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Chemical Residues in Irish Farmed Finfish, 2011

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CHEMICAL RESIDUE IN IRISH FARMED FINFISH 2011

Report on monitoring of aquaculture undertaken in accordance with Council Directive 96/23/EC of 29 April 1996 *on measures to monitor certain substances and residues thereof in live animals and animal products.*

April 2013

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Front Cover: Polar Circle fish cages, Clew Bay, Westport, Co. Mayo

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Department of Agriculture, Food & Marine
Food Safety Authority of Ireland
Sea-Fisheries Protection Authority
The ongoing co-operation of the aquaculture industry

Summary

On behalf of the Department of Agriculture, Food and Marine (DAFM), the Marine Institute carries out monitoring of chemical residues in aquaculture 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.* The main objectives of the Aquaculture National Residue Control Plan (NRCP) is 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.

In 2011, in excess of 630 tests and 1,566 individual measurements for substances were carried out on 140 samples of farmed finfish taken on farms and at processing plants for a range of residues. In accordance with Council Directive 96/23/EC, the following species were sampled and tested: Atlantic salmon (*Salmo salar*), freshwater and sea-reared trout (*Oncorhynchus mykiss*). Tests were carried out for banned substances such as growth promoters, and other unauthorised substances such as malachite green, which should not be present. Harvested fish were also tested for authorised veterinary treatments such as antibiotics and sea lice treatments, environmental contaminants such as trace metals, polychlorinated biphenyls and organochlorine pesticides, to check for compliance with Maximum Residue Levels (MRL) where available.

As in previous years, **no non-compliant results** were reported in the surveillance monitoring programme for farmed finfish. Overall, in recent years the outcome for aquaculture remains one of consistently low occurrence of residues in farmed finfish, with 0.23% non-compliant results from routine targeted monitoring in 2004, 0.09% in 2005 and **0% for the period 2006-2011.**

CONTENTS

1.0 BACKGROUND

The Marine Institute monitors aquaculture finfish for the presence of chemical residues 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*, otherwise known as the Residues Directive; on behalf of the Department of Agriculture, Food and Marine (DAFM). The main objectives of the National Residue Control Programme (NRCP) for aquaculture is 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.

There are several agencies and a department involved in the Residue Monitoring Programme for Aquaculture (Fig. 1). The Food Safety Authority of Ireland (FSAI) co-ordinates the activities of the department and various agencies involved in delivering the overall NRCP for all relevant food groups including aquaculture. For the aquaculture sector, the Sea Fisheries Protection Authority (SFPA), with technical support from the Marine Institute (MI), is responsible for residue controls on farmed finfish. In 2011, DAFM, FSAI SFPA and MI further improved coordination on implementation of the Directive as it pertains to aquaculture; outlined below is a summary of each current role.

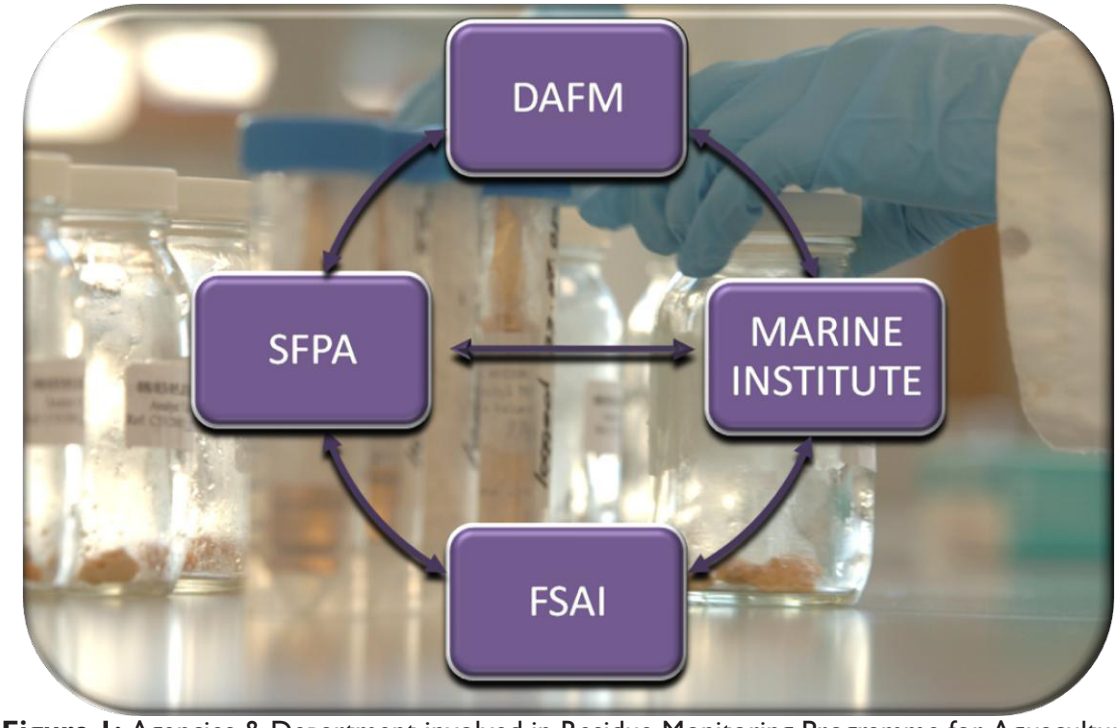


Figure 1: Agencies & Department involved in Residue Monitoring Programme for Aquaculture

- **DAFM**

DAFM Veterinary Inspectors (VIs) carry out routine inspections on finfish farms. These inspections are carried out to verify compliance with Fish Health, Animal Remedies, Feedstuffs, Animal-By Products and Food hygiene regulations. If an issue or non-compliance arises from a routine inspection, DAFM VIs will conduct a follow-up investigation. Assistance may be required from the MI with respect to sampling and analysis and from the SFPA in closing out the follow-up.

- **SFPA**

SFPA is responsible for the follow-up of samples that are non-compliant under the NRCP. If a non-compliant result were to arise from sampling under the NRCP the SFPA will carry out a follow-up investigation with the assistance of the MI to carry out sampling and analysis. Assistance may be required from DAFM VIs in closing out the follow-up investigation.

- **MI**

MI is the official laboratory for residue sampling and analysis of farmed finfish under the NRCP. The MI aids and assists the SFPA in follow-up investigations and provides scientific advice as required. MI officers are authorised under Section 10(1) of the Animal Remedies Act, 1993. MI is also a National Reference Laboratory (NRL) for certain substances under the overall NRCP.

2.0 MONITORING PROGRAMME FOR AQUACULTURE

Annually, the MI prepares the Aquaculture National Residue plan[1], which is reviewed and finalised by SFPA, FSAI and DAFM and then submitted to the European Commission for approval. This sets out the national surveillance monitoring plan, including species, sample numbers, target substances and analytical methods in line with the specific requirements of Directive 96/23/EC. Figure 2, details the Annual Cycle of National Residue Control Programme for Aquaculture.

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. For further details of the main legislation concerning the Irish finfish aquaculture industry for monitoring of NRCP see the legislation section of this document.

3.0 SCOPE OF AQUACULTURE NRCP

The scope of this testing under the Aquaculture Plan is comprehensive covering 4 broad categories: **banned substances; unauthorised substances; authorised substances and environmental contaminants**. These substances are classed into 2 categories: Group A and Group B. Details are given in Table 1.

[1]Reference Appendix 3 for 2011 NRCP for Finfish

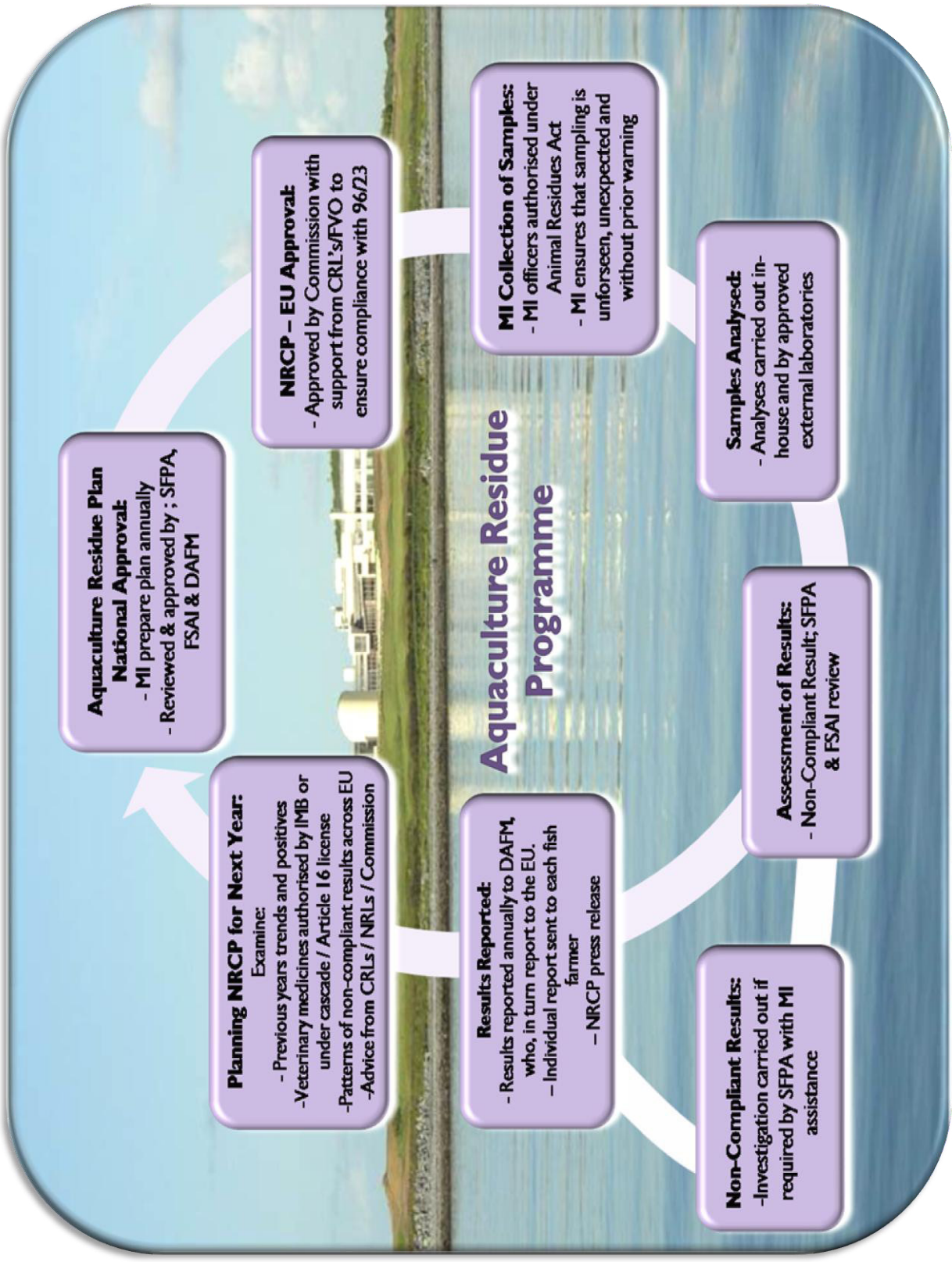


Figure 2: Annual cycle of National Residue Control Programme for Aquaculture

Table 1: List of substances included in the Residue plan for farmed finfish

Group A–Substances having anabolic effect		Group B- Veterinary drugs and contaminants	
A3	Steroids	**B1**	Antimicrobials (Antibacterial)
A6	Compounds included in Annex IV of Council Regulation 2377/90/EC	**B2a**	Anthelmintics (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 such as beta-oestradiol and methyltestosterone which occur naturally but also could be used for growth promotion
- A6 compounds such as nitrofurans and nitroimidazole which are antibacterial drugs; chloramphenicol which has a broad spectrum of antibiotic properties

Group B:

Group B substances are classed into 3 categories: **unauthorised substances, authorised substances and environmental contaminants**. As with other intensively farmed animals farmed finfish can be subject to disease and infestation which can have animal welfare, environmental and commercial implications. Similar procedures are in place for the treatment of farmed finfish as for other farmed animals, with approved veterinary medicines, under the direction of a veterinarian. These include antibiotics and anthelminthics to prevent or treat disease or infestation e.g: antibacterial agents, antifungal agents, antiparasitic treatments. To ensure compliance with Maximum Residue Limits (MRL), operators are obliged to adhere to the specified withdrawal periods after treatment before harvesting.

Veterinary substances are used to control sea lice infestations of farmed salmon

Figure 3: Sea lice on fish

A particular issue for the finfish aquaculture sector is the requirement to control ecto-parasitic sea lice (*Lepeophtheirus salmonis, Caligus elongatus)* infestations on fish farms (Fig. 3). Sea lice levels are monitored on fish farms with a requirement to treat if trigger levels are exceeded. Targeted treatment regimes can include synchronous treatments, i.e. "Single Bay Management". Further information is available in: "A strategy for the improved pest control on Irish salmon farms" (DAFF, 2008), which

outlines a comprehensive range of measures to provide for enhanced sea lice control; and in "The Farmed Salmonid Health Handbook" (Marine Institute, 2011), which assists producers in establishing a framework which will protect animal health and promote fish welfare on Irish farms. Table 2 details the veterinary medicines that are available for use in Ireland in 2011 for farmed finfish.

Table 2: Veterinary medicines authorised for use in Finfish 2011^

Medicine	Active Ingredient	Treatment	Group	Licensing Status
Alphamax ®	Deltamethrin	Bath	Pyrethroid	Full Medical Authorisation
Ektobann ®	Teflubenzuron	In-Feed	Insect Growth regulator	Full Medical Authorisation
Florocol ®	Florfenicol	In-Feed	Antibiotic	Full Medical Authorisation
Aqualet ®	Oxtetracycline Hydrochloride	In-Feed	Antibiotic	Full Medical Authorisation
Pyceze ®	Bronopol	Bath	Antimicrobial	Full Medical Authorisation
Slice ®	Emamectin Benzoate	In-Feed	Avermectin	Full Medical Authorisation
Calicide ®	Teflubenzuron	In-Feed	Insect Growth regulator	Full Medical Authorisation
Salmosan ®	Azamethiphos	Bath	Organo-phosphate	Article 16 ^^
Excis ®	Cypermethrin	Bath	Pyrethroid	Full Medical Authorisation

^Further details on veterinary medicines reference report prepared by the Marine Institute for SWRBD March 2007
^^ Certain veterinary medicines are authorised by DAFM in accordance with the provisions of national legislation (Regulations 16 & 17, S.I. No. 786 of 2007). These authorisations are viewed as being 'exceptional' and are subject to certain conditions. Information relating to veterinary medicines which have been authorised by the Dept. do not appear on the IMB website.

Fish, including farmed finfish, can accumulate trace metals and persistent organic pollutants from the environment or the feed; therefore these contaminants are also examined. An assessment of contaminants and residues in Irish seafood (2004 -2008) was carried out by the Marine Institute (McGovern *et al*, 2011). This identified that Irish seafood (fish and shellfish) was compliant with standards. Furthermore, the report established that estimates of intake of mercury, hexachlorbenzene and dioxins and dioxin-like PCBs from seafood consumption for the Irish seafood consumer were within safe limits as established by the European Food Safety Authority (EFSA). Moreover, the report highlighted the well established health benefits of eating fish, especially oily fish such as salmon, herring and mackerel.

4.0 SAMPLING

Residue sampling is carried out in accordance with Council Directive 96/23/EC by MI authorised sampling officers (Authorised under the Animal Remedies Act 1993). The following species are sampled and analysed as part of the NRCP (Fig. 4): Atlantic salmon (*Salmo salar*), freshwater and sea-reared trout (*Oncorhynchus mykiss*). Typically, five individual fish are collected per farm by an MI authorised officer from a producer at either the processing plant or fish farm. Figures 6 - 10 illustrate the locations of farmed finfish sites registered in Ireland in 2011; it is important to note that not all of these sites are active within a given year. The MI ensures that sampling is unforeseen, unexpected, without prior warning and that a strict chain of custody is maintained in accordance with Article 3 of Regulation 882/2004; *"Member States shall ensure that official controls are carried out regularly, on a risk basis and with appropriate frequency"* and Article 12 of Council Directive 96/23/EC; *"The checks provided for in this Directive must be carried out by the competent national authorities without prior notice"*.

Atlantic salmon (*Salmo salar*)

Freshwater trout (*Oncorhynchus mykiss*)

Figure 4: 2 species sampled as part of NRCP (BIM, 1999)

Samples are taken throughout the year in an effort to spread sampling across different sites and are taken in accordance with the National Residue Plan i.e.

- One third of the samples are taken 'on farm' at the smolt stage (Table 3) for salmon and at the Other Stages of Production (OSOP) for freshwater trout. This is aimed at detection of illegal treatment (prohibited substances/Group A and unauthorised substances /Group B3 (e)-Dyes).

Table 3: Autumn or spring smolts

Spring smolts	Autumn smolts
S1's : Smolts introduced to the sea in March	**S0's**: Smolts introduced to the sea in November

- Two thirds of the samples are taken at harvest stage which is aimed at ensuring compliance with the MRLs and for detection of illegal treatment (prohibited substances Group A and unauthorised substances Group B). These harvest samples are taken primarily at processing plants (Table 4) for salmon and sea-reared trout, and 'on farm' for freshwater trout.

Table 4: 2011 Active processing plants for salmon and sea-reared trout

Eany Fish Products Ltd.
Irish Seafood Producers Group (ISPG)
Marine Harvest Ireland Ltd.
Murphy's Irish Seafood Ltd.

In 2011, a total of 140 target (surveillance) samples were collected from fish farms and processing plants in accordance with the 2011 National Residues Control Plan (Appendix 3) as follows:

- 48 target samples (i.e. 41 salmon smolts and 7 freshwater trout) were taken at other stages of production from twelve farms for Group A and malachite green analysis

- 92 target samples were taken at harvest which comprised of 64 farmed salmon, 19 sea-reared trout and 9 freshwater trout

These target samples were collected from farmed finfish producers (Table 5) during 27 sampling events (samples collected from a given site at a given time) throughout the year. Salmon were collected on 21 occasions, freshwater trout twice and sea-reared trout four times. Generally 5 fish were taken from each producer and each individual fish was treated as one sample. Where an individual fish was not large enough to provide sufficient test material, a number of fish were pooled to provide a sample.

Table 5: 2011 Registered smolt and freshwater trout (OSOP) Sites*

Salmon Smolts	Freshwater Trout (OSOP)
ESB Salmon Hatchery (Parteen & Carraigadrohid)	Salmo Nova Ltd. (Garyhill)
Murphy's Irish Seafood Ltd. (Borlin)	Araglen Valley Trout Farm Ltd. (Kilworth)
Marine Harvest Ireland Ltd. (Pettigo, Lough Altan & Clare Island)	Douglas Valley Hatchery Ltd. (Kilworth)
Millbrook Salmon Hatcheries Ltd. (Drumcavney)	Goatsbridge Trout Farm Ltd. (Goatsbridge & Ballyduff)
Salmo Salar Ltd. (Ballyshannon)	ESB Fisheries Conservation (Parteen & Carraigadrohid)
Delphi Fishery Ltd.	IDAS Trout Ltd. (Lower-farm Woodenbridge, Main-farm Woodenbridge, Coatsbridge, Augrim & Gortdrom)
Derrylea Holdings Ltd. (Screebe, Lough Ahalia & Poulmounty)	Inland Fisheries Ireland (Cullion-Mullingar & Roscrea)
Mannin Bay Salmon Co. Ltd. (Lake Beaghcauneen)	Santa Cruise Salmon Farm Ltd. (Nenagh)
Stofnfiskur Ireland Ltd. (Bunatober)	
Inland Fisheries Ireland (Cong)	
Marine Institute Newport	

* not all sites are active in a given year

No suspect sampling took place in 2011 as there was no requirement for this arising from surveillance monitoring findings.

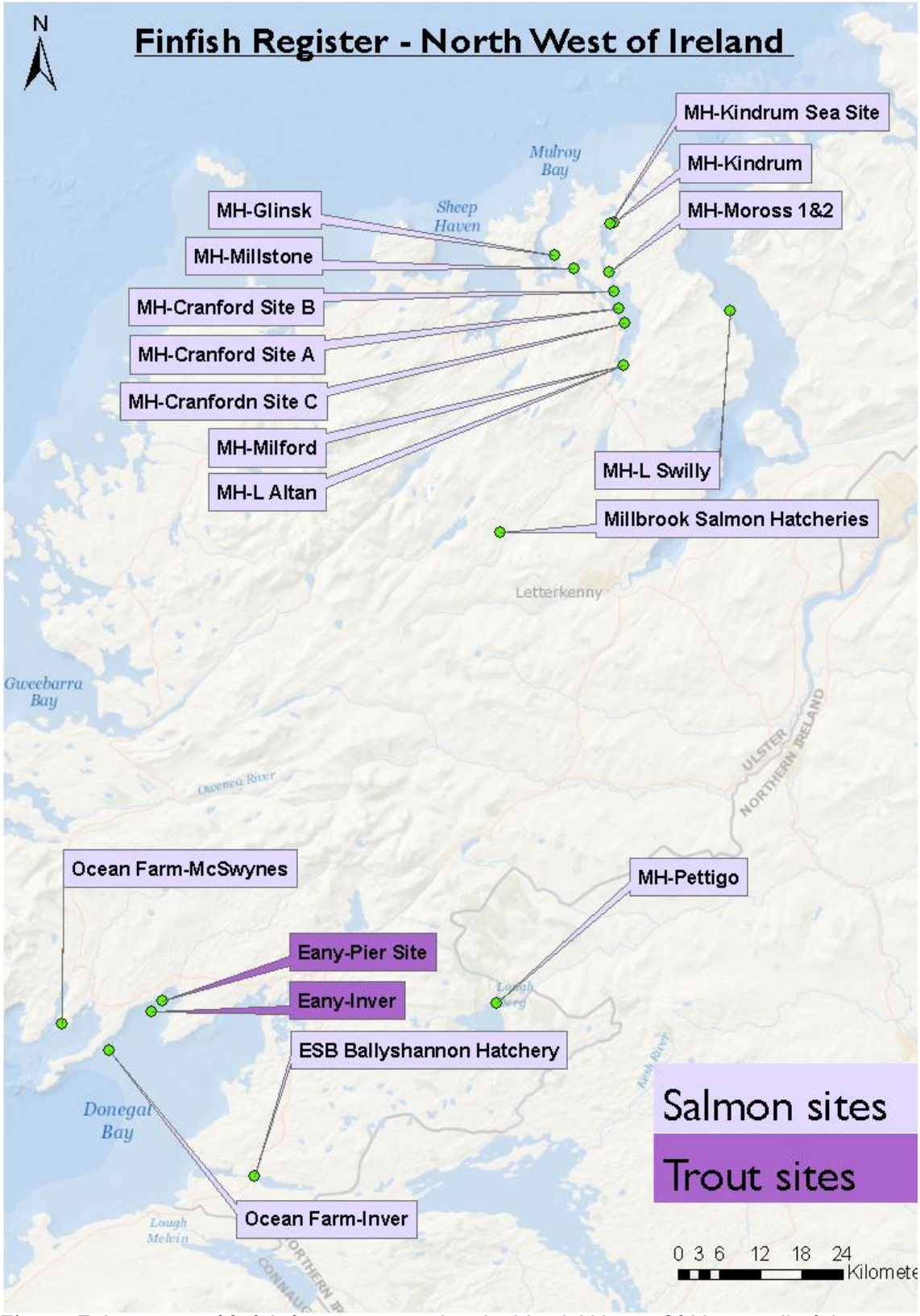


Figure 7: Locations of finfish farms registered in the North West in 2011 - not all of the sites are active in a given year (Marine Institute, 2012)

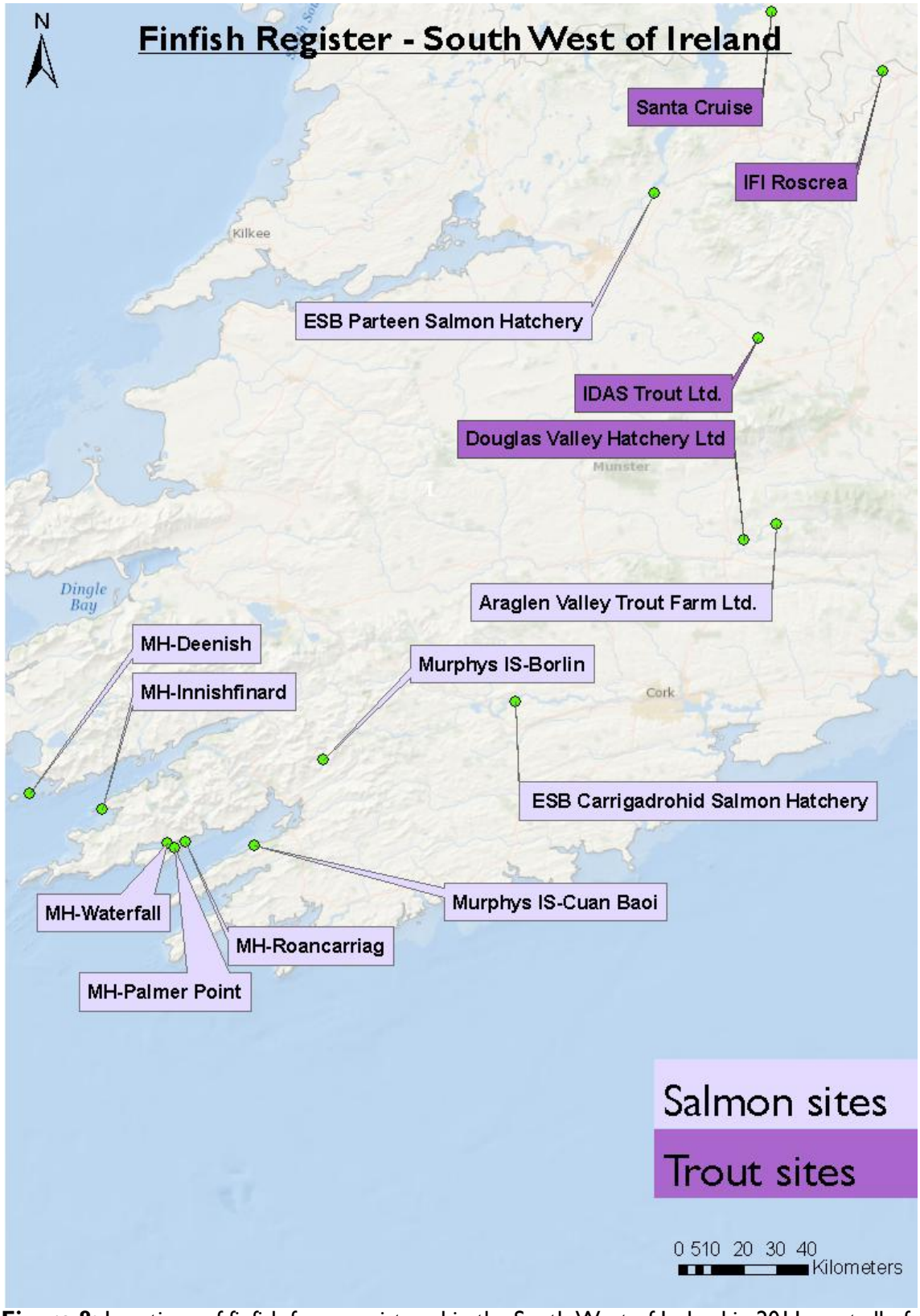


Figure 8: Locations of finfish farms registered in the South West of Ireland in 2011 - not all of the sites are active in a given year (Marine Institute, 2012)

Figure 9: Locations of finfish farms registered in the East of Ireland in 2011 - not all of the sites are active in a given year (Marine Institute, 2012)

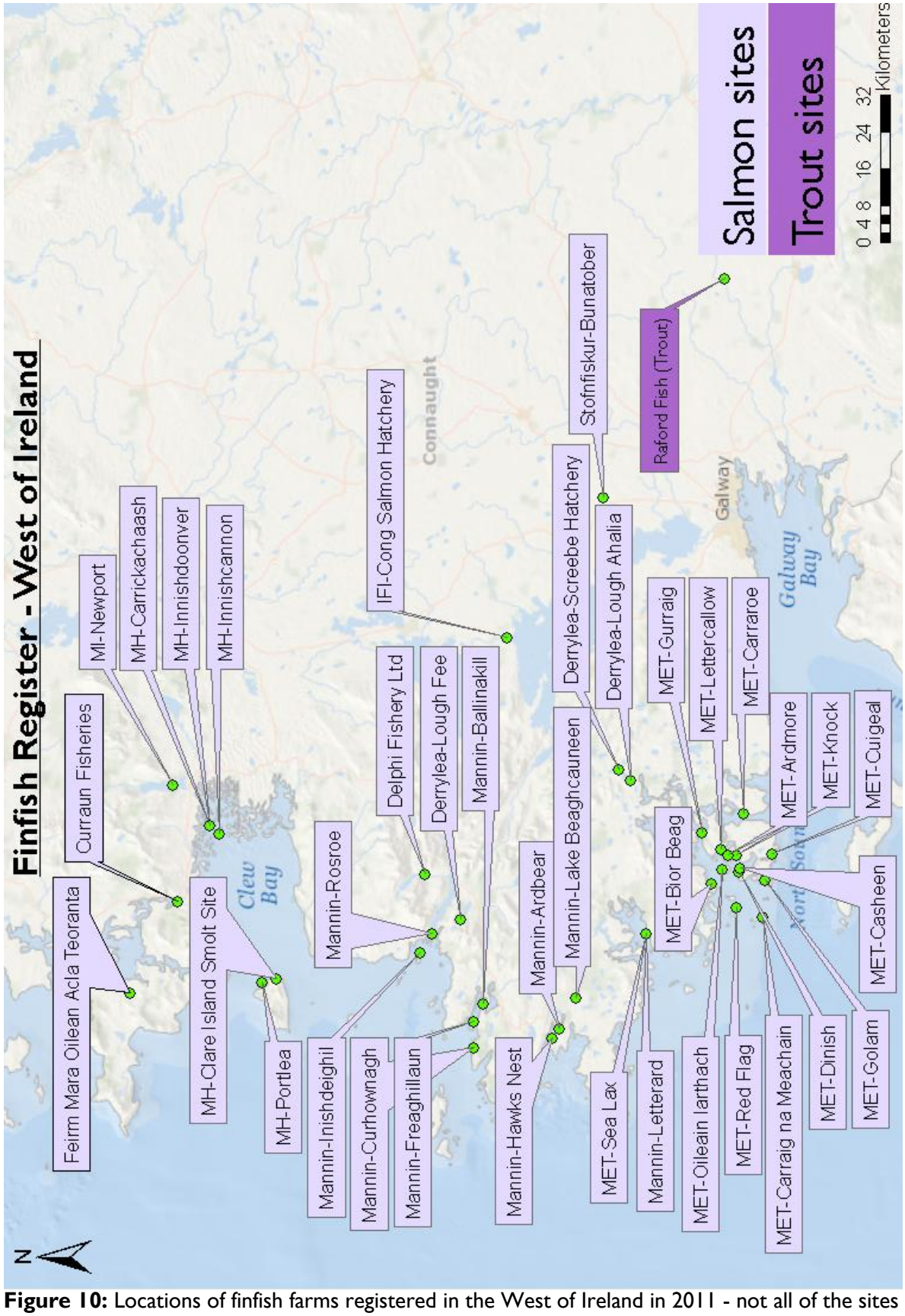


Figure 10: Locations of finfish farms registered in the West of Ireland in 2011 - not all of the sites are active in a given year (Marine Institute, 2012)

5.0 RESIDUE TESTING AND MI QUALITY SYSTEM

5.1 Chemical Analysis

Preparation of the samples is carried out upon arrival in the laboratory (Fig. 12), prior to testing for a broad range of substances and co-factors (Table 6). This testing utilises a variety of modern analytical techniques (Fig. 13) and employs both screening and confirmatory techniques. Depending on the parameter, the testing is carried out in the MI laboratories or in approved external laboratories that meet the strict validation requirements (Appendix I for analytical methodology utilised).

Figure 11: The Residues Directive for finfish aquaculture - Sampling process

A comprehensive quality assurance programme supports the monitoring programme as outlined below.

Table 6: List of substances included in the 2011 Residue plan for farmed finfish

Group A–Substances having anabolic effect		Group B- Veterinary drugs and contaminants	
A3	Steroids: Methyltestosterone 17 Betaoestradiol	**B1**	Antimicrobials (Antibacterial): Quinolones Tetracyclines Sulphonamides Florfenicol
A6	Compounds included in Annex IV of Council Regulation 2377/90/EC: Chloramphenicol Nitrofurans Nitroimidazoles	**B2a**	Anthelmintics (Antiparasitic): Ivermectin Emamectin B1a
		B2c	Pyrethroids: Cypermethrin Deltamethrin
		B2f	Other pharmacologically active substances: Teflubenzuron Diflubenzuron Corticosteroids
		B3a	Organochlorine compounds: PCBs OCPs
		B3c	Chemical elements: Lead Cadmium Mercury
		B3d	Mycotoxins: Aflatoxin
		B3e	Dyes: Malachite green Leuco malachite green

On return to the MI, the integrity of the samples is checked to ensure chain of custody has been maintained. Samples are filleted, scored & homogenised. (Note: Archive sample is set aside and stored. This can be used where dispute over results occurs.)The homogenised sample is sub- divided into several jars & stored in a locked freezer until analysis. The chain of custody remains intact from farm to final end of year report.

Figure 12: Sample preparation for NRCP samples

Samples are weighed; homogenised with solvent to extract analyte(s) of test; followed by sample clean-up techniques such as liquid or SPE; analysed by various scientific analytical equipment.

(Left to right): GC, HPLC and LCMSMS.

Figure 13: Laboratory analysis for NRCP samples

5.2 Quality Assurance

The Marine Institute implements a Quality System in line with ISO 17025 to underpin provision of high quality monitoring data. MI is accredited for the residue tests carried out in MI laboratories and is the National Reference Laboratory (NRL) for certain substances relevant to the NRCP (Table 7).

Table 7: MI National Reference Laboratory (NRL's)

Group B (2a)	Anthelmentics – Emamectin in Aquaculture only
Group B (2f)	Teflubenzuron & Diflubenzuron in Aquaculture only
Group B (3e)	Malachite Green and Leuco Malachite Green in Aquaculture only

It is a requirement of the NRCP that all screening and confirmatory methods are validated to Commission Decision 2002/657/EC[2]. All MI in-house test methods carried out as part of the NRCP are validated to Commission Decision 2002/657/EC and have obtained accreditation to ISO17025 from the Irish National Accreditation Board (INAB) as detailed in Scope Registration Number 130T and detailed in Appendix 2. Quality Control data for MI residue analysis in 2011 is reported in Appendix 2.

For some substances (Appendix 3) analysis is carried out in externally approved laboratories. Approved laboratories also must employ appropriate methods that are validated to the required specifications of Commission Decision 2002/657/EC and accredited to ISO17025 or equivalent standard.

2. Commission Decision 2002/657/EC establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories.

6.0 RESULTS OF ANALYSIS

6.1 Interpretation of Results

Samples are deemed **compliant** (i.e. negative) if authorised compounds do not exceed the MRL prescribed by the EC and if unauthorised substances are not detected above a defined analytical method action/decision limit. The MRLs and action levels are specified in Table 8.

Samples are deemed **non-compliant** (i.e. positive) if concentrations of a given residue are confirmed to be present in excess of the MRL, where the MRL has been set. Where no MRL is set, (e.g. for banned substances including steroids and compounds listed in Table 1 of Commission Regulation (EU) No 37/2010 and for unauthorized substances), an action level or decision limit is used and samples are reported as non-compliant where residues are confirmed to be present.

Generally, MRLs will not be exceeded if good husbandry practices are in place and the withdrawal periods are adhered to i.e. the animal is not slaughtered for a set period of time after treatment. A non-compliant result (i.e. a confirmed positive result) is assessed and reviewed by SFPA and FSAI and an investigation is carried out if required by SFPA with MI assistance.

6.2 Summary of 2011 results

In 2011, in excess of 630 analytical tests and a total of 1,566 individual measurements were carried out on 140 samples of farmed finfish. As in the recent years, no non-compliant results for target samples were reported in the national monitoring programme for farmed finfish in 2011.

Summary target results from sampling of farmed finfish for residues from 2004 - 2011 are outlined in Table 9, Figure 14, and Tables 10a and 10b respectively. Overall, the outcome for aquaculture remains one of consistently low occurrence of residues in farmed finfish, with 100% compliance for surveillance monitoring residue results for the period 2006-2011.

Table 8: Maximum Residues Limits, Action Limits & Guideline values used for assessing compliance with residues directive

Parameter	Maximum Level or Action Level (F)	Source
Group A Compounds[A] : Corticosteroids, Methyltestosterone, Betaestradiol, Chloramphenicol and Nitrofurans, Nitroimidazoles	These are banned substances and should not be detected.	
Ivermectin	**0.4 μg kg^{-1}**	Decision Limit[C]
Emamectin B1a	**100 μg kg^{-1}**	Maximum Residue Limit[B]
Cypermethrin	**50 μg kg^{-1}**	Maximum Residue Limit[B]
Deltamethrin	**10 μg kg^{-1}**	Maximum Residue Limit[B]
Teflubenzuron	**500 μg kg^{-1}**	Maximum Residue Limit[B]
Diflubenzuron	**1000 μg kg^{-1}**	Maximum Residue Limit[B]
Sulphadiazine	**100 μg kg^{-1}**	Maximum Residue Limit[B]
Oxytetracycline (Tetracyclines)	**100 μg kg^{-1}**	Maximum Residue Limit[B]
Oxolinic Acid (Quinolones)	**100 μg kg^{-1}**	Maximum Residue Limit[B]
Flumequine (Quinolones)	**600 μg kg^{-1}**	Maximum Residue Limit[B]
Sarafloxacin (Quinolones)	**30 μg kg^{-1}**	Maximum Residue Limit[B]
Florfenicol	**1000 μg kg^{-1}**	Maximum Residue Limit[B]
ICES PCB 6 [G]	**75 μg kg^{-1}**	EC Maximum Limit[H]
HCB	**50 μg kg^{-1}**	Norway (G)[D]
γ HCH	**100 μg kg^{-1}**	Finland (S)[D]
p,p'DDT and metabolites	**500 μg kg^{-1}**	Finland (S)[D]
Aldrin + Dieldrin	**100 μg kg^{-1}**	Finland (S)[D]
Endrin	**50 mg kg^{-1}**	Finland(S)[D]
Malachite Green	**1.0 μg kg^{-1}**	Decision Limit[C]
Leuco Malachite Green	**1.0 μg kg^{-1}**	Decision Limit[C]
Lead	**0.3 mg kg^{-1}**	EC Maximum Limit[E]
Cadmium	**0.05 mg kg^{-1}**	EC Maximum Limit[E]
Mercury	**0.5 mg kg^{-1}**	EC Maximum Limit[E]

Notes

A. Commission Regulation (EU) No 37/2010 (Table 2) and Directive 2008/97/EC: *Substances banned and should not be detected*
B. Commission Regulation No 37/2010 (Table 1) on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
C. These compounds are not authorised for use in finfish, concentrations above the analytical methods decision limit are non-compliant.
D. 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.
E. Commission Regulation (EC) No 1881/2006 *setting maximum levels for certain contaminant in foodstuffs and its amendments* Commission Regulation 629/2008/EEC and 565/2008/EEC.
F. Maximum Residue Limits and Action Levels concentration are on a wet weight basis.
G. ICES PCB 6: sum of the following 6 CB congeners –PCB 28, 52, 101, 138, 153, 180.
H. Commission Regulation No 1259/2011 of 2nd December 2011 amending Regulation No. 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. These limits applied from 1st January 2012

Table 9: Summary total target results for residue program for 2004-2011.

	2004	2005	2006	2007	2008	2009	2010	2011
No. Target samples^	183 (124, 59)	164 (105, 59)	162 (104, 58)	161 (103, 58)	162 (103, 59)	146 (98,48)	141 (92,49)	140 (92,48)
Total Group A^^	145/0	163/0	162/0	148/0	144/0	128/0	109/0	105/0
Total Group B^^	130/5	164/0	162/0	161/0	162/0	146/0	141/0	140/0
Total No. of Results^^^	2214/5	2251/2	2207/0	2219/0	2073/0	1750/0	1569/0	1566/0
% non - compliant results	0.23	0.09	0	0	0	0	0	0

Notes

^ Target samples (sampled at harvest, sampled at other stages of production)

^^ No. of samples tested/No. of samples non-compliant

^^^ Total no. of results: samples taken for Group A and Group B substances which are tested for multiple residue categories within each group

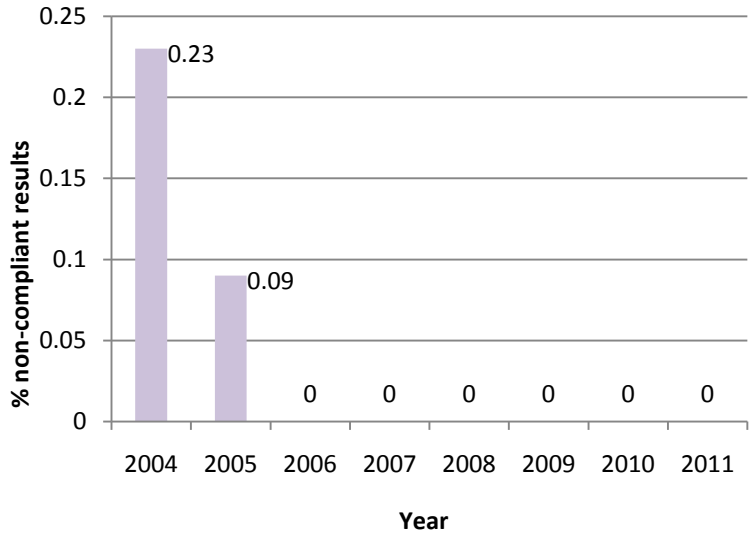


Figure 14: Percentage (%) non-compliant residue results from surveillance monitoring of farmed finfish 2004-2011

Table 10a: Summary of 2011 Residue Monitoring Results for Target farmed fish samples (salmon and trout). All tests preformed on muscle tissue

Residue	Group	Number examined	Non-Compliant^	Detection Limit^^ (ppb)
Corticosteroids	A3	49	0	1.5
Methyltestosterone	A3	36	0	1.5
17β-oestradiol	A3	10	0	1.5
Chloramphenicol	A6	47	0	0.25
Nitrofurans	A6	12	0	0.06
Nitroimidazole	A6	12	0	3.0
Tetracyclines	B1	92	0	Various
Quinolones	B1	92	0	Various
Sulphonamides	B1	92	0	Various
Florfenicol	B1	92	0	Various
Emamectin B1a	B2a	92	0	9.0
Ivermectin	B2a	92	0	0.4
Cypermethrin	B2c	86	0	4.0
Deltamethrin	B2c	92	0	4.0
Teflubenzuron	B2f	92	0	80
Diflubenzuron	B2f	92	0	86
ICES PCB 6^^^	B3a	19	0	-
Pentachlorobenzene	B3a	9	0	0.02
Hexachlorobenzene	B3a	9	0	0.09
Heptachlor	B3a	9	0	0.09
HCP-cis	B3a	9	0	0.02
HCP-trans	B3a	9	0	0.09
Aldrin	B3a	9	0	0.09
Toxaphene 26	B3a	9	0	0.05
Toxaphene 50	B3a	9	0	0.09
Toxaphene 62	B3a	9	0	0.17
Octachlorstyrene	B3a	9	0	0.02
Dieldrin	B3a	9	0	0.02
Eindrin	B3a	9	0	0.02
Mirex	B3a	9	0	0.02
Endosulphane sulphate	B3a	9	0	0.06
α-Endosulphane	B3a	9	0	0.09
β-Endosulphane	B3a	9	0	0.06
Chlordane- α	B3a	9	0	0.02
Chlordane-oxy	B3a	9	0	0.02

Notes

^ Action limit reference Table 8.

^^Limit of Detection (LOD) for organochlorine compounds are averages as LOD is sample dependant.

^^^ICES 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 food.*

Table 10b: Summary of 2011 Residue Monitoring Results for Target farmed fish samples (salmon and trout). All tests preformed on muscle tissue

Residue	Group	Number examined	Non-Compliant^	Detection Limit^^ (ppb)
α-HCH	B3a	9	0	0.02
β-HCH	B3a	9	0	0.02
γ-HCH	B3a	9	0	0.02
σ-HCH	B3a	9	0	0.07
DDD-o,p'	B3a	9	0	0.02
DDT-o,p'	B3a	9	0	0.02
DDT-p,p'	B3a	9	0	0.02
DDE-o,p'	B3a	9	0	0.02
DDE-p,p'	B3a	9	0	0.02
DDD-p,p'	B3a	9	0	0.02
Lead	B3c	9	0	8
Cadmium	B3c	9	0	2
Mercury	B3c	9	0	8
Aflatoxins	B3d	7	0	0.1
Malachite Green	B3e	75	0	1.0
Leuco Malachite Green	B3e	75	0	1.0

Notes

^ Action limits Ref. Table 8.

^^Limit of Detection (LOD) for organochlorine compounds are averages as LOD is sample dependant.

Liquid Chromatography

6.3 Breakdown of 2011 Results

Group A

A total of 105 samples were tested for Group A substances and **no non-compliant results were obtained for these banned compounds;**

- Group A3:

Of these 95 samples were tested for Group A3 steroids

- Corticosteroids: 49 samples including dexamethasone, flumethasone and betamethasone
- Methyltestosterone: 36 samples
- 17β-oestradiol: 10 samples

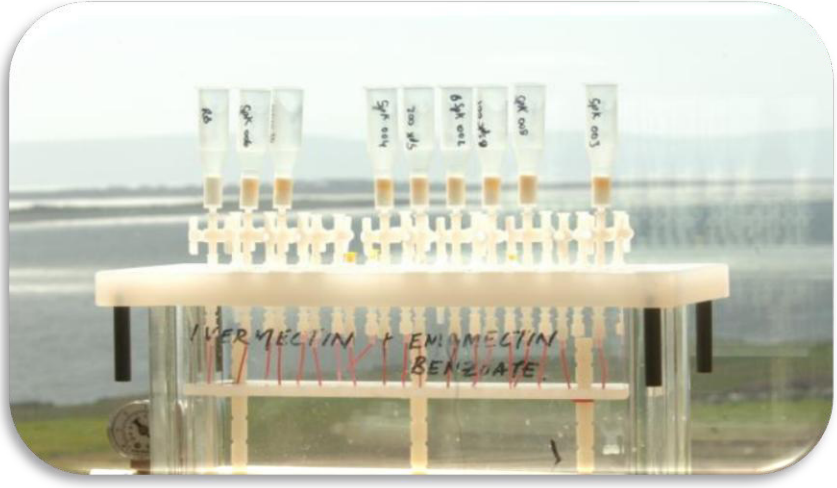
Screening was carried out using the ELISA method. **No non-compliant (i.e. no positive) results were reported for Group A3 samples.**

- Group A6:

71 samples were tested for the following Group A6 compounds:

- Chloramphenicol: 47 samples analysed for chloramphenicol by screening ELISA method
- Nitrofurans: 12 samples analysed for the marker metabolites of the nitrofurans; furazolidone, furaltadone, nitrofurantoin and nitrofurazone using a quantitative test (LCMSMS)
- Nitroimidazole: 12 samples analysed for nitroimidazole and its metabolites by a quantitative test (LCMSMS)

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



SPE clean up technique for analysis

Group B

A total of 140 samples were tested for Group B substances which can be classed as authorised substances, unauthorised substances or environmental contaminants. **No non-compliant results were obtained for these compounds;**

- Group B1:
  Antibiotic residues were screened for using a qualitative screening method for the detection of quinolones, tetracyclines, sulphonamide and florfenicol. **No non-compliant (i.e. no positive) results were obtained out of the 92 samples tested for antibiotic residue.**

- Group B2:
  Group B2 contains treatments that are classed as other veterinary drugs. In aquaculture these veterinary drugs are all generally authorised or unauthorised sea lice treatments. These include cypermethrin, deltamethrin, ivermectin, emamectin B1a, teflubenzuron and diflubenzuron. 92 samples were analysed for each of these analytes. **No non-compliant (i.e. no positive) results were obtained for Group B2 compounds.**

- Group B3a: Environmental Contaminants
  Persistent Organic Pollutants: Persistent organic pollutants (POPs) are primarily anthropogenic toxic substances that are persistent in the environment. They can be transported long distances in air and water and consequently tend to be globally ubiquitous pollutants. They bioaccumulate in animal tissue, especially fatty tissues such as oily fish, and may biomagnify through the food chain. Seafood is one of the key dietary sources for humans. Examples of POPs include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) such as DDT, dioxins, certain brominated flame retardants and perfluorinated compounds. Consequently POPs are widely monitored in seafood to ensure consumer safety. Measures to control or phase out POPs have been implemented at national, regional and global scales over recent decades (OSPAR 2010, EPA 2012) and environmental concentrations of many POPs are generally decreasing, although given their persistence this is a slow process. POPs are found in the tissue of farmed fish, primarily due to uptake from fishfeed, and this is associated with the POP burdens in the wild fish oil/meal component of the feed.

  The section below details the outcome of surveillance monitoring of indicator PCBs and OCPs in farmed salmon in 2011. More information on POPs in Irish seafood, including farmed finfish, is available in McGovern *et al*, 2011.

  Polychlorinated Biphenyls (PCBs) are a group of homologous man-made substances with a molecular structure comprising of a chlorinated biphenyl ring. PCBs are used in a wide range of industrial applications due to their thermal and electrical insulation properties. Despite being banned since the 1980s they are still being found in long life equipment such as electrical transformers and capacitors (EPA 2012). They are persistent environmental contaminants that accumulate in lipids and as such 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. Recent European legislation (Commission Regulation (EU) No 1259/2011 which came into force 1st Jan 2012 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 ug kg^{-1} wet eight has been set for the sum of these six congeners. **None of the 19 samples analysed exceeded the standard for NDL- PCB.**

Organochlorine pesticides (OCPs) are synthetic substances used for pest control that are persistent and widespread in the marine environment. 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 with high levels especially found in marine mammals. A number of OSPAR contracting countries have set levels and Annex 1 shows the available standards/guidance values in so far as MI is aware of these**. All the samples analysed for chlorinated pesticides were below these levels and were reported as compliant.**

The FSAI, in conjunction with the MI, carried out a discrete study in 2010-2011 to survey a broad range of halogenated POPs in Irish fishery products. 6 farmed salmon and 2 sea reared trout were included in this survey. Substances tested for included Group B substances, namely polychlorinated dibenzo-*p*-dioxanes and polychlorinated dibenzofurans (PCDD/Fs – dioxins), PCBs including 12 WHO 'dioxin-like' PCBs, polybrominated diphenyl ethers (PBDEs) and other brominated flame retardants such as polybrominated biphenyls (PBBs). A number of mixed halogenated compounds such as mixed halogenated dioxins and biphenyls were also included in the survey. The study showed that levels of PCDDs, PCDFs and PCBs (both DL-PCBs and indicator PCBs) in Irish fish and other seafood are well below the limits laid down for these POPs in Council Regulation 1881/2006, as amended. Other halogenated POPs including brominated flame retardants were also detected in seafood although limits have not been established for these substances as yet. The FSAI concluded that, taking into consideration recent risk assessments carried out by the EFSA on a number of brominated POPs, it is unlikely that intake of any of these brominated flame retardants, brominated or mixed halogenated dioxins, furans or biphenyls from fish is of health concern for the Irish population. With the banning and introduction of restrictions on the use of all BDE (brominated diphenyl ethers) commercial flame retardant mixtures and certain other brominated flame retardants e.g. HBCD (Hexabromocyclododecane) in the European Union, the use and production of alternative substances is predicted to increase. Future surveillance programs will have to closely monitor any trends in Irish produce (FSAI 2013).

- Group B3b: Chemical elements
  Levels of mercury, cadmium and lead were all very low and well below the relevant European maximum limits as described in Table 8.

  The highest mercury concentration obtained for the 9 samples analysed was 0.07 mg kg^{1} (wet weight). This is approximately seven times lower than the maximum limit for mercury in fishery products i.e. 0.5 mg kg^{-1} (wet weight) thus **all samples were reported as compliant for mercury.**

  Cadmium, also an environmental contaminant, has a maximum limit set in fish of 0.05 mg kg^{-1} (wet weight). Cadmium was not detected in any of the samples above the limit of quantification of 0.005 mg kg^{-1}. **All 9 samples were reported as compliant.**

  Lead has a maximum limit set in fish of 0.3 mg kg^{-1} (wet weight). All 9 samples analysed had concentrations less than 0.3 mg kg^{-1} (wet weight), the highest lead concentration obtained for the 9 samples was 0.09 mg kg^{-1}; thus **all samples were reported as compliant for lead.**

  2011 results confirm previous studies which show that Irish farmed finfish have low concentrations of these elements. More information on trace elements in Irish seafood, including farmed finfish, is available in McGovern *et al*. (2011).

- Group B3c: Mycotoxins
  A mycotoxin is a toxic by-product of mold 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 aquaculture plan in 2011 analysed for the following mycotoxins: aflatoxin B1 aflatoxin, B2, aflatoxin G1 and aflatoxin G2. **All samples analysed were found to have concentrations of less that the limit of detection.**

- Group B3d: Dyes-Malachite green
  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). Its use had been primarily associated with freshwater farms and hatcheries; therefore over recent years, monitoring has been scaled up with freshwater installations particularly targeted. Recent results suggest that as a result of increased industry awareness that it is an unauthorised substance[3], supported by monitoring and enforcement, the use of malachite green has ceased and **all target samples tested for malachite green and its metabolite leuco malachite green in 2011 were found to be compliant i.e. negative.**

[3] Article 6 of Directive 2001/82/EC requires the inclusion of pharmacologically active substances in Annex I, II or III of Regulation 2377/90. Malachite green and leuco malachite green are not included in these Annexes and have never been evaluated according to this regulation. The use of this substance in food producing animals is therefore illegal.

7.0 CONCLUSION

Veterinary treatments, environmental contaminants, unauthorised and banned substances in farmed finfish were analysed in 2011 and found to be 100% compliant with the requirements of the Residues Directive; with no non-compliant results detected for samples analyses as part of the routine targeted monitoring in 2011. This suggests that there is continued awareness and improved practices within the industry for the past number of years as there have been no non-compliant results for target samples since 2005; i.e. 100% compliance for surveillance monitoring residue for the period 2006-2011. It also indicates that the use of medicines and treatments is been managed with proper use of approved treatments including the observation of extended withdrawal periods.

Concentrations of environmental contaminants such as trace metals were low and well within EC maximum limits. Contaminant levels for PCBs and OCPs analysed as part of 2011 NRCP for farmed finfish, were within regulatory available limits where such limits are set for the protection of consumers. McGovern *et al*. (2011) provides further information of organohalogen compounds such as PCBs, dioxins and furans, organochlorine pesticides and brominated flame retardants in Irish seafood.

In order to comply with EU legislation, it is necessary that the monitoring of contaminants and veterinary residues in farmed fish continues into the future. In addition to legislation this monitoring programme provides ongoing assurance of consumer safety and enables ongoing consumer risk assessments; it provides data to support setting of new and practical standards at international level; it provides factual information as a basis for considered response to food safety scares relating to farmed finfish and this programme promotes good practice within the fish farming industry on an ongoing basis.

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OSPAR (2010) Quality Status Report 2010. OSPAR Commission. London. ISBN 978-1-907390-38-8 http://qsr2010.ospar.org

Maps

Finfish register – North West of Ireland in 2011. Scale 1:672,016. Locations of farmed finfish sites registered in Ireland in 2011, 2013. Using ArcGIS Desktop 9.3; Marine Institute, 2013.

Finfish register –South West of Ireland in 2011. Scale 1:1,346,943. Locations of farmed finfish sites registered in Ireland in 2011, 2013. Using ArcGIS Desktop 9.3; Marine Institute, 2013.

Finfish register – East of Ireland in 2011. Scale 1:755,033. Locations of farmed finfish sites registered in Ireland in 2011, 2013. Using ArcGIS Desktop 9.3; Marine Institute, 2013.

Finfish register – West of Ireland in 2011. Scale 1:924,039. Locations of farmed finfish sites registered in Ireland in 2011, 2013. Using ArcGIS Desktop 9.3; Marine Institute, 2013.

Legislation

This section lists some of the main legal instruments concerning the Irish finfish aquaculture industry for monitoring of NRCP.

European

Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC.

Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories

Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin.

Commission Decision 2004/25/EC of 22 December 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin

Commission Implementing Decision 2011/717/EU of 27 October 2011 amending Decision 2006/130/EC amending Decision 98/536/EC establishing the list of national reference laboratories for the detection of residues

Directive 2008/97/EC of the European Parliament and of the Council of 19 November 2008 amending Council Directive 96/22/EC concerning the prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of beta-agonists

REGULATION (EC) No 470/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council

COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

COMMISSION REGULATION (EU) No 420/2011 of 29 April 2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs

COMMISSION REGULATION (EU) No 594/2012 of 5 July 2012 amending Regulation (EC) 1881/2006 as regards the maximum levels of the contaminants ochratoxin A, non dioxin-like PCBs and melamine in foodstuffs

COMMISSION REGULATION (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs

National
Animal Remedies Act, 1993 (Act No 23 of 1993).

European Communities Animal Remedies Regulations 2007. S.I. 786 of 2007 as amended SI12/2009 & SI262/2012

European Communities Control of Animal Remedies and their Residues) Regulations 2009 S.I. 183 of 2009 as amended SI263/2012

Useful websites

- Department of Agriculture, Fisheries and Food (Ireland): http://www.agriculture.gov.ie/; http://www.agriculture.gov.ie/foodsafetyconsumerissues/veterinarymedicinesresidues/
- Food Safety Authority of Ireland: www.fsai.ie; http://www.fsai.ie/legislation/food_legislation/veterinary_medicines/monitoring_of_residues.html
- European Food Safety Authority: www.efsa.eu
- European Agency for the Evaluation of Medicinal Products: www.emea.eu.int; http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/landing/veterinary_medicines_regulatory.jsp&mid=WC0b01ac058001ff8a
- European Commission: http://ec.europa.eu/health/veterinary-use/index_en.htm
- BIM www.bim.ie; http://www.bim.ie/about-the-seafood-industry/
- South Western River Basin District http://www.swrbd.ie/

Appendix 1: Methods of Analysis

1.1 Sample Collection and Preparation.

In accordance with the 2011 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 (<-20°C).

1.2 Screening for Group A Compounds.

Group A compounds were screened using the ELISA method, this testing was subcontracted out to Irish Diagnostic Laboratory Services (IDLS). This method is qualitative in nature and is used to detect residues of Corticosteroids, Beta-estradiol, Chloramphenicol and Methyltesterone.

1.3 Nitrofuran Analysis.

The analysis of Nitrofurans is carried out by the Ashtown Food Centre (AFC). Tissue bound residues of Nitrofurans are hydrolysed with acid and both the released and the free metabolites are derivatised with 2-Nitrobenzaldehyde. The nitrophenyl derivatives are extracted with ethyl acetate and determined by LC-MS/MS using deuterated analogues as internal standards for quantification. Metabolites of Furazolidone, Furaltadone, Nitrofurantoin and Nitrofurazone are analysed.

1.4 Nitroimidazoles

Analysis of nitroimidazoles is carried out by a subcontracted laboratory (LGC, UK). Samples are homogenised with dichloromethane and the supernatants filtered and collected. The extracts are cleaned up using silica Solid Phase Extraction (SPE) cartridges before analysis by LC-MSMS.

1.5 Analysis for Cypermethrin and Deltramethrin by Gas Chromatography Electron Capture Detection (GC-ECD).

Approximately 2g of sample was extracted using a hexane/acetone mixture, followed by liquid/liquid partition and solid phase extraction techniques. The extract was then evaporated to dryness and reconstituted in 2, 2, 4-trimethylpentane. The analysis was carried out using Varian CP-3800 Gas Chromatography coupled with electron capture detector (GC-ECD) with a Chrompack 15m CpSil 8 column.

1.6 Analysis of Ivermectin and Emamectin B1a by High Performance Liquid Chromatography (HPLC) with Fluorescence Detection.

A representative sample (5g) 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 high performance liquid chromatography (HPLC) with fluorescence detection.

1.7 Analysis of Teflubenzuron and Diflubenzuron by HPLC 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 HPLC using an acetonitrile/water mobile phase and UV detection. Confirmation and peak purity is evaluated using a photodiode array detector.

1.8 Antimicrobial Screening.

Antimicrobial screening is carried by the Fish Health Unit (FHU) of the Marine Institute, using a modification of the Three 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 is used to detect residues of Quinolones, Tetracyclines, Sulphonamides and Florfenicol.

1.9 Analysis for Malachite Green and Leuco Malachite Green.

Analysis of Malachite Green (MG) and Leuco Malachite Green (LMG), are extracted from samples by homogenisation with acetonitrile: McIlvain Buffer pH 3 (9:1). The sample extract is cleaned up using solid phase extraction techniques. The eluant is evaporated to dryness and the subsequent determination of MG and LMG was achieved using Liquid Chromatography with tandem Mass Spectrometry detection (LC-MS/MS).

1.10 Analysis for Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCPs).

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

1.11 Determination of Cadmium and Lead using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS).

Concentrated nitric acid (4ml) and hydrogen peroxide (4ml) were added to approximately 0.2g freeze-dried tissue. Samples were then digested with the aid of microwave (CEM Mars5). Lead and cadmium concentrations were determined using Graphite Furnace Atomic Absorption Spectrometry with Zeeman background correction (Varian SpectrAA 220Z).

1.12 Determination of Mercury using Cold Vapour Atomic Fluorescence Spectroscopy (CVAFS).

Concentrated nitric acid (4ml) was added to 0.2g of accurately weighed wet tissue, which was then digested in a laboratory microwave oven (CEM Mars5). Mercury concentrations were determined by Cold Vapour Atomic Fluorescence Spectroscopy (CV-AFS) using a PSA Merlin Analyser.

1.13 Determination of Percentage Moisture Content.

Samples for metal analysis were freeze dried prior to analysis at approximately –10°C and with a vacuum of less than 10 microns of Hg. The percentage moisture content was calculated by determining the loss on freeze drying.

1.14 Analysis for Mycotoxins.

Analysis of Aflatoxins B1, B2, G1 and G2 is carried out under contract with the LGC UK. The method involves the extraction of about 25g of muscle using dichloromethane and the extract 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.

Appendix 2: Quality Control

MI Methods for Residue Programme Accredited by INAB

Scope Registration Number 130T	**Test Method**
Ivermectin	(CHE-8)
Emamectin	(CHE-8)
Mercury	(CHE-32)
Teflubenzuron	(CHE-42)
Diflubenzuron	(CHE-42)
Cadmium	(CHE-85)
Lead	(CHE-84)
Cypermethrin	(CHE-25)
Deltamethrin	(CHE-25)
Screening of Antibiotic Residues in Fish	(FHU-1)
Malachite Green and Leuco Malachite Green	(CHE-125)
Note	
When Collecting Samples the Laboratory Complies with Council Directive 96/23/EC	(CHE-6)

To check the quality of the data produced during the 2011 National Surveillance Scheme for chemical residues in farmed fish, Quality Control (QC) samples in the form of either reagent blanks, replicates, spiked samples or Certified Reference Materials (CRMs) were analysed with each batch of samples. The quality assurance results obtained, as shown below $\pm$ 1 Standard Deviations (SD), 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.

QC control data 2011

Analyte	QC Type	Target Value	Result ± SD	Mean Z – Score
Anthelmintics (μg kg^{-1})				
Ivermectin		*2.0*		–
Emamectin B1a		*100*		–
Pyrethroids (μg kg^{-1})				
Cypermethrin		*50*		–
Deltamethrin		*10*		–
Benzoylurea (μg kg^{-1})				
Teflubenzuron		*500*		–
Diflubenzuron		*1000*		–
Malachite Green (μg kg^{-1})				
Malachite Green		*2*		–
Leuco Malachite Green		*2*		–
Chemical Elements (mg kg^{-1})				
Lead		1.19		*0.46*
Cadmium		*2.48*		*0.85*
Mercury		*4.64*		*1.79*

Note: n is the sample number

Appendix 3: 2011 Plan for the Monitoring and Detection 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, Fisheries and Food
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 the 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 and the CRL – Bilthoven

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

4. Approved laboratories:
Marine Institute (MI),
Rinville,
Oranmore,
Co. Galway.

Laboratory of the Government Chemist,
Queens Road,
Teddington,
Middlesex,
TW11 OLY,
England

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

RILILT-CRL,
Laboratory for Residue analysis,
NL-3720 BA BILTHOVEN,
Netherland

Eurofins GfA GmbH,
D-48161 Münster,
Germany

Ashtown Food Research Centre,
Teagasc,
Ashtown
Dublin 15

Wessling GmbH,
Bochum,
Germany

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 2011

Sampling levels and frequency:
Minimum number of fish from which samples must be taken.

Finfish:

Total Tonnes Produced 2009	**Total min. no. to be tested****	**Min. no. Group A**	**Min. no. Group B**
13,584	Production (tonnes)/100 =136	1/3 Total Tested = 45	2/3 Total Tested =91

** min no. to be tested will be based on 2009 finfish production numbers as production numbers not available for 2010

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**
Group A								
A 3 Steroids	***Corticosteroids*** Betamethasone	Muscle & Skin	(1)ELISA (2) LCMS	1)1.5 ug kg^{-1}	2)1.5 ug kg^{-1}	Presence	45*	(1) IEC (2) CRL
	Methyltestosterone		(1) ELISA (2) GCMS	1)1.5 ug kg^{-1}	2)0.5 ug kg^{-1}	Presence	30*	(1) IEC (2) CRL
	17 Betaoestradiol		(1) ELISA∞ (2) GCMS	1)1.5 ug kg^{-1} 1)1.0 ug kg^{-1}∞	2)0.5 ug kg^{-1}	0.5 ug kg^{-1}	10*	(1) IEC∞ (2) CRL
A 6 Compounds included in Annex IV Council Reg. 2377/90	***Chloramphenicol***	Muscle & Skin	(1) ELISA∞ (2) GCMS	1)0.25 ug kg^{-1} 1)0.3 ug kg^{-1}∞	2)0.19 ug kg^{-1}	Presence	45*	(1) IEC∞ (2) LGC
	Nitrofurans AOZ AMOZ AHD ***SEM***	Muscle & Skin	LCMSMS	-	0.5 ug kg^{-1} 0.5 ug kg^{-1} 1.0 ug kg^{-1} 1.0 ug kg^{-1}	Presence	12*	AFC
	Nitroimidazoles Dimetridazol hydroxyl- Dimetridazol Ronidazol Metronidazol hydroxyl- Metronidazol	Muscle & Skin	LCMSMS	-	2.2 ug kg^{-1} 1.1 ug kg^{-1} 3.0 ug kg^{-1} 2.6 ug kg^{-1} 2.9 ug kg^{-1}	Presence	12*	LGC

* At least 50% of Group A are "on farm" samples

Column 4: Screening is No. (1) and Confirmatory is No. (2)

∞For screened positive samples for Chloramphenicol using the Elisa, these samples will be sent to subcontract lab LGC for further screening (LCMS).

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: Quinolones: -Oxolinic acid -Flumequine Tetracyclines: -oxytetracycline Sulphonamides -Sulphadiazine Florfenicol	Muscle & Skin	Modified EC 3-plate method.	Zone size > 2mm	N/A	>MRL	91	MI
	Tetracycline Chlortetracycline Epi-Chlortetracycline Oxytetracycline Epi-Oxytetracycline Tetracycline Epi-Tetracycline Doxycycline	Muscle & Skin	1)LC-TOF 2)LCMSMS	50 ug kg-1 50 ug kg-1 50 ug kg-1 50 ug kg-1 50 ug kg-1 50 ug kg-1 50 ug kg-1	120 ug kg-1 135 ug kg-1 124 ug kg-1 130 ug kg-1 122 ug kg-1 146 ug kg-1 135 ug kg-1	120 ug kg-1 135 ug kg-1 124 ug kg-1 130 ug kg-1 122 ug kg-1 146 ug kg-1 135 ug kg-1	Confirmation and post screening identification of positive Microbiological Samples	LGC
	Quinolones Ciprofloxacin Enrofloxacin Danofloxacin Difloxacin Flumequine Oxolinic acid Sarafloxacin	Muscle & Skin	1)LC-TOF 2)LCMSMS	50 ug kg-1 50 ug kg-1 50 ug kg-1 150 ug kg-1 200 ug kg-1 50 ug kg-1 50 ug kg-1	128 ug kg-1 125 ug kg-1 134ug kg-1 401 ug kg-1 700 ug kg-1 124 ug kg-1 40 ug kg-1	128 ug kg-1 125 ug kg-1 134ug kg-1 401 ug kg-1 700 ug kg-1 124 ug kg-1 40 ug kg-1		LGC

Column 4: Screening is No. (1) and Confirmatory is No. (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		1)LC-TOF 2)LCMSMS	 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1} 50 ug kg^{-1}	**	**		LGC
B2 Other veterinary drugs								
B2 (a) Anthelmintics	Ivermectin	Muscle & Skin	HPLC-Flu	-	0.4 ug kg^{-1}	0.4 ug kg^{-1}	91	MI
	Emamectin B1a			-	120 ug kg^{-1}	120 ug kg^{-1}		
B2(b) Nitroimidazoles++	hydroxyl-ipronidazole		LCMSMS	-	2.2 ug kg^{-1}	Presence	12*	LGC
B2 (c) Carbamates / Pyrethroids	Cypermethrin		1)GC-ECD 2)GC-MS	1)42 ug kg^{-1}	2)65 ug kg^{-1}	65 ug kg^{-1}	91	1)MI 2)LGC
	Deltamethrin			1)8 ug kg^{-1}	2)15 ug kg^{-1}	15 ug kg^{-1}		
B2 (f) Other Pharmacologically active substances	Teflubenzuron		HPLC-DAD	-	571 ug kg^{-1}	571 ug kg^{-1}	91	MI
	Diflubenzuron			-	1154 ug kg^{-1}	1154 ug kg^{-1}		
	Corticosteroids Dexamethasone		(1)ELISA (2) LC-MS	1.5 ug kg^{-1}	0.5 ug kg^{-1}	0.5 ug kg^{-1}	45*	(1) IEC (2) CRL
	Flumethasone			1.5 ug kg^{-1}	0.5 ug kg^{-1}	0.5 ug kg^{-1}		

At least 50% are "on farm" samples / Column 4: Screening is No. (1) and Confirmatory is No. (2)

**LGC 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

++Included in testing for nitromidazoles see A6 compound

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 Other substances and environmental contaminants							
B3 (a) Organochlorine compounds including PCBs	PCBs CB Congener 28 CB Congener 52 CB Congener 101 CB Congener 118 CB Congener 138 CB Congener 153 CB Congener 180	Muscle & Skin	GCHRMS	 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1	Excess of Guideline value.	 18	Eurofins
	Chlorinated Pest. α-HCH □-HCH γ-HCH □-HCH DDT-o,p' DDT-p,p' DDD-o,p' DDD-p,p' DDE-o,p' DDE-p,p' HCB Aldrin Dieldrin Endrin cis-Chlordane trans-Chlordane oxychlordane trans-Nonachlordane		GCHRMS	 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1 0.1 ug kg-1	Excess of Guideline value.	9	
B3 (c) Chemical elements	Lead		GFAAS	8 ug kg -1	300 ug kg -1	9	MI
	Cadmium		GFAAS	2 ug kg -1	50 ug kg –1	9	
	Mercury		CVAFS	8 ug kg -1	500 ug kg -1	9	
B3 (d) Mycotoxins	Aflatoxin B1	Muscle & Skin	HPLC-Flu	0.01 ug kg-1	-	7	Wessling
	Aflatoxin B2			0.01 ug kg-1			
	Aflatoxin G1			0.01 ug kg-1			
	Aflatoxin G2			0.01 ug kg-1			

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)		LCMSMS	- -	1.0 ug kg^{-1}## 1.0 ug kg^{-1}##	1.0 ug kg^{-1}##	74# (16 salmon/sea trout, 18 Freshwater trout, 40 salmon smolts)	MI

#58 of the 74 samples for Malachite Green and Leuco Malachite Green are "on farm" samples.
Reporting limit for MG and LMG.

List of Acronyms

AFC - Ashtown Food Centre
BDE - Brominated diphenyl ethers
CRM's - Certified Reference Materials
CVAFS - Cold Vapour Atomic Fluorescence Spectroscopy
DAFM - Department of Agriculture, Fisheries & Marine
DDD - Dichlorodiphenyldichloroethane
DDE - Dichlorodiphenyldichloroethylene
DDT - Dichlorodiphenyltrichloroethane
EC – European Commission
EEC – European Economic Community
EFSA – European Food Safety Authority
ELISA method - Enzyme-Linked ImmunoSorbent Assay
ESB - Electricity Supply Board
EU – European Union
FHU - Fish Health Unit
FSAI - Food Safety Authority of Ireland
GC-ECD - Gas Chromatography Electron Capture Detection
GFAAS - Graphite Furnace Atomic Absorption Spectroscopy
HBCD - Hexabromocyclododecane
HCB - Hexachlorobenzene
HCH - Hexachlorocyclohexane
HPLC - Performance Liquid Chromatography
HRGC - High Resolution Gas Chromatography
HRMS - High Resolution Mass Spectrometry
ICES - International Council for Exploration of the Sea
IDLS - Irish Diagnostic Laboratory Services formerly IEC - Irish Equine Centre
IFI- Inland Fisheries Ireland
INAB - Irish National Accreditation Board
ISO - International Organization for Standardization
ISPG - Irish Seafood Producers Group
LCMSMS - Liquid Chromatography with tandem Mass Spectrometry detection
LGC, UK - Laboratory of the Government Chemist, United Kingdom
LMG - Leuco Malachite Green
LOD - Limit of Detection
MET - Meitheal Éisc Teo
MG - Malachite Green
MH - Marine Harvest Ireland Ltd.
MI - Marine Institute
MRL - Maximum Residue Limit
NDL-PCB non dioxin-like Polychlorinated Biphenyls
NRCP - National Residue Control Plan
NRL - National Reference Laboratory
OCP's - Organochlorine pesticides
OSOP - Other Stages of Production
OSPAR - OSPAR Commission, protecting and conserving the North-East Atlantic and its resources
PBB - Polybrominated biphenyls
PCB - Polychlorinated Biphenyls
PDBE - Polybrominated diphenyl ethers
POP - Persistant organic pollutants
QC - Quality Control
QUASIMEME - Quality Assurance of Marine Environment and Monitoring in Europe
SFPA - Sea-Fisheries Protection Authority
SI - Statutory Instrument
SPE - Solid Phase Extraction
TPT - Three Plate Test
UV - Ultraviolet
VIs - Veterinary Inspector's
WHO – World Health Organisation

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