	0.1	
	Min	0.03	-	0.03	
	Max	0.1	0.07	0.1	
PBDE-183	Mean	-	-	-	0.009
	Min	-	-	-	
	Max	<LOQ	<LOQ	<LOQ	
UB-Sum PBDE-7	Mean	1.1	-	1.1	--
	Min	0.5	-	0.5	
	Max	3.2	0.7	3.2	
PBDE-66	Mean	0.03	0.03	0.03	0.006
PBDE-119	Mean	0.01	0.01	0.01	0.006
PBDE-138	Mean	<LOQ	<LOQ	<LOQ	0.006
α-HBCD	Max	<LOQ N=30	<LOQ N=1	<LOQ N=31	1.0
β-HBCD	Max	<LOQ N=30	<LOQ N=1	<LOQ N=31	1.0
γ-HBCD	Max	<LOQ N=30	<LOQ N=1	<LOQ N=31	1.0
TBBPA	Max	<LOQ N=28	--	<LOQ N=28	1.0
UB: All sums and averages are ”upper bound” calculations. LOQ: All LOQ values are related to the individual congeners					

6.2.8.2 Perfluorinated organic compounds

The perfluorinated organic compounds (PFC) were included in the surveillance programme on undesirable substances in farmed fish for 2010. A total of 33 samples were analysed, of which one sample was trout and the rest were salmon, results are given in Table 9. The only PFC levels in farmed fish above the LOQ were for PFHxA with a maximum concentration of 1.4 µg/kg, the remaining 17 PFCs levels in farmed fish were below the LOQ.

Table 9. Perfluorinated organic compounds (µg/kg wet weight) in individual farmed fish samples*

Compound	LOQ	UB-Mean	N	#Values above LOQ	Max value
PFBS	1.5	-	33	0	<LOQ
PFHxS	0.3	-	33	0	<LOQ
PFOS	0.3	-	33	0	<LOQ
PFDS	0.3	-	33	0	<LOQ
PFOSA	0.9	-	33	0	<LOQ
PFBA	1.5	-	33	0	<LOQ
PFPeA	0.3	-	33	0	<LOQ
PFHxA	0.3	-	33	5	1.40
PFHpA	0.3	-	33	0	<LOQ
PFOA	0.3	-	33	0	<LOQ
PFNA	0.3	-	33	0	<LOQ
PFDA	0.3	-	33	0	<LOQ
PFDUdA	0.3	-	33	0	<LOQ
PFDoA	0.3	-	33	0	<LOQ
PFTrDA	0.3	-	33	0	<LOQ
PFTeDA	0.3	-	33	0	<LOQ
PFHxDA	24	-	33	0	<LOQ
PFODA	24	-	33	0	<LOQ

* N=32 salmon N=1 trout

6.2.8.3 PAH

Table 10 summarises the results for the PAH compounds analysed in farmed fish in 2010. A total of 33 samples were analysed, one Atlantic halibut, four cod, seven trout and twenty-one salmon. Benzo[a]pyrene (BaP) is currently the only PAH compound included in EU regulations for food, as an indication of PAH contamination with a maximum limit for BaP in fish fillets of 2 µg/kg w.w. None of the farmed fish samples analysed in 2010 had detectable levels of BaP, which is consistent with the finding from the surveillance programme in previous years, except in 2007 when one of 84 samples contained a quantifiable level of BaP, although below the maximum limit for BaP in fish. This indicated local contamination in feed or water on the fish farm from which it was taken. Of the 13 PAH compounds analysed only fluorene and phenantrene were measurable in more than 50% of the farmed fish samples, which is consistent with the findings of the surveillance programme since 2007.

Table 10. Polyaromatic hydrocarbons (µg/kg wet weight) in individual farmed fish samples*						
PAH compound	LOQ	UB-Mean	#values	Max	Min	EU Limit
Anthracene	0.5	-	1	1.8	<LOQ	
Benzo(a)antracene	0.5	-	0	<LOQ	<LOQ	
Benzo(a)pyrene	0.5	-	0	<LOQ	<LOQ	2.0
Benzo(b)fluoranthene	0.5	-	0	<LOQ	<LOQ	
Benzo(ghi)perylene	0.5	-	0	<LOQ	<LOQ	
Benzo(k)fluoranthene	0.5	-	0	<LOQ	<LOQ	
Chrysene/Triphenylene	0.5	-	0	<LOQ	<LOQ	
Dibenzo(a,h)anthracene	0.5	-	0	<LOQ	<LOQ	
Fluoranthene	0.5	-	4	0.8	<LOQ	
Fluorene	0.5	1.7	18	8.7	<LOQ	
Indeno(1,2,3-cd)pyrene	0.5	-	0	<LOQ	<LOQ	
Phenatrene	0.5	1.28	18	3.6	<LOQ	
Pyrene	0.5	-	2	0.8	<LOQ	
UB-sum	-	4.5	-	8.3	-	

* N=32 salmon N=1 trout

6.2.8.4 BHA, BHT and Ethoxyquin (synthetic antioxidants)

All of the samples analysed for synthetic antioxidants were individual, farmed salmon and the results are summarised in Table 11. The concentration range for BHT in farmed salmon in 2010 was LOQ-8.9 mg/kg and the mean was 3.7 mg/kg fillet. In the previous years the range and mean values were respectively: <LOQ-13, 3.0 mg/kg (2009), 0.4-8.9, 3.7 mg/kg (2008), 0.4-15.4, 4.8 mg/kg (2007), 0.8 - 9.5, 3.8 mg/kg (2006). There is currently no EU or national MRL for BHT in food of animal origin following its use as a feed additive. However, Japan has an MRL of 10 mg BHT/kg w.w. In 2010 none of the 33 samples analysed had concentrations of BHT above this MRL. In 2008 10% of the samples in this surveillance programme were above the MRL while in 2007 3% of the samples of farmed fish had BHT levels which exceeded the Japanese MRL.

The range of BHA concentrations in farmed salmon fillets was between the LOQ-0.09 mg/kg w.w., and the mean concentration was 0.02 mg/kg, tcomparable with the concentrations found in 2008 and 2009. There is currently no EU or national MRL for BHA in food. However, Japan has an MRL for BHA of 0.5 mg/kg, which is more than five fold higher than the maximum concentration in farmed salmon from the monitoring programme in 2010.

Only two samples of farmed salmon were analysed for ethoxyquin and its dimer in 2010. There is no EU or national MRL value for ethoxyquin in food however, some provincial states in Germany have established an MRL for ethoxyquin of 0.01 mg/kg, with a legal basis in the EU pesticide directive which does not apply to fish. In contrast Japan has an MRL of 1 mg/kg. Both limits concern only the parent compound, not the dimer or their sum. The levels

of ethoxyquin in the fish samples were less than 0.004 mg/kg w.w., well below the Japanese MRL for ethoxyquin.

The EU maximum limit for the sum of synthetic antioxidants in feed is 150 mg/kg w.w. Since 1996 NIFES has conducted a surveillance programme on fish feeds and fish feed ingredients on behalf of the Norwegian Food Safety Authority. None of the fish feed samples analysed to date have exceeded the maximum limit.

Table 11. Synthetic antioxidants (mg/kg wet weight) in fillets of individual farmed salmon samples

		Salmon	Trout	All
BHA	Mean	0.04	0.04	0.04
	N	31	1	32
	Min	0.01		0.01
	Max	0.09	0.04	0.09
BHT	Mean	3.72	0.6	3.98
	N	31	1	32
	Min	<LOQ		<LOQ
	Max	8.9	0.6	8.9
EQ	Mean	0.003		0.003
	N	2	0	2
	Min	<LOQ		<LOQ
	Max	0.004		0.004
EQDM	Mean	0.4		0.4
	N	2	0	2
	Min	0.3		0.3
	Max	0.6		0.6
Values for ethoxyquin are analytically overestimated, and should be read as “less than” values.				

CONCLUSIONS

None of the substances with anabolic effect (group A1 and A2) were detected in any of the samples analysed for 2010. Samples examined under group A6 in 2010, did not contain metronidazol and its hydroxyl metabolite or metabolites of the substances in the nitrofuran group. Chloramphenicol was detected in one of the 194 pooled samples analysed for this component in a concentration between the Limit of Detection (0.25 ng/g) and the Limit of Quantification (1.0 ng/g), which is well below the MRL.

None of the group B compounds - veterinary drugs exceeded the maximum residue limits (MRL) established for fish, in the monitoring programme in 2010. Similarly, none of the environmental contaminants (organochlorine compounds and chemical elements) in the farmed fish analysed in 2010 were present at levels above 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 2010 were comparable to those found in the monitoring programme for Directive 96/23 for the years 2003 to 2009.

The maximum PBDE-7 concentration in farmed salmon in 2010 was 3.2 µg/kg. The concentrations of the other brominated flameretardants (α-, β- and γ-hexabromocyclododecane (HBCD) and Tetrabromobisphenol A (TBBPA)) in farmed fish were all below their LOQ value of 1.0 µg/kg wet weight. The only PFC levels in farmed fish above the LOQ were for PFHxA with a maximum concentration of 1.4 µg/kg, the remaining 17 PFCs levels in farmed fish were below the LOQ. There are no maximum limits in the EU for these compounds in food.

Benzo[a]pyrene (BaP) is currently the only PAH with an established maximum limit in food. The concentration of BaP in all of the samples of farmed fish fillet analysed since 2007 has been below the LOQ value.

Levels of the synthetic antioxidants ethoxyquin, BHT and BHA in farmed fish in 2010 were lower than those found in previous years, and all the samples were compliant with the Japanese maximum residue limits. There is currently no EU maximum residue limit for these compounds in food of animal origin as a result of their use as feed additives.

	Annex I Summary of analytical methods							
Group of substances	**Compounds**	**Matrix**	**Method principle**	**Screen-ing method LOD (wet weight) (µg/kg)**	**Analytical method LOD (wet weight in muscle) (µg/kg)**	**Analytical method LOQ (wet weight) (µg/kg)**	**Level of action**	**Laboratory**
A1 Stilbenes	Diethylstilboestrol	Muscle	GC-MS	n.a.	0.4	Use LOD	Presence	AUS
	Dienoestrol	Muscle	GC-MS	n.a.	0.7	Use LOD	Presence	AUS
	Hexoestrol	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
A3	α-nandrolon	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
	β-nandrolon	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
	α-trenbolon	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
	β-trenbolon	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
A6 Annex IV substances	Chloramphenicol	Muscle	LC-MS	n.a.	0,30 (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.	Use LOQ (MRPL =1.0)	0.5	Presence	Eurofins
	Nitrofuran 1-Aminohydrantoin	Muscle	LC-MS/MS	n.a.	Use LOQ (MRPL =1.0)	0.5	Presence	Eurofins
	Nitrofuran 3-Amino-5-morpholinomethyl-2-oxazol	Muscle	LC-MS/MS	n.a.	Use LOQ (MRPL =1.0)	1	Presence	Eurofins
	Nitrofuran Semicarbazide	Muscle	LC-MS/MS	n.a.	Use LOQ (MRPL = 1.0)	1	Presence	Eurofins
B1 Antibacterial substances	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
	Quinolones	Muscle	LC-MS/MS	n.a.	Use LOQ	10	600	Eurofins
	Oxytetracycline	Muscle	LC-MS/MS	n.a.	Use LOQ	50	100	Eurofins
	Chlorotetracycline	Muscle	LC-MS/MS	n.a.	Use LOQ	50		Eurofins
	Tetracycline	Muscle	LC-MS/MS	n.a.	Use LOQ	50		Eurofins
	Doxycycline	Muscle	LC-MS/MS	n.a.	Use LOQ	50		Eurofins
	Florfenicol	Muscle	LC-MS	n.a.	0.2	0.5	1000	NIFES
B2a Anthelmintics	Praziquantel	Muscle	LC-UV (DAD)	n.a.	50.0	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.	10	10	50 µg/kg	Eurofins
	Deltamethrin	Muscle	GC-EC	n.a.	10	10	10 µg/kg	Eurofins
B2f Other active substances	Diflubenzuron	Muscle	LC-MS	n.a.	10	20	1000 µg/kg	NIFES
	Teflubenzuron	Muscle	LC-MS	n.a.	5	15	500 µg/kg	NIFES
B3a Organo-chlorine compounds	PCDD	Muscle	GC-HRMS	n.a.	<0.08 ng/kg	0.2 ng/kg	4 ng TEQ/ kg	NIFES
	non- and mono-orto PCB	Muscle	GC-HRMS	n.a.	<0.08 ng/kg	0.2 ng/kg	8 ng TEQ /kg (dioxin + dl dioxin)	NIFES
	PCDF	Muscle	GC-HRMS	n.a.	<0.08 ng/kg	0.2	0.2 pg/kg	NIFES
	PCB 28	Muscle	GC-MS	n.a.	0.02	0.06	n.a.	NIFES
	PCB 52	Muscle	GC-MS	n.a.	0.03	0.09	n.a.	NIFES
	PCB 101	Muscle	GC-MS	n.a.	0.03	0.09	n.a.	NIFES
	PCB 118	Muscle	GC-MS	n.a.	0.03	0.09	n.a.	NIFES
	PCB 138	Muscle	GC-MS	n.a.	0.04	0.12	n.a.	NIFES
	PCB 153	Muscle	GC-MS	n.a.	0.03	0.09	n.a.	NIFES
	PCB 180	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES
	α-HCH	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	β-HCH	Muscle	GC-MS	n.a.	0.4	1.2*	n.a.	NIFES
	γ-HCH	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	HCB	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	Pentachlorobenzene	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	Heptachlor	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	Heptachlor epoxide	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	Aldrin	Muscle	GC-MS	n.a.	0.3	0.9	n.a.	NIFES
	Isodrin	Muscle	GC-MS	n.a	0.3	0.9	n.a	NIFES
	Dieldrin	Muscle	GC-MS	n.a	0.05	0.15	n.a	NIFES
	Oxy-chlordane	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	trans-chlordane	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	cis-chlordane	Muscle	GC-MS	n.a.	0.3	0.9	n.a.	NIFES
	α-endosulfan	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES
	β-endosulfan	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES
	Endosulfansulphate	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES
	cis-nonachlor	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES
	trans-nonachlor	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES
	Toxaphene 26	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Toxaphene 32	Muscle	GC-MS	n.a.	0.5	1.5	n.a.	NIFES
	Toxaphene 40+41	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Toxaphene 42	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Toxaphene 50	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Sum Toxaphene 62	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Mirex	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	DDT-op DDT-pp DDD-op DDD-pp DDE-op DDE-pp	Muscle	GC-MS	n.a.	0.3	0.9	n.a.	NIFES

	PBDE, Polybrominated diphenylethers	Muscle	GC-MS	n.a.	0.02/0.03	0.045/0.10		NIFES
	α, β, + γ-HBCD	Muscle	GC-MS	n.a.	0.2	0.5		NIFES
	TBBPA	Muscle	GC-MS	n.a.				NIFES
B3b Organo-phosphorous compounds	Azametiphos	Muscle	GC-FPD			0.2		Eurofins
	Dichlorvos	Muscle						Eurofins
B3c Chemical elements	Pb	Muscle	ICPMS	n.a.	0.01 mg/kg d.w.	0.04 mg/kg d.w.	0.3 mg/kg	NIFES
	Cd	Muscle	ICPMS	n.a.	0.004 mg/kg dry w.	0.01 mg/kg d.w.	0.05 mg/kg.	NIFES
	As	Muscle	ICPMS	n.a.	0.01 mg/kg d.w.	0.03 mg/kg d. w.	n.a.	NIFES
	Hg	Muscle	ICPMS	n.a.	0.01 mg/kg d.w.	0.03 mg/kg d.w.	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.	0.17 (MRPL=2.0) ∑ malachite green and leuco-malachite green= 2.0	0.5	Presence	NIFES
	Leuco malachite green	Muscle	LC-MS/MS	n.a.	0.18/0.30 ∑ malachite green and leuco-malachite green= 2.0	0.5	Presence	NIFES
	Crystal violet	Muscle	LC-MS/MS	n.a.	0.19	0.5	Presence	NIFES
	Leucocrystal violet	Muscle	LC-MS/MS	n.a.	0.28	0.5	Presence	NIFES
	Brilliant green	Muscle	LC-MS/MS	n.a.	0.25	0.5	Presence	NIFES
B3f, other pharmacol-ogically active substances	Ethoxyquin (EQ)	Muscle	LC-UV	n.a.	0.2	0.6	n.a.	NIFES
	Ethoxyquin-dimer	Muscle	LC-UV	n.a.	0.1	0.3	n.a.	NIFES
	BHT	Muscle	LC-UV	n.a.	14	45	n.a.	NIFES
	BHA	Muscle	LC-UV	n.a.	2.4	7.3	n.a.	NIFES
	PFOS. perfluoroctane sulphonate	Muscle	GC-MS	n.a.	1-3	3		NIFES
	PAH, benzo(a)pyrene	Muscle	GC-MS	n.a.		0.5	2.0 µg/kg	Eurofins