MONITORING PROGRAM FOR RESIDUES OF THERAPEUTIC AGENTS, ILLEGAL SUBSTANCES, POLLUTANTS AND OTHER UNDESIRABLES IN FARMED FISH

(In accordance with Council Directive 96/23/EC)

ANNUAL REPORT FOR 2009

(Revised version of this report.)

Helge Torbjørn Hove, Bjørn Tore Lunestad, Rita Hannisdal and Kåre Julshamn

07. December 2010

National Institute of Nutrition and Seafood Research
Address: P.O. Box 2029 Nordnes, 5817 Bergen, Norway
Phone: +47 55 90 51 00 **Fax:** +47 55 90 52 99
E-mail: postmottak@nifes.no

N I F E S
NASJONALT INSTITUTT FOR ERNÆRINGS- OG SJØMATFORSKNING

Tables

Introduction

The aim of this program is to monitor residues of therapeutic agents, illegal substances, pollutants and other undesirable substances in Norwegian farmed fish in accordance with the Directive 96/23/EC "On measures to monitor certain substances and residue thereof in live animal and animal products" and specified in the 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 for this directive in Norway. The activity is focused on both terrestrial and marine food producing animals. On behalf of NFSA, The National Institute of Nutrition and Seafood Research (NIFES) has been given the responsibility to carry out the analytical work and to elaborate the report on species in aquaculture. According to the directive 96/23/EC Annex I, substances are grouped into A and B components. These two groups differ in their requirement to the sampling plan, thus this report also refers to group A and B samples. For the samples collected in 2009 these compound groups have been analyzed for in fish:

Group A: Substances having anabolic effect and unauthorized substances:

- A1: Stilbenes, derivatives and their salts and esters.
- A3: Steroids
- A6: Substances included in Annex IV of Regulation (EEC) No. 2377/90.

Group B: Veterinary drugs and contaminants:

- B1: Antibacterial agents
- B2a: Anthelmintics
- B2c: Carbamates and pyrethroids
- B2f: Other pharmacologically active substances
- B3a: Organochlorine compounds
- B3d: Chemical elements
- B3d: Mycotoxins
- B3e: Dyes
- B3f: Others

Group A-compounds are illegal to use in animals intended for food production. The group A-samples were collected at the farms and fish in all stages of growth are sampled. Sampling was carried out by official inspectors with no prior notification.
The plan is designed to ensure samples representative of the fish that are in production.

Group B-compounds are drugs for which a Maximum Residue Limit (MRL) is established. Group B also comprise organic and inorganic contaminants of food safety concern. The group B-samples were taken from slaughtered fish at the slaughterhouses or the packing plants. According to the plan, these samples should be representative of the commercially sold fish.

In 2009, NIFES used subcontractors for analysis of some parameters. The Hormone Laboratory of Oslo University Hospital (AUS), Aker was subcontractor for the

determinations of stilbenes and steroids, The National Veterinary Institute (VI), Oslo was for the determination of mycotoxins, and Eurofins was subcontractor for PAH analysis and the analysis of selected therapeutic compounds.

Annette Bjordal was in charge of the analytical work, while Elin Kronstad was responsible for the work related to sample reception, storage, preparation and distribution within the institute. Manfred Torsvik, Anne Margrethe Aase and Vidar Fauskanger carried out the sample pre-treatment. Rita Hannisdal, Felicia D. Couliard, Eva Torgilstveit, Edel Erdal, Lina Beyer Vågenes and Rosini Ngyen were responsible for chemical analysis of residues of therapeutics. Karstein Heggstad, Tadesse T. Negash, Jannicke A. Berntsen, Dagmar Nordgård, John Nielsen, Lene H. Johannessen, Britt Elin Øye, Pablo Cortez, Kari Breisten Sæle, Kjersti Pisani, Tina C. Rossvold, Joseph Malaiamaan, Betty Irgens and Elilta Hagos were responsible for extraction, clean-up and determination of the organic contaminants. Jorun Haugsnes, Siri Bargård, Tonja Lill Eidsvik, Berit Solli, Edel Erdal and Laila Sedal carried out the analysis of the chemical elements. Eva Torgilstveit, Annette Bjordal, Tina C. Rossvold, Lina B.Vågenes, Rosini Ngyen, Anette Kausland, Annbjørg Bøkevoll and Anne Karin Syversen were responsible for the day to day reports to the NFSA. Tone Galluzzi, Lene Skålevik and Anne Karin Godvik were responsible for the bioassay for determination of the antibacterial agents. Determination of the synthetic antioxidants BHT, BHA and Ethoxyquin was performed by Kjersti Ask.

The scope for the project in 2009

According to current legislation, the minimum number of samples to be collected each year must be at least 1 per 100 tons produced fish. In 2009 this applied for salmon and trout, whereas as a national measure the sample frequency for other farmed species was increased to one sample per 25 tons fish produced. One-third of the fish were sampled according to the requirements of the group A components, and two-thirds of the samples according to group B. Farm sites from all regions with aquaculture activity, and at least 10% of the total number of sites were covered by the sampling plan. The sampling plan was designed to ensure statistical randomness over all seasons and regions. The samples were made anonymous at the sample reception in NIFES. Thus the person performing the analysis was unable to identify the sample or the producer. The samples submitted to the laboratory were for most parameters frozen muscle or liver tissue, as for other parameters gutted fish stored on ice were the sample material. The muscle samples were filets with skin or they were chops with skin and backbone. Except for the single fish, the samples were shipped frozen to NIFES in isolated boxes. These samples were kept frozen until prepared for analysis. The single fish samples were intended for analysis for synthetic antioxidants. These single fish samples were immediately filleted and prepared for analysis on arrival, or discarded if this could not be done. Only the fillet part of each single fish was analysed. For practical reasons some single fish samples were analysed for other chemical parameters as well, but then only for parameters within the scope of the Norwegian

National measures. Except for the antioxidants where there are only single fish samples, the pooled samples data and the single fish data are considered equivalent. Both kinds of data are combined in the statistical parameters with the same weighting. The parameter "the number of fish" is calculated from both the single and the pooled fish samples to give a correct total number of fish. In 2009 there were 51 single fish samples.

On arrival to NIFES the fillets or chops from the five-fish samples were homogenized to proper pooled samples with equal contribution from each fish. The advantage of pooled samples is that a large number of fish can be included in the surveillance. The drawback is that the individual variability between the fish is levelled out in the pooling. The observed standard deviations then reflect the variability between the farms. The liver samples were all analysed individually.

Table 1 provides an overview of the number of fish that were the basis for each chemical and microbiological examination, whereas Table 3 gives an overview on the number of fish in each species that is the basis for the samples examined in this program. Information in both tables is as analysed pr 11. August 2010. The total number of fish in the data base was 10 851, analysed in a total of 1470 pooled filet samples, 51 single fillet samples and 3 450 liver samples. The total number of analytical measurements is 11 726.

All sample collection, submission and selection of fish to each analytical method were done according to a detailed plan set up by the NFSA. The plan was tailored to ensure statistical independent and representative samples. Since some samples are included in the data for more than one parameter, the total sum of the samples is less than the sum of the samples analysed for each parameter. For the data interpretation the significant number is the sum of samples analysed for each parameter. As a general rule each sample was to be analysed for only one parameter. However, some analytical methods inevitably give results on several parameters during one run. All such additional data are utilised in the current report. Thus, occasionally sample splitting for analysis by more than one analytical method was prescribed in the sampling plan. The statistical basis for the results for each parameter (number of samples and fish) are shown in Table 1.

Table 1. Sampling data.

Substance group		Parameter	Number of fish	Number of samples	Number of determinations
Samples taken from the farms with no pre-notice	A1 Stilbenes	Diethylstilboestrol Dienoestrol Hexoestrol	230	46	46 46 46
	A3 Steroids	Nandrolon alfa Nandrolon beta Trenbolon alfa Trenbolon beta	220	44	44 44 44 44
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	550	110	110
		Metronidazole Hydroxy metronidazole	800	160	160 160
		Nitrofuran metabolites 3-Amino-2-oxazolidone 1-Aminohydrantion 3-Amino-5-Morpholinomethyl-2-oxazol Semicarbazide	565	113	113 113 113 113
	Sum of group A		2 365	473	1 196
Samples taken from the slaughter House	B1 Anti bacterial agents	Flumequine	10	2	2x14=28
		Oxolinic acid			
		Cinoxacin			
		Ciprofloxacin			
		Danofloxacin			
		Difloxacin			
		Enoxacin			
		Enrofloxacin			
		Lomefloxacin			
		Marbofloxacin			
		Nalidixic acid			
		Norfloxacin			
		Ofloxacin			
		Sarafloxacin			
		Florfenicol	20	4	4
		Oxytetracycline	90	18	18
	B2 Other veterinary drugs	Teflubenzuron	160	32	32
		Diflubenzuron	160	32	32
		Cypermethrine	90	18	18
		Praziquantel	445	89	89
		Fenbendazole	195	39	39
		Emamectin	395	79	79
		Ivermectin	30	6	6
		Deltamethrin	65	13	13
	B3a Organochlorine Compounds	HCB	390	78	78x26=2028
		α-HCH			
		β-HCH			
		γ-HCH			
		Pentachlorobenzene			
		Hexachlorobenzene			
		Heptachlor			
		Heptachlor-a			
		Aldrin			
		Dieldrin			
		Isodrin			
		Oxy-Chlordane			
		Trans-Chlordane			
		Cis-Chlordane			
		α-Endosulfan			
		Endosulfansulfate			
		β-Endosulfan			
		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	390	78	78x6=468
		Dioxins and Dioxin like PCBs	320	64	66x29=1856
		PCB-7 (+2)	315	63	63x7=441
		PBDE (10)+ total HBCD	5	1	1x11=11
	B3c Chemical elements	Pb Cd Hg As	905	181	181x4=724
	B3d Mycotoxins	Ochratoxin A	90	18	18
	B3e, Dyes	Malachite green Leuco malachite green	870	174	5x174=870
		Chrystal violet Leuco Chrystal violet			
		Brilliant green			
	B3f Others	BHT BHA Ethoxyquin+ dimer	51	51	51x2=102 50x2=100
		PFC (10)	0	0	0

Table 1. Sampling data.

Substance group		Parameter	Number of fish	Number of samples	Number of determinations
		PAH (13)	40	8	8x13=104
	Sum B fillets, pooled and from single fish, chemical method		5036	1048	7080
B (liver)	B1 Microbiological screening of liver	Quinolones	1150	1150	1150
		Tetracyclines and amphenicols	1150	1150	1150
		Sulphonamides	1150	1150	1150
Total sum liver, three plate inhibition assay			3450	3450	3450
Total sum B			8486	4498	10530
Total sum fillet A+B			7401	1521	8276
Total sum, fillets, pooled and single fish and liver			10851	4971	11726

Terminology

Tissue: The samples examined in this program were either liver or muscle with skin and bone. In the text this is referred to as "fillets" or "muscle", as distinct from "liver samples".

Parameter: A chemical or physical property whose value describes or characterises the sample. In this report the term is used exclusively for the concentrations of chemical species of drugs, pollutants or additives contaminating the sample.

Analyte: The chemical substance that is the subject or target of the chemical analysis.

Matrix: The non-analyte part of the sample.

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 any detection of the compound (i.e. with > 95% probability) would be important information. For other compounds quantification is required. The LOQ is the lower limit for a reliable quantitative measurement. Levels that are so low that they cannot be quantified with acceptable reliability will be reported as "less than LOQ", for example: <2.0 µg/kg. On the other hand, illegal compounds will, depending on the concentration, be reported as: "ND" (Not detected), "detected"[1], or the result will be reported as the value of the measured concentration if it was above the LOQ value.

Congener: In this context "congeners" refers to analogous compounds within the classes PCB, PBDE, dioxins, furans and thoxaphenes. The congeners in each class are closely related in molecular structure. A congener is usually not referred to by a unique name, but an ID number is used for identification: PCB-147 or Tox-62. Congeners are molecules with several halogen atoms (chlorine or bromine) in their structure. The differences between the congeners within a class are the number and the position of the halogen atoms attached to an otherwise identical molecular structure.

Upper bound (UB) calculation: or "upper bound LOQ" calculation is a principle for the calculation of sums and mean values from analytical data. 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 sums, like if the number zero were used for these values in the sum (Lower bound calculation). When UB calculation is required, methods with the most advanced instruments will give lower (and also more correct) sums. Thus if the UB sum is required there is an incentive to have the samples analysed by the most advanced methods. Previously lower bound (LB) calculations were the norm. Cheap methods

[1] "Detected" for illegal compounds thus means that the concentration is in the range from LOD to LOQ.

with high LOQ values gave the lowest LB-sums and LB-mean values. Thus a food producer would benefit if he chose an unfit laboratory to document his products.
UB calculation is in accordance with requirements from the EU for dioxin data[2]. For all contaminants an UB calculation will give a "worst case" value. UB calculation is therefore a good choice for the assessment of risks associated with an undesirable compound in food. In this report UB calculations are used for many types of contaminants. UB calculation is documented 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 producing animals intended for human consumption. The MRLs are established in accordance with the Council Regulation (EEC) 2377/90. The MRL is set for muscle and skin in natural proportions for all agents, except for oxytetracycline where only muscle concentrations are considered. The EU regulations on pharmacologically active agents in food are currently under revision.

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

TEF and TEQ: WHO has established two lists of toxicity factors for dioxin and PCB congeners. These are called TEF values, toxic equivalent factors. One list was published in 1998 and one in 2005. When a concentration is weighted with its corresponding TEF factor it is no longer called a concentration, but a toxic equivalent, a TEQ. The TEQ values have the same unit of measurement as a concentration, in this case ng/kg wet weight.

Sum of TEQ: The sum of the TEQ values for all congeners of the relevant kind.

Analytical methods

All data in this report are acquired from analytical methods using modern and advanced equipment. The 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 a basis from fewer validation experiments. Accreditation of these methods is an on-going process, priority given to group A parameters and to the methods with the highest number of samples analyzed.

[2] Commission directive 2002/69/EC

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. The exception is the dioxin method. This method is of very high quality based on the isotope dilution principle. This provides a quality assurance integral to the method. Thus the frequency of the QCS analysis is lowered 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. This is the full chemical analysis of a fictitious sample, a sample without any sample material. Any positive value found for this “sample” will reveal a contamination of reagents or equipment that could affect the results of real 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 aggregated or pooled samples made from five fish each, with the exception of 51 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 negative test ("not detected") for five individual fish provides a more reliable and significant result than a negative test on a pooled sample since any individual variation would have been levelled out in the homogenisation step. Any individual value above the LOD will be identified in the individual analysis. A summary of the analytical methods used is shown in Table 2.

Table 2. Summary of analytical methods.

Group of substances	Compounds	Matrix	Method principle	Screening 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.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	Nandrolon alpha	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
	Nandrolon beta	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
	Trenbolon alpha	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
	Trenbolon beta	Muscle	GC-MS	n.a.	0.6	Use LOD	Presence	AUS
A6 Annex IV substances	Chloramphenicol	Muscle	LC-MS	n.a.	0.3 (MRPL=0.3)	1.0	presence	NIFES
	Metronidazole	Muscle	LC-MS/MS	n.a.	2.0	6.0	Presence	NIFES
	Metronidazole-hydroxy	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	Muscle	LC-	n.a.	Use LOQ	1	Presence	Eurofisn

	Table 2. Summary of analytical methods.							
Group of substances	Compounds	Matrix	Method principle	Screening 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
	Semicarbazide		MS/MS		(MRPL = 1.0)			
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
	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	Cypermethrine	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 Organochlorine compounds	PCDD	Muscle	GC-HRMS	n.a.	<0.08 ng/kg	0.2 ng/kg	4 ng TE/ kg	NIFES
	non- and mono-orto PCB	Muscle	GC-HRMS	n.a.	<0.08 ng/kg	0.2 ng/kg	8 ng TE /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
	HCH-alfa	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	HCH-beta	Muscle	GC-MS	n.a.	0.4	1.2*	n.a.	NIFES
	HCH-gamma	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
	Heptachlor	Muscle	GC-MS	n.a.	0.1	0.3	n.a.	NIFES
	Heptachlor-a	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
	Alfa-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
	Beta-endosulfan	Muscle	GC-MS	n.a.	0.05	0.15	n.a.	NIFES

	Table 2. Summary of analytical methods.							
Group of substances	Compounds	Matrix	Method principle	Screening 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
	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 50	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Toxaphene 62	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Toxaphene 42a	Muscle	GC-MS	n.a.	0.2	0.6	n.a.	NIFES
	Sum Toxaphene 40+41	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.	0.01	0.03		NIFES
	HBCD, hexabromocyclododecane	Muscle	GC-MS	n.a.	0.2	0.5		NIFES
	TBBPA tetrabrombisphenol-A	Muscle	GC-MS	n.a.				NIFES
B3c Chemical elements	Pb	Muscle	ICPMS	n.a.	0.01 mg/kg dry w.	0.04 mg/kg dry w.	0.2 mg/kg	NIFES
	Cd	Muscle	ICPMS	n.a.	0.004 mg/kg dry w.	0.01 mg/kg dry w.	0.05 mg/kg.	NIFES
	As	Muscle	ICPMS	n.a.	0.01 mg/kg dry w.	0.03 mg/kg dry w.	n.a.	NIFES
	Hg	Muscle	ICPMS	n.a.	0.01 mg/kg dry w.	0.03 mg/kg dry w.	0.5 mg/kg	NIFES
B3d Mycotoxins	Ochratoxine A	Muscle	Immuno affinity/ HPLC	n.a.	0.06 µg/kg		n.a.	VI
B3e, dyes	Malachite green	Muscle	LC-MS/MS	n.a.	0.15 (MRPL=2.0)	0.5	Presence	NIFES
	Leuco malachite green	Muscle	LC-MS/MS	n.a.	0.15 (MRPL=2.0)	0.5	Presence	NIFES
	Crystal violet	Muscle	LC-MS/MS	n.a.	0.15	0.5	Presence	NIFES
	Leuco crystal violet	Muscle	LC-MS/MS	n.a.	0.15	0.5	Presence	NIFES
	Brilliant green	Muscle	LC-MS/MS	n.a.	0.15	0.5	Presence	NIFES
B3f, other pharmacologically 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.	not available	not available	2.0 µg/kg	Sub contractor

* LOD and LOQ for HCH-beta vary from series to series.

The Group A-compounds

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

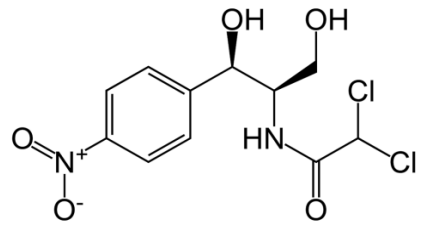
Group A1 and A3

The A1 and A3 compounds diethylstilbestrol, dienesterol, hexosterol and steroid compounds were analyzed at the Hormone Laboratory, Oslo University Hospital, Aker. The analytical method consists of an enzymatic hydrolysis of the samples, followed by extraction and fat removal. The hydrolyzed substances are further cleaned-up with liquid / liquid extraction steps and solid phase extraction before derivatization and analytical measurement on a GC / MS. Any positive findings would be confirmed by a confirmatory method.
In the confirmatory methods the samples are then given an additional clean-up by HPLC before a derivatization step. The final analytical determination is on a GC/MS also for the confirmatory method.
This method is based on the Hormone Laboratory's accredited method for detection of steroids and stilbenes in muscle tissue from terrestrial animals.

Group A6, Annex IV substances to council regulation EEC 2377/90, 26. June 1990.

Chloramphenicol and metronidazole were analyzed by NIFES. The nitrofurans and relevant metabolites were analyzed by Eurofins.

Chloramphenicol
Chloramphenicol is an antibiotic agent 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.



Chemical structure of chloramphenicol.

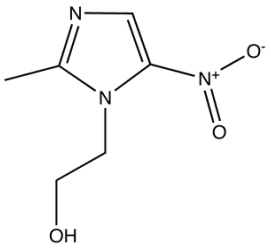
Procedure (NIFES method 143):
An internal standard (chloramphenicol-D5) is added to the sample before extraction with ethyl acetate. Fat is removed from the extract by a liquid/liquid extraction between aqueous saline and heptane. To separate the analytes from the water-soluble components, a liquid/liquid extraction step with aqueous saline and ethyl acetate is performed. The sample is analyzed by LC-MS, with a reversed phase C18 column for separation. The components are ionized by ESI and detected as negative ions using the SIM mode. Quantification is based on an external calibration curve.

Nitrofurans
This group of synthetic antibacterial agents are derivatives of nitrofurane. The compounds have previously been widely used in veterinary medicine. These agents are

rapidly metabolized in the tissue, thus in this surveillance program the metabolites 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semicarbazide (SEM) have been included.

Procedure (Eurofins): The analytes are extracted with aqueous hydrochloric acid and derivatized with nitrobenzaldehyde. Solid phase extraction is then applied for sample clean up. The analytes are 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.



Chemical structure of metronidazole.

Procedure *(NIFES method 351)*:
Internal standard (dimetronidazole-D3) is added to a homogenized sample. The analytes are extracted by ethyl acetate in an alkaline environment. The solution is evaporated and re-dissolved in heptane. Sample clean up is performed by solid phase extraction. The solution is evaporated to dryness and re-dissolved in water, before analysis 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 is based on an external calibration curve.
Dyes(Malachite green and crystal violet with relevant metabolites and brilliant green)
The method is described in the next section, under the group B compounds.

The Group B compounds

NIFES performed most of the group B analysis. However, sub contractors were used for these groups of compounds:

B3d: Mycotoxins: The National Veterinary Institute in Oslo.
B3f: PAH: Eurofins

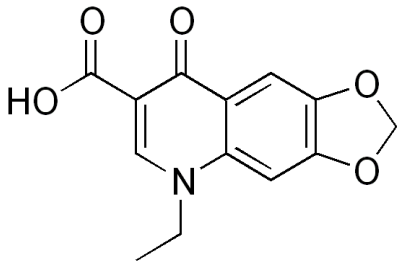
B1, Antibacterial agents (antibiotics)

Procedure: In this assay for antibacterial agents, a three-plate microbiological inhibition method was applied (NIFES methods no. 106, 107 and 108). Each plate contains growth agar and are added a specific bacterial strain that is particularly sensitive to these analytes. Pieces of liver samples measuring 6 x 5 mm from five

different fish, were carefully deposited in the agar surface, before the plate is set to incubation. If the samples contain residues of antibacterial agents, the bacterial growth will be inhibited in a zone around each piece of liver tissue. A transparent zone with no bacterial growth surrounding the liver sample indicates a positive sample. The liver has a central function in 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 sensitivity of this bioassay is beyond those found for chemical methods, the higher concentrations found in liver enhance the detection of antibiotics. Moreover, the method is able to detect a wider range of antibiotics than the more specific chemical methods. This makes the bioassay a useful screening method. Any detection by the inhibition assay will be verified by chemical analysis of the corresponding fillet sample received together with the liver 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.



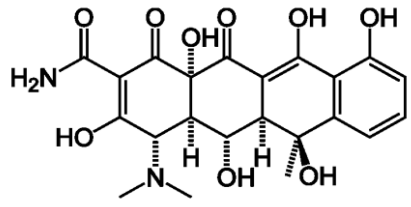
Chemical structure of oxolinic acid.

Procedure (Eurofins):

The analytes are extracted with acetonitrile, and analysis is 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.

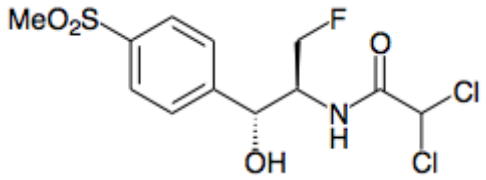


Chemical structure of oxytetracycline.

Procedure (Eurofins):

The analyte is extracted with EDTA-succinate buffer. Solid phase extraction is used for sample clean up. The analyte is 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.



Chemical structure of florfenicol.

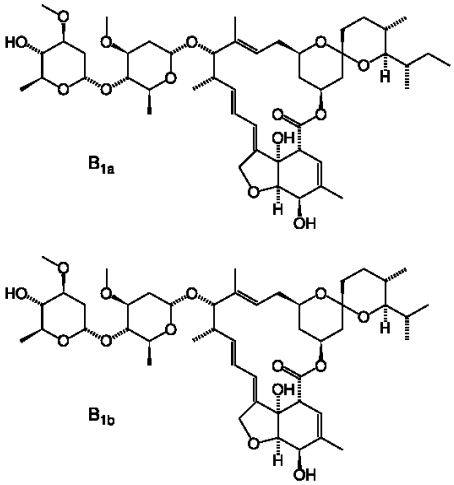
Procedure *(NIFES method 290)*:
Internal standard (chloramphenicol-D5) is added to the sample, and the analytes are extracted with ethyl acetate. To remove fat from the extract a liquid/liquid extraction between aqueous saline and heptane are conducted. Furthermore, to separate the water-soluble components a liquid/liquid extraction step with saline and ethyl acetate is performed. The samples are analyzed by LC-MS. A reversed phase C18 column is used for separation, and the molecules were ionized by ESI, and detected as negative ions using the SIM mode. Quantification is based on an external calibration curve.

B2a, Anthelmintics

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

Procedure *(NIFES method 138 and 139)*: Internal standard (diflubenzuron when teflubenzuron is determined, and teflubenzuron when diflubenzuron is determined) is added to the sample, and the analytes are extracted with acetone. Heptane is used to remove fat. A solid phase extraction is applied for further purification of the sample. The samples are analyzed by LC-MS. A reversed phase column is used for separation, and the components were ionized by ESI and detected as negative ions using the SIM mode. Quantification is based on an external calibration curve.

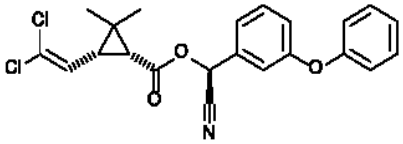
Ivermectin and Emamectin Ivermectin and emamectin belong to the class of avermectins. Emamectin is used against external parasites on fish.



Molecular structures of two ivermectin forms.

Procedure *(NIFES method 130 and 131)*:
Internal standard is added to the sample (ivermectin is used when emamectin is determined, and emamectin is used when ivermectin is determined) , and the analytes are extracted with acetonitrile. Fat is removed by extraction with heptane, and the sample is further purified by solid phase extraction. The sample is analyzed by LC-MS. A reversed phase C18 column is used for separation, and the components are ionized by APCI and detected as positive ions using the SIM mode. Quantification is based on an external calibration curve.

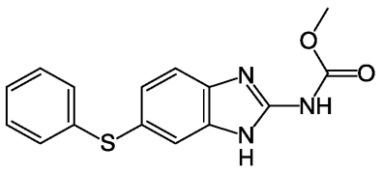
Cypermethrine and deltamethrin
Cypermethrine and deltamethrin are synthetic pyrethroids used in bath treatment against sea lice.



Chemical structure of cypermethrine.

Procedure (Eurofins):
Cypermethrine and deltamethrin are extracted from the samples with acetone. The extracts are cleaned up by a liquid/liquid extraction, and further by gel permeation chromatography. The samples are analyzed by gas chromatography-electron capture (GC-EC).

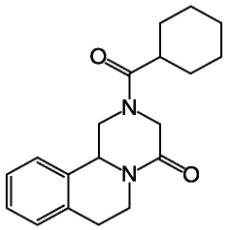
Fenbendazole
Fenbendazole is a broad spectrum benzimidazole anthelmintics used against intestinal parasites in fish.



Chemical structure of fenbendazole.

Procedure *(NIFES method 141)*:
The homogenized sample is extracted with a solution of methanol and water. Petroleum ether is used to remove fat from the extract. Sodium dihydrogen phosphate and a mixture of diethyl ether/ethyl acetate are added to the polar extract before shaking and centrifugation, and the upper layer is collected and vaporized. The sample is then dissolved in a solution of acetonitrile and water prior to analysis on LC-MS/MS. A reversed phase column is used for separation, and the components were ionized by ESI and detected as positive ions in the MRM mode. Quantification is based on an external calibration curve.

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



Chemical structure of praziquantel.

Procedure (NIFES method 140):
Praziquantel is extracted from the homogenized sample by acetone. Diethyl ether and hexane are then added to the extract. The organic phase is transferred and evaporated. The sample is then dissolved in methanol, and fat is removed. A reversed phase column is used for separation, and praziquantel is detected by LC-UV at a wavelength of 205 nm. Quantification is based on an external calibration curve.

B3a, Organochlorine compounds

This is a heterogeneous group of compounds that exhibit a range of chemical and pharmacological properties. Several classes of compounds like PCBs, dioxins and brominated flames retardants are found in this group. They have in common a tendency to persist in the environment and to accumulate in the food chains. For this reason they are not only of environmental concern, they are food safety issues as well. Regarding their chemical properties they are of lipophilic and hydrophobic character. For this reason they are found in fatty tissues and they are extracted from the sample with organic solvents.

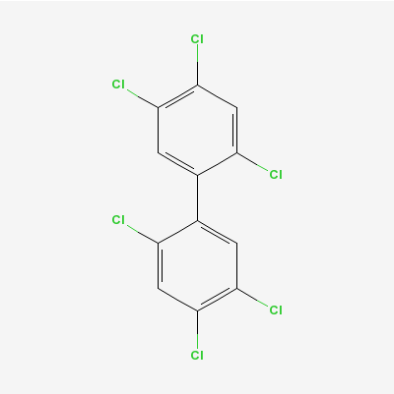
Polychlorinated biphenyls (PCB)

PCBs are a group of compounds with low biodegradability in the environment. Commercial PCB mixtures were previously produced on a large scale for a variety of industrial applications. The total global production of PCBs has been estimated to 1.5 million tons[3]. PCB production was banned by the United States Congress in 1976 and by the Stockholm Convention on Persistent Organic Pollutants in 2001. Today there are regulations like EU directive 96/59/EC for the safe disposal of remaining PCB or PCB contaminated waste. In Europe PCB levels in food and feed are monitored and regulated according to a number of EU regulations:

[3] Breivik K, Sweetman A, Pacyna JM, Jones KC (2002). "Towards a global historical emission inventory for selected PCB congeners - a mass balance approach 1. Global production and consumption". The Science of the Total Environment 290: 181–198. doi:10.1016/S0048-9697(01)01075-0

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

Theoretically there are 209 possible congeners of PCBs. Most of these were present only in minor quantities in the commercial mixtures. The International Council for the Exploration of the Sea (ICES) has selected seven congeners for the monitoring of the marine environment. This list is known as PCB-7, PCB7, or ICES-7 and consists of these PCB compounds: PCB-28, -52, -101, -118, -138, -153 and -180. Other congeners, with a higher toxicity are determined as part of the Dioxin method described in the next section. In addition to the individual values of the PCB-7 compounds NIFES also report their “upper bound” sum, calculated as described in a previous section. The PCB-7 list overlaps with the list of dioxin like PCBs (next section) in that PCB 118 exists in both lists. Thus it has been suggested that the PCB-7 sum should be replaced by a PCB-6 sum since both PCB-7 and the DLPCBs are used to monitor food safety. There is still no consensus on this.

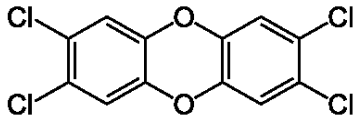


The chemical structure of PCB-153.

Procedure (NIFES method 137):
The sample is weighed, and PCB-53 is added as the internal standard. The sample is homogenized and freeze dried. Hydromatrix® is mixed in before extraction to aid the solvent penetration. Extraction with hexane under elevated pressure and temperature is performed in an Accelerated Solvent Extractor (ASE 300®, Dionex, Sunnyvale, CA, USA). Fat and other matrix components are removed by oxidation in a separate layer of sulphuric acid on silica in the extraction column. The solvent is evaporated in a TurboVap® Concentration workstation (Zymark, USA) and replaced with a small volume of isooctane. The sample is analysed on GC/MS in SIM mode with electron impact ionization. Quantification is based on the internal standards method. The method determines the PCBs no. 28, 52, 101, 118, 138, 153 and 180. The LOQ values for the compounds are listed in Table 5. The method is not accredited.

Dioxins, furans, and the non-orto and mono-orto PCBs.
The dioxins and furans (PCDDs and PCDFs) have never been industrially produced. These compounds are created as unwanted by-products in various chemical processes, or in the combustion in waste incineration plants. The EU regulation cited for the PCBs are in effect for dioxins as well. Like the PCBs, dioxins are a class of organic compounds and the derivatives of the group are called congeners. Like PCBs these are identified by numbers. But structural names like in the figure below are used as well. The congeners vary in toxicity. A small number of them are highly toxic. Also some PCBs have a structure similar to these toxic dioxins. These are the so called dioxin-like PCBs (DLPCB). The UN health organization (WHO) created in 1998 a list of the most toxic PCDDs, PCDFs and DLPCBs (a total of 29 compounds), and a related list of their relative toxicity values (TEF-98). These lists are the basis for the monitoring of these compounds in most countries. The TEF-98 values are relative to the most toxic dioxin: The 2,3,7,8 TCDD. In 2005 the TEF list vas revised and from January 2009 the TEF-05 list replaces the TEF-98 list. Unlike other compounds the results are reported as TEQ values rather than concentrations. The TEQ value is the measured concentration multiplied by the corresponding TEF value. Thus all 29 congeners are reported in 2,3,7,6 PCDD equivalents. The “sum of TEQ” is the sum of the 29 TEQ values, usually split in the sum TEQ for the dioxins and furans, and the sum TEQ

values of the PCBs. These sums provide easy to interpret values for the effective amount of these compounds in the sample.



Chemical structure of 2,3,7,8 TCDD, the most toxic dioxin.

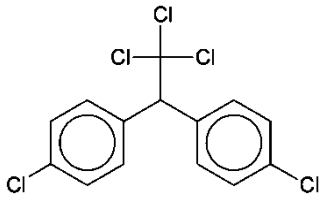
Procedure: NIFES method no. 228. The method is an adaptation to modern clean-up equipment of the US-EPAs (Environmental Protection Agency) methods No. 1613 and 1668. A mixture of 27 different ^{13}C-labelled internal standard compounds is mixed in with a homogenized sample corresponding to 3 g of fat. The sample is freeze dried. Hydromatrix® is mixed in before extraction to aid the solvent penetration. Extraction with hexane under elevated pressure and temperature is performed in an Accelerated Solvent Extractor (ASE 300®, Dionex, Sunnyvale, CA, USA). Fat and other matrix components are removed by oxidation in a separate layer of sulphuric acid on silica in the extraction column. Further clean-up is performed in a Power-Prep® instrument (FMS-USA) where successive chromatographic steps are carried out in three columns: "Multi-layered silica, basic alumina and activated charcoal. The mobile phase is changed successively from hexane, 2% dichloromethane (DCM) in hexane, 50% DCM in hexane, ethyl acetate and finally back-flush with toluene. The PCDD / PCDF and the non-orto PCBs are collected with the toluene fraction. The mono-orto PCBs are collected with the 50% DCM/hexane fraction. The two collected fractions are each evaporated to 10 ml in a TurboVap ® concentration Workstation (Zymark, USA). Two ^{13}C-labelled congeners are added to serve as "recovery standards". They are then separately analysed in a HRGC / HRMS instrument.

The method determines all 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.The internal standard method is based on the isotope dilution method according to the EPA 1613 and 1668 standard methods mentioned above. The concentrations are weighted by the TEF-98 values to give TEQ values. And the sums of the TEQ values are calculated for all 29 congeners, as an “upper bound” sum (UB-Sum TEQ ng / kg). 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. The LOQ values for all the congeners are in the range of 0.006 to 0.2 ng / kg.

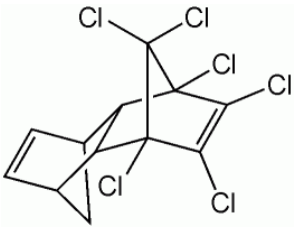
Polyhalogenated pesticides, including DDT and its metabolites.

This is a group of compounds that include a wide range of complex molecular structures. These molecules are designed by researchers, to give a compound with a high biological activity. Thus they are unwanted in both feed and food. They typically have several chlorine atoms attached to one or more positions in the structure. Many of

them are persistent in the environment and may have a profound potential for accumulation in the food chain.



Chemical structure of para-para DDT.



Chemical structure of chlordane.

Procedure (NIFES method 380): A mixture of ^{13}C-labelled internal standard compounds is added to a homogenized and freeze dried sample. Then Hydromatrix® is mixed in before extraction to aid the solvent penetration. Extraction with hexane under elevated pressure and temperature is performed in an Accelerated Solvent Extractor (ASE 300®, Dionex, Sunnyvale, CA, USA). Evaporation aided by nitrogen until approximately 0,5 ml is carried out in a TurboVap ® (Zymark, USA) The concentrated sample is then split into two equal fractions for separate clean up and analysis.

The first fraction is cleaned up using concentrated sulphuric acid. Acid is added to the sample extract, the sample is mixed, and the dark coloured acid fraction is removed. This procedure is repeated until no coloured oxidation products can be seen in the sulphuric acid. Prior to analysis, a recovery standard is added to the extract. Final analysis is carried out by GC/MS in electron ionization and SIM mode.

Solid phase extraction (SPE) clean up is performed for the second sample fraction. Chem Elut (Varian, Palo Alto, CA, USA), QuEChERS (Restek, Bellefonte, PA, USA) and C18 (Varian) cartridges are used, and the procedure is automated on ASPEC XL4 (Gilson, Middleton, WI, USA). Prior to analysis, a recovery standard is added to the extract. Final analysis is carried out by GC/MS in negative chemical ionization and SIM mode.

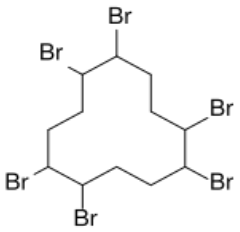
In both methods, quantification is based on an internal standard method with isotope labelled internal standards. The analytical recovery is estimated for each sample based on the recoveries of the internal standards relative to the two recovery standards. There are individual LOQ values for each of the compounds reported. These are the pesticides determined: Pentachlorobenzene, Hexachlorobenzene, three Hexachlorocyclohexanes; alfa-HCH, beta-HCH and gamma-HCH, DDT and its metabolites (pp-DDT, op-DDT, pp-DDD, op-DDD, pp-DDE and op-DDE), heptachlor, heptachlor -A, aldrin, dieldrin, isodrin, mirex, oxy-chlordane, trans-chlordane, cis-chlordane, endosulfan-A, endosulfan-B, 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. The LOQ values are listed in Table 6.

Polybrominated flames retardants (BFR)

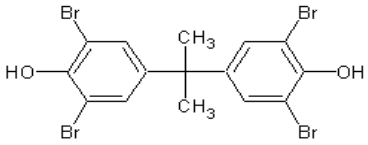
There are four main classes BFR: Polybrominated diphenyl ethers (PBDE), Tetrabromobisphenol-A (TBBPA), Hexabromocyclododecane (HBCD) and Polybrominated biphenyls (PBB). The molecular structures of the PBBs and the

PBDEs are very close to the PCBs. Like the PCBs both the PBDEs and the PBBs have 209 possible congeners. The only chemical differences between the structures of PCB and PBB are that in the latter bromine has replaced chlorine. When production changed from PBBs to the PBDE an oxygen atom was inserted in the molecular structure to reduce toxicity. Despite this safety measure the PBDEs are still an environmental and food safety concern. The PBBs have not been used much in Europe and are now banned in the United States. 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. In addition to the individual levels of these compounds NIFES report their 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 toxicology. As can be seen from the different compound ID numbers the substitution patterns for the compounds in the two lists are different. However, due to the chemical and toxicological similarities of the PCB and the PBDE classes it is still natural to compare the values of these sums.

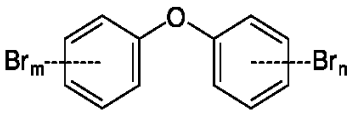
HBCD exists in three molecular isomers (isomers= otherwise identical molecules with different spatial arrangements of their structures). They are: α-, β-, γ-HBCD. Tetrabromobisphenol-A (TBBPA) is the most widely used BFR. And it is more common in Asia than in Europe and America. Due to the increasing trade and transport the levels in the environment are expected to increase also in Europe. "TBBPA" is written by some authors as TBBP-A.



Chemical structure of HBCD



Chemical structure of TBBPA



Chemical structure of PBDE

Procedure for PBDE. (NIFES method 238):
PBDE-139 is added as the internal standard to the freeze dried sample. Hydromatrix® is added to aid the solvent penetration. The sample is then thoroughly homogenized. Extraction with hexane/Dichloromethane under elevated pressure and temperature is performed in an Accelerated Solvent Extractor (ASE 300®, Dionex, Sunnyvale, CA, USA). Fat and other matrix components are removed by oxidation in a separate layer of sulphuric acid on silica in the extraction cell. The solvent is evaporated in a TurboVap ® concentration workstation (Zymark, USA) and replaced with a small volume of isooctane. The sample is analysed on GC / MS in SIM mode with negative

chemical ionizing. Quantification is according to the internal standard method based on a five point linear dose-response curve.

Procedure for HBCD and TBBPA (NIFES metode350)
A mixture of ^{13}C-labeled α-, β-, -γ-HBCD and γ-TBBPA is thoroughly mixed in with a pre-homogenized,feeze dried sample. A sequence of the solvents acetone, Cyclo-hexane and saline water is used to extract the analytes. After centrifugation the organic phase is isolated, collected and partially evaporated before the sample is re-dissolved in hexane. Fat and other matrix components are removed by oxidation with sulphuric acid. A ^{13}C-labelled "recovery standard" is added to the solution prior to the instrumental analysis. The α-, β-, γ-HBCD and TBBPA are determined on LC / MS / MS with electro spray (ES) in the negative ionization mode with Multiple Reaction Monitoring (MRM) mode. Quantification is based on the "isotope dilution" method using ^{13}C-labeled internal standards. The analytical recovery is estimated for each sample based on the recovery of the internal standards relative to the recovery standard.

B3b, Organophosphorous compounds

No B3b compound was part of the program in 2009.

B3c, Chemical elements

Heavy metals

Living organisms require small amounts of some metals in their diet. Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans in minute quantities. Excessive levels of these elements can damage the organism. "Heavy metals" usually refer to toxic elements such as mercury, plutonium, and lead. Heavy metals occur naturally in the environment with large geographical variations in their concentrations. Today, anthropogenic sources of heavy metals, i.e. pollution, have a significant contribution to the environment. From the environment heavy metals find their way to food and feed production. From a food safety concern contamination introduced during feed production is potentially the most serious source in farmed fish since this may unexpectedly contaminate a previously pure product. In Norway there has been one incident where an inorganic mineral feed ingredient contaminated fish feed with cadmium. In this study we focus on the toxic elements arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb).

Procedure (NIFES method 197)
Two parallels are weighed from a homogenate of the sample. They are then "digested", that is broken down and dissolved, with a mixture of extra pure nitric acid and hydrogen peroxide. The digestion is performed by heating in a closed container in a microwave heater (Milestone-MLS-1200). The analytes are measured quantitatively on an Agilent 7500C inductively coupled plasma mass spectrometer (ICPMS). These

elements were measured: 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 National Research Council (Ottawa, Canada) were analyzed in each analytical series: Tort-2 (hepatopankreas of lobster) and Dorm-2 (muscle of grayfish).

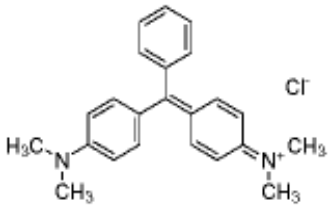
B3d, Mycotoxins

Many foods and feeds are susceptible to mould contamination when stored under inappropriate conditions. Mycotoxins may be formed in foods, raw materials for food production, or in animal feeds. Most moulds can grow at high salt concentrations or in relatively dry products, at water activities down to 0.8 (a_w value). A diverse range of moulds are able to produce toxic secondary metabolites, collectively known as mycotoxins. Three genera are particularly important in feeds and foods: *Aspergillus*, *Penicillium*, and *Fusarium*. In terms of acute toxicity the most commonly encountered mycotoxins in food and feed are less toxic than the Botulinum toxins and many of the algal toxins. However, long term low level exposure is of concern, since several of the mycotoxins are carcinogenic and may influence the human immune response if ingested.

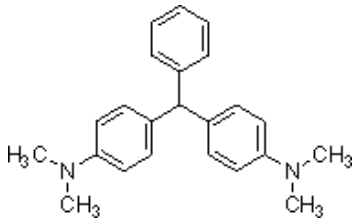
Mycotoxins from the feed can be carried over to the fish. As a part of this monitoring program our subcontractor the National Veterinary Institute (VI) has performed annual analysis of mycotoxins. An improved method specially developed by VI to determine mycotoxins with the highest relevance for marine feed products was introduced this year. A total of 18 samples of fish muscle from 2009 were examined with respect to Ochratoxine A. Procedure: The extract is cleaned up chromatographically by an immunoaffinity column. The analytical determination is performed on HPLC with fluorescence detection.

B3e, Dyes

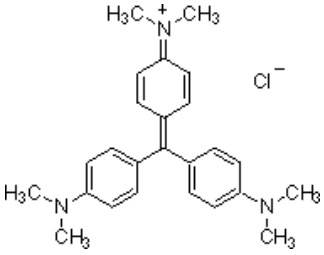
Malachite green (MG), crystal violet (CV), brilliant Green (BG) and their metabolites. These are triphenylmethane compounds. Historically some of these compounds have been used to treat fish and fish eggs against fungal infections in the fresh water phase. In Norway MG have formerly been used to treat fish eggs. 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*.



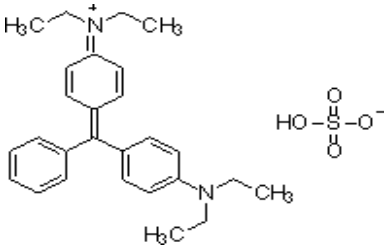
Chemical structure of MG



Chemical structure of LMG



Chemical structure of CV



Chemical structure of BG

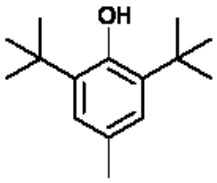
Procedure (NIFES method 264, Dyes):
The samples are extracted with acetonitrile and dichloromethane in an acidic environment. Solid phase extraction is used for sample clean-up. The samples are analyzed by LC-MS/MS. A reversed phase C18 column is used for separation and the components is ionized by ESI and detected as positive ions using the MRM mode. Quantification is based on an external calibration curve.

B3f, Others

This is a heterogeneous group of compounds that is analyzed within the scope of Norwegian national measures. This work is thus not mandatory according to the Directive 96/23/EC. It includes the synthetic antioxidants BHA, BHT and ethoxyquin, the perfluorinated organic compounds (PFC) and polycyclic aromatic hydrocarbons (PAH).

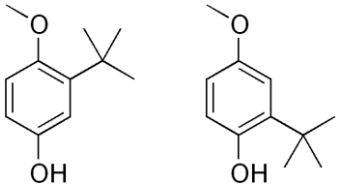
Synthetic anti-oxidants
The synthetic antioxidants ethoxyquin, BHT (4-methyl-2,6-ditert-butyl-phenol) and BHA (4-amino-2-hydroxy-benzoic acid) are approved for use in animal feed.

Antioxidants are necessary in feed ingredients to prevent s self ignition during bulk transport and because oxidized fats in feed may have adverse nutritional effects. Antioxidants protect the feed ingredients sacrificially, they are themselves oxidised. Their oxidation products are primarily "dimers" of the parent compounds (two molecules that are hooked together). For ethoxyquin there is also to some extent formed the de-ethylated ethoxyquin. The relative quantities of these molecular species change from the feed as they are digested, absorbed and metabolised in the fish. In fish both the oxidized derivatives and the parent compounds should be measured. In this report the parent ethoxyquin and its dimer is reported, but for BHT and BHA only the parent compounds. For all published data on synthetic antioxidants the "speciation" should be taken into account, i.e. the exact variants of these compounds that are measured. However, this is not always possible due to ambiguous data presentation.

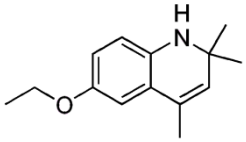


Molecular structure of BHT.

The



The two molecular structures of BHA.



Chemical structure of Ethoxyquin.

Ethoxyquin (NIFES method 229)

Ethoxyquin and its dimer are very unstable compounds, making it an efficient antioxidant. Analysis must be performed carefully, ensuring adequate shielding against light and atmospheric oxygen. Pyrogallol, ascorbic acid and EDTA are added from the start to protect ethoxyquin against oxidation. The samples are extracted with acetonitrile mixed with ascorbic acid. The fat in the extract is then saponified in a mixture of ethanol, NaCl and NaOH at 100 °C. The analyts and the other un-saponifiables are separated from the polar phase with hexane. The solvent is then evaporated and the sample is re-dissolved in acetonitrile containing 0.1% ascorbic acid. Ethoxyquin and its dimer are quantified by analysis on reverse phase HPLC with fluorescence detection. The method is no longer accredited since our quality assurance program revealed that it overestimated the results for ethoxyquin and its dimer. Quantification is based on the external standard method.

BHT (NIFES method 250)

The compound is easily destroyed by light and by air. A thorough shielding against light and atmospheric oxygen is required for any analytical step after the extraction from the sample. Pyrogallol, ascorbic acid and EDTA are added from the start of the procedure to protect against oxidation. The analyte is extracted with acetonitrile containing 0.1% ascorbic acid. After centrifugation the extract is filtered through a micro-filter before quantification using reverse phase HPLC and fluorescence detection. Quantification is based on the external standard method. The method measures the BHT mother compound.

BHA (NIFES method 294)

The compound is easily destroyed by light and by air. A thorough shielding against light and atmospheric oxygen is required for any analytical step after the extraction from the muscle tissue. BHA is extracted directly with acetonitrile with 0.1% ascorbic acid. The BHA concentration in the extract is measured using reverse phase HPLC with acetonitrile as the mobile phase and fluorescence detection. Quantification is based on the external standard method. The method measures the BHA mother compound.

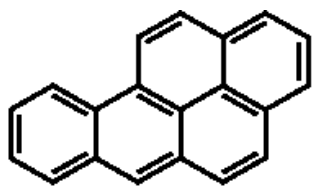
PAH

"Polycyclic aromatic hydrocarbons" (PAH) is a class of many compounds. The name is a description of their molecular structures; large molecules of multiple "aromatic" rings. PAHs are found in fossil fuels, in the smoke from burning wood and in the smoke and ash from industrial or incineration processes. In food PAH can be formed from excessive heat treatment. Also smoked food products have high levels of PAH. The class includes some of the most carcinogenic (cancer causing) compounds that are known, as for example, Benzo (a) pyrene (BaP).

The largest contributors to the environment are smoke and ash from industry, vehicles and households. Farmed fish can be exposed through contaminated feed. Wild fish can be exposed to PAHs from mineral oil products pollution. Mineral oil can be distinguished from other PAH sources by chemical analysis since some of their PAH molecules will have ethyl or methyl groups like branches attached to their molecular structure.

The large number of compounds in the class makes any "total PAH" analytical method impractical or inaccurate. A selection of compounds is required. There are several such lists in use. The US Environmental Protection Agency published a list of 16 compounds that is in wide use. The Norwegian Standard Organisation has published two lists, one for industrial PAH sources and one for PAH from combustion sources. The EPA-16 is designed to target combustion sources as well. But due to its popularity it is sometimes applied to samples and purposes for which it is wholly unsuited. A well-compiled list of compounds will enable conclusions to be drawn about the source of the contamination.

The most commonly reported single PAH is Benzo(a)Pyrene. BaP is often used as an analytical indicator or target for "PAH content". Of course its carcinogenicity also makes BaP a subject of interest itself. The EU has set an upper limit for BaP at 2 µg/kg. In 2007 a single sample in this monitoring program had values of PAH that indicated a local contamination in feed or water on the fish farm from which it was taken. As for the rest, this monitoring program has so far documented that the levels of PAH and BaP are low in Norwegian farmed fish.



Chemical structure of benzo(a)pyrene

Procedure: The samples were analyzed for PAH by Norsk Matanalyse / Eurofins. We have not received answers to our inquiries on the analytical principle underlying the method.

Table 3. Number of fish of each species and parameter.

Class of compounds			# fish	Salmon	Trout/ Rainbow trout	Turbot	Halibut	Cod	Arctic char	Saithe
Samples taken from the farms with no pre-notice	A1 Stillebenes	Diethylstilboestrol	230	215	5			10		
		Dienoestrol								
		Hexoestrol								
	A3 Steroids	Nandrolon alfa	220	200	20					
		Nandrolon beta								
		Trenbolon alfa								
		Trenbolon beta								
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	550	500	25			25		
		Metronidazole	800	690	65		5	25	15	
		Metronidazole-OH	800	690	65		5	25	15	
		Nitrofuran metabolites 3-Amino-2-oxazolidone	565	480	35		15	35		
		1-Aminohydrantion								
		3-Amino-5-morpholinomethyl-2-oxazol								
		Semicarbazide								
Samples taken from the slaughter House	B1 Chemical method on muscle	Flumequine	10					10		
		Oxolinic acid								
		Cinoxacin								
		Ciprofloxacin								
		Danofloxacin								
		Difloxacin								
		Enoxacin								
		Enrofloxacin								
		Lomefloxacin								
		Marbofloxacin								
		Nalidixic acid								
		Norfloxacin								
		Ofloxacin								
		Sarafloxacin								
		Florfenicol	20					20		
		Oxytetracycline	90	30	10	10		40		
	B1 Microbiological assay on liver	Quinolones	1150	1105	45					
		Tetracyclines and amphenicols	1150	1105	45					
		Sulphonamides	1150	1105	45					
	B2 Other veterinary drugs	Teflubenzuron	160	145	10			5		
		Diflubenzuron	160	160						
		Cypermethrine	90	85	5					
		Praziquantel	445	400	30		5	10		
		Fenbendazole	195	155	25			15		
		Emamectin	395	360	20			15		
		Ivermectin	30	30						
		Deltamethrin	65	65						
	B3a Organochlorine compounds	HCB	390	355	25			10		
		α-HCH								
		β-HCH								
		γ-HCH								
		Heptachlor								
		Heptachlor-a								
		Aldrin								
		Oxy-Chlordane								
		trans-Chlordane								
		Cis-Chlordane								
		α-Endosulfan								
		Endosulfan-sulfate								
		β-Endosulfan								
		Cis-Nonachlor								
		Trans-Nonachlor								
		Toxaphene 26								
		Toxaphene 32								
		Toxaphene 50								
		Toxaphene 62								
		Mirex								
		DDT, DDE og DDD orto-para + para-para	390	355	25			10		
		Dioxins + Dioxin like PCBs	320	265	40		5		10	
		PCB-7	315	260	40		5		10	
		PBDE (10)	5	5						
		Total HBCD	5	5						
		HBCD, α , β, γ	0							
		TBBPA								
	B3c Chemical elements	Pb	905	735	95			75		
		Cd								
		Hg								
		As								
	B3d	Mycotoxins	90	75	5			5		5
	B3e, Dyes	Malachite green Leuco Malachite green	870	755	95			20		
		Crystal violet Leuco Crystal violet								

Table 3. Number of fish of each species and parameter.

Class of compounds			# fish	Salmon	Trout/ Rainbow trout	Turbot	Halibut	Cod	Arctic char	Saithe
		Brilliant green								
	B3f Others	BHT								
		BHA								
		Ethoxyquin+ dimer	255	251						
		PAH	40	40						

Results and discussion

Group A

A total of 473 fillet samples from 2365 fish were collected for analysis of pharmacologically active substances under group A. The samples were taken at the farm site without prior notice. The samples in this group are collected of fish in all growth phases, not only from fish ready to be slaughtered. Group A was analyzed for substances that are not legal in food producing animals. A total of 1196 determinations were carried out in group A.

Group A1

The levels of the group A1 substances diethylstilboestrol, dienoestrol and hexoesterol were examined in 46 pooled samples from a total of 230 fish from three species. The determinations was conducted by the Hormone Laboratory, Oslo University Hospital, Aker. The detection limits (LOD) are listed in Table 2, the number of fish from each species is listed in Table 3. None of the substances were detected.

Group A3

The levels of group A3 substances nortestosterone (nandrolon) and trenbolon were analyzed in 44 pooled samples from 220 fish from two species. Analysis was performed by the Hormone Laboratory, Oslo University Hospital, Aker. The detection limits (LOD) are listed in Table 2, the number of fish from each species is listed in Table 3. None of the substances were detected.

Group A6 (annex IV to EEC 2377/90)

A total of 383 pooled samples from 1915 fish were analysed in this group. The detection limits (LOD) are listed in the Table 2, the number of fish from each species is listed in Table 3. No residues of A6/Annex IV drugs were detected in any sample.

Group B

There were a total of 1398 pooled and single fish samples of fillets from a total of 6786 fish for chemical analysis, and 1150 individual fish liver samples for the inhibition test. Samples were taken at the slaughterhouses from fish that were ready for the market. A total of 10549 analytical determinations were conducted in class B.

Group B1, antibacterial agents

The antibacterial agents in class B1 was determined by a combination of chemical and the three plate bioassay: Quinolones, amphenicols, tetracyclines and sulphonamides were measured in liver from 1150 fish using three plate bioassay, giving a total of 3465 determinations. These parameters were also analyzed in 24 pooled fillet samples, representing 120 fish by three chemical methods. The compounds were not detected in any of the samples. In the microbiological assay the detection limit, LOD, is estimated to be between 200 and 400 µg/kg for each of these compounds. LOD for each compound in the chemical analysis is listed in the Table 2. No positive samples were found in the B1 group.

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

The levels of the B2 substances teflubenzuron (B2f), diflubenzuron (B2f), cypermethrine (B2c), praziquantel, fenbendazole (B2a), emamectin benzoate (B2a), ivermectin (B2a) and deltamethrin (B2c) were determined in 308 pooled fillet samples representing 1540 fish from five species. Emamectin benzoate could be detected in one of the totally 79 examined samples included in the monitoring program of 2009. According to the analytical protocol, any detection of drug residues would be followed by a re-analysis of the same sample material in three replicates, and also analysis of a backup-sample when available. This program was followed for this positive sample, giving a mean concentration of 12.6 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 the Table 2.

Group B3a, Organochlorine compounds

In this group we find several well known contaminants and groups of environmental contaminants. In a context of food safety these compounds usually are given much focus and the consumers have taken a special interest in them. All together there are 284 samples from 1 420 fish in this group and 4 804 analytical determinations have been carried out. The results are summarised in the Tables 4 to 8.

DDT and its metabolites

The values are listed in Table 4. In the Table op-DDT and pp DDT means the orto-para and para-para DDT compounds. For several of the parameters there are values below the LOQ. "Upper-bound LOQ" (UB) sum calculation is used in these cases. There is a significant variation between the species, consistent with the variation in their fat content. The chemical compounds of this group are lipophilic and hydrophobic. UB-sum of DDT and its metabolites in 2009 show a variation from the theoretical minimum UB sum of 5.4 µg/kg to a value of 14.6 µg/kg wet weigh. The

UB- mean value is highest in salmon: 10.2 µg/kg wet weight followed by rainbow trout at 9.4. In 2008 UB-sum of DDT in salmon was 8.8 µg/kg and 7.8 µg/kg wet weights in rainbow trout. This is consistent with the fat content in their fillets. The use of UB calculation in the reports since 2007 must be taken into account when comparing results with previous years. Correcting for this there is a slight decrease in DDT values since 2003. The highest values in the whole period since 2003 have consistently been found for the pp-DDE compound. Generally the para-para compounds are found in higher concentration levels than the orto-para compounds.

Table 4. Concentration (µg/kg wet weight) of DDT, DDD and DDE in the fillet. Pooled samples (µg/kg w.w.).

	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	5.4
Salmon							
N	71	71	71	71	71	71	71
UB-Mean	-	-	-	1.7	-	4.8	10.2
Min	<LOQ	<LOQ	<LOQ	1.0	<LOQ	2.6	7.3
Max	<LOQ	1.0	<LOQ	2.8	<LOQ	8.5	14.6
Rainbow trout							
N	5	5	5	5	5	5	5
UB-Mean	-	-	-	1.5	-	4.2	9.4
Min	<LOQ	<LOQ	<LOQ	1.1	<LOQ	2.9	8.0
Max	<LOQ	<LOQ	<LOQ	1.7	<LOQ	6.4	11.7
Cod							
N	2	2	2	2	2	2	2
UB-Mean	-	-	-	-	-	-	5.4
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.4
Max	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.4
All groups							
N	78	78	78	78	78	78	78
UB-Mean	-	-	-	1.7	-	4.7	10
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5.4
Max	<LOQ	1.0	<LOQ	2.8	<LOQ	8.5	14.6

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

PCB_7

The values for the PCBs selected for marine monitoring are given in Table 5. The sums, calculated as the "upper bound-LOQ" (UB) in 63 samples ranged from the theoretical limit of 0.7 to 18 µg/kg wet weight. In 2006 and 2007 the maximum values were 18 and 15.5 µg/kg wet weight, respectively. The maximum values are typically each year from salmon samples, thus this number is not influenced by the varying number of lean fish in each year's data. The effect of the change to UB calculation from 2007 is negligible in PCB_7 data because there are so few values below the LOQ. For each year since 2003 the congeners PCB-138 and PCB-153 have been the primary contributors to the sum (error in the report for 2004.) The differences between the

species in the UB- mean values reflect their difference in fat content. In particular the lower value for the arctic char of 4.2 µg/kg wet weight versus the values of 7.8 (salmon), 6.5 (trout) and 10 µg/kg wet weight (Atlantic halibut). There is no obvious trend in the data for the period since 2003 for sum PCB_7 values. The EU has not set any upper limit for these compounds in fish. However, the Netherlands has set an upper limit for both individual congeners and for the sum PCB_7. The limit is set to 620 µg/kg wet weight in filet for the sum. The highest sum value in this dataset was 18 µg/kg wet weight.

Table 5. Concentration of the ICES PCB-7 compounds in the fillets of pooled farmed fish samples (µg/kg w.w.).

	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	UB Sum PCB-7
LOQ	0.06	0.09	0.09	0.09	0.12	0.09	0.15	0.7
Salmon								
N	52	52	52	52	52	52	52	52
UB-Mean	0.2	0.6	1.2	1.0	2.1	2.2	0.7	7.8
Min	0.07	0.2	0.5	0.4	0.8	0.8	0.2	3.6
Max	0.5	1.1	2.6	2.2	4.9	5.2	2.3	18
Rainbow trout								
N	8	8	8	8	8	8	8	8
UB-Mean	0.2	0.5	1.0	0.9	1.9	1.8	0.6	6.5
Min	0.08	0.4	0.6	0.5	1.1	0.7	0.4	4.2
Max	0.5	0.9	1.7	1.3	2.6	3.2	0.7	7.6
Atlantic Halibut								
N	1	1	1	1	1	1	1	1
Value	0.1	0.5	1.4	1.4	2.9	3	0.9	10
Arctic Char								
N	2	2	2	2	2	2	2	2
UB-Mean	0.1	0.3	0.7	0.5	1.1	1.0	0.3	4
Min	0.1	0.2	0.5	0.3	0.8	0.9	0.2	3.2
Max	0.2	0.3	0.8	0.6	1.3	1.2	0.3	4.7
All groups								
N	63	63	63	63	63	63	63	63
UB-Mean	0.2	0.6	1.2	1.0	2.1	2.1	0.7	7.6
Min	0.07	0.2	0.5	0.3	0.8	0.7	0.2	3.2
Max	0.5	1.1	2.6	2.2	4.9	5.5	2.3	18

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

Hexachlorcyclohexane (HCH) and hexachlorbenzene (HCB)

The data for these compounds are summarised in Table 6. A total of 76 pooled samples from 380 fish were analysed for alfa-, and beta and gama-HCH. In salmon there are enough measurable values to give a meaningful UB-mean for beta-HCH and a maximum value of 3.0 (µg/kg w.w.) In gama-HCH the maximum value is 0.4 µg/kg w.w. HCB is measurable in a number of salmon and trout samples, with a maximum value of 2.6 µg/kg w.w. However, the number of samples with values <LOQ is considerable and the levels must be considered low also in those samples with measurable values. The higher number of positives this year is a result of lower LOQ values due to analytical improvements to the method. Thus they do not reflect a rising trend. The difference in concentrations between the species reflects their different levels of fat, as expected since these are lipophilic compounds.

Other pesticides

A number of 81 samples were analysed for 23 other pesticides as well. The results for these compounds are summarised in Table 6. Half of these compounds: Aldrin, beta-HCH, cis-chlordane, cis-nonachlor, dieldrin, oxy-chlordane, heptachlor-A, toxaphene-26, toxaphene-50, toxaphene-62 and trans-nonachlor had measurable concentrations in one or more sample of the total 78 samples. The values ranged from <LOQ to 3.3 µg/kg w.w., the highest value for dieldrin. Also for these compounds a significant number of samples had values less than their respective LOQ values. Thus, for most of them a representative mean value could not be calculated. The other half of the compounds were below their LOQ values in all samples and all analysed species. These low levels are consistent with the findings from previous years. No measurable concentration was found in cod fillets for any of these compounds. This is consistent with cod being a lean fish.

Table 6. Concentration of other pesticides in fillets of pooled fish samples (µg/kg w.w.)

		Atlantic salmon	Rainbow Trout	Cod	All Grps	LOQ
Aldrin	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	0.9-
	Max	<LOQ	<LOQ	<LOQ	<LOQ	1.5
Alpha-endosulfane	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.15
Alpha-HCH	Mean	-	-	-	-	
	N	68	3	2	74	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.3
Beta-endosulfane	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.15
Beta-HCH	Mean	-	-		-	
	N	47	3	0	50	
	Min	<LOQ	<LOQ		<LOQ	1.2-
	Max	3.0	<LOQ		3.0	3.0
Cis-chlordane	Mean	1.0	1.4	-	1.0	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	2.0	2.0	<LOQ	2.0	0.9
Cis-nonachlor	Mean	0.5	0.4	-	0.5	
	N	71	5	2	78	
	Min	<LOQ	0.2	<LOQ	<LOQ	
	Max	1.1	0.6	<LOQ	1.1	0.15
Dieldrin	Mean	1.8	1.4	-	1.7	
	N	71	5	2	78	
	Min	0.7	1.1	<LOQ	<LOQ	
	Max	3.3	2.1	<LOQ	3.3	0.15
Oxy-chlordane	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	0.4	<LOQ	<LOQ	0.4	0.3
Pentachlorobenzene	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.3

Endosulfane-Sulfate	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.15
Gamma-HCH	Mean	-	-	-	-	
	N	67	3	2	72	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	0.4	<LOQ	<LOQ	0.4	0.3
HCB	Mean	0.9	0.8	-	0.9	
	N	71	5	2	78	
	Min	0.3	0.6	<LOQ	0.3	
	Max	2.6	1.2	<LOQ	2.6	0.3
Heptachlor	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.3
Heptachlor A	Mean	-	-	-	0.3	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	0.3-
	Max	<LOQ	<LOQ	<LOQ	<LOQ	1.5
Isodrin	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.9
Mirex	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.3
Toxaphene-26	Mean	0.8	0.7	-	0.8	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	1.8	0.8	<LOQ	1.8	0.6
Toxaphene-32	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	1.5
Toxaphene-40+41	Mean	0.8	0.7	-	0.8	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	1.7	0.8	<LOQ	1.7	0.6
Toxaphene-42a	Mean	-	-	-	0.6	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	0.9	<LOQ	<LOQ	0.9	0.6
Toxaphene-50	Mean	1.3	1.0	-	1.3	

	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	3.2	1.6	<LOQ	3.2	0.6
Toxaphene-62	Mean	0.8	0.8	-	0.8	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	2.1	1.3	<LOQ	2.1	0.6
Trans-chlordane	Mean	-	-	-	-	
	N	71	5	2	78	
	Min	<LOQ	<LOQ	<LOQ	<LOQ	
	Max	<LOQ	<LOQ	<LOQ	<LOQ	0.3
Trans-nonachlor	Mean	1.1	0.8	-	1.0	
	N	73	5	2	78	
	Min	0.5	0.4	<LOQ	<LOQ	
	Max	2.3	1.3	<LOQ	2.3	0.15

Dioxins, furans and dioxin like PCBs

There are a total of 29 compounds in this class. The sums of the TEQ values of these 29, the sum TEQ (ng/kg w.w.) weighted by the WHO TEF-1998 toxicity factors are summarised in Table 7. The discussion in this report will be based on the TEF-1998 weighted data since no maximum limits yet are established for the TEF-2005 weighted data. All figures in the table are calculated as the "upper bound-LOQ" sum (UB-sum). A total of 64 pooled samples were analysed for a total of 320 fish from Atlantic halibut, salmon, rainbow trout and Arctic char.

For the 17 dioxin and furan compounds (PCDD + PCDF) the sum values ranged from 0.18 ng TEQ/kg to 0.57 ng TEQ/kg w.w. The mean of their sums for each species was in the range 0.25-0.30 ng TEQ/kg w.w. Both the means and the range are very consistent with the values found each year since 2004, even if the data from all these years are not split up in separate figures for each fish species. The means in these years are respectively: 0.25, 0.32, 0.3, 0.3, 0.3 ng TEQ/kg w.w. and 0.3 in 2009. Thus there seems to be no trend in this period. The maximum value of 0.57 ng TEQ/kg w.w is below the EU's upper limit of 4.0 ng TEQ/kg w.w with a fair margin. The difference between the species are consistent with their fat content as would be expected since these compounds are lipophilic. Thus the mean levels are salmon> trout > arctic char.

The dioxin-like PCBs (DLPCB) are PCB congeners with non-and mono-orto molecular structure. There are a total of 12 of these compounds. The figures for the sum of the DLPCB levels range from 0.16 to 1.07 ng TEQ/kg wet weight. Both mean and range are consistent with the values found each year since 2004. The means in these years for all analysed species are respectively: 0.8, 1.0, 1.2, 0.9 and 0.7 ng TEQ/kg w.w compared to 0.6 ng TEQ/kg w.w in 2009. Thus there is no obvious trend in this period. There is no upper limit for the sum of the DLPCBs. As regards the difference between the species also the DLPCBs mean data levels follow the sequence: Salmon> trout > arctic char.

The figures for the total sum of dioxins and DLPCBs (total sum of TEQ) ranged from 0.44 to 1.52 ng TEQ/kg w.w. The mean has a range from 0.65 to 0.88 ng TEQ/kg w.w in these species. This is a factor of about 10 below the EU's upper limit. The means for all species in the years since 2004 were respectively: 1.2, 1.1, 1.5 1.3, and 1.0 ng TEQ/kg w.w. as compared to 0.9 in 2009. The upper limit in the EU for the total sum of TEQs is 8.0 ng TEQ/kg w.w. No single value in this project since 2004 is close to or above the EU's legal limit. The figures show no apparent trend in this period. The results correspond well with figures reported in NIFES online database "Seafood Data". Since 2004 the levels of DLPCB have been found to be higher than the PCDD/PCDF levels.

Table 7. Levels of dioxins, furans (PCDDs/ DFs) and the dioxins-like PCBs (ng TEQ-98/kg w. w.) in fillets of farmed fish.

		Atlantic Halibut	Atlantic Salmon	Rainbow Trout	Arctic Char	All Groups	EU Limit
	N	1	53	8	2	64	
Sum DLPCB	Mean	-	0.58	0.51	0.40	0.57	
	Min	-	0.16	0.29	0.26	0.16	
	Max	0.76	1.07	0.66	0.54	1.07	
Sum PCDD	Mean	-	0.14	0.15	0.15	0.14	
	Min	-	0.07	0.10	0.08	0.07	
	Max	0.07	0.35	0.21	0.22	0.35	
Sum PCDF	Mean	-	0.16	0.13	0.10	0.16	
	Min	-	0.08	0.11	0.09	0.08	
	Max	0.16	0.30	0.16	0.10	0.30	
SUM PCDD-PCDF	Mean	-	0.30	0.28	0.25	0.29	
	Min	-	0.18	0.22	0.18	0.18	
	Max	0.23	0.57	0.37	0.31	0.57	4.0
SUM DLPCB + SUM Dioxins	Mean	-	0.88	0.79	0.65	0.87	
	Min	-	0.44	0.56	0.57	0.44	
	Max	0.99	1.52	1.03	0.72	1.52	8.0

UB: All sums and averages are"upper bound" calculations.
LOQ: All LOQ values are related to the individual congeners, NIFES has not established a LOQ values for their sums.

Table 8. Concentrations of the brominated flame retardants, PBDE, HBCD (µg/kg wet weight) in the fillet, pooled samples.

	LOQ	value	N
PBDE-28	0.006	0.14	1
PBDE-47	0.006	1.84	1
PBDE-99	0.006	0.22	1
PBDE-100	0.006	0.40	1
PBDE-153	0.006	0.04	1
PBDE-154	0.006	0.10	1
PBDE-183	0.006	0.06	1
UB-Sum PBDE-7	-	2.96	1
PBDE-66	0.006	0.09	1
PBDE-119	0.003	0.02	1
PBDE-138	0.003	0.06	1
Sum HBCD	0.5	1.40	1

UB: All sums and averages are "upper bound" calculations.
LOQ: All LOQ values are related to the individual congeners, NIFES has not established a LOQ values for their sums.

Brominated flame retardants (BFR)

The results for the brominated flame retardants are summarised in Table 8. One sample of five pooled salmon was analysed. HBCD is determined in two methods: Sum HBCD is determined as part of the PBDE method. Total HBCD in the salmon sample is 1.4 µg/kg w.w. The value for the sum $PBDE_7$ is 2.96 µg/kg w.w. In 2008 the range was from 0.8 to 2.0. Thus the single value in 2009 is above the highest value of 2008. The similarity in structures from the PBDE class to the PCB class makes it natural to compare the data from these classes. For this single sample the sum $PBDE_7$ levels is 38% of the sum PCB_7 level.

Group B3b, Organophosphorous compounds

No B3b compound was part of the project in 2009.

Group B3c, Chemical elements

The analytical values for the elements are summarised in Table 9. They were determined in 181 pooled fish samples made from the filets of 905 fish.

Arsenic (As)

In Table 9 we see that the arsenic levels in the fillet of farmed fish ranged from 0.19 to 2.4 mg/kg w.w. The UB-mean ranged from 1.09 mg/kg w.w. in salmon to 1.3 in cod. In the period 2004-2008 the average levels for all species analysed were respectively: 2.1, 2.0, 1.4, 1.6 and 1.5 mg/kg w.w. (all before 2007 were calculated as LB-average).

In 2009 the average for all species was 1.1 mg/kg w.w. Thus the level of arsenic in fillets has in this period been fairly constant. The levels in the lean species cod are not significantly different from the others.

Table 9. Concentrations of the heavy metals (As, Cd, Hg and Pb) (mg/kg w.w.) in the fillet, pooled fish samples.

		Salmon	Trout	Cod	All Grps	EU-Limit	LOQ
As	UB-Mean	1.09	1.27	1.28	1.12		
	N	147	19	15	181		
	Min	0.19	0.79	0.74	0.19		
	Max	2.10	2.40	2.00	2.40	-	0.03
Cd	UB-Mean	-	-	-	-		
	N	147	19	15	181		
	Min	<LOQ	<LOQ	<LOQ	<LOQ		
	Max	0.02	<LOQ	<LOQ	0.02	0.05	0.01
Hg	UB-Mean	0.03	0.04	0.07	0.04		
	N	147	19	15	181		
	Min	0.01	0.02	0.03	0.01		
	Max	0.06	0.08	0.13	0.13	0.5	0.03
Pb	UB-Mean	-	-	-	-		
	N	147	19	15	181		
	Min	<LOQ	<LOQ	<LOQ	<LOQ		
	Max	0.05	0.02	<LOQ	0.05	0.3	0.015

Cadmium

None of the samples analysed in this project in 2005 and 2006 showed measurable concentrations of cadmium (all <LOQ), and in 2007, 26 of a total of 184 values were measurable and in 2008 13 of a total of 175 were measurable The 2009 data show that only 6 of a total of 186 samples are measurable. The maximum measured value was this year 0.02 mg/kg w.w. The EU's upper limit is 0.05 mg/kg w.w. Thus all samples were compliant.

Mercury

Table 9 shows that the concentration of total mercury ranged from 0.01 to 0.13 mg/kg w.w in 2009. The UB-average over the analysed species ranged from 0.03 to 0.09

mg/kg w.w. The highest value was found in cod. In contrast the EU's upper limit is 0.5 mg/kg w.w. for these products.

Lead

The data are summarised in Table 9. Only fifteen samples out of 181 had measurable concentrations. The highest value was found in salmon, 0.05 mg/kg w.w. The EU has from 2006 in the "Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs" set the limit for lead at 0.3 mg/kg, in "muscle meat of fish".

Trends since 2002

In the class of heavy metals the measured levels have been more or less stable over the years since 2002. In this time period a total of 1211 single or pooled fish samples have been analysed. For all these samples the maximum value for arsenic was 6.3 mg/kg w.w. There is currently no EU upper limit for total arsenic in fish fillet. The concentrations of cadmium were in all samples since 2002 less than or equal to 0.02 mg/kg w.w., except for one single sample from 2008. That sample was, within the analytical uncertainty, found at the value of the EU's upper limit for cadmium of 0.05 mg/kg w.w. For mercury, all values since 2002 were less or equal to 0.23 mg/kg w.w. The EU's upper limit for mercury is 0.5 mg/kg w.w. The levels of lead were in all samples since 2002 less than or equal to 0.1 mg/kg w.w. The EU's upper limit for lead is 0.3 mg/kg w.w. Thus, based on 1211 samples made from more than five thousand fish we conclude that all samples were in compliance with EU's upper limit. And that Norwegian farmed fish are well below the EU's upper limits for the heavy metals.

Group B3d, Mycotoxins

The 18 samples from 2009 were analysed for ochratoxine-A, by an analytical method adapted to marine samples. Of the 18 samples, 15 were salmon, one was rainbow trout, one cod and one sample was saithe. All samples were pooled with material from five fish each. Ochratoxin-A was not detected in any of the samples examined.

Group B3e, Dyes

The components in the B3e group were collected and examined both as A and B samples. In total 174 pooled samples from 870 fish representing three species were examined with respect to malachite green and its leuco form, crystal violet and its leuco form, and brilliant green. No residues of any of these agents were detected.

Group B3f, Others

BHA, BHT and Ethoxyquin (synthetic antioxidants)
The results are summarised in Table 10. All samples were salmon. All data for the synthetic antioxidants are on a wet weight basis. The concentration range for BHT in 2009 was LOQ-13.0 mg/kg and the mean was 2.96. The concentration range for BHT in 2008 was: 0.4-24.5 mg/kg and the mean was 5.4 mg/kg. In 2007 the range was 0.4-15.4 mg/kg and the mean was 4.8 mg/kg. In 2006, the range was 0.8 - 9.5 mg/kg. and the mean was 3.8 mg/kg. In 2005, the range was 0.10-3.8 mg/kg and the mean was 2.3 mg/kg. The ranges reflect an unexpected high variability. But unlike the other parameters in this project these are all based on single fish samples. For that reason alone a higher variability should be expected. There is no EU or national Norwegian MRL for BHT in food. However, Japan has an MRL of 10 mg BHT/kg w.w. In 2009 only one sample was above this MRL. In 2008 10% of the samples in this project were above the MRL while in 2007 3% were above and in 2006 the highest value was just below the MRL.

The range of BHA concentrations was LOQ-0.10 mg/kg w.w., and the mean was 0.02 mg/kg like the two previous years. There is no EU or national MRL for BHA in food. However, Japan has an MRL for BHA of 0.5 mg/kg.

There is no EU or national Norwegian 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 EU pesticide regulations. 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 analysed fish are all less than 0.24 mg/kg w.w. and the mean is 0.04 mg/kg. The concentrations of the ethoxyquin dimer are higher than those of the parent compound.

The EU has established an upper limit for the sum of antioxidants in feed at 150 mg/kg w.w. Since 1996 NIFES has conducted a surveillance program on fish feeds and fish feed ingredients on behalf of the Norwegian Food Safety Authority. None of the feed samples analysed so far exceeded the upper limit.

Table 10. Concentrations of synthetic antioxidants in the fillet of individual salmon samples. (mg/kg w.w.).		
		Salmon
BHA	Mean	0.02
	N	51
	Min	<LOQ
	Max	0.10
BHT	Mean	2.96
	N	51
	Min	<LOQ
	Max	13.0
EQ	Mean	0.04
	N	50
	Min	<LOQ
	Max	0.24
EQDM	Mean	0.81
	N	50
	Min	0.09
	Max	1.99
SUM EQ	Mean	0.84
	N	50
	Min	0.09
	Max	2.04
All values for ethoxyquin are analytically overestimated, and should be read as "less than" values.		

The PFC class

The perfluorinated organic compounds were not included in the surveillance in 2009.

The PAH class

Table 11 summarises the results for the PAH compounds. Eight samples were analysed, all of them from salmon. Calculation of the mean is performed by the UB procedure. Benzo[a]pyrene (BaP) is the most focused single PAH due to its carcinogenic effect. The EU has an upper limit for fish fillet at 2 µg/kg w.w. for this compound. None of the samples had measurable levels of BaP. This is consistent with the results from 2007 when only one of 84 samples had a measurable level and in 2008 when none of 21 samples had measurable levels. Most of the 13 PAH compounds have no measurable value in the eight samples. Only phenantrene are measurable in more than 50% of the samples, followed by fluorine measurable in three samples. These compounds have lower carcinogenic effects than Benzo[a]pyrene.

These data are consistent with the results from 2007 and 2008.

Table 11. Concentrations of PAH compounds (µg/kg wet weight) in the fillet, pooled fish samples, all salmon.

	LOQ	UB-Middelverdi	N	Maks	Min
Anthracene	0.5	-	8	<LOQ	<LOQ
Benzo(a)antracene	0.5	-	8	<LOQ	<LOQ
Benzo(a)pyrene	0.5	-	8	<LOQ	<LOQ
Benzo(b)fluoranthene	0.5	-	8	<LOQ	<LOQ
Benzo(ghi)perylene	0.5	-	8	<LOQ	<LOQ
Benzo(k)fluoranthene	0.5	-	8	<LOQ	<LOQ
Chrysene/Triphenylene	0.5	-	8	<LOQ	<LOQ
Dibenzo(a,h)anthracene	0.5	-	8	<LOQ	<LOQ
Fluoranthene	0.5	0.7	8	1.2	<LOQ
Fluorene	0.5	1.5	8	7.9	<LOQ
Indeno(1,2,3-cd)pyrene	0.5	-	8	0.5	<LOQ
Phenatrene	0.5	2.4	8	4.9	<LOQ
Pyrene	0.5	-	8	<LOQ	<LOQ
UB-sum	7.5	9.5	8	18.6	7.5

Summary

The number of samples in the 96/23 monitoring program is defined by the production volume. With the high production volume of farmed fish in Norway the marine part of the EU Directive 96/23/EC monitoring program is extensive. This report is based on a total of 10851 fish and 11 726 analytical determinations. Like in the report for last year, all sums and mean values found in the tables are calculated according to the "upper bound-LOQ" principle (UBLOQ): the value of the relevant LOQ is substituted in the calculation for all values below the LOQ value. Individual values are still reported as "<LOQ", less than the LOQ. UBLOQ calculations give a "worst case" figure that is a good basis for contaminants risk assessment. As a result of the UB-calculation procedure the sums, and mean values in this report will be systematically higher than the corresponding values found in the reports prior to the 2007 report.

The group-A samples are intended for analysis of drugs that are banned from use in food producing animals. For that reason these samples are collected by official inspectors on the farm location with no prior notification. Samples for group-A determinations are taken in all growth stages of the fish and should be representative for the fish in production. The group-B samples are analyzed for compounds for which an upper limit is established, or for compounds with a mandatory withdrawal period from medication or for compounds that for other reasons are monitored. The group B-samples are taken from fish in the slaughterhouses or the packing plants. These samples should be representative for the commercially available Norwegian farmed fish.

No detectable residues of the pharmacologically active agents in group A were found. For the veterinary drugs in group B, one of 79 examined samples was found to contain emamectin benzoate in a concentration of 12.6 µg/kg wet weight (w.w.). The present MRL for this agent is 100 µg/kg. For all other therapeutic agents in group B (antibacterials, anthelmintics, other sea lice agents and dyes), no residues were detected.

The mycotoxin ochratoxine A was not detected in any of the 18 pooled samples made from 90 individual fish.

The persistent organic pollutants (POPs) have been part of this program for years. The number of compounds and compound classes are increased in this period. The levels found for the dioxins (PCDDs and PCDFs), DLPCBs, PCB-7 and the organic pesticides were similar to the results from this program for the years 2003 to 2008, and also to the corresponding values found in the online database: "Seafood data" (www.nifes.no).

The brominated flame retardant compounds and compound classes PBDE, HBCD and TBBP-A are included in the program. The PBDEs are structurally similar to the PCBs. The sum $PBDE_7$ amount to <40% of the mean value for the sum PCB_7. Their toxicity is, however, lower. Total HBCD has the value of 0.5 µg/kg w.w.

In the class of heavy metals the measured levels have been stable over the years since 2002. In this period of time a total of approximately 1200 single or pooled fish samples have been analysed. This is equivalent to more than five thousand fish. For all these samples the maximum value for arsenic was 6.3 mg/kg w.w. There is currently no EU upper limit for arsenic. The concentrations of cadmium were in all samples since 2002 less than or equal to 0.02 mg/kg w.w. accept for one single sample from 2008. For mercury, all values since 2002 were less or equal to 0.25 mg/kg w.w. The EU upper limit for mercury is 0.5 mg/kg w.w. The levels of lead were in all samples since 2002 less than or equal to 0.1 mg/kg w.w. The EU upper limit for lead is 0.3 mg/kg w.w.

The synthetic antioxidants are legal additives to fish feed. It can be concluded from the results that there is a transfer of synthetic antioxidants from the feed to the fish fillets, in particular for BHT. A national upper limit in Japan for BHT is exceeded by fish in the present data set. The analytical levels of BHA and ethoxyquin are lower than those of BHT. There is no EU or national Norwegian upper limit for these compounds in seafood.

Thirteen PAH compounds are included in the program. This class is of interest because some of the compounds are carcinogenic. The most potent carcinogenic of the analysed compounds is benzo[a] pyrene (BaP). This compound is the only one with an established upper limit. The concentrations of BaP are below the limit and also below the LOQ value in all of the samples analysed in 2009. In the majority of the samples only fluorene and phenantrene are found in measurable concentrations. These are far less potent health hazards than BaP.