MONITORING PROGRAM FOR RESIDUES OF THERAPEUTIC AGENTS, ILLEGAL SUBSTANCES, POLUTANTNS AND OTHES UNDESIERABLES IN FARMED FISH

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

ANNUAL REPORT FOR 2008

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Tables

Introduction

The aim of this program is an ongoing monitoring of residues of therapeutic agents, illegal substances, pollutants and other undesirable substances in Norwegian farmed fish. The Norwegian Food Safety Authority (NFSA) is responsible for the enforcement of this directive in Norway, and 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, whereas The National Veterinary Institute are responsible for the terrestrial part. On this program NFSA has planned and carried out the sampling for both parts of the program.

The activity as described in this report is conducted in accordance with the requirements in Directive 96/23/EC "On measures to monitor certain substances and residue thereof in live animal and animal products" and further as specified in the directive 2002/657/EC on the implementation of the above mentioned directive.

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 2008 these compound groups have been analyzed 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 to 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
- B3b: Organophosphorous 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 samples should be representative of the fish that are in production in the Norwegian aquaculture sector.

Group B-compounds are drugs for which a Maximum Residue Limit (MRL) is established or a mandatory quarantine period after medication is established, and also contaminants of food safety concern. The group B-samples were taken from slaughtered fish at the slaughterhouses or the packing plants. These samples should be representative of the commercially sold fish.

In 2008, NIFES used subcontractors for some parameters. The Hormone Laboratory of Oslo University Hospital, Aker was subcontractor for the determinations of stilbenes and steroids, The National Veterinary Institute, 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, Lina Beyer Vågenes and Rosini Ngyen were responsible for chemical analysis of the medicine residues. 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 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 2008

According to current legislation, the minimum number of samples to be collected each year must be at least 1 per 100 tons produced. In 2008 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. Farms from all regions with aquaculture activity, and at least 10% of the total number of plants 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 either frozen muscle tissue from five

fish, frozen liver from five fish or one gutted, single fish stored on ice. 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 environmental pollutants in group B. 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 2008 there were 60 single fish samples.

On arrival to NIFES the filets 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 lost in the pooling. The observed standard deviations than reflect the variability between the farms. The liver samples were all analyses individually.

Table 1 provides an overview of the number of fish that were the basis for each chemical and microbiological examination. The total number of fish in the data base was 9066. From these 1692 pooled filet samples and 975 liver samples from individual fish were prepared. The total number of analytical measurements reported here is 9785.

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 analyzed 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 analyzed for only one parameter. This was implemented to mean analysed by only one analytical method. Each analytical method often determines a whole class of compounds or several closely related compounds at the same time. The one sample - one analysis rule was given second priority to the other design criteria for the sampling plan. 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. As far as practical considerations and the number of samples allowed for each data basis included fish from several regions and most seasons.

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	260	52	52
		Dienoestrol			52
		Hexoestrol			52
	A3 Steroids	Nandrolon alfa	280	56	56
		Nandrolon beta			56
		Trenbolon alfa			56
		Trenbolon beta			56
	A6 Illegal drugs: Annex IV to EEC 2377/90	Chloramphenicol	510	102	102
		Metronidazole	665	133	133
		Hydroxy metronidazole			133
		Furazolidone	500	100	100
		Furaltadone			100
		Nitrofurantoine			100
		Nitrofurazone			100
		Malachite green	120	24	24
		Leuco malachite green			24
	Sum of group A		2335	467	1196
Samples taken from the slaughter House	B1 Anti bacterial agents	Flumequine	50	10	10
		Florfenicol	45	9	9
		Oxolinic acid	50	10	10
		Oxytetracycline	35	7	7
	B2 Other veterinary drugs	Teflubenzuron	95	19	19
		Diflubenzuron	95	19	19
		Cypermethrine	95	19	19
		Praziquantel	360	72	72
		Fenbendazole	150	30	30
		Emamectin	280	56	56
		Ivermectin	35	7	7
		Deltamethrin	105	21	21
	B3a Organochlorine Compounds	HCB α-HCH β-HCH γ-HCH Heptachlor Heptachlor-a Aldrin Oxy-Chlordane Trans-Chlordane Cis-Chlordane α-Endosulfan Endosulfansulfate β-Endosulfan Cis-Nonachlor Trans-Nonachlor Toxaphene 26 Toxaphene 32 Toxaphene 50 Toxaphene 62	224	52	52x19=988
		DDT, DDE og DDD : orto-para and para-para congeners	754	162	162x6=972
		Dioxins and Dioxin like PCBs	458	98	98x29=2842
		PCB-7 (+2)	467	100	100x7=700
		PBDE (11)	23	15	15x11=165
		HBCD, α , β and γ	21	9	9x3=27
		TBBPA	21	9	9
	B3c Chemical elements	Pb Cd Hg As	851	175	175x4=700
	B3d Mycotoxins	Ochratoxin A	175	35	35
	B3e, Dyes	Malachite green	320	64	64
		Leuco malachite green			64
		Chrystal violet	320	64	64
		Leuco Chrystal violet			64
		Brilliant green	320	64	64
	B3f Others	BHT BHA Ethoxyquin+ dimer	320	64	50x4=200
		PFC (10)	22	10	10x10=100
		PAH (13)	45	21	21*13=273
		Nitrosamines	20	4	4
	Sum[4] B fillets, pooled and from single fish, chemical method		5756	1225	7614
B (liver)	B1 Microbiological screening of liver	Quinolones	325	325	325
		Tetracyclines and amphenicols	325	325	325
		Sulphonamides	325	325	325
Total sum liver, three plate inhibition assay			975	975	975
Total sum B			6731	2200	8589
Total sum fillet A+B			8091	1692	8810
Total sum, fillets, pooled and single fish and liver			9066	2667	9785

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 UB is required there is an incentive to have the samples analyzed by the most advanced methods. Previously lower bound (LB) calculations were the norm. Cheap methods with high

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

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 a 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.

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

Minimum required performance level (MRPL): This is a maximum permitted detection limit for methods used to determine this analyte in public surveillance. The MRPL value is established in accordance with the EU Commission Decision 2002/657/EC

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.

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 comparative

[2] Commission directive 2002/69/EC

analytical 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 60 individual fish samples. In the microbiological assay for antibiotics, liver samples are tested individually. The assay is a qualitative analysis 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+ skin	GC-MS	MRPL=2.0	MRPL=2.0	Use LOD	Presence	AUS[3]
	Dienoestrol	Muscle	GC-MS	MRPL=1,0	MRPL=1.0	Use LOD	Presence	AUS
	Hexoestrol	Muscle	GC-MS	MRPL=2,0	MRPL=2.0	Use LOD	Presence	AUS
A3	Nandrolon alpha	Muscle	GC-MS	MRPL=1,0	MRPL=1.0	Use LOD	Presence	AUS
	Nandrolon beta	Muscle	GC-MS	MRPL=1,0	MRPL=1.0	Use LOD	Presence	AUS
	Trenbolon alpha	Muscle	GC-MS	MRPL=3,0	MRPL=3.0	Use LOD	Presence	AUS
	Trenbolon beta	Muscle	GC-MS	MRPL=3,0	MRPL=3.0	Use LOD	Presence	AUS
A6 Annex IV substances	Chloramphenicol	Muscle	LC/MS	n.a.	0.3 (MRPL)	(1.0) Use LOD	presence	NIFES
	Metronidazole	Muscle	LC/MS	n.a.	1.0	(10.0) Use LOD	Presence	NIFES
	Metronidazole-hydroxy	Muscle	LC/MS	n.a.	2.0	(10.0) Use LOD	Presence	NIFES
	Furazolidone	Muscle	LC/MS/MS	n.a.	0.5 (MRPL[2] =1,0)	(1.5) Use LOD	Presence	NIFES
	Furaltadone	Muscle	LC/MS/MS	n.a.	0.5 (MRPL=1,0)	(1.5) Use LOD	Presence	NIFES
	Nitrofurantoine	Muscle	LC/MS/MS	n.a.	0.5 (MRPL =1.0)	(1.5) Use LOD	Presence	NIFES
	Nitrofurazone	Muscle	LC/MS/MS	n.a.	0.5 (MRPL = 1,0)	(1.5) Use LOD	Presence	NIFES
B1 Antibacterial substances	Oxolinic acid	Liver	3-plate Screening method and HPLC-MS	200	10	30	100 µg/kg[3)]	NIFES
	Flumequine	Liver		200	10	20	600 µg/kg[3)]	NIFES
	Tetracyclines	Liver		200	2.0	5.0	100 µg/kg[3)]	NIFES
	Florfenicol	Liver		200	0.2	0.5	1000 µg/kg[3)]	NIFES
	Sulfonamides	Liver		400	n.a.	n.a.	100 µg/kg[3)]	NIFES
B2a Anthelmintics	Praziquantel	Muscle	LC-UV (DAD)	n.a.	50.0	100	n.a.	NIFES

[1] MRPL = "Minimum required performance limit", gives the minimum requirements for the capability of the analytical method in terms of detection limits, in accordance with the Commission Decision 2002/657/EC; [2] Aker University Hospital's Hormonlaboratoriet; [3] MRL = "Maximum residue limit", the highest permitted concentration of a legally applied agents in products from food producing animals, in accordance with the Council Regulation (EEC) 2377/90. The MRL is set for muscle and skin I natural proportions for all agents except for oxytetracycline, where muscle is the material for analysis.

	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
	Fenbendazole	Muscle	HPLC-MS	n.a.	2.5	5,0	n.a.	NIFES
	Emamectin	Muscle	LC-MS	n.a.	2.5	5,0	100 µg/kg[3)]	NIFES
	Ivermectin	Muscle	LC-MS	n.a.	25.0	50	n.a.	NIFES
B2c Carbamates and pyrethroids	Cypermethrine	Muscle	GC-MS	n.a.	5.0	10,0	50 µg/kg[3)]	NIFES
	Deltamethrin	Muscle	GC/MS	n.a.	10	20	10 µg/kg[3)]	NIFES
B2f Other active substances	Diflubenzuron	Muscle	LC-MS	n.a.	10	20	1000 µg/kg[3)]	NIFES
	Teflubenzuron	Muscle	LC-MS	n.a.	5	15	500 µg/kg[3)]	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.	0,2	0.6	n.a.	NIFES
	HCH-beta	Muscle	GC-MS	n.a.	0.4	1.2[4]	n.a.	NIFES
	HCH-gamma	Muscle	GC-MS	n.a.	2,0	2	n.a.	NIFES
	HCB	Muscle	GC-MS	n.a.	0,02	0,07	n.a.	NIFES
	Heptachlor	Muscle	GC-MS	n.a.	0.8	2,5	n.a.	NIFES
	Heptachlor-a	Muscle	GC-MS	n.a.	0.17	0,5	n.a.	NIFES
	Aldrin	Muscle	GC-MS	n.a.	0,2	0,6	n.a.	NIFES
	Oxy-chlordane	Muscle	GC-MS	n.a.	0.4	1,3	n.a.	NIFES
	Trans-chlordane	Muscle	GC-MS	n.a.	0.2	0,7	n.a.	NIFES
	Cis-chlordane	Muscle	GC-MS	n.a.	0.17	0,5	n.a.	NIFES
	Alfa-endosulfan	Muscle	GC-MS	n.a.	0.1	0,3	n.a.	NIFES
	Endosulfansulphate	Muscle	GC-MS	n.a.	0.17	0,5	n.a.	NIFES
	Beta-endosulfan	Muscle	GC-MS	n.a.	0.1	0,3	n.a.	NIFES
	Cis-nonachlor	Muscle	GC-MS	n.a.	0.2	0,7	n.a.	NIFES
	Trans-nonachlor	Muscle	GC-MS	n.a.	0,2	1,5	n.a.	NIFES
	Toxaphene 26	Muscle	GC-MS	n.a.	0.8	1	n.a.	NIFES
	Toxaphene 32	Muscle	GC-MS	n.a.	0.5	0,7	n.a.	NIFES
	Toxaphene 50	Muscle	GC-MS	n.a.	0.8	2,5	n.a.	NIFES
	Toxaphene 62	Muscle	GC-MS	n.a.	0.5	1,5	n.a.	NIFES
	DDT-op DDT-pp DDD-op DDD-pp DDE-op DDE-pp	Muscle	GC-MS	n.a.	0.06 0.08 0.03 0.03 0.05 0.04	0.2 0.2 0.1 0.1 0.2 0.1	n.a.	NIFES

[4] LOD and LOQ for HCH-beta vary from series to series.

	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
	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.to 0.12 µg/kg		n.a.	VI[5]
B3e, dyes	Malachite green Crystal violet	Muscle	LC-MS	n.a.	1.0 (MRPL=2.0)	2.0	Presence	NIFES
	Malachite green-Leuco Crystal violet-Leuco	Muscle	LC-MS	n.a.	1.0 (MRPL = 2.0)	2.0	Presence	NIFES
B3f, other pharmacologically active substances	Ethoxyquin (EQ)	Muscle	HPLC-UV	n.a.	0,2	0.6	n.a.	NIFES
	Ethoxyquin-dimer	Muscle	HPLC-UV	n.a.	0,1	0.3	n.a.	NIFES
	BHT	Muscle	HPLC-UV	n.a.	14	45	n.a.	NIFES
	BHA	Muscle	HPLC-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	2007: sub contractor

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.

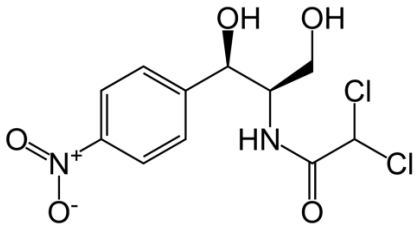
[5] The National Veterinary Institute

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 four nitrofurans, furazolidone, furaltadone, nitrofurantoine and nitrofurazone were analyzed by Eurofins.

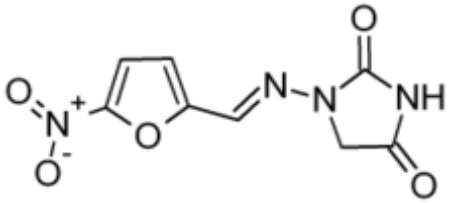
Chloramphenicol (NIFES method 143)
Chloramphenicol is an antibiotic agent with activity against a broad spectrum of microorganisms. It has been used in human and veterinary medicine since 1949, but due to a rare but serious dose-independent adverse effect (aplastic anaemia); this agent is no longer authorized in the treatments of food-producing animals.



Chemical structure of chloramphenicol.

Procedure: An internal standard (chloramphenicol-D5) was added to the sample before extraction with ethyl acetate. Fat was subsequently removed from the extract by a liquid/liquid extraction between aqueous saline and heptane. To separate the analytes from the water-soluble components, a new liquid/liquid extraction step with aqueous saline and ethyl acetate was performed. The sample was analyzed by LC-MS, with a reversed phase C18 column for separation. The components were ionized by electro spray ionization (API-ES) and detected as negative ions using the selected ion monitoring (SIM) mode. Quantification was based on the internal standard method with a three point calibration curve.

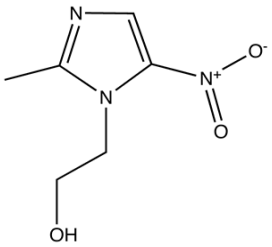
Nitrofurans
This group of synthetic antibacterial agents are derivatives of nitrofurane. The compounds have previously been widely used in veterinary medicine. In this report the nitrofurans furazolidone, furaltadone, nitrofurantoine and nitrofurazone are included. These agents are rapidly metabolized in the tissue, and thus the metabolites 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semicarbazide (SEM) are included in the assay as well.



Chemical structure of nitrofurantoine.

Procedure: The analytes were extracted with aqueous hydrochloric acid and derivatized with nitrobenzaldehyde. Solid phase extraction was then applied for sample clean up. The analytes were determined by LC-MS/MS in the positive mode.

Metronidazole and its metabolite (NIFES method 351)
Metronidazole is a synthetic antimicrobial compound that is used against infections caused by anaerobic bacteria and certain parasites.



Chemical structure of metronidazole.

Procedure: Internal standard (dimetronidazole-D3) was added to a homogenized sample. The analytes were extracted by ethyl acetate in an alkaline environment. The solution evaporated and re-dissolved in heptane. Sample clean up was performed by solid phase extraction. The solution was then evaporated to dryness and re-dissolved in water, before analysis by LC-MS. A reversed phase C18 column was used for separation, and the components ionized by electro spray ionization (API-ES) and fragments detected as positive ions using the selected ion monitoring (SIM) mode. Quantification was based on the internal standard method with a three point calibration curve.

Malachite green and its metabolite (NIFES method 264)
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:

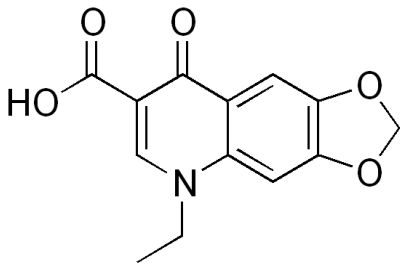
B3d: Mycotoxins: The National Veterinary Institute in Oslo.
B3f: PAH: Eurofins

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 test. The liver has a central function in distribution and elimination of drugs from fish and 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.

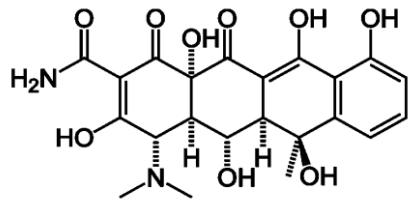


Chemical structure of oxolinic acid.

Procedure: The analytes were extracted with acetonitrile, where after analysis was performed by LC-MS/MS in the positive mode.

Oxytetracycline

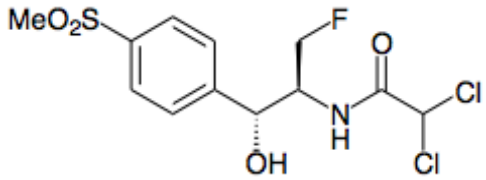
Oxytetracycline belongs to the tetracycline antibiotics. It is a broad spectrum antibiotic that is active against a wide range of bacteria.



Chemical structure of oxytetracycline.

Procedure: The analytes were extracted with EDTA-succinate buffer, and solid phase extraction was used for sample clean up. The analytes were determined by LC-MS/MS in the positive mode.

Florfenicol (NIFES method 290)
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: Internal standard (chloramphenicol-D5) was added to the sample, and the analytes were extracted with ethyl acetate. To remove fat from the extract a liquid/liquid extraction between aqueous saline and heptane were conducted. Furthermore, to separate the water-soluble components a liquid/liquid extraction step with saline and ethyl acetate was performed. The sample was analyzed by LC-MS. A reversed phase C18 column was used for separation, and the molecules were ionized by electro spray ionization (API-ES), and detected as negative ions using the selected ion monitoring (SIM) mode. Quantification was based on the internal standard method with a three point calibration curve.

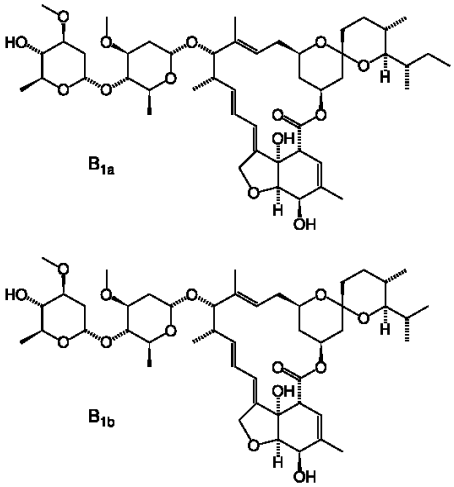
B2a, Anthelmintics

Diflubenzuron and teflubenzuron (NIFES method 138 and 139)
Diflubenzuron and teflubenzuron are both chitin synthesis inhibitors used in treatment against sea lice.

Procedure: Internal standard (diflubenzuron when teflubenzuron is determined, and teflubenzuron when diflubenzuron is determined) was added to the sample, and the analytes were extracted with acetone. Heptane was used to remove fat. A solid phase extraction was applied for further purification of the sample. The samples were analyzed by LC-MS. A reversed phase column was used for separation, and the components were ionized by electro spray ionization (API-ES) and detected as

negative ions using the selected ion monitoring (SIM) mode. Quantification was based on the internal standard method with a three point calibration curve.

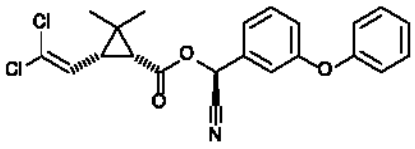
Ivermectin and Emamectin (NIFES method 130 and 131)
Ivermectin and emamectin belong to the class of avermectins. Emamectin is presently used against external parasites on fish.



Molecular structures of two ivermectin forms.

Procedure: Internal standard (ivermectin when emamectin is determined, and emamectin when ivermectin is determined) was added to the sample, and the analytes were extracted with acetonitrile. Fat was removed by extraction with heptane, and the sample was further purified by solid phase extraction. The sample was analyzed by LC-MS. A reversed phase C18 column was used for separation, and the components were ionized by atmospheric pressure chemical ionization (APCI) and detected as positive ions using the selected ion monitoring (SIM) mode. Quantification was based on the internal standard method with a three point calibration curve.

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

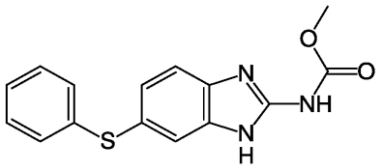


Chemical structure of cypermethrine.

Procedure, cypermethrine and deltamethrin: The samples were extracted with acetone. The extracts were then cleaned up by a liquid/liquid extraction, and further by gel

permeation chromatography. The samples were analyzed by gas chromatography-electron capture (GC-EC).

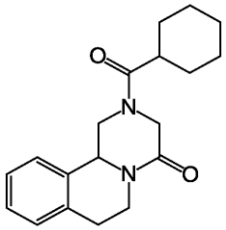
Fenbendazole (NIFES method 141)
Fenbendazole is a broad spectrum benzimidazole anthelmintics used against intestinal parasites in fish.



Chemical structure of fenbendazole.

Procedure fenbendazole: The homogenized sample was extracted with a solution of methanol and water. Petroleum ether was used to remove fat from the extract. Sodium dihydrogen phosphate and a mixture of diethyl ether/ethyl acetate were then added to the polar extract before shaking and centrifugation, and the upper layer was collected and vaporized. The sample was then dissolved in a solution of acetonitrile and water prior to analysis on LC-MS. A reversed phase column was used for separation, and the components were ionized by atmospheric pressure chemical ionization (APCI) and detected as positive ions using the selected ion monitoring (SIM) mode. Quantification was based on the external standard method with a three point calibration curve.

Praziquantel (NIFES method 140)
Praziquantel is an isoquinolin agent used against intestinal parasites in fish.



Chemical structure of praziquantel.

Procedure: Praziquantel was extracted from the homogenized sample by acetone. Diethyl ether and hexane were then added to the extract. Afterwards the organic phase was transferred and evaporated, following methanol addition to remove fat. A reversed phase column was used for separation, and praziquantel was detected by LC-UV at a wavelength of 205 nm. Quantification was based on the external standard method with a three point calibration curve.

B3a, Organochorine 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 lipofilic and hydrophobic character. For this reason they are found in fatty tissues and they are extracted from the sample with organic solvents.

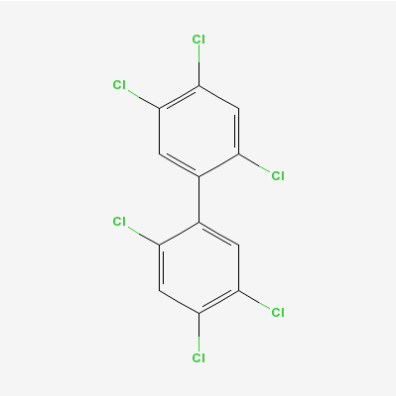
Polychlorinated biphenyls (PCB)

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[6]. 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:

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.

[6] 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

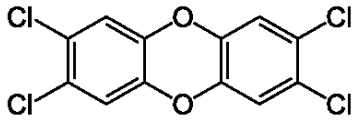


The chemical structure of PCB-153.

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 7. 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.

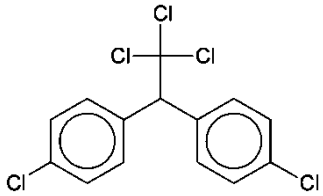


Chemical structure of 2,3,7,8 TCDD, the most toxic dioxin.

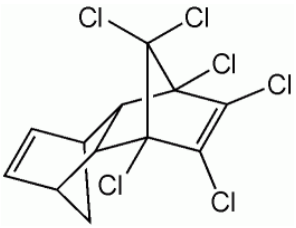
Procedure: NIFES method no. 228. The method is an adaptation to modern clean-up equipment of the US-EPAs (Environmental Protection Agency) methods No. 1613 and 1668. A mixture of 27 different ^{13}C-labelled internal standard compounds is mixed in with a homogenized sample corresponding to 3 g of fat. 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 have 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.

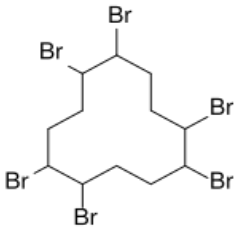
Procedure (NIFES method 263): A mixture of ^{13}C-labelled internal standard compounds is mixed in with a homogenized sample. The sample is freeze dried. Then Hydromatrix® is mixed in before extraction to aid the solvent penetration. Extraction with hexane under elevated pressure and temperature is performed in an Accelerated Solvent Extractor (ASE 300®, Dionex, Sunnyvale, CA, USA). Evaporation aided by nitrogen until approximately 0,4 ml is carried out in a TurboVap ® concentration Workstation (Zymark, USA) The concentrated sample is then mixed with a drop of sulphuric acid, and the dark coloured acid is removed. This is repeated until no coloured oxidation products can be seen in the sulphuric acid. Two ^{13}C-labelled pesticides are added to serve as "recovery standards". Final analysis is carried out by GC/MS in negative chemical ionization and SIM mode. Quantification occurs by 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 labelled recovery standards. There are individual LOQ values for each of the compounds reported. These are the pesticides determined: Pentachlorobenzene, three Hexachlorobenzenes: 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 8.

Polybrominated flames retardants (BFR)

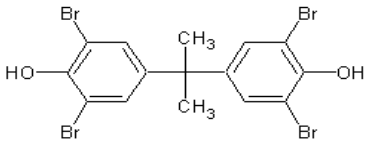
There are four main classes BFR: Polybrominated diphenyl ethers (PBDE), Tetrabromobisphenol-A (TBBPA), Hexabromocyclododecane (HBCD) and Polybrominated biphenyls (PBB). The molecular structures of the PBBs and the PBDEs are very close to the PCBs. Like the PCBs both the PBDEs and the PBBs have 209 possible 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.

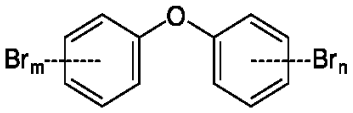
HBCD exists in three molecular isomers (isomers= otherwise identical molecules with different spatial arrangements of their structures). They are: α-, β-, γ-HBCD. Tetrabromobisphenol-A (TBBPA) is the most widely used BFR. And it is more common in Asia than in Europe and America. Due to the increasing 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. Hydromatrix® is added to aid the solvent penetration. The sample is then homogenized and freeze dried. 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 sample. A sequence of the solvents acetone, Cyclo-hexane and saline water is used to extract the analyts. After centrifugation the organic phase is isolated, collected and partially evaporated before it 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 analysis of the α-, β-, γ-HBCD and TBBPA using LC / MS / MS with electro spray (ES) negative ionization mode and

with Multiple Reaction Monitoring (MRM). Quantification is based on the "isotope dilution" method using 13C-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 2008.

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 more serious since this may occur unexpected in a previously pure product. In Norway there has been an 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 “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

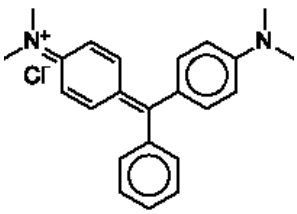
moulds are able to produce toxic secondary metabolites, collectively known as mycotoxins. Three genera are particularly important in feeds and foods: *Aspergillus*, *Penicillium*, and *Fusarium*. In terms of acute toxicity the most commonly encountered mycotoxins in food and feed are less toxic than 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 and also our monitoring program for fish feed, 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 15 samples of fish muscle from 2007 and 20 samples from 2008 were examined with respect to Ochratoxine A. Both sets of data are included in the current report. This parameter was not included in the 2007 report, since the method was not ready in time for the report.

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 use in food-producing animals is 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 malachite green.

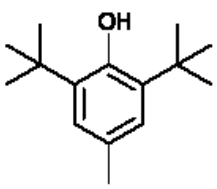
Procedure (NIFES method 264, MG and LMG):
The samples are extracted with acetonitrile and dichloromethane in an acidic environment. Solid phase extraction is used for sample clean-up. The method includes a first scanning for presence of LMG by LC-MS. If any LMG is present, it is oxidized to MG with PbO_2. Total MG (MG+LMG) is quantified with LC-UV. A reversed phase column is used for separation, and MG is detected at a wavelength of 620 nm. Quantification is based on the external standard method with a three point calibration curve.

Procedure (Eurofins, MG, LMG, CV, LCV and BG):
The samples are extracted with acetonitrile under acidic conditions. This is followed by a sample clean up. The purified extract is analyzed by LC-MS/MS in a positive ionisation mode.

B3f, Others

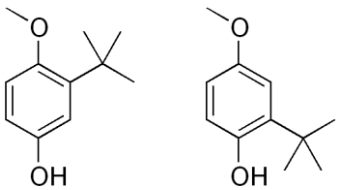
This is a heterogeneous group of compounds that is analyzed as part of Norwegian national measures. It includes the synthetic antioxidants BHA, BHT and ethoxyquin, the perfluorinated organic compounds (PFC) and polycyclic aromatic hydrocarbons (PAH).

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.

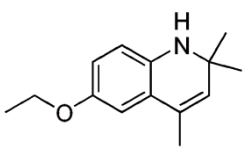


Molecular structure of BHT.

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 the 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 accredited with regards to Ethoxyquin and its dimer. The method is so far not accredited for the de-ethylated Ethoxyquin and these data are not reported. 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.

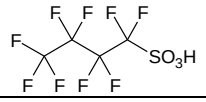
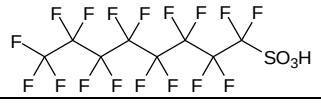
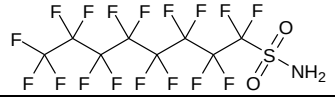
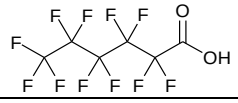
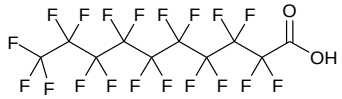
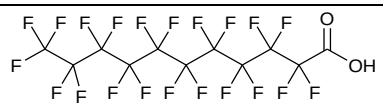
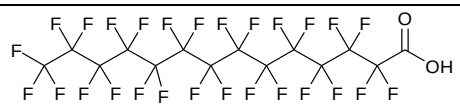
BHA (NIFES method *294)*
The compound is easily destroyed by light and by air. A thorough shielding against light and atmospheric oxygen is required for any analytical step after the extraction from the muscle tissue. BHA is extracted directly with Acetonitrile with 0.1% ascorbic acid. The BHA concentration 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.

Perfluorinated compounds (PFCs).
These synthetic organo-fluorine molecules are a class of compounds that have fluorine rather than hydrogen attached to their chemical structure. But they also contain at least one different atom or functional group. Thus, they have properties similar to fluorocarbons as they are fluorocarbon derivatives. They have unique properties to make materials stain, oil, and water resistant, and they are widely used in diverse applications. Due to the exceptionally strong C-F bond they are very chemically inert, useful for applications like waterproofing coats, lubricants, surfactants, preservatives and coatings on frying pans. From all these sources the compounds will eventually find their way into the environment. The cold ecosystems in the Northern and the Southern hemispheres will be their final recipients. These compounds bio-accumulate in the higher trophic levels of the ecosystems. Their inertness makes them very

persistent in the environment. They have a low acute toxicity, but there is a rising concern for effects from continuous exposure. The chemical properties of these compounds are an analytical challenge. Today analytical data with increasing degree of reliability are being published. These compounds have been determined in domestic as well as wild animals and also in human tissues like blood [7] and breast milk [8]. There is a growing interest in these data from consumers as well as researchers. There is now an effort to change the use from compounds with long molecular chains to those with a shorter one to reduce the potential for bio-accumulation [9]. So far no MRL or MRPL values have been established for their levels in fish. In May 2009 Perfluorooctanesulfonic acid (PFOS) was included in the Stockholm Convention on persistent organic pollutants. The use of PFOS is restricted in Europe [10] In Canada there is a proposed a ban on PFOS, the second chemical proposed for a complete ban under the Canadian Environmental Protection Act [11].
The structures of common PFC compounds are shown in Table 3.

[7] Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL (Nov 2007). "Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000". Environ Health Perspect. 115 (11): 1596–602.
[8] LIN TAO (1) ; KANNAN Kurunthachalam; WONG Chung M. ; ARCARO Kathleen F. ; BUTENHOFF Iohn L. , "Perfluorinated Compounds in Human Milk from Massachusetts, U.S.A", Environmental science & technology, 2008, vol. 42, no8, pp. 3096-3101
[9] Renner R (January 2006). "The long and the short of perfluorinated replacements". Environ. Sci. Technol. 40 (1): 12–13.
[10] Directive 2006/122/EC of the European Parliament and of the Council of 12 December 2006
[11] Environmental Defence: "Stain Repellant Chemical, PFOS, Listed for "Virtual Elimination"" News Release. (April 21, 2008).

Table 3. The structure of common PFC compounds.

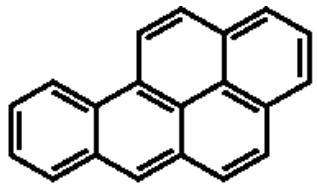
Abbreviation	Name	Chemical structure
PFBS	Perfluorobutane Sulfonic Acid	
PFOS	Perfluorooctane Sulfonic Acid	
PFOSA	Perfluorooctane Sulfonic Amide	
PFHxA	Perfluorohexanoic Acid	
PFHpA	Perfluorohepanoic Acid	
PFOA	Perfluorooctanoic Acid	
PFNA	Perfluorononanoic Acid	
PFDcA	Perfluorodeanoic Acid	
PFUnA	Perfluoroundecanoic Acid	
PFTeA	Perfluorotetradecanoic Acid	

Procedure *(NIFES method 349)*: A mixture of internal standards, eight of them ^{13}C labelled analogues of the corresponding PFC, is mixed in with a homogenized sample. Extraction with methanol takes place in an ultrasonic bath. Solid remains are removed from the extract by centrifugation and by the use of a 0.45 μm nylon syringe filter as the liquid sample is transferred to a clean vial. Clean-up is performed on the ASPEC™ XL4 (Gilson, Middleton WI, USA) analytical robot using an OASIS® WAX chromatographic column. The purified extract is analyzed by LC / MS / MS and quantified using the internal standard method.

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 4. Number of fish of each species and parameter, counting single fish and fillets.

Class of compounds			# fish	Salmon	Rainbow Trout	Turbot	Halibut	Cod	Arctic char	Pollock
Samples taken from the farms with no pre-notice	A1 Stillebenes	Diethylstilboestrol	260	220	10			30		
		Dienoestrol	260							
		Hexoestrol	260							
	A3 Steroids	Nandrolon alfa	280	245	25			5		5
		Nandrolon beta	280							
		Trenbolon alfa	280							
		Trenbolon beta	280							
	A6 Illegal drugs:	Chloramphenicol	510	440	30		15	25		
	Annex IV to EEC 2377/90	Metronidazole + metabolite	672	581	40		5	25	11	
		Furazolidone	500	400	35		15	50		
		Furaltadone	500	400	35		15	50		
		Nitrofurantoine	500	400	35		15	50		
		Nitrofurazone	500	400	35		15	50		
		Malachite green + metabolite	120	70	35	5		10		
Samples taken from the slaughter House	B1 Chemical method on muscle	Flumequine	50	20	5			10		15
		Florfenicol	45	5			5	30		5
		Oxolinic acid	50	20	5			10		15
		Oxytetracycline	35	35						
	B1 Microbiological assay on liver	Quinolones	325	280	10	35				
		Tetracyclines and amphenicols	325	280	10	35				
		Sulphonamides	325	280	10	35				
	B2 Other veterinary drugs	Teflubenzuron	95	95						
		Diflubenzuron	95	95						
		Cypermethrine	95	95						
		Praziquantel	360	340	15		5			
		Fenbendazole	150	125	25					
		Emamectin	280	250	5		20	5		
		Ivermectin	35	35						
		Deltamethrin	105	90	15					
	B3a Organochlorine compounds	HCB	224	199	15			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								
		DDT, DDE og DDD orto-para + para-para	754	649	75	5		25		
		Dioxins + Dioxin like PCBs	458	402	50				6	
		PCB-7	467	407	55			5		
		PBDE (10)	23	18	5					
		HBCD, α , β, γ	21	16	5					
		TBBPA								
	B3c Chemical elements	Pb	851	701	70	5	5	55		15
		Cd								
		Hg								
		As								
	B3d	Mycotoxins	175	160	10			5		
	B3e, Dyes	Malachite green: MG+LMG	320	265	55					
		Crystal violet + metabolite	320	265	55					
		Brilliant green	320	265	55					
	B3f Others	BHT	50	50						
		BHA								
		Ethoxyquin+ dimer								
		PFC-10	22	17	5					
		PAH	45	40	5					

Results and discussion

Group A

A total of 467 fillet samples from 2335 fish were collected for analysis of pharmacologically active substances under group A. The samples were taken on the facility 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 52 pooled samples from a total of 260 fish from four species. Analysis 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 4. None of the substances were detected.

Group A3

The levels of group A3 substances nortestosterone (nandrolon) and trenbolon were analyzed in 56 pooled samples from 280 fish from five species. No single fish sample is part of the data. 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 4. None of the substances were detected.

Group A6 (annex IV to EEC 2377/90)

A total of 359 pooled samples from 1795 fish were analysed in this group. No residues of A6/Annex IV drugs were detected in any sample. The detection limits (LOD) are listed in the Table 2.

Group B

There were a total of 1225 pooled and single fish samples of fillets from a total of 5756 fish for chemical analysis, and 975 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 8589 analytical determinations were conducted in class B.

Group B1, antibacterial agents

The antibacterial agents in class B1 was analyses by a combination of chemical and the three plate bioassay: Quinolones, amphenicols, tetracyclines and sulphonamides

were measured in liver from 325 fish using three plate bioassay, giving a total of 975 determinations. These parameters were also analyzed in 36 pooled fillet samples, representing 180 fish by four 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 pooled fillet samples. Emamectin benzoate could be detected in nine of the totally 56 examined samples included in the monitoring program of 2008. According to the analytical protocol, any detection of drug residues above the LOD 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 the nine positive samples. Table 5 gives more information on the positive samples. Residues of other agents or their metabolites were not found in any of the samples. Detection limits (LOD) for the substances are specified in the Table 2.

Journal No.	Geographic area of sampling	Initial analysis (µg/kg)	Re-analysis, average of three parallels (µg/kg)	Backup sample (µg/kg)
2008-960/1	Hardanger	4,9 and 2,1	6,6	7,9
2008-836/4	Bergen	4,4	6,2	6,2
2008-971/2	Bergen	6,3	8,9	9,0
2008-940/7	Ytre Helgeland	2,8	0	-
2008-781/6	Søre Sunnmøre	4,1	3,5	3,0
2008-673/3	Fosen	2,8	0	-
2008-878/6	Sunnhordland	2,8	1,7	2,7
2008-1718/7	Namsos	7,5	9,1	-
2008-1736/3	Namsos	7,9	8,0	-

Table 5. Information on samples positive for emamectin benzoate.

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 445 samples from 1968 fish in this group and 5703 analytical determinations have been carried out. The results are summarised in the Tables 6 to 10.

DDT and its metabolites

The values are listed in Table 6. 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 such cases. In this report the data are for the first time presented for each fish species separately. There is a significant variation between the species. This is consistent with their variation in fat content. The chemical compounds of this group are lipofilic and water phobic. They are found in fatty tissues.

UB-sum of DDT and its metabolites show a variation from the theoretical minimum UB sum of 0.9 µg/kg to a value of 17.3 µg/kg wet weight. The UB-sum mean value is highest in salmon: 8.8 µg/kg wet weight followed by rainbow trout 7.8, turbot 1.1 and cod 0.9. This sequence is consistent with the fat content in the fillets. The use of UB calculation in the reports of 2007 and 2008 must be taken into account when comparing results with previous years: In 2003-2006 the overall mean values (including all species) varied from 10 to 15 µg/kg wet weight. Thus there is a slight decrease since 2003. The results correspond to figures reported in NIFES database "Seafood Data" (www.nifes.no/sjomatdata). The highest values in the whole period since 2003 have been found for the pp-DDE compound. The para-para compounds have higher concentration than the orto-para compounds.

Table 6. Concentration (µg/kg wet weight) of DDT, DDD and DDE in the fillet. Pooled and single samples (µg/kg w.w.).

	op-DDT	pp-DDT	op-DDD	pp-DDD	op-DDE	pp-DDE	UB-sum DDT
LOQ	0.18	0.24	0.09	0.09	0.15	0.12	0.9
Salmon							
N	141	141	141	141	141	141	
UB-Mean	0.3	1.0	0.3	2.3	0.2	4.7	8.8
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.1	2.2
Max	1.3	2.2	1.0	5.5	3.7	9.7	17.3
Rainbow trout							
N	15	15	15	15	15	15	
UB-Mean	0.2	1.0	0.2	2.3	0.2	4.0	7.8
Min	0.2	0.49	<LOQ	1.1	<LOQ	2.0	4.1
Max	0.2	1.6	0.6	3.9	0.2	6.5	12.2
Cod							
N	5	5	5	5	5	5	
UB-Mean	-	-	-	-	-	-	0.9
Min	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.9
Max	<LOQ	<LOQ	<LOQ	0.1	<LOQ	0.2	0.9
Turbot							
N	1	1	1	1	1	1	
Value	0.2	<LOQ	<LOQ	0.1	<LOQ	0.24	1.0

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

PCB_7

The values for the seven PCBs selected for marine monitoring by ICES are given in Table 7. The sums, calculated as the "upper bound-LOQ" (UB) in the 162 samples ranged from the theoretical limit of 0.7 to 15.5 µg/kg wet weight. In 2007 the range was from 3.0 µg/kg to 18 µg/kg wet weight. In previous years the mean values were calculated for the entire dataset rather than for each species. In 2007 the UB-mean was 8.5 µg/kg. Prior to 2007 the lower bound sums (LB) were reported: In 2006, 2005, 2004 and 2003 the LB-means were respectively, 8.9 µg/kg, 8.5 µg/kg, 9.2 µg/kg and 8.3 µg/kg. The effect of the change to UB calculation is negligible because there are so few data below the LOQ value. From 2003 to 2008 the congeners PCB-138 and PCB-153 were the largest contributors to the sum (error in the report for 2004.) Like the other lipofilic contaminants there is for PCB a significant difference in levels between the examined species. The UB-sum PCB7 found in this study are similar to those that have been reported in salmon fillet in the "Seafood Data"[12]databank. The effect of the change to UB calculation is negligible because there are so few values <LOQ. There is no obvious trend in the data for the period 2003-2008 for sum PCB. 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 PCB7. The limit is set to 620 µg/kg wet weight in filet for the sum. The highest value in this dataset was 15.5

Table 7. Concentration of the ICES PCB-7 compounds in the fillets of pooled and single 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	87	87	87	87	87	87	87	
UB-Mean	0.3	0.6	1.2	0.8	1.6	1.7	0.4	6.7
Min	0.09	0.2	0.4	0.3	0.5	0.5	<LOQ	2.1
Max	0.9	1.1	2.4	1.6	4.3	4.5	1.4	15.5
Rainbow trout								
N	11	11	11	11	11	11	11	
UB-Mean	0.3	0.6	1.1	0.8	1.6	1.7	0.4	6.4
Min	0.1	0.3	0.6	0.4	0.9	0.9	0.2	3.4
Max	0.4	0.9	1.9	1.2	2.7	2.7	0.7	10.4
Cod								
N	1	1	1	1	1	1	1	
Value	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.7

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

[12] Online database: www.nifes.no

Hexachlorcyclohexane (HCH) and hexachlorbenzene (HCB)
The data for these two compounds are summarised in Table 8. In 2008 a total of 52 pooled and single fish samples from 224 fish were analysed for alfa-, beta and gamma-HCH. The levels of all were below their LOQ value for each of them. This is consistent with the results from previous years. The data for HCB in the same samples are summarised in Table 8. Only the two cod samples of the 52 samples were below the LOQ value. On a wet weight basis there is a concentration range from less than LOQ value in cod (<LOQ) to 1.9 µg/kg in salmon. The range in 2007 was from <LOQ to 3 µg/kg and in 2006 from <LOQ to 2 µg/kg and in 2005 to 1.5 µg/kg. There is no obvious trend in this time series. The sequence of the mean values follows the fat content in the fillets: Salmon ≈ Rainbow trout > Cod, as expected.

Other pesticides
These 52 samples were analysed for other pesticides as well. The results for the compounds heptachlor, aldrin, chlordane, nonachlor, endosulfan and toxaphene and molecular variants of these are summarised in Table 8. All except the five compounds Cis chlordane, trans-nonachlor, toxaphene 26, toxaphene 50 and toxaphene 62 were below their LOQ values in all samples and all the species. Also among these five a significant number of values were less than their LOQ values. Thus even in salmon and rainbow trout the UB-mean values are close to the sum of the LOQ values except for cis-chlordane and trans-nonachlor. These low levels are consistent with the findings from previous years. No measurable concentration was found in cod for any of these compounds.

Table 8. Concentration of other pesticides in fillets of pooled and single fish samples (µg/kg w.w.)

	Alfa-HCH	Gama-HCH	HCB	Heptachlor	Heptachlor-A	Aldrin	Oxy-chlordane	Trans-chlordane	Cis-chlordane
LOQ	0.6	2.0	0.07	2.5	0.5	0.6	1.3	0.7	0.5
Salmon									
N	47	47	47	47	47	47	47	47	47
UB-Mean	-	-	1.3	-	-	-	-	-	1.0
Min	<LOQ	<LOQ	0.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.6
Max	<LOQ	<LOQ	1.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2.1
Rainbow trout									
N	3	3	3	3	3	3	3	3	3
UB-Mean	-	-	1.3	-	-	-	-	-	0.8
Min	<LOQ	<LOQ	1.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.7
Max	<LOQ	<LOQ	1.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.9
Cod									
N	2	2	2	2	2	2	2	2	2
Values	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

	Endo-sulfan-a	Endo-sulfan-sulphate	Endo-sulfan-b	Trans-nonachlor	Cis-nonachlor	Toxa-phene-26	Toxa-phene-32	Toxa-phene-50	Toxa-phene-62
LOQ	0.3	0.5	0.3	0.5	1.5	1.0	0.7	2.5	1.5
Salmon									
N	47	47	47	47	47	47	47	47	47
UB-Mean	-	-	-	1.6	-	1.2	-	2.6	1.5
Min	<LOQ	<LOQ	<LOQ	0.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	2.6	<LOQ	2.0	<LOQ	3.9	2.0
Rainbow trout									
N	3	3	3	3	3	3	3	3	3
UB-Mean	-	-	-	1.4	-	1.1	-	2.6	-
Min	<LOQ	<LOQ	<LOQ	1.0	<LOQ	1.0	<LOQ	<LOQ	<LOQ
Max	<LOQ	<LOQ	<LOQ	1.7	<LOQ	1.3	<LOQ	2.8	<LOQ
Cod									
N	2	2	2	2	2	2	2	2	2
Values	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Dioxins, furans and dioxin like PCBs

There are a total of 29 compounds in this class. The weighted sums of these 29, the sum WHO-TEQ (ng/kg) w.w. are summarised in Table 9. All figures in the table are calculated as the "upper bound-LOQ" sum (UB-sum) and they are weighted with toxicity factors to give TEQ values. A total of 98 single fish and pooled samples were analysed for a total of 458 fish from salmon, rainbow trout and arctic char. For the 17 dioxin and furan compounds (PCDD + PCDF) the sum values ranged from 0.1 ngTEQ/kg to 0.5 ngTEQ/kg w.w. The upper limit in the EU for the sum is 4 ngTEQ/kg w.w. The mean of their sums was in the range 0.2-0.3 ngTEQ/kg w.w for each of the three species. Both the means and the range are very consistent with the values found each year since 2004, even if the data from 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 and 0.3 ngTEQ/kg w.w. There seems to be no trend in this period. No single fish data in this period were close to or above the EU's upper limit of 4.0 ngTEQ/kg w.w.

The dioxin-like PCBs compounds (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 0.3 to 1.7 ngTEQ/kg wet weight. Their mean was 0.7 ngTEQ/kg in all three species. Both mean and range are consistent with the values found each year since 2004. The means in these years are respectively: 0.8, 1.0, 1.2 and 0.9 ngTEQ/kg w.w. There is no obvious trend in this period. There is no upper limit for the sum of the DLPCBs.

The figures for the total sum of TEQ range from 0.4 to 2.2 ngTEQ/kg w.w. The mean has a range from 0.8 to 1.0 ngTEQ/kg w.w in these species. This is a factor of 10 below the limit. The means in the years 2004-2007 were respectively: 1.2, 1.1, 1.5 and 1.3 ngTEQ/kg w.w. The upper limit in the EU for the total sum of TEQs is 8.0 ngTEQ/kg w.w. No single fish data in this period were 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".

The results confirm that the levels of DLPCB are higher than the PCDD/PCDF levels.

Table 9. levels of dioxins, furans (PCDDs/ DFs) and the dioxins-like PCBs (ng TEQ/kg w. w.).

	Sum PCDDs	Sum PCDFs	Sum PCDD+ PCDF	Non orto PCBs	Mono orto PCBs	Sum DLPCBs	Total sum TEQ
LOQ**	--	--	--	--	--	--	--
EU-limit			**4.0**				**8.0**
Salmon							
N	**86**	**86**	**86**	**86**	**86**	**86**	
UB-Mean	**0.09**	**0.2**	**03**	**0.5**	**0.2**	**0.7**	**1.0**
Min	**0.04**	**0.07**	**0.1**	**0.2**	**0.07**	**0.3**	**0.4**
Max	**0.2**	**0.3**	**0.5**	**1.2**	**0.5**	**1.7**	**2.2**
Rainbow trout							
N	**10**	**10**	**10**	**10**	**10**	**10**	
UB-Mean	**0.09**	**0.2**	**0.2**	**0.2**	**0.5**	**0.7**	**1.0**
Min	**0.07**	**0.1**	**0.2**	**0.2**	**0.7**	**0.5**	**0.7**
Max	**0.12**	**0.2**	**0.3**	**0.3**	**0.4**	**0.9**	**1.2**
Arctic Char							
N	**2**	**2**	**2**	**2**	**2**	**2**	
UB-Mean	**0.06**	**0.1**	**0.2**	**0.5**	**0.2**	**0.7**	**0.8**
Min	**0.06**	**0.1**	**0.2**	**0.6**	**0.2**	**0.5**	**0.7**
Max	**0.07**	**0.1**	**0.2**	**0.4**	**0.2**	**0.8**	**1.0**

*** 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 10. Concentrations of the brominated flame retardants, PBDE, HBCD and TBBP-A (µg/kg wet weight) in the fillet of single and pooled fish samples.

	PBDE-28	PBDE-47	PBDE-99	PBDE-100	PBDE-153	PBDE-154	PBDE-183	UB-Sum PBDE-7
LOQ*	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.021
Salmon								
N	14	14	14	14	14	14	14	14
UB-Mean	0.04	0.8	0.15	0.16	0.02	0.10	0.01	1.3
Min	0.02	0.53	0.09	0.10	0.02	0.06	<LOQ	0.8
Max	0.07	1.3	0.30	0.26	0.04	0.15	0.02	2.0
Rainbow trout								
N	1	1	1	1	1	1	1	1
Value	0.04	0.64	0.13	0.13	0.03	0.08	<LOQ	1.0

	PBDE-66	PBDE-119	PBDE-138	Sum HBCD	Alfa HBCD	Beta HBCD	Gama HBCD	TBBP-A
LOQ	0.003	0.003	0.003	0.5	1	1	1	1
Salmon								
N	14	14	14	14	8	8	8	8
UB-Mean	0.04	-	-	0.5	-	-	-	-
Min	0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max	0.10	<LOQ	<LOQ	0.6	<LOQ	<LOQ	<LOQ	<LOQ
Rainbow trout								
N	1	1	1	1	1	1	1	1
Value	0.03	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

* 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 10. A total of 15 samples of single and pooled salmon and rainbow trout samples from a total of 23 fish were analysed. HBCD is determined in two methods: Sum HBCD is determined as part of the PBDE method. But, the individual HBCD compounds with names alpha- beta- and gamma-HBCD are determined in a separate method. Unfortunately, this second method has higher LOQ values, higher than the levels in the measured samples. Thus no actual figures were reported for the individual HBCD compounds. Total HBCD in salmon shows the UB-average of 0.5 µg/kg and a range from <LOQ to 0.6 µg/kg w.w. Like the individual HBCD data the TBBP-A values are below the LOQ value in all samples. The values for the sum PBDE-7 range from 0.8 to 2.0 µg/kg w.w in salmon. In rainbow trout the single value was 1.0 µg/kg w.w. The data from 2007 include a few lean fish. Still the 2007 UB-average is 1.5 µg/kg w.w, close enough to the 2008 value of 1.3 µg/kg w.w. given the analytical uncertainty. The similarity in structures from the PBDE class to the PCB class makes it natural to compare the data from these classes. Sum PBDE-7 levels are less than 20% of the sum PCB-7 levels. Also, the PBDE compounds are the least toxic of the two classes.

Group B3b, Organophosphorous compounds

No B3b compound was part of the project in 2008.

Group B3c, Chemical elements

The analytical values for the elements are summarised in Table 11. They were determined in 175 pooled and single fish samples made from the filets of 851 fish.

Arsenic (As)

In Table 11 we see that the arsenic levels in the fillet of farmed fish ranged from <LOQ to 3.1 mg/kg w.w. The UB-mean ranged from 1.4 mg/kg w.w. in pollock to 1.9 in cod. In the period 2004-2007 the average levels were respectively: 2.1, 2.0, 1.4, and 1.6 mg/kg w.w. (all except 2007 were calculated as LB-average). Thus the level of arsenic in fillets has in this period been fairly constant. The levels in the lean species cod and pollock are not significantly different from the others.

Table 11. Concentrations of the heavy metals (As, Cd, Hg and Pb) (mg/kg w.w.) in the fillet of single and pooled fish samples.

	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)
LOQ**	0.03	0.01	0.03	0.015
EU-limit	--	0,05	0,5	0,3
Salmon				
N	145	145	145	145
UB-Mean	1.5	-	0.04	-
Min	<LOQ	<LOQ	0.02	<LOQ
Max	2.80	0.06	0.17	0.02
Rainbow trout				
N	14	14	14	14
UB-Mean	1.7	-	0.05	0.02
Min	1.10	<LOQ	0.04	<LOQ
Max	3.10	<LOQ	0.08	0.10
Cod				
N	11	11	11	11
UB-Mean	1.7	-	0.10	-
Min	0.77	<LOQ	0.03	<LOQ
Max	2.40	<LOQ	0.14	<LOQ
Pollock				
N	3	3	3	3
UB-Mean	1.4	-	0.08	-
Min	1.20	<LOQ	0.08	<LOQ
Max	1.60	<LOQ	0.09	<LOQ
Halibut				
N	1	1	1	1
Value	1.8	<LOQ	0.08	<LOQ
Turbot				
N	1	1	1	1
Value	1.7	<LOQ	0.09	<LOQ

Cadmium

In 2005, a Cd contaminated feed ingredient led to Norwegian farmed fish being banned from the Russian market from alleged elevated values of Cd found in Norwegian farmed salmon. But none of the samples analysed in the framework of this project in 2005 and 2006 showed measurable concentrations of cadmium (all <LOQ), and in 2007, we found that 158 of a total of 184 values were <LOQ. Now, the 2008 data show that 162 of a total of 175 are below measurable concentrations. The maximum measured value was this year 0.06 mg/kg w.w. (actually 0.55 mg/kg). In 2007 the maximum value was 0.01 mg/kg w.w.. The EU's upper limit is 0.05 mg/kg w.w. Taking into consideration the measurement uncertainty of ±25% in this concentration range this value does not qualify for a legally non-compliant status. It is still the highest value we have measured in a Norwegian farmed fish as part of this project. The value was found in a salmon sample. The UB-average value in 2007 and 2008 exaggerates the apparent difference compared to previous years where lower bound calculations were used. Still the possibility of an increasing trend in Cd levels cannot be ruled out from these findings.

Mercury

Table 11 shows that the concentration of total mercury ranged from 0.02 to 0.2 mg/kg w.w in 2008. This is identical to the range in 2007. The UB-average ranged over the analysed species from 0.04 to 0.09 mg/kg w.w. The highest value was found in the single turbot. In contrast the EU's upper limit is 0.5 mg/kg w.w. for these products.

Lead

The data are summarised in Table 11. Only three samples out of 175 had measurable concentrations, all rainbow trout. The UB-calculated mean for trout was 0.02 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 to 0.3 mg/kg, in "muscle meat of fish".

Trends since 2003

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 1030 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 arsenic. The concentrations of cadmium were in all samples since 2002 less than or equal to 0.01 mg/kg w.w. accept for one single sample from 2008. That sample was, within the analytical uncertainty, found at the value of the EU 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 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. Thus, based on 1030 samples made from more than four thousand fish we conclude that no incompliant sample was found. And that Norwegian farmed fish are well below the EU upper concentration limits for the heavy metals.

Group B3d, Mycotoxins

The 20 samples from 2008 were analysed for ochratoxine A by a new analytical method adapted to marine samples. Of the 20 samples, 17 were salmon, 2 were rainbow trout and one sample was from cod. In addition15 samples for fish muscle from 2007, not previously reported are included here. 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 88 pooled samples from 440 fish were examined with respect to malachite green and its leuco form. Crystal violet, leuco crystal violet and brilliant green were each examined for in 64 samples from 320 pooled fish. No residues of any of these agents were detected.

Group B3f, Others

BHA, BHT and Ethoxyquin (synthetic antioxidants)
The results are summarised in Table 12. All samples were salmon. All data for the synthetic antioxidants are on a wet weight basis.The concentration range for BHT 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 range reflects an unexpected high variability. But unlike the other parameters these are all 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/kg w.w. for BHT. 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 0.006-0.08 mg/kg w.w., and the mean was 0.02 mg/kg like last year. There is no EU or national Norwegian MRL for BHA in food. However, Japan has an MRL for BHA of 0.5 mg/kg.

The data for ethoxyquin are summarised in Table 12. It was recently established that this (accredited) method overestimates the true analytical value. Thus the true concentrations are below the values listed in the table. This is the case for the 2007 and 2006 reports as well. There is no EU or national Norwegian MRL in food for ethoxyquin. 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 0.2 mg/kg w.w. The concentration of ethoxyquin dimer is higher than that of the parent compound.

Table 12. Concentrations of synthetic antioxidants in the fillet of individual salmon samples. (mg/kg w.w.).

	BHT	BHA	Ethoxyquin	Ethoxyquin dimer	Sum Ethoxyquin
N	50	50	50	50	50
UB-Mean	5.4	0.02	0.04*	0.6*	0.7*
Min	0.41	0.006	0.003*	0.2*	0.2*
Max	24.5	0.08	0.2*	2.2*	2.5*

***) All values for ethoxyquin are overestimated and should be read as "less than" values.**

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.

The PFC class

Table 13 lists the results for the Perfluorinated organic compounds. There are seven single fish and two pooled samples in the data, all samples were salmon. No quantifiable concentration was found for any of the compounds in the nine samples analysed. The LOQ values are listed in the table. This is consistent with the results from the 84 samples in 2007. The LOQ values range from 1-4.5 µg/kg w.w.

Table 13. Concentrations of PFC compounds in the fillets of single and pooled samples of fish (µg/kg wet weight); N=9 salmon and 1 rainbow trout.

Compound	Max value	LOQ
PFBS	<LOQ	4,5
PFOS	<LOQ	3
PFOSA	<LOQ	1,5
PFHxA	<LOQ	6
PFHpA	<LOQ	4.5
PFOA	<LOQ	1
PFNA	<LOQ	1
PFDcA	<LOQ	1
PFUnA	<LOQ	1
PFTeA	<LOQ	1.5

The PAH class

Table 14 summarises the results for the PAH compounds. Twenty-one samples were analysed, twenty of them salmon and one rainbow trout. 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 twenty samples had measurable levels of BaP. This is consistent with the results from 2007 when only one of 84 samples had a measurable level. Most of the 13 PAH compounds reported have no measurable value in the 20 samples. Only phenantrene and fluorene are measurable in more than 50% of the samples. These two have lower carcinogenic effects than Benzo[A]Pyrene.

If these results are compared to the 2007 data, keep in mind that one of the samples in 2007 was obviously contaminated, to the point that it affected all the maximum values in Table 16 of the 2007 report.

Table 14. Concentrations of PAH compounds in fillets (µg/kg wet weight) ; N=20 Salmon and 1 Rainbow trout.

Compound	LOQ	Min. Value salmon	Max. value salmon	UB-mean salmon	Rainbow trout 1 sample	EU-Limit
Flurene	0,5	<LOQ	5.2	2.8	1.4	
Phenantrene	0,5	<LOQ	5.7	1.8	2.0	
Antracene	0,5	<LOQ	0.6	0.5	<LOQ	
Fluranthene	0,5	<LOQ	0.8	0.5	0.9	
Pyrene	0,5	<LOQ	1.2	0.6	<LOQ	
Benzo[a]antracene	0,5	<LOQ	<LOQ	--	<LOQ	
Chrysene/ trifenylene	0,5	<LOQ	<LOQ	--	<LOQ	
Benzo[b]fluoranthene	0,5	<LOQ	<LOQ	--	<LOQ	
Benzo[k]fluoranthene	0,5	<LOQ	<LOQ	--	<LOQ	
Benzo[a]pyrene	0,5	<LOQ	<LOQ	--	<LOQ	2.0
Indeno[123-cd]pyrene	0,5	<LOQ	<LOQ	--	<LOQ	
Dibenzo[ah]anthracene	0,5	<LOQ	<LOQ	--	<LOQ	
Benzo[ghi]perylene	0,5	<LOQ	<LOQ	--	<LOQ	
UB-Sum of PAH	--	6	14.7	9.5	9.4	

Summary

With the increasing production volume of farmed fish in Norway the marine part of the EU Directive 96/23/EC monitoring program is now extensive. This report is based on a total of 9066 fish and 9785 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 the sums, and mean values in this report will be systematically higher than 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, nine out of 56 examined samples were found to contain emamectin benzoate. The highest concentration found in one individual sample was 9.1 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 35 pooled samples made from 175 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 2007, 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. But the sum PBDE-7 amount to less than 20% of the value for the sum PCB-7. Their toxicity is also lower. All samples analyzed for TBBPA had levels below 1.0 µg/kg w.w. Total HBCD has the UB-mean of 0.5 and a maximum value of 0.6 µg/kg w.w.

In the class of heavy metals over the years since 2002 a total of 1030 samples have been analysed. For all these samples the maximum value for arsenic was 6.3 mg/kg w.w. The concentrations of cadmium were in all these samples less than or equal to 0.01 mg/kg w.w. accept for one single sample that, within the analytical uncertainty, was found at the value of the EU upper limit. The levels of mercury were below or equal to 0.23 mg/kg w.w. The levels of lead were less than or equal to 0.1 mg/kg w.w. The EU upper limits for cadmium, mercury and lead are 0.05, 0.5 and 0.3 mg/kg w.w. respectively. Thus, based on 1030 samples made from more than four thousand fish we conclude that no incompliant sample was found. And that Norwegian farmed fish are well below the EU upper concentration limits for the heavy metals.

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 now 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 food.

Ten PFC compounds (perfluorinated organic compounds) are included in the program. This class is characterized by a low acute toxicity and very high degree of persistence in the environment. For all the ten compounds the levels in the nine samples were less than their LOQ values. This confirms that the levels of PFCs are low in Norwegian farmed fish.

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 below the LOQ in all of the twenty samples analysed in 2008. In the majority of the samples only fluorene and phenantrene are found in measurable concentrations. These are far less potent health hazards than BaP.