Summary Report on 2016 Residue Monitoring of Irish Farmed Finfish

&

2016 Border Inspection Post Fishery Product Testing undertaken at the Marine Institute

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Marine Institute
Foras na Mara

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Marine Institute

Rinville, Oranmore, County Galway

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Foras na Mara

ISO 17025 INAB ACCREDITED TESTING DETAILED IN SCOPE REG NO.130T

The MI scope of accreditation for analysis in this report is detailed in Appendix 2

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Part A

Summary Report on 2016 Residue Monitoring of Farmed Finfish

Carried out under Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products.

1. 2016 OVERALL SUMMARY

In 2016, in excess of 691 tests and a total of 1,933 measurements were carried out on 136 samples (i.e. 126 target samples & 10 suspect samples) of farmed finfish for a range of residues. Implementation of the Aquaculture 2016 Plan involves taking samples at both farm and processing plant:

- 92 target samples taken at harvest [84 farmed salmon and 8 freshwater trout]
- 34 target samples were taken at other stages of production [26 salmon smolts and 8 freshwater trout]
- 10 suspect harvest salmon samples were taken as part of SFPA on-farm investigations

All 2016 samples were compliant with the exception of two harvest salmon samples taken from one farm which were found to have oxytetracycline (Group B1- antibacterial substance) present in excess of the Decision Limit. As part of the on-farm investigation carried out by the SFPA the Marine Institute collected an additional 10 suspect samples, all of which were found to be compliant.

For target sampling of farmed fish, a summary table of the residue results from 2005 - 2016 is outlined in Table 1. Overall, the outcome for aquaculture remains one of consistently low occurrence of residues in farmed finfish, with 0% non-compliant target residues results for the period 2006-2014, 0.11% and 0.10% non-compliant target residues results in 2015 and 2016 respectively.

Table 1: Summary Target Results for Residue program 2005-2016

Year	No. of Target Samples[1]	Total Group A[2]	Total Group B[2]	No. of Results[3]	Non-Compliant Results %
2005	164 (105 , 59)	163/0	164/0	2251/2	**0.09**
2006	162 (104 , 58)	162/0	162/0	2207/0	**0**
2007	161 (103 , 58)	148/0	161/0	2219/0	**0**
2008	162 (103 , 59)	144/0	162/0	2073/0	**0**
2009	146 (98 , 48)	128/0	146/0	1750/0	**0**
2010	141 (92 , 49)	109/0	141/0	1569/0	**0**
2011	140 (92 , 48)	105/0	140/0	1566/0	**0**
2012	169 (112 , 57)	101/0	169/0	1596/0	**0**
2013	137 (91 , 48)	83/0	137/0	1494/0	**0**
2014	136 (91 , 45)	83/0	136/0	1882/0	**0**
2015	124 (91 , 33)	71/0	124/2	1841/2	**0.11**
2016	126 (92 , 34)	65/0	126/2	1933/2	**0.10**

[1]Target samples (sampled at harvest, sampled at other stages of production)
[2] No. of samples tested/No. of samples non-compliant
[3]Total no. of results as target samples taken for Group A and Group B substances are tested for multiple residue categories within each group/No. of non-compliant results

2. BACKGROUND

As with other farmed animals, farmed finfish can be subject to disease and infestation which can have animal welfare, environmental and commercial implications. Therefore, authorised veterinary medicines and treatments may be used, and sometimes must be used, to control disease and infestation as part of health control plans e.g. antibacterial and antiparasitic treatments. The National Residues Control Plan (NRCP) sets out the monitoring requirements for residues in animal products in accordance with Council Directive 96/23/EC of 29 April 1996 *on measures to monitor certain substances and residues thereof in animals and animal products.* On behalf of the Department of Agriculture, Food and Marine (DAFM), the Marine Institute carries out monitoring of chemical residues for aquaculture. The main objectives of the NRCP for Aquaculture are to ensure farmed fish are fit for human consumption, to provide a body of data showing that Irish farmed fish is of high quality, to promote good practices in aquaculture and to comply with EU Directive 96/23/EC. The Food Safety Authority of Ireland (FSAI) co-ordinates the activities of the various departments and agencies involved in delivering this programme. For the aquaculture sector, the Sea Fisheries Protection Authority (SFPA) with technical support from the Marine Institute is responsible for residue controls on farmed finfish to ensure compliance with the Residue Directive (96/23/EC). A summary of each department and agencies' role with respect to the NRCP is outlined in Table 2.

Table 2: Department and Agency Roles

Department of Agriculture Food and Marine (DAFM) - Implements the overall residues controls in Ireland
Food Safety Authority of Ireland (FSAI) - Coordinates the activities of the departments and agencies involved
Sea Fisheries Protection Authority (SFPA) - Ensures compliance with the Directive for finfish aquaculture
Marine Institute - Implements the surveillance monitoring programme for farmed fish and is the official laboratory for residue sampling and analysis. The MI is National Reference Laboratory (NRL) for a number of substances in aquaculture
DAFM Veterinary Inspectors - Carry out routine on-farm inspections to verify compliance with various regulations including fish health, animal remedies, feedstuffs, etc

2.1 National Residue Control Plan (NRCP)

Annually, the Marine Institute (MI) prepares the NRCP for Aquaculture, which is reviewed and finalised by SFPA, FSAI and DAFM. The NRCP once agreed is then submitted to the European Commission (EC) for approval, this sets out the monitoring plan, including species, sample numbers and target substances in line with the specific requirements of the Directive. The national legal basis for the Residue Monitoring Plan is provided for in the Animal Remedies Act, 1993 and other relevant legislation in particular, the Control of Animal Remedies and their Residues Regulations, 2009. Figure 1 illustrates the National Aquaculture Residue Control Cycle. The 2016 NRCP is available in Appendix 5.

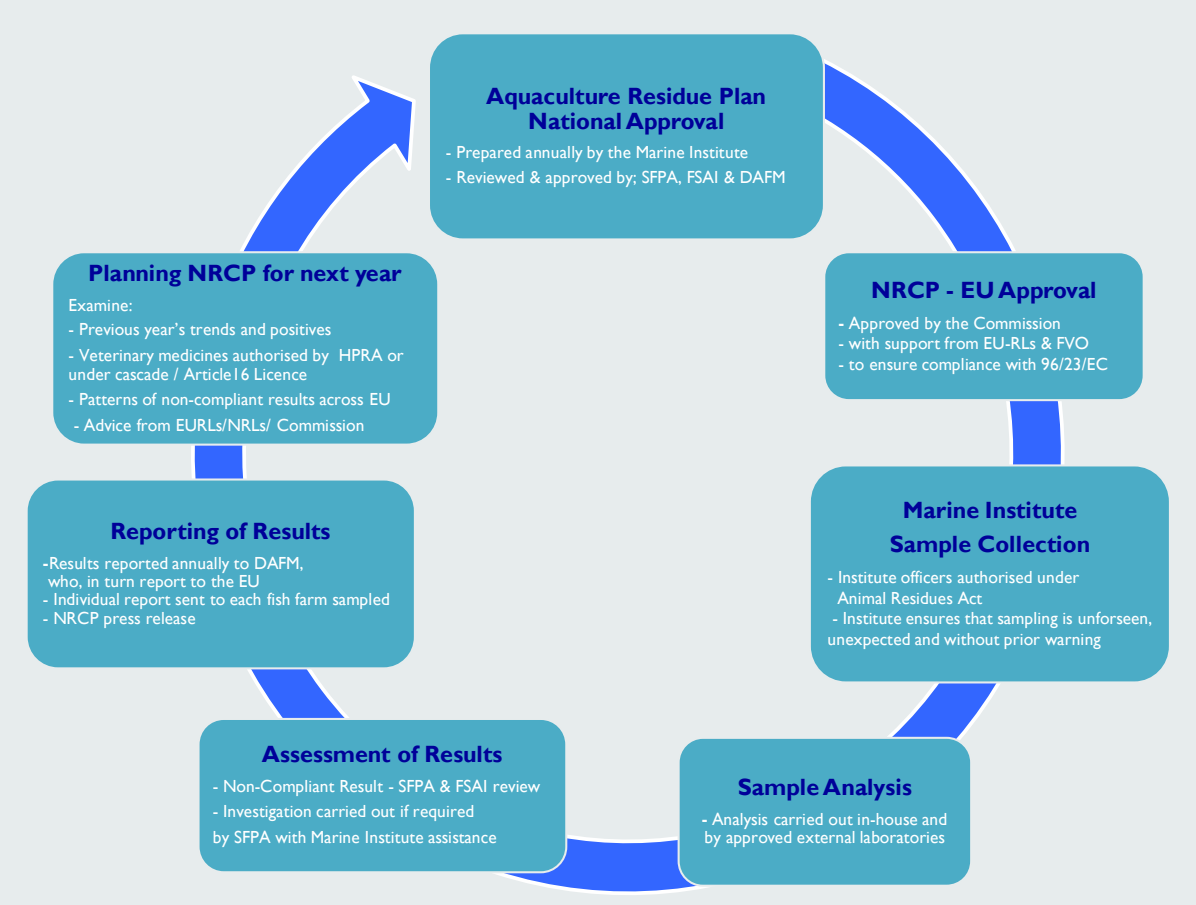

Figure 1: National Aquaculture Residue Control Cycle

2.2 Scope of NRCP

The scope of this testing under the NRCP is comprehensive covering the following broad categories outlined in Table 3.

Table 3: **NRCP testing categories**

Category	Details
Banned	These compounds should **not** be present as no safe limit can be set for their residue e.g. steroids, chloramphenicol, nitroimidazoles
Authorised	Authorised medicines which may be used in aquaculture and should be **below statutory limit** (i.e. Maximum Residue Limit – MRL*) e.g. Sea lice treatments- emamectin, deltamethrin
Unauthorised	These compounds should **not** be present as these treatments should **not be used** in aquaculture. e.g. malachite green
Environmental contaminants	Certain contaminants occur naturally in the environment but they may also be introduced inadvertently and may accumulate in fish e.g. polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), heavy metals

*MRL = maximum concentration allowable in the edible portion of the animal which should not be exceeded at the time of harvest.

These substances are classed into 2 categories: Group A & Group B. Details are given in Table 4.

Table 4: List of substances included in the NRCP for farmed finfish

Group A - Substances having an anabolic effect	
A3	Steroids
A6	Compounds included in Annex IV of Council Regulation 2377/90/EC
Group B - Veterinary drugs and contaminants	
B1	Antimicrobials (Antibacterial)
B2a	Anthelminthics (Antiparasitic)
B2c	Pyrethroids
B2f	Other pharmacologically active substances
B3a	Organochlorine compounds
B3c	Chemical elements
B3d	Mycotoxins
B3e	Dyes

Group A

Group A substances are banned substances and should not be present in farmed finfish. These can be categorised as the following:

- A3 steroids, beta-oestradiol and methyltestosterone which occur naturally but also could be used for growth promotion.
- A6 compounds, nitrofurans and nitroimidazole which are antibacterial drugs, and chloramphenicol a broad spectrum antibiotic.

Group B

Group B substances can be categorised into unauthorised substances, authorised substances and environmental contaminants. Farmed finfish can be subject to disease and infestation which can have animal welfare, environmental and commercial implications. Therefore, similar procedures are in place for farmed finfish as for other farmed animals which may involve treatment with approved veterinary medicines such as antibiotics or anthelminthics to prevent or treat disease or infestation e.g. antibacterial agents, antifungal agents, antiparasitic treatments. Farmed finfish can also accumulate trace metals and persistent organic pollutants from their feed or the environment; therefore, levels of these contaminants are also determined.

3. SAMPLING

In 2016, samples were taken in accordance with Council Directive 96/23/EC by Marine Institute Authorised Sampling Officers (Authorised under the Animal Remedies Act 1993). The Institute ensures that sampling is unforeseen, unexpected and without prior warning in accordance with Article 3 of Regulation 882/2004 and Article 12 of Council Directive 96/23/EC and a strict chain of custody is maintained. Samples are taken throughout the year in an effort to spread sampling across different sites and are taken in accordance with the NRCP i.e.

- One third of the samples are taken 'on farm' at the smolt stage which is aimed at detection of illegal treatment (prohibited substances Group A and unauthorised substances Group B3 (e) - Dyes).

- Two thirds of the samples are taken at harvest stage which is aimed at controlling the compliance with the Maximum Residue Limits (MRL) and for detection of illegal treatment (prohibited substances Group A and unauthorised substances-e.g. Group B3 (e) - Dyes). These harvest samples are taken primarily at processing plants for salmon and 'on farm' for freshwater trout.

In 2016, a total of 126 target (surveillance) samples were taken from fish farms and processing plants in accordance with the NRCP for Aquaculture 2016 (Appendix 5).

- 34 target samples were taken at other stages of production (OSOP); 26 salmon smolts and 8 freshwater trout were collected from seven farms for Group A substances and dyes.

- 92 target samples were taken at harvest which comprised of 84 farmed salmon and 8 freshwater trout. These harvest samples were collected during 19 sampling events (samples collected from a given site at a given time) throughout the year. Salmon were collected on 17 occasions and freshwater trout on 2 occasions. In 2016 no sea reared trout samples were taken. Samples were collected from the same producers on a number of occasions due to the small number of active harvest sites in the given year.

Generally, 5 fish were taken from each producer and each individual fish was treated as a sample. However, where an individual fish was not large enough to provide sufficient test material, a number of fish were pooled to provide a sample. Samples were further subsampled as multiple tests were typically performed on individual samples.

Suspect sampling took place in 2016 following the confirmation of oxytetracycline (Group B1-antibacterial substance) in excess of the Maximum Residue Limit (MRL) in two harvest salmon samples from one farm. As a result of this finding, an on-farm investigation was carried out by the SFPA which included further sampling by the Marine Institute where 10 suspect samples were taken in June 2016.

4. RESULTS OF ANALYSIS

4.1 Interpretation of Results

Samples are tested for a broad range of substances using a variety of modern analytical techniques. The scope of testing under the Aquaculture Plan is comprehensive covering four broad categories: banned substances, unauthorised substances, authorised substances (approved substances i.e. veterinary substances) and environmental contaminants. Details of the methods and subcontract laboratories used are provided in Appendix 4.

Where a Maximum Residue Limit (MRL) has been set, samples are deemed non-compliant (i.e. positive) if concentrations of a given residue are confirmed to be in excess of the MRL.

Where no MRL is set, {e.g. for banned substances including steroids and compounds listed in Commission Regulation (EU) No 37/2010 (Table 4) and for unauthorized substances}, a Decision Limit (action level) is used. Samples are deemed non-compliant if concentrations of a given residue are confirmed to be in excess of the Decision limit (action level).

Follow up action is taken on confirmed positive samples. The sources of MRLs and Decision Limits (Action Level) are specified in Appendix 1.

Organochlorine compounds including Polychlorinated Biphenyls (PCBs) are persistent environmental contaminants that accumulate in lipid-rich animal tissue. For PCBs, typically, a group of indicator congeners are measured "EFSA PCB 6" which is the sum of the following 6 CB congeners – PCB 28, 52, 101, 138, 153, 180 and the Commission have set a Maximum Level (ML) of 75 µg kg^{-1} wet weight. For Organochlorine Pesticides (OCPs) there are no MRL/MLs; however, a number of OSPAR contracting countries have set levels that are presented in Appendix 1.

Maximum levels for mercury, cadmium and lead in fisheries products are set out in Commission Regulation (EC) No 1881/2006 as amended *setting maximum levels for certain contaminants in foodstuffs*. For salmon and trout, the levels specified are 0.3 mg kg^{-1} for lead, 0.05 mg kg^{-1} for cadmium and 0.5 mg kg^{-1} for mercury. These are taken as the "action levels" in this report.

A comprehensive quality assurance programme supports the monitoring programme and is detailed in Appendix 2 and 3.

4.2 Breakdown of 2016 Results

In 2016, in excess of 691 tests and a total of 1,933 measurements were carried out on 126 target samples of farmed finfish. **All 2016 samples were compliant with the exception of two harvest salmon samples taken from one farm which were confirmed as having oxytetracycline (Group B1- antibacterial substance) present in excess of the Maximum Residue Limit (MRL).**

Table 5: Summary of 2016 residue monitoring results for target farmed fish samples (salmon and trout). All tests performed on muscle and skin.

RESIDUE	NUMBER TESTED	NON-COMPLIANT[1]	DETECTION LIMIT[2] (μg kg $^{-1}$)
Group A3 - Steroids			
Methyltestosterone	38	0	1.5
17β-oestradiol	7	0	1.5
Group A6 - Compounds included in Annex IV of Council Regulation 2377/90/EC			
Chloramphenicol	38	0	0.25
Nitrofurans	9	0	See Appendix 5 for cc alphas
Nitroimidazole	9	0	See Appendix 5 for cc alphas
Group B1 - Antibacterial Substances			
Tetracyclines: oxytetracycline	92	2	50 (Screening)
Quinolones: Oxolinic acid Flumequine	92	0	 75 150
Florfenicol	92	0	750
Sulphonamides: Sulphadiazine	92	0	50
Group B2a - Anthelmintics			
Emamectin B1a	92	0	9.0
Ivermectin	92	0	0.1
Doramectin	92	0	0.1
Group B2c - Pyrethroids			
Cypermethrin	92	0	5[3]
Deltamethrin	92	0	10[3]
Group B2f - Other pharmacologically active substances			
Corticosteroids	26	0	1.5
Teflubenzuron	92	0	80
Diflubenzuron	92	0	86
Group B3a- Organochlorine Compounds			
EFSA PCB 6 (incl. LOQ)[4]	19	0	0.13
DDT and metabolites[5]	10	0	0.12
α-HCH	10	0	0.04
β-HCH	10	0	0.04
γ-HCH (lindane)	10	0	0.04
δ -HCH	10	0	0.04

Table 5 (continued): Summary of 2016 residue monitoring results for target farmed fish samples (salmon and trout). All tests performed on muscle and skin.

RESIDUE	NUMBER TESTED	NON-COMPLIANT[1]	DETECTION LIMIT[2] (μg kg $^{-1}$)
Group B3a- Organochlorine Compounds			
hexachlorobenzene	10	0	0.08
Pentachlorobenzene	10	0	0.08
Aldrin + dieldrin[6]	10	0	0.04
endrin	10	0	0.05
Toxaphene 26	10	0	0.08
Toxaphene 50	10	0	0.08
Toxaphene 62	10	0	0.16
heptachlor	10	0	0.02
mirex	10	0	0.02
cis-heptachlorepoxide	10	0	0.02
trans-heptachlorepoxide	10	0	0.05
octachlorostyrene	10	0	0.01
trans-nonachlor	10	0	0.01
oxychlordane	10	0	0.08
trans-chlordane (γ- chlordane)	10	0	0.02
cis-chlordane (α-chlordane)	10	0	0.02
Group B3c – Chemical Elements			
Lead	10	0	7
Cadmium	10	0	1
Mercury	10	0	2
Group B3d - Mycotoxins			
Aflatoxins	6	0	0.01
Group B3e - Dyes			
Malachite Green	59	0	0.5
Leuco Malachite Green	59	0	0.5
Crystal Violet	59	0	0.5
Leuco Crystal Violet	59	0	0.5
Victoria Blue	59	0	0.5
Brilliant Green	59	0	0.5

[1] Action limit reference Appendix 1
[2] Limit of Detection (LOD) for organochlorine compounds are averages as LOD is sample dependent.
[3] LOQ value
[4] EFSA PCB 6: sum of the following 6 non dioxin like PCBS–PCB 28, 52, 101, 138, 153, 180. Commission Regulation No 1259/2011 (came into force 1st Jan 2012) amending Regulation No. 1881/2006 *setting maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs.*
[5] DDT and metabolites – sum of individual DDT metabolites (o,p'DDT, p,p' DDT, o,p'DDE, p,p' DDE o,p'DDD, and p,p' DDE) – sum of individual LODs also included.
[6]Aldrin + dieldrin sum - sum of individual LODs also included.

4.2.1 Group A – Banned Substances

A total of 65 samples consisting of other stage of production and harvest samples were tested for at least one Group A compound.

Group A3: Steroids

43 individual samples were tested for Group A3 Steroids.

- **Methyltestosterone –** 38 samples screened for methyltestosterone by Enzyme-Linked ImmunoSorbant Assay (ELISA) method.
- **17β-oestradiol –** 7 samples screened for 17β-oestradiol by ELISA method.

No non-compliant (i.e. no positive) results were reported for Group A3 compounds.

Group A6: Compounds included in Annex IV of Council Regulation 2377/90/EC

43 individual samples were tested for Group A6 Compounds.

- **Chloramphenicol –** 38 samples screened for chloramphenicol by ELISA method.
- **Nitrofurans** – 9 samples analysed for the marker metabolites of the nitrofurans; furazolidone, furaltadone, nitrofurantoin and nitrofurazone using a quantitative test (LCMSMS)
- **Nitroimidazole –** 9 samples analysed for nitroimidazole and its metabolites[1] by a quantitative test (LCMSMS)

No non-compliant (i.e. no positive) results were reported for Group A6 compounds.

4.2.2 Group B – Veterinary Drugs and Contaminants

A total of 136 samples (i.e. 126 target samples & 10 suspect samples) of farmed finfish were tested for Group B compounds which can be classed as authorised substances, unauthorised substances or environmental contaminants. All samples were compliant with the exception of two harvest salmon samples from one farm which were confirmed as having oxytetracycline (Group B1- antibacterial substance) present in excess of the Maximum Residue Limit (MRL).

Group B1: Antibacterial Substances

- **Sulphonamides –** 92 samples were screened for sulphonamides by ELISA method.

No non-compliant (i.e. no positive) results were obtained for sulphonamides.

[1] The following nitroimidazole metabolites are listed on the NRCP-dimetridazol, ronidazol, metronidazol, hydroxyl-dimetridazol, hydroxyl-metronidazol

Quinolones, tetracyclines, florfenicol – 92 samples were screened for the following antibacterial substances quinolones, tetracyclines and florfenicol using a qualitative method.

2 harvest samples were non- compliant for oxytetracycline i.e. oxytetracycline was confirmed to be present in excess of the MRL.

Confirmatory analysis was carried out by RIKILT using LCMSMS. An investigation was carried out by the SFPA which included the Marine Institute taking 10 suspect samples. These 10 samples were tested by the Marine Institute and found to be compliant.

Group B2: Other veterinary drugs

With the exception of corticosteroids, these are authorised and unauthorised substances that could be used in treating sea-lice infestation.

- **B2(a) Anthelmintics** (Ivermectin, emamectin B1a, doramectin) – 92 harvest samples were analysed for the above anthelmintics using UPLC-FLU

No non-compliant results were obtained.

- **B2(c) Pyrethroids** (Cypermethrin, deltamethrin) **–** 92 harvest samples were analysed for the above pyrethroids using GC-MS

No non-compliant results were obtained.

- **B2(f) Other pharmacologically active substances**

Teflubenzuron, diflubenzuron – 92 harvest samples were analysed for teflubenzuron and diflubenzuron using UPLC with UV detection.

No non-compliant results were obtained.

Corticosteroids (dexamethasone, flumethasone and betamethasone) – 26 samples (other stage of production and harvest) were screened for the above corticosteroids using the ELISA method.

No non-compliant results were obtained.

Group B3a: Organochlorine Compounds

- **Polychlorinated Biphenyls**

Polychlorinated Biphenyls are a group of homologous man-made substances with a molecular structure comprising of a chlorinated biphenyl ring. PCBs are persistent environmental contaminants that accumulate in lipid and can be present at levels of concern in fish. PCBs can be divided into groups

according to their toxicological properties e.g. dioxin-like PCBs, non dioxin-like PCBs. As part of the NRCP, it is primarily the following six non dioxin-like PCBs (NDL-PCB) which are monitored; PCB 28, 52, 101, 138, 153 and 180. These NDL-PCBs are routinely used as a monitoring indicator as they are generally presumed to be the most persistent in fish tissue and comprise about half of the amount of total PCB present in feed and food. European legislation (Commission Regulation (EU) No 1259/2011 amending Regulation (EC) 1881/2006) has fixed maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs. In the case of NDL-PCBs the maximum level of 75 µg kg $^{-1}$ wet weight has been set for the sum of these six congeners. The mean and maximum concentrations measured for the sum of 6 indicator PCBs was 8.1 and 14.9 µg kg $^{-1}$ wet weight respectively.

None of the 19 harvest samples analysed exceeded the standard for the sum of EFSA 6 PCBs (reference Table 6 for details of the number of samples tested and the concentration range).

- **Organochlorine pesticides**

Organochlorine pesticides are synthetic substances used for pest control that are persistent and widespread in the marine environment despite the fact that their use has largely been phased out over recent decades. A number of OCPs are included in residues testing including DDT and its breakdown products. Chlorinated pesticides behave similarly to PCBs in the environment and do not have maximum concentrations in fish set by the EC. Due to their chemical properties (fat solubility) these substances bio-accumulate in fish tissue and also bio-magnify through the marine food chain. A number of OSPAR contracting countries have set standards/guidance values for certain OCPs and Appendix 1 presents these in so far as Marine Institute is aware.

All the harvest samples analysed for chlorinated pesticides were below these levels and were reported as compliant.

Group B3c: Chemical elements

Levels of mercury, cadmium and lead were all very low and well below the relevant European maximum limits in all of the samples tested (Appendix 1). Mercury has a maximum limit set in fish of 0.5 mg kg^{-1} wet weight. The highest mercury concentration obtained for the 10 samples analysed was 0.07 mg kg^{-1} wet weight. Cadmium, also an environmental contaminant, has a maximum limit set in fish of 0.05 mg kg^{-1} wet weight. The highest cadmium concentration obtained for the 10 samples analysed was 0.003 mg kg^{-1} wet weight. Lead has a maximum limit set in fish of 0.3 mg kg^{-1} wet weight. The highest lead concentration obtained for the 10 samples analysed was <0.02 mg kg^{-1} wet weight.

All 10 harvest samples were reported as compliant for mercury, lead and cadmium.

Table 6 provides a breakdown of the number of samples tested and the concentration range for the samples tested.

Table 6: Trace metal (mg kg^{-1}) and PCB (µg kg^{-1}) concentrations

Parameter	Median / Mean	Range	EC Max Limit	Number tested
Mercury	0.05 / 0.04	0.02 – 0.07	0.5	10
Cadmium	nd (<0.001)	nd (<0.001) – 0.003	0.05	10
Lead	nd (<0.007)	nd (<0.007) – <0.02	0.3	10
EFSA PCB 6[1]	7.9 / 8.1	2.4 – 14.9	75	19

For values reported as "nd", substances were not detected above the Limit of Detection (LOD is given in brackets)
[1]EFSA PCB 6: sum of the following non-dioxin like PCBS-PCB 28, 52, 101, 138, 153, 180

Group B3d: Mycotoxins

A mycotoxin is a toxic by-product of mould growth in feed and can remain as a residue in meat tissue. The amount and type of mycotoxin varies with environmental conditions such as temperature and humidity. The NRCP for Aquaculture 2016 analysed for the following mycotoxins: aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2. Aflatoxin B1 is the most common in food and amongst the most potent genotoxic and carcinogenic aflatoxins. All aflatoxins were reported as <0.01 µg kg^{-1} (wet weight) in the 6 samples tested.

Currently there are no maximum limits set for aflatoxins in fish.

Group B3e: Dyes

The following triphenylmethane dyes are analysed as part of Group B3e substances, malachite green and its metabolite leuco malachite green, brilliant green, crystal violet, leuco crystal violet, and victoria blue. These dyes could be used illegally in aquaculture as they exhibit antimicrobial and antiparasitic properties.

Malachite green is a common commercial fabric dye which had been widely used both prophylactically and in the treatment of fungal infection of both fish and eggs for over 60 years. It is also effective against several protozoal infestations, including agents causing proliferative kidney disease (PKD) and ichthyophthiriosis (white dot disease). Malachite green was regularly detected in aquaculture samples during the early years of the residues monitoring but as a result of increased industry awareness of its status as an unauthorised substance, supported by monitoring and enforcement, the use of malachite green has ceased with no non-compliant results reported since 2004. Its use had been primarily associated with freshwater farms and hatcheries; therefore, freshwater sites are particularly targeted by the NRCP. Malachite green is possibly both carcinogenic and genotoxic (i.e. damaging to DNA).

A minimum required performance level (MRPL) has been set for the sum of malachite green and its metabolite leuco malachite green[2] at 2 µg kg $^{-1}$ and MI has set a decision limit of 0.5 µg kg $^{-1}$ for malachite green and leuco malachite green individually i.e. a sample is deemed non-compliant if detected above the decision limit of 0.5 µg kg $^{-1}$.

There has been no evidence of brilliant green, crystal violet, leuco crystal violet, victoria blue being used in aquaculture in Ireland; however, these dyes have the potential to be used to treat Saprolegnia (fungus) either when present on the fish or as a prophylactic treatment to protect fish eggs from infection. No MRPL has been set for brilliant green, crystal violet, leuco crystal violet, victoria blue. However as these dyes are unauthorised, a decision limit of 0.5 µg kg $^{-1}$ has been set for all dyes.

All 59 target samples [25 harvest and 34 other stage of production] tested for malachite green and its metabolite leuco malachite green, crystal violet and its metabolite leuco crystal violet, brilliant green and victoria blue were found to be compliant i.e. negative.

[2] The MRPL of 2µg kg $^{-1}$ was reaffirmed by EFSA in 2016 https://www.efsa.europa.eu/de/efsajournal/pub/4530

PART B

Summary Report on 2016 Border Inspection Posts Fishery Product Testing undertaken at the Marine Institute

Carried out under Council Directive 97/78/EC of 18 December 1997 laying down the principles governing the organisation of veterinary checks on products entering the Community from third countries
&
Commission Regulation (EC) No 136/2004 of 22 January 2004 laying down procedures for veterinary checks at Community border inspection posts on products imported from third countries

Third Countries (non-EU) wishing to export animal products to the EU are required to satisfy the European Commission that their residue surveillance measures provide equivalent guarantees for EU consumers similar to EU residue surveillance 96/23/EC. Therefore, food imports of animal origin from a Third country may only be brought into the European Community through a Border Inspection Post (BIP) that has been approved for importation. In Ireland, the responsibility for carrying out checks at the BIP (Dublin Port and Shannon Airport) is with the DAFM BIP Officers.

In 2016, BIP samples were collected by DAFM Sampling Officers and samples for testing of antibacterials (B1a), anthelminthics (B2a), heavy metals (B3d) and dyes (B3e) were sent to the Marine Institute for testing in accordance with 2016 BIP plan. In total 15 random samples were sent to the Institute by the DAFM Sampling Officers at Dublin Port and Shannon Airport. The 2016 BIP results as tested at the Marine Institute are presented in Table 7 – **All 15 samples were reported as compliant.**

In addition, **1 Safeguard sample from India** was received from DAFM and required testing for tetracyclines under Commission Decision 2010/381/EU '*on emergency measures applicable to consignments of aquaculture products imported from India and intended for human consumption*' and its amendment Commission Implementing Decision 2012/690/EU. **This safeguard sample was reported as compliant**.

Table 7: 2016 Border Inspection Posts results for fishery products tested at the Marine Institute

MI CODE	DAFM Sample code	BIP Office	Product type	Substances for Identification	Result
RESBIP2016-5001	ARA0983068	Shannon Airport	Lobster (Tail & Claw)	Lead, cadmium and mercury	**Compliant**
RESBIP2016-5002	ARA718402	Dublin Port	Whole gutted hilsa	Cadmium	**Compliant**
RESBIP2016-5005	ARA718446	Dublin Port	Aqua salmon fillet	Avermectins	**Compliant**
RESBIP2016-5006	ARA718464	Dublin Port	Raw prawn	Malachite Green and dyes	**Compliant**
RESBIP2016-5007	ARA718465	Dublin Port	Raw prawn	[1]Antibacterials	**Compliant**
RESBIP2016-5008	ARA0983073	Shannon Airport	Frozen lobster tail	Lead, cadmium and mercury	**Compliant**
RESBIP2016-5009	ARA718490	Dublin Port	Panga fillet	[1]Antibacterials	**Compliant**
RESBIP2016-5010	ARA718491	Dublin Port	Panga fillet	Malachite Green and dyes	**Compliant**
RESBIP2016-5012	ARA718534	Dublin Port	Canned salmon	Mercury	**Compliant**
RESBIP2016-5014	ARA718564	Dublin Port	Canned salmon	Mercury	**Compliant**
RESBIP2016-5015	ARA718537	Dublin Port	Frozen shrimp	Avermectins	**Compliant**
RESBIP2016-5016	ARA718513	Dublin Port	Frozen shrimp	Malachite Green and dyes	**Compliant**
RESBIP2016-5017	ARA718538	Dublin Port	Frozen shrimp	Malachite Green and dyes	**Compliant**
RESBIP2016-5018	ARA718512	Dublin Port	Frozen shrimp	[1]Antibacterials	**Compliant**
[2]RESBIP2017-5001	ARA0983074	Shannon	Live Lobster	Lead, cadmium and mercury	**Compliant**

[1] Antibacterials – Agar Plate Method (tetracyclines, florfenicol and quinolones) and Evidence Investigator (sulphonamides)
[2] Note – Sample taken 21/12/2016, received in MI 13/01/2017 and given 2017 number

Table 8: 2016 Safeguard results for fishery products tested at the Marine Institute

MI CODE	DAFM Sample code	BIP Office	Product type	Substances for Identification	Result
RESBIP 2016-5013	ARA718530	Dublin Port	Shrimp muscle	Tetracyclines	**Compliant**

Appendix 1: Source of Maximum Residues Limits, Decision Limits and Guideline Values used for comparison with the results for 2016

Parameter	Maximum Level or Decision Limit (6)	Source
Group A Compounds[1]: Methyltestosterone, 17β-Oestradiol, Chloramphenicol, Nitrofurans & Nitroimidazoles	These are banned substances and should not be detected.	
Ivermectin	0.4 µg kg^{-1}	Decision Limit[3]
Doramectin	0.4 µg kg^{-1}	Decision Limit[3]
Emamectin B1a	100 µg kg^{-1}	Maximum Residue Limit[2]
Cypermethrin	50 µg kg^{-1}	Maximum Residue Limit[2]
Deltamethrin	10 µg kg^{-1}	Maximum Residue Limit[2]
Teflubenzuron	500 µg kg^{-1}	Maximum Residue Limit[2]
Diflubenzuron	1000 µg kg^{-1}	Maximum Residue Limit[2]
Antibacterial Substances		
Sulphonamides	100 µg kg^{-1}	Maximum Residue Limit[2]
Oxytetracycline (Tetracyclines)	100 µg kg^{-1}	Maximum Residue Limit[2]
Oxolinic Acid (Quinolones)	100 µg kg^{-1}	Maximum Residue Limit[2]
Flumequine (Quinolones)	600 µg kg^{-1}	Maximum Residue Limit[2]
Sarafloxacin (Quinolones)	30 µg kg^{-1}	Maximum Residue Limit[2]
Florfenicol	1000 µg kg^{-1}	Maximum Residue Limit[2]
EFSA PCB 6 [7]	75 µg kg^{-1}	EC Maximum Limit[8]
HCB	50 µg kg^{-1}	Norway (G)[4]
γ HCH	100 µg kg^{-1}	Finland (S)[4]
DDT and metabolites	500 µg kg^{-1}	Finland (S)[4]
Aldrin + Dieldrin	100 µg kg^{-1}	Finland (S)[4]
Endrin	50 µg kg^{-1}	Finland(S)[4]
Malachite Green	0.5 µg kg^{-1}	Decision Limit[3]
Leuco Malachite Green	0.5 µg kg^{-1}	Decision Limit[3]
Brilliant Green	0.5 µg kg^{-1}	Decision Limit[3]
Crystal Violet	0.5 µg kg^{-1}	Decision Limit[3]
Leuco Crystal Violet	0.5 µg kg^{-1}	Decision Limit[3]
Victoria Blue	0.5 µg kg^{-1}	Decision Limit[3]
Lead	0.3 mg kg^{-1}	EC Maximum Limit[5]
Cadmium	0.05 mg kg^{-1}	EC Maximum Limit[5]
Mercury	0.5 mg kg^{-1}	EC Maximum Limit[5]

Notes:

1. Commission Regulation (EU) No 37/2010 (Table 2) and Directive 2008/97/EC: *Substances banned and should not be detected*
2. Commission Regulation No 37/2010 (Table 1) *on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin*.
3. These compounds are not authorised for use in finfish, concentrations above the analytical methods decision limit are non-compliant.
4. OSPAR: *A compilation of standards and guidance values for contaminants in fish, crustaceans and molluscs for the assessment of possible hazards to human health*, Update 1993, JMP 17/3/10-E. (S) standard; (G) guidance value.
5. Commission Regulation (EC) No 1881/2006 *setting maximum levels for certain contaminant in foodstuffs and its amendments* Commission Regulation 629/2008/EC, Commission Regulation 420/2011/EC and Commission Regulation 488/2014/EC.
6. Maximum Residue Limits and Decision Limits concentration are on a wet weight basis.
7. EFSA PCB 6: sum of the following 6 CB congeners –PCB 28, 52, 101, 138, 153, 180.
8. Commission Regulation No 1259/2011 amending Regulation No. 1881/2006 *as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin like PCBs in foodstuffs.*

Appendix 2: Accreditation to ISO 17025

The table below outlines the parameters for which the Marine Institute is accredited by the Irish National Accreditation Board (INAB) to ISO 17025 as detailed in Scope Registration Number 130T.

Test	SOP
Ivermectin, Emamectin B1a , Doramectin[3]	CHE-8
Mercury[4]	CHE-32
Teflubenzuron , Diflubenzuron[3]	CHE-42
Dyes[3]: Malachite Green, Crystal Violet, Victoria Blue, Leuco Crystal Violet, Leuco Malachite Green and Brilliant Green	CHE-167
Cadmium[4]	CHE-178
Lead[4]	CHE-178
Screening of Antibiotic Residues in Fish[3]	FHU-1
Screening of sulphadiazine[3]	FHU-119
Moisture %[4]	CHE-52
When collecting samples the laboratory complies with Council Directive 96/23/EC	CHE-6

[3] Accreditation is for finfish only
[4] Accreditation is for Marine Biota

Appendix 3: Quality Control

To check the quality of the data produced during the 2016 National Surveillance Scheme for chemical residues in farmed fish, Quality Control (QC) samples in the form of either reagent blanks, spiked samples or Certified Reference Materials (CRMs) were analysed with each batch of samples tested by the Marine Institute. The quality assurance results as shown below were considered sufficient for the purpose of the monitoring programme. For CRMs, z-scores were calculated using the methodology of QUASIMEME (Quality Assurance of Marine Environment and Monitoring in Europe). A Z-score of between –2 and +2 is generally considered satisfactory for the purpose of environmental monitoring programmes.

Analyte	QC Type	Target Value	% Recovery ± SD
Group B2a Anthelmintics (µg kg^{-1})			
Ivermectin	Spike (n=22)	2	87.7 ± 7.2
Emamectin B1a	Spike (n=16)	100	94.7 ± 6.8
Doramectin	Spike (n=22)	2	98.1 ± 7.6
Group B2f other pharmacologically active substances (µg kg^{-1})			
Teflubenzuron	Spike (n=22)	500	78.6 ± 8.0
Diflubenzuron	Spike (n=22)	1000	83.9 ± 5.8
Group B3e Dyes (µg kg^{-1})			
Brilliant Green	Spike (n=18)	2	88.7 ± 19.6
Crystal Violet	Spike (n=18)	2	99.3 ± 4.6
Leuco Crystal Violet	Spike (n=15)	2	103.4 ± 9.8
Leuco Malachite Green	Spike (n=18)	2	102.9 ± 5.9
Malachite Green	Spike (n=18)	2	96.1 ± 6.9
Victoria Blue	Spike (n=18)	2	95.1 ±15.7
Group B3c Chemical Elements (mg kg^{-1} dry weight) Recovery for Analytical Batch QC			
Lead	SRM 2976 (n=1)	1.19	110
Cadmium	SRM 2976 (n=1)	0.82	101
Mercury	DORM2 (n=2)	4.64	99.5
Dry weight (%)	QTMO58BT (n=1)	23.56	98.2

Note: n = sample number

Appendix 4: Methods of Analysis

1.1 Sample Collection and Preparation

In accordance with the 2016 National Residues Control Plan for Aquaculture under Council Directive 96/23/EC, Staff authorised under the *Animal Remedies Act 1993*, collected samples at farms or at processing plants. All samples were transported to the laboratory under controlled conditions, while ensuring an unbroken chain of custody. Sub-samples were taken for both analytical and archive purposes and all sub-samples were stored frozen (< -18°C).

1.2 Analysis of Ivermectin, Doramectin and Emamectin B1a by Ultra-Fast Liquid Chromatography (UFLC) with Fluorescence Detection

Approximately 5g of sample from each fish was homogenised and extracted with methanol. The extract was cleaned up by liquid/liquid partition and solid phase extraction techniques. The resultant residue was derivatised and analysed by liquid chromatography (UFLC) with fluorescence detection.

1.3 Analysis of Teflubenzuron and Diflubenzuron by Ultra-Fast Liquid Chromatography (UFLC) with Ultraviolet (UV) Detection

This method involves the extraction of approximately 3g of tissue with acetonitrile followed by clean up using liquid/liquid partition and silica SPE. Quantification was carried out by reverse phase UFLC using an acetonitrile/water mobile phase and UV detection. Confirmation and peak purity was evaluated using a photodiode array detector.

1.4 Analysis for Cypermethrin and Deltamethrin by Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis was performed in co-operation with a Eurofins sister-laboratory accredited for this test. After addition of internal standards an extraction was performed with appropriate organic solvents. Subsequently the extract was subjected to a clean-up procedure using gel permeation chromatography (GPC), followed by dispersive solid phase extraction (dSPE) using PSA. The measurement was performed by gas chromatography and mass spectrometry (GC/MS). The quantification was carried out with the use of internal and external standards. The analytical system was calibrated using a multi-point calibration.

1.5 Analysis of Dyes by Ultra-Fast Liquid Chromatography (UFLC) with MS/MS detection

Samples were extracted for Dyes analysis with Acetonitrile by shaking in the presence of hydroxylamine and magnesium sulphate. The eluant is evaporated to dryness followed by reconstitution in a mixture of acetonitrile/water /ascorbic acid solution. This solution is centrifuged, filtered and analysed for brilliant green, crystal violet, leuco crystal violet, leuco malachite green, malachite green and victoria blue by Ultra-Fast Liquid Chromatography coupled to Mass Spectrometry (UFLC-MS/MS).

1.6 Screening for Antibacterial Substances (Quinolones, Tetracylines and Florfenicol) using modified Two Plate Test

Antimicrobial screening was carried by the Fish Health Unit (FHU) of the Marine Institute, using a modification of the Two Plate Test (TPT). The aim of this method is to reveal residues of substances with antibacterial activity by testing the fish tissue using agar plates that have been seeded with suitably sensitive bacterial cultures. This method is qualitative in nature and was used to detect residues of Quinolones, Tetracyclines and Florfenicol. Where confirmatory analysis was required for oxytetracyclines the samples were tested by RIKILT.

1.7 Screening for sulphonamides by Evidence Investigator

Screening for sulphonamides was carried by the Fish Health Unit (FHU) of the Marine Institute using Immunoassay. This method is qualitative in nature and tested on the Evidence Investigator instrument.

1.8 Screening for Group A Compounds by Elisa method

Screening for Group A compounds was carried out by the Irish Equine Centre (IEC) using the Enzyme-Linked Immuno Sorbant Assay (ELISA) method. This method is qualitative in nature and was used to detect residues of 17β-oestradiol, chloramphenicol and methyltesterone.

1.9 Screening for Group B - Cortiscosteroids by Elisa method

Screening for corticosteroids was carried out by the Irish Equine Centre (IEC) using the Enzyme-Linked Immuno Sorbant Assay (ELISA) method.

1.10 Analysis of Nitrofurans by Ultra Performance Liquid Chromatography with Mass Spectrometry detection (UPLC-MS/MS)

Analysis of nitrofurans was carried out by Teagasc Food Research Centre (TFRC). Tissue bound residues of nitrofurans are hydrolysed with acid and derivatised with 2-nitrobenzaldehyde. The nitrophenyl derivatives are extracted with ethyl acetate and determined by Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS) using deuterated analogues as internal standards for quantification. Metabolites of furazolidone, furaltadone, nitrofurantoin and nitrofurazone are analysed.

1.11 Analysis of Nitroimidazoles by UPLC-MS/MS

Analysis of nitroimidazoles was carried out by Teagasc Food Research Centre (TFRC). Samples are extracted with acetonitrile, water, magnesium sulphate and sodium chloride; defatted with n-hexane and concentrated. The residue content is determined by Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS) and analysed for dimetridazole and its metabolite, ipronidazole and its metabolite, metronidazole and its metabolite, ornidazole and ronidazole.

1.12 Analysis for Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCPs) by HRGC/HRMS

Analysis for PCBs and OCPs was carried out by a subcontracted laboratory (Eurofins). Prior to the extraction, ^{13}C-UL-labeled internal standards were added, followed by an extraction using a solid/lipid extraction and clean up by a multicolumn system. Concentration levels were determined by (high resolution gas chromatography and high resolution mass spectrometry (HRGC/HRMS) using a DB-5 capillary column.

1.13 Analysis of Cadmium and Lead by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

Concentrated nitric acid (4 ml) and hydrogen peroxide (4 ml) were added to approximately 0.2 g freeze-dried tissue, wh
ich was then digested in a laboratory microwave oven (CEM Mars Xpress). After cooling, samples were diluted to 50mls with deionised water. Concentrations were determined by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS, Agilent 7700x with High Matrix Introduction (HMI) system).

1.14 Analysis of Mercury by Cold Vapour Atomic Fluorescence Spectroscopy (CV-AFS)

Concentrated nitric acid (4 ml) was added to approximately 0.2 g freeze-dried tissue, which was then digested in a laboratory microwave oven (CEM Mars Xpress). After cooling, potassium permanganate was added until the purple colour of the solution stabilized. Sufficient hydroxylamine sulphate/sodium chloride solution was added to neutralise the excess potassium permanganate and potassium dichromate was added as a preservative. The solution was diluted to 100mls using deionised water. Following reduction of the samples with tin (II) chloride, total mercury concentration was determined by Cold Vapour Atomic Fluorescence Spectroscopy (CV-AFS) using a PSA Merlin Analyser.

1.15 Determination of Moisture Content

The moisture content was determined by drying approximately 1g of tissue overnight in an oven at 104ºC to constant weight.

1.16 Analysis of Mycotoxins

Analysis of Aflatoxins B1, B2, G1 and G2 was carried out by Wessling. The method involved the extraction of about 25g of muscle using dichloromethane and the extract was cleaned up on an immunoaffinity column. The subsequent determination of aflatoxins B1, B2, G1 and G2 was achieved using Liquid Chromatography with Fluorescence Detection after post column derivatisation.

1.17 Confirmatory Method for Tetracyclines by Ultra HPLC-MS/MS

Confirmatory testing for oxytetracylines was carried out by RIKILT. A test portion of 2g is used. After the internal standards are added the components of interest (tetracyclines) are extracted from the matrix using 0.1M EDTA-Mcllvain buffer (pH 4). Further purification is done by Solid Phase Extraction (Oasis HLB) with compounds eluted using methanol. The residues are evaporated to dryness and re-dissolved for analysis on a (UH)PLC-MS/MS system.

Appendix 5: 2016 Plan for the Monitoring of Residues in Aquaculture products

1. National Legislation on use of substances listed in Annex I of Directive 96/23/EC

Animal Remedies Act, 1993 (No. 23 of 1993)
Animal Remedies Regulations, 2007 (SI No. 786 of 2007)
Control of Animal Remedies and their Residues Regulations 2009(SI No. 183 of 2009)

2. Relevant Departments and their infrastructure

Dept of Agriculture, Food and Marine
Agriculture House
Kildare Street
Dublin 2

Sea-Fisheries Protection Authority
Block B
Clogheen
Clonakilty
Co. Cork

Marine Institute
Rinville
Oranmore
Co. Galway

3. Staff resources to carry out plan
Authorised Officers will collect all samples.

Group A substances will be performed by the Irish Equine Centre- Kildare, Laboratory of the Government Chemist-UK, Ashtown Food Research Centre-Dublin & EU-RL –RIKILT

Analyses for Group B substances will be performed within the Marine Institute with the exception of those indicated in the plan.

4. Approved laboratories

Marine Institute (MI)
Rinville
Oranmore
Co. Galway

Irish Equine Centre (IEC)
Johnstown,
Naas,
Co. Kildare.

Teagasc Food Research Centre (TFRC)
Teagasc, Ashtown
Dublin 15

Eurofins GfA GmbH,
D-48161 Münster
Germany

RIKILT EU-RL
Laboratory for Residue analysis,
Akkermaalsbos 2,
6708 WB Wageningen,
Netherlands

Laboratory of the Government Chemist (LGC)
Queens Road
Teddington Middlesex
TW11 OLY, UK

Wessling GmbH,
Kohlenstraße 51-55,
44795 Bochum,
Germany

ANSES EU-RL
Fougères
10B rue Claude Bourgelat, Javené CS 40608
35306 Fougères Cedex

5. Additional Information

For Group A analysis more than half the samples are 'on farm' samples, taken at various stages of production, the remainder are samples taken at harvest.

DIRECTIVE 96/23/EC ANNUAL PLAN FOR THE EXAMINATION FOR RESIDUES IN FARMED FINFISH FOR THE YEAR 2016

Sampling levels and frequency:

Minimum number of finfish from which samples must be taken.

Total Tonnes Produced 2014	Total min. no. to be tested[(a)]	Min. no. Group A	Min. no. Group B
10,176	Production (tonnes)/100 =102	1/3 Total Tested = 34	2/3 Total Tested = 68

[(a)] min no. to be tested will be based on 2014 finfish production figures as 2015 figures are not available

1	2	3	4	5	6	7	8	9
Group of Substances	**Compounds**	**Matrix**	**Laboratory Method**	**CCbeta (screening) Detection capability**	**CCalpha (confirmatory) decision limit**	**Level of action**	**Number of samples**	**Laboratory**
A 3 Steroids[d]	Methyltestosterone	Muscle & Skin	(1) ELISA (2) GCMS	1)1.5 µg kg^{-1}	2)0.05 µg kg^{-1}	Presence	38[b]	(1) IEC (2) EU-RL RIKILT
	17β-Oestradiol	Muscle & Skin	(1) ELISA (2) GCMSMS	1)1.5 µg kg^{-1}	2)0.17 µg kg^{-1}	0.5 µg kg^{-1}	7[b]	(1) IEC (2) EU-RL RIKILT
A 6 Compounds included in Annex IV Council Reg. 2377/90	**Chloramphenicol**	Muscle & Skin	(1) ELISA (2) LCMSMS	1)0.25 µg kg^{-1} 1)0.3 µg kg^{-1}[c]	2)0.05 µg kg^{-1}	Presence	38[b]	(1) IEC[c] (2) EU-RL ANSES- Fougeres
	Nitrofurans AOZ AMOZ AHD SEM	Muscle & Skin	UPLCMSMS		 0.041 µg kg^{-1} 0.061 µg kg^{-1} 0.057 µg kg^{-1} 0.064 µg kg^{-1}	Presence	9[b]	TFRC
	Nitroimidazoles Dimetridazole HMMNI Metronidazole Hydroxyl- Metronidazole Ornidazole Ronidazole Ipronidazole Hydroxyl-ipronidazole	Muscle & Skin	UPLCMSMS		 0.12 µg kg^{-1} 1.0 µg kg^{-1} 0.10 µg kg^{-1} 0.15 µg kg^{-1} 0.29 µg kg^{-1} 0.10 µg kg^{-1} 0.15 µg kg^{-1} 0.10 µg kg^{-1}	Presence	9[b]	TFRC

[b] At least 50% of Group A are "on farm" samples

Column 4: (1) Screening Method, (2) Confirmatory Method

[c] For screened positive samples for Chloramphenicol using the Elisa, these samples will be sent to subcontract laboratory LGC for further screening (LCMSMS).

[d] Corticosteroids: re-categorised as B2f

1	2	3	4	5	6	7	8	9
Group of Substances	**Compounds**	**Tissue**	**Laboratory Method**	**CCbeta (screening) Detection capability**	**CCalpha (confirmatory) decision limit**	**Level of action**	**Number of samples**	**Laboratory**
B 1 Antibacterial substances	Microbiological screening: <u>Quinolones</u>: -Oxolinic acid -Flumequine <u>Tetracyclines:</u> -oxytetracycline <u>Florfenicol</u>	Muscle & Skin	Modified EC 2-plate method.	 75 150 50 250	N/A	 100 µg kg^{-1} 600 µg kg^{-1} 100 µg kg^{-1} 1000 µg kg^{-1}	91	MI
	Screening: <u>Sulphonamides</u> -Sulphadiazine	Muscle & Skin	1)Immunoassay	50 µg kg^{-1}	N/A	100 µg kg^{-1}	91	MI
	<u>Tetracycline</u> Chlortetracycline Epi-Chlortetracycline Oxytetracycline Epi-Oxytetracycline Tetracycline Epi-Tetracycline Doxycycline	Muscle & Skin	1)LC-TOF 2)LCMSMS	 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1}	(f)	100 µg kg^{-1}	Confirmation and post screening identification of positive Microbiological Samples/ Bioassay	1)LGC([e]) 2) EU-RL ANSES- Fougeres
	<u>Quinolones</u> Ciprofloxacin Enrofloxacin Danofloxacin Difloxacin Flumequine Oxolinic acid Sarafloxacin		1)LC-TOF 2)LCMSMS	 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 150 µg kg^{-1} 200 µg kg^{-1} 50 µg kg^{-1} 15 µg kg^{-1}	(f)	 600 µg kg^{-1} 100 µg kg^{-1} 30 µg kg^{-1}		1)LGC([e]) 2) EU-RL ANSES- Fougeres

Column 4: (1) Screening Method, (2) Confirmatory Method

[e]For screened positive samples for tetracyclines, quinolones, sulphonamides using MI in-house methods, these samples will be sent to subcontract laboratory LGC for further screening by LC-TOF

[f] EU-RL calculates on the day of confirmatory analysis under ISO11843-2

1	2	3	4	5	6	7	8	9
Group of Substances	**Compounds**	**Tissue**	**Laboratory Method**	**CCbeta (screening) Detection capability**	**CCalpha (confirmatory) decision limit**	**Level of action**	**Number of samples**	**Laboratory**
	Sulphonamides Sulphathiazole Sulphaquinoxaline Sulphapyridine Sulphamethoxy-pyridazine Sulphamonomethoxine Sulphamethazine Sulphamerazine Sulphisoxazole Sulphadimethoxine Sulphadiazine Sulphachlorpyridazine Sulphamethizole Florfenicol	Muscle & Skin	1)LC-TOF 2)LCMSMS	 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} (k)	(h) (k)	(h) 100 µg kg^{-1} (k)1000 µg kg^{-1}		1)LGC(e) 2) LGC
B2 (a) Anthelmintics	Ivermectin	Muscle & Skin	UFLC-Flu	-	0.4 µg kg^{-1}	0.4 µg kg^{-1}	91	MI
	Emamectin B1a			-	124 µg kg^{-1}	124 µg kg^{-1}		
	Doramectin			-	0.4 µg kg^{-1}	0.4 µg kg^{-1}		
B2 (c) Carbamates / Pyrethroids	Cypermethrin	Muscle & Skin	GC-MS	-	(i)	(i)	91	MI
	Deltamethrin			-	(i)	(i)		
B2 (f) Other Pharmacologically active substances	Teflubenzuron	Muscle & Skin	UFLC-DAD	-	574 µg kg^{-1}	574 µg kg^{-1}	91	MI
	Diflubenzuron			-	1136 µg kg^{-1}	1136 µg kg^{-1}		
	Corticosteroids Betamethasone Dexamethasone Flumethasone	Muscle & Skin	(1) ELISA (2) LC-MS	1)1.5 µg kg^{-1} 1.5 µg kg^{-1} 1.5 µg kg^{-1}	(j)	Presence	26(g)	(1) IEC (2) EU-RL RIKILT

(g) At least 50% are "on farm" samples

Column 4: (1) Screening Method, (2) Confirmatory Method

(h) LGC under flexible scope of accreditation has an accredited procedure for the development and validation of methods in place in the event that a fish sample tested screen positive using the LC-TOF method. CC alpha will be calculated at that point.

(i) MI pending validation and accreditation under ISO17025

(j) EU-RL can provide confirmation under flexible scope. CCalpha will be calculated at that point.

(k) Validation pending

1	2	3	4	5	7	8	9
Group of Substances	**Compounds**	**Tissue**	**Laboratory Method**	**Detection limit**	**Level of action**	**Number of samples**	**Laboratory**
B3(a) Organochlorine compounds including PCBs	PCBs Sum of 6 PCBs [PCB28, 52, 101, 138, 153, 180]	Muscle & Skin	GCHRMS	[m] 0.07 µg kg^{-1} per individual congener	[l] 75 µg kg^{-1}	19	Eurofins
	Chlorinated Pesticides [o] γ-HCH DDT and metabolites HCB Endrin Aldrin + Dieldrin		GCHRMS	 [m] 0.0625 µg kg^{-1} [m] 0.125 µg kg^{-1} [m] 0.125 µg kg^{-1} [m] 0.075 µg kg^{-1} [m] 0.0625 µg kg^{-1}	Excess of Guidance value [n] 100 µg kg^{-1} 500 µg kg^{-1} 50 µg kg^{-1} 50 µg kg^{-1} 100 µg kg^{-1}	10	
B3(c) Chemical elements	Lead		ICP-MS	7 µg kg^{-1}	[l]300 µg kg^{-1}	10	MI
	Cadmium		ICP-MS	1 µg kg^{-1}	[l]50 µg kg^{-1}	10	
	Mercury		CVAFS	2 µg kg^{-1}	[l]500 µg kg^{-1}	10	
B3(d) Mycotoxins	Aflatoxin B1	Muscle & Skin	HPLC-Flu	0.01 µg kg^{-1}	-	6	Wessling
	Aflatoxin B2			0.01 µg kg^{-1}			
	Aflatoxin G1			0.01 µg kg^{-1}			
	Aflatoxin G2			0.01 µg kg^{-1}			

[l] Commission Regulation No. 1881/2006 as amended setting maximum levels for certain contaminants in foodstuffs
[m] Detection limit is at limit of quantification for PCBs and OCPs
[n] There are no national or European maximum limits for organochlorine pesticides in fish. The guidance values used represent the strictest national limits applied by contracting parties to the OSPAR convention and as compiled by OSPAR (1992), in so far as they are known. These values have no statutatory basis and are used in the absence of other criteria.
[o] Additional chlorinated pesticides are also included in routine testing but no action level or guidance values are available

1	2	3	4	5	6	7	8	9
Group of Substances	**Compounds**	**Tissue**	**Laboratory Method**	**CCbeta (screening) Detection capability**	**CCalpha (confirmatory) decision limit**	**Level of action**	**Number of samples**	**Laboratory**
B3(e) Dyes	Malachite Green (MG) Leuco Malachite Green (LMG) Brilliant Green (BG) Crystal Violet (CV) Leuco Crystal Violet (LCV) Victoria Blue (VB)	Muscle & Skin	UFLCMSMS	- - 	0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1}	0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1} 0.5 µg kg^{-1}	59[(q)] 17 x salmon/sea trout 8 x freshwater trout (harvest) 8 x freshwater trout (osop) 26 x salmon smolts	MI

[(q)] 42 of the 59 samples for dyes are “on farm” samples