

VETERINARY RESIDUES COMMITTEE ANNOUNCEMENT ON THE RESULTS OF THE BRAND NAMING SURVEY OF DYES, TETRACYCLINES, NITROFURANS, CHLORAMPHENICOL, FLORFENICOL, ANTIMICROBIALS AND QUINOLONES IN IMPORTED FARMED FISH

A survey carried out by the Veterinary Medicines Directorate (VMD) on behalf of the Veterinary Residues Committee (VRC) found no evidence of the use of dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones in samples of imported farmed fish.

The Committee recommended that imported farmed fish should be tested because they noted that a wide range of farmed fish species were being imported and the previous brand name survey of fish in 2004 had concentrated on salmon. It was agreed that 100 samples should be collected as a brand name survey and should concentrate on fish other than salmon.

104 samples of imported farmed fish were analysed for dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones in the survey which was carried out between October 2009 and January 2010. The Committee decided on the majority of these substances based on reports issued under the Rapid Alert System for Food and Feed (RASFF).

The VRC welcomes the findings of this survey, which showed no evidence of the use of the substances tested for in the imported farmed fish sampled.

Results of the brand naming survey for dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones in imported farmed fish

Summary

A survey of dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones in samples of imported farmed fish was carried out by the Veterinary Medicines Directorate (VMD) between October 2009 and January 2010. The purpose of the survey was to assess the incidence of residues of these substances in imported farmed fish available from retail outlets and Border Inspection Posts (BIPs).

A total of 104 samples of imported farmed fish were analysed for residues of dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones. Fifty six samples for the survey were purchased from a variety of retailers by shoppers employed by the market research organisation Mintel International Group Ltd. Port Health Officers at four BIPs collected 33 samples and market inspectors provided 15 samples from Billingsgate Market. All the samples were analysed by the Food and Environment Research Agency (Fera) using validated methods of analysis.

All samples tested compliant.

Annex 1 gives details of all the samples collected as part of the survey. All available sample information has been included. Annex 2 gives details of sample preparation and the method of analysis.

Background

Fish has been tested under the non-statutory surveillance scheme for many years, including this year. The last time it was surveyed was in 2004 when home-produced and imported salmon was tested for malachite green and leucomalachite green as a brand naming survey.

When the options for brand naming were being considered in June 2009, three notifications had been sent out to Member States under the Commission's Rapid Alert System for Feed and Food (RASFFs) so far that year for fish. Two RASFFs had been issued for malachite green and/or leucomalachite green and one for enrofloxacin which was detected under the non-statutory programme in 2008. There were four RASFFs issued for fish in 2008; two were for malachite green and two for nitrofurans (AOZ/SEM¹). Fifteen RASFFs were issued for fish in 2007; nine for malachite green and/or leucomalachite green; two for nitrofurans (AOZ/AMOZ); one for enrofloxacin; one for oxytetracycline and two for crystal violet. Full details of the detection of residues in fish in recent years are provided in Table 1 below.

¹ Key to nitrofuran abbreviations: AOZ, 3-amino-2-oxazolidone (the metabolite of furazolidone); SEM, semicarbazide (the metabolite of nitrofurazone); AMOZ, 3-amino-5-morpholinomethyl 2-oxazolidone (the metabolite of furaltadone).

Table 1

Year	Country of origin	Residue detected (number of detections)	Fish type
2007	China	Leucomalachite green (2)	Eel
		Nitrofuran – AOZ (1)	Tilapia
		Crystal violet (1)	Tilapia
	Greece	Nitrofuran – AMOZ (1)	Sea bass
	Jamaica	Crystal violet (1)	Tilapia
	Spain	Malachite green (1)	Trout
	Thailand	Leucomalachite green (2)	Catfish
		Enrofloxacin (1)	Tilapia
		Oxytetracycline (1)	Tilapia
	Vietnam	Malachite green/leucomalachite green (3)	<i>Pangasius</i>
Leucomalachite green (1)		<i>Pangasius</i>	
2008	China	Nitrofuran – AOZ (1)	Tilapia
	Germany	Malachite green (1)	Trout
	Vietnam	Nitrofuran – SEM (1)	<i>Pangasius</i>
		Malachite green (1)	Yellow catfish
2009	China	Nitrofuran – SEM (1)	Tilapia
	Italy	Malachite green/leucomalachite green (1)	Trout
	Poland	Malachite green (1)	Trout
	Turkey	Enrofloxacin (1)	Trout
	Vietnam	Chloramphenicol (1)	Golden threadfin bream
		Leucomalachite green (1)	Mudfish
		Trifluralin (1)	<i>Pangasius</i>
2010 (to date)	Vietnam	Malachite green (1)	<i>Pangasius</i>
		Neomycin (2)	<i>Pangasius</i>

The Committee noted that it had not carried out a brand name survey of fish for four years. Also, a wide range of farmed fish species were being imported and the previous survey had concentrated on salmon. It agreed that 100 samples from those allocated to the programme should be collected as a brand naming survey and would exclude salmon.

Dyes

The triarylmethane dye, malachite green, has been used extensively in the past by the aquaculture industries as an anti parasite treatment in Europe and throughout the world in the absence of any authorised veterinary medicine alternative. It is banned for use in food-producing species in the EU and in food imported into the EU. UK expert committees have concluded that malachite green and its metabolite leucomalachite green should be regarded as *in vivo* mutagens and that it would be prudent to regard leucomalachite green as a genotoxic carcinogen. Crystal (or gentian) violet is of the same family of dyes as malachite green and may be expected to pose similar risks.

The phenothiazine dye methylene blue has been used therapeutically for the treatment of protozoal infections and as an antifungal agent. Methylene blue and its metabolite are considered to be mutagens.

Tetracyclines

These are a group of broad-spectrum antibiotics. Oxytetracycline, one of the tetracycline antibiotics, is authorised in the UK for the treatment and control of furunculosis (*Aeromonas salmonicida*) and columnaris disease in Atlantic salmon and furunculosis and enteric red mouth disease in rainbow trout.

Nitrofurans

The nitrofurans are a group of antibiotic drugs which were banned from use in food animal production in the European Union in the mid 1990s because of concerns about their carcinogenicity and mutagenicity (Commission Regulation 1442/95)².

Chloramphenicol

Chloramphenicol is a powerful broad-spectrum antibiotic used for a wide range of bacterial infections in many species. Chloramphenicol also has various uses in human medicine and can be contained in products such as eye drops and topical creams. Chloramphenicol is **not authorised** for use in the treatment of animals for food production in the EU and **may not be used** in the production of food of animal origin which is imported into the EU. Chloramphenicol is considered to be a potential genotoxic carcinogen. Oral and intravenous use of chloramphenicol is associated with a rare idiosyncratic aplastic anaemia in humans which can be fatal. (There are no data to associate the use of chloramphenicol eye drops with aplastic anaemia.)

The use of chloramphenicol and nitrofurans in the treatment of food-producing animals is prohibited in the EU and residues should not be present in food products, whether home-produced or imported, intended for human consumption.

Florfenicol

Florfenicol is a broad spectrum antibiotic which is active against both Gram positive and Gram negative bacteria isolated from domestic animals. It is authorised in the UK for the treatment of furunculosis (*Aeromonas salmonicida*) infection of Atlantic salmon.

Antimicrobials

Antimicrobials are compounds that, at low concentrations, exert an action against micro-organisms and exhibit selective toxicity towards them. The term includes any substance of natural, synthetic or semi-synthetic origin that is used to kill, or inhibit the growth of, micro-organisms (bacteria, fungi, protozoa and viruses). Antimicrobials include antibiotics, disinfectants, preservatives and other substances. The antimicrobial screen element of this survey is concerned with antibiotics (specifically sulphonamides, penicillins

² Commission Regulation (EC) No 1442/95 of 26 June 1995 amending Annexes I, II, III and IV of Council Regulation (EEC) No 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin

and cephalosporins; additional classes of antibiotics are covered by other methods) and the analytical methodology employed has been optimised accordingly. The antimicrobials method used detects a broad range of antibiotics but has a low sensitivity to biocides such as disinfectants and preservatives. Penicillins and cephalosporins belong to a broad class of antibiotics known as β -lactams. Amoxicillin, a β -lactam antibiotic is authorised in the UK for the treatment of furunculosis (*Aeromonas salmonicida*) infection of Atlantic salmon.

Quinolones

Quinolones are synthetic broad-spectrum antibiotics. None are authorised in the UK for the treatment of fish.

Legislation

EC legislation requires that all active substances used in veterinary medicines for food-producing animals must be assessed so that a Maximum Residue Limit (MRL) can be set if required. Nitrofurans and chloramphenicol are listed in Table 2 of Commission Regulation 37/2010³ (previously Annex IV of Council Regulation 2377/90) and their use in the treatment of food-producing animals is prohibited in the EU. Countries that wish to export food of animal origin to the EU must respect the requirements of this legislation and therefore all exports to the EU should not contain residues of these substances.

Commission Decision 2002/657/EC⁴ introduced the concept of Minimum Required Performance Limits (MRPLs). This recognises the need for EU harmonisation of analytical detection limits, particularly for banned substances, and is the concentration at which regulatory laboratories should be able to detect and confirm the presence of particular substances. In March 2003, Commission Decision 2003/181/EC⁵ set an MRPL of 0.3 µg/kg for chloramphenicol in meat, milk, eggs, aquaculture products and honey and 1 µg/kg for nitrofurans metabolites in poultry meat and aquaculture products. In December 2003, Commission Decision 2004/25/EC⁶ set an MRPL of 2 µg/kg for the sum of malachite green and leucomalachite green in meat of aquaculture products. Article 2 of Commission Decision 2005/34/EC⁷, states that the MRPLs laid down in Annex II to Decision 2002/657/EC shall be used as reference points for action irrespective of the matrix tested.

³ 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

⁴ Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

⁵ 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

⁶ 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

⁷ Commission Decision 2005/34/EC of 11 January 2005 laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries

MRLs have been set in fish muscle for florfenicol, sulphonamides and some of the tetracyclines, quinolones and β -lactams as follows:

Table 2

Substance group	Substance	MRL ($\mu\text{g}/\text{kg}$)
Tetracyclines	Chlortetracycline	100
	Doxycycline	Not set
	Oxytetracycline	100
	Tetracycline	100
Florfenicol	Florfenicol amine	1000
Quinolones	Sarafloxacin	30 (<i>Salmonidae</i>)
	Ciprofloxacin	
	Enrofloxacin	100 (Sum + Ciprofloxacin)
	Danofloxacin	100
	Nalidixic acid	Not set
	Norfloxacin	Not set
	Oxolinic acid	100
	Marbofloxacin	Not set
	Flumequine	600
	Difloxacin	300
Antimicrobial screen (β -lactams)	Amoxicillin	50
	Ampicillin	50
	Benzylpenicillin	50
	Cloxacillin	300
	Dicloxacillin	300
	Methicillin	Not set
	Oxacillin	300
	Phenethicillin	Not set
	Ticarcillin	Not set
Antimicrobial screen (sulphonamides)		100 (total)

All methods were validated at Fera according to the requirements of Commission Decision 2002/657/EC. This required the determination of a number of key parameters including "CC α " (decision limit). Where values for CC α are available, these are given in Annex 2. A confirmed residue with a measured concentration at or above the CC α indicates that the sample is non-compliant.

Sampling

In this survey, 104 samples of imported fish products were analysed. The countries of origin of the samples were as follows:

Table 3

EU	Non-EU	More than 1 country of origin
1 Italy	1 Ecuador	10 Greece & Turkey
1 Ireland	1 Iceland	
3 Spain	1 India	
9 Greece	1 Sri Lanka	
	1 Jamaica	
	2 Zimbabwe	
	5 Indonesia	
	6 China	
	9 Thailand	
	14 Turkey	
	39 Vietnam	

Fifty six samples were purchased by Mintel International Group Ltd from retail outlets in England, Ireland, Scotland and Wales. Shoppers were asked to collect samples according to a sampling protocol and were advised not to purchase salmon. Port Health Officers at Felixstowe, Grimsby, Southampton and Tilbury BIPs collected 33 samples. Market inspectors purchased 15 samples from Billingsgate Market. The sample size was a minimum of 600 g.

Results

Full results of the survey are detailed in Annex 1. The survey found that all of the 104 samples tested were compliant.

Conclusions

The purpose of this survey was to assess the incidence of residues of dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones in samples of imported farmed fish. The substances to test for were chosen mainly because of the reports issued under the European Commission's Rapid Alert System for Feed and Food over recent years. The results of this survey found no evidence of the use of dyes, tetracyclines, nitrofurans, chloramphenicol, florfenicol, antimicrobials and quinolones in any of the samples tested.

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N0877	Vietnamese River Cobbler	Not provided	05/10/2009	6 Oct	Vietnam	Tesco Arena Park Phoenix Way Coventry CV6 6EW	-	-	Compliant
09N0878	Fresh Gilthead Sea Bream	Not provided	05/10/2009	07/10/2009	Greece	Morrisons Holyhead Road Coventry CV5 8BX	-	-	Compliant
09N0879	Skinless & Boneless 8 Basa Fillets	Young's	05/10/2009	Mar 2010	Vietnam	Tesco Arena Park Phoenix Way Coventry CV6 6EW	-	Young's PO Box 51 Grimsby DN31 3TJ	Compliant
09N0880	Tilapia Fillets	Not provided	05/10/2009	06 Oct	Jamaica	Tesco Arena Park Phoenix Way Coventry CV6 6EW	-	-	Compliant
09N0881	Seabass Fillet	Not provided	05/10/2009	07.10.2009	Greece	Morrisons Holyhead Road Coventry CV5 8BX	-	-	Compliant
09N0895	Sea Bass	Not provided	05/10/2009	07/10/2009	Turkey	Asda Bugsby's Way Charlton London SE7 7ST	-	-	Compliant
09N0896	Trout	Ocean Pearl	05/10/2009	02/12/2010	Spain	Makro Anchor & Hope Lane Charlton London SE7 7RZ	-	Sea Products International Ltd Ocean House Wholesale Markets Precinct Pershore Street Birmingham B5 6UU	Compliant

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09N0897	Sea Bass Fillets	Extra Special	05/10/2009	09 Oct	Farmed in Greece or Turkey	Asda Bugsby's Way Charlton London SE7 7ST	-	Packed in the UK for Asda Stores Ltd Leeds LS11 5AD	Compliant
09N0898	Sea Bass Fillets	Not provided	05/10/2009	06-10-2009	Greece	Sainsbury's 55 Bugsby's Way Charlton London SE10 0QJ	-	Sainsbury's Supermarkets Ltd 33 Holborn London EC1N	Compliant
09N0899	Basa Fillets	Not provided	05/10/2009	06-10-2009	Vietnam	Sainsbury's 55 Bugsby's Way Charlton London SE10 0QJ	-	Sainsbury's Supermarkets Ltd 33 Holborn London EC1N	Compliant
09N0913	Whole Sea Bass	Asda Fish	04/10/2009	05-10-09	Farmed in Greece or Turkey	Asda Kingswood Retail Park Bude Road Hull HU7 4HS	-	Asda Stores Limited Leeds LS11 5AD	Compliant
09N0914	Whole Sea Bream	Asda Fish	04/10/2009	05-10-09	Farmed in Greece or Turkey	Asda Kingswood Retail Park Bude Road Hull HU7 4HS	-	Asda Stores Limited Leeds LS11 5AD	Compliant
09N0915	Tilapia Fillets	Waitrose	04/10/2009	06/10/09	Zimbabwe	Waitrose Willerby Shopping Park Grange Park Lane Willerby Hull HU10 6EB	-	Waitrose Ltd Bracknell Berkshire	Compliant

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09N0931	Whole Farmed Sea Bass	Not provided	04/10/2009	5 Oct	Greece	Tesco Nant Y Ffin Road Llansamlet Swansea SA7 7DE	-	-	Compliant
09N0932	River Cobbler	Asda Fish	04/10/2009	05-10-09	Farmed in Vietnam	Asda Upper Forest Way Morrison Swansea SA6 8PS	-	Asda Stores Limited Leeds LS11 5AD	Compliant
09N0933	Whole Sea Bream	Asda Fish	04/10/2009	05/10/2009	Turkey	Asda Upper Forest Way Morrison Swansea SA6 8PS	-	Asda Stores Limited Leeds LS11 5AD	Compliant
09N0934	Sea Bass Fillets	Asda Fish	04/10/2009	05-10-09	Farmed in Greece/ Turkey	Asda Upper Forest Way Morrison Swansea SA6 8PS	-	Asda Stores Limited Leeds LS11 5AD	Compliant
09N0950	4 Whole Sea Bream	The Fishmonger	09/11/2009	17.06.2010	Farmed In Turkey	Aldi 9-10 Boulevard Retail Park Aberdeen AB24 5EZ	-	Specially packed for Aldi Stores PO Box 26 Atherstone Warwickshire CV9 2SH / Aldi Stores (Ireland) Ltd P O Box 736 Naas Co Kildare	Compliant

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09N0951	Whole Sea Bass	The Fishmonger	09/11/2009	13.02.2010	Farmed In Turkey	Aldi 9-10 Boulevard Retail Park Aberdeen AB24 5EZ	-	Specially packed for Aldi Stores PO Box 26 Atherstone Warwickshire CV9 2SH / Aldi Stores (Ireland) Ltd P O Box 736 Naas Co Kildare	Compliant
09N0965	2 White Rainbow Fillets Trout	Tesco	09/11/2009	Dec 09	Spain	Tesco Ffordd Pengam Cardiff CF24 2HP	-	-	Compliant
09N0966	Sea Bass Fillets	Tesco Finest	09/11/2009	13 Nov	Turkey	Tesco Ffordd Pengam Cardiff CF24 2HP	-	Produced in the UK for Tesco Stores Ltd Cheshunt EN8 9SL	Compliant
09N0967	Fresh Gilthead Sea Bream	Not provided	09/11/2009	11.11.2009	Greece	Morrisons 8 - 12 Ty Glas Road Cardiff CF14 5EP	-	-	Compliant
09N0968	River Cobbler	Asda Fish	09/11/2009	10-11-09	Vietnam	Asda Longwood Drive Cardiff CF14 7EW	-	-	Compliant
09N0969	Mediterranean Sea Bass Fillets	Extra Special	09/11/2009	14 Nov	Turkey	Asda Longwood Drive Cardiff CF14 7EW	-	-	Compliant

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09N0982	Farmed Sea Bass Fillets	Not provided	09/11/2009	10/11/2009	Italy	Tesco Aachen Way Halifax HX1 3TU	-	-	Compliant
09N0983	River Cobbler	Not provided	09/11/2009	10/11/2009	Vietnam	Tesco Aachen Way Halifax HX1 3TU	-	-	Compliant
09N0984	Whole Sea Bass	The Fishmonger	09/11/2009	01/2011	Turkey	Aldi Crossley Retail Park Halifax HX1 5RA	-	Specially packed for Aldi Stores PO Box 26 Atherstone Warwickshire CV9 2SH / Aldi Stores (Ireland) Ltd P O Box 736 Naas Co Kildare	Compliant
09N0985	Sea Bass	Not provided	09/11/2009	10-11-09	Greece	Sainsbury's 21 Park Drive Halifax HX1 7NP	-	Sainsbury's Supermarkets Ltd 33 Holborn London EC1N	Compliant
09N0986	Tilapia Fillet	Not provided	09/11/2009	10-11-09	Zimbabwe	Sainsbury's 21 Park Drive Halifax HX1 7NP	-	Sainsbury's Supermarkets Ltd 33 Holborn London EC1N	Compliant
09N0999	White Fish Fillets	Booker Basics for Caterers	09/11/2009	Aug 2011	Vietnam	Booker Boundary Industrial Estate Stafford Road Fordhouses Wolverhampton WV10 7EL	-	-	Compliant

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09N1000	8 Basa Fillets	Young's	09/11/2009	Nov. 2009	Vietnam	Tesco Heath Way Cannock Staffordshire WS12 3YY	-	Young's PO Box 51 Grimsby DN31 3TJ	Compliant
09N1001	2 Aegean Sea Bass Fillets	Extra Special	09/11/2009	Sep 2010	Greek/ Turkish	Asda Avon Road Cannock Staffordshire WS11 1LH	-	-	Compliant
09N1002	4 Rainbow Trout Fillets	Young's	09/11/2009	05.2010	Thailand	Morrisons Mill Street Cannock Staffordshire WS11 0DR	-	Young's PO Box 51 Grimsby DN31 3TJ	Compliant
09N1015	Tilapia Fillets I.Q.F.	Holmes	07/10/2009	14/05/2011	China	CT Holmes & Co Shop 17/Office 46 Billingsgate Market London E14 5ST	-	Packed for: Holmes Seafoods P.O. Box No 2345 London E14 5ST	Compliant
09N1016	Snakehead	Not provided	07/10/2009	Not provided	Sri Lanka	Asian Fresh Stand No A6/7/8 Billingsgate Market London E14 5ST	-	-	Compliant
09N1017	Tilapia Steak	Forest Springs	07/10/2009	03.2011	Thailand	John Koch Shop 11/12 Billingsgate Market London E14 5ST	-	-	Compliant
09N1018	Red Tilapia	Not provided	07/10/2009	Not provided	Indonesia	Fawsitt Fish Shop 8 Billingsgate Market London E14 5ST	-	-	Compliant

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09N1019	Black Tilapia	Not provided	07/10/2009	Not provided	Thailand	Fawsitt Fish Shop 8 Billingsgate Market London E14 5ST	-	-	Compliant
09N1020	Not provided	Not provided	10/11/2009	25/08/2011	China	J & P Exotics Shop 5 Billingsgate Market E14 5ST	-	-	Compliant
09N1021	Milkfish	Sea Pride	10/11/2009	21.07.2011	Indonesia	John Koch Shop 11/12 Billingsgate Market London E14 5ST	-	-	Compliant
09N1022	Catfish Gutted	Forest Springs	10/11/2009	07.2011	Thailand	Billingsgate Exotic Stand G3 & G4 Billingsgate Market London E14 5ST	-	Imported by: SFI UK PO Box 235	Compliant
09N1023	Milkfish Gutted	Forest Springs	10/11/2009	03.2011	Thailand	Billingsgate Exotic Stand G3 & G4 Billingsgate Market London E14 5ST	-	Imported by: SFI UK PO Box 235	Compliant
09N1024	Pangasius	Not provided	10/11/2009	17/11/2009	Vietnam	Fawsitt Fish Shop 8 Billingsgate Market London E14 5ST	-	-	Compliant

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09N1128	Milkfish	Not provided	16/11/2009	Not provided	Indonesia	-	Swallow Foods International Ltd Scandia-Hus Business Park Felcourt Road Felcourt East Grinstead West Sussex RH19 2LP	-	Compliant
09N1129	Tilapia	Not provided	16/10/2009	Not provided	Indonesia	-	Ruskim Seafoods Ltd Marine House Stafford Park 15 Telford Shropshire TF3 3BB	-	Compliant
09N1130	Pangasius Fillet	Danish Brand	27/10/2009	11/09/2011	Vietnam	-	MFS Sea Foods Ltd Unit 39 Walsall Enterprise Park Regal Drive Walsall WS2 9HQ	-	Compliant
09N1131	Pangasius Fillet	Not provided	10/11/2009	15.09.2011	Vietnam	-	Swallow Foods International Ltd Scandia-Hus Business Park Felcourt Road Felcourt East Grinstead West Sussex RH19 2LP	-	Compliant
09N1132	Black Tilapia	Not provided	12/11/2009	Not provided	China	-	Milegate Ltd 20 Somerleyton Road London SW9	-	Compliant

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09N1133	Tilapia	Asian Star	12/11/2009	Not provided	Thailand	-	Nortrade Foods Ltd Hawkhurst Station Business Park Gills Green Hawkhurst Cranbrook Kent TN18 5BD	-	Compliant
09N1134	Black Tilapia	Blue Ocean	17/11/2009	05/09/2011	China	-	Milegate Ltd 20 Somerleyton Road London SW9	-	Compliant
09N1135	Basa Fillets	Kaitak	27/10/2009	20.07.2011	Vietnam	-	Immediate Transportation Co Chapel Street Liverpool L2 8TX	-	Compliant
09N1136	Pangasius Fillets	Not provided	29/10/2009	Not provided	Vietnam	-	Mitsubishi Corporation UK Plc Mid City Place 71 High Holborn London WCV1	-	Compliant
09N1140	Pangasius Fillets	Not provided	12/11/2009	Not provided	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1141	Pangasius Fillets	Not provided	14/10/2009	14.01.11	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant

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09N1142	Pangasius Portions	Not provided	14/10/2009	24.02.11	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1143	Pangasius Fillet	Not provided	20/10/2009	12.08.11	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1274	Tilapia	Ash	17/11/2009	10.2011	Thailand	-	Ash Poultry Wholesalers Ltd Units 65/2-65/3 Sienna Park White Hart Avenue Thamesmead London SE28 0GW	-	Compliant
09N1275	White Pangasius Fillet	Indus Fish Co	02/12/2009	Not provided	Vietnam	-	Indus Fish Co 2-4 Langley Road Small Heath Birmingham B10 0TL	-	Compliant
09N1276	Tilapia	Ash	05/12/2009	Not provided	Thailand	-	Ash Poultry Wholesalers Ltd Units 65/2-65/3 Sienna Park White Hart Avenue Thamesmead London SE28 0GW	-	Compliant
09N1277	Pangasius Fillets	Bloomsbury	07/12/2009	Not provided	Vietnam	-	Bloomsbury International Ltd 1 Knightsbridge London SW 1X	-	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1278	Catfish	Not provided	06/12/2009	Not provided	Thailand	-	Milegate Ltd 20 Somerleyton Road London SW9	-	Compliant
09N1279	Mekong Catfish Chunk	Kim Son	19/11/2009	14/10/2011	Vietnam	-	Kim Son Ltd 4 Estate Way London E10 7JN	-	Compliant
09N1280	Pangasius	Not provided	08/12/2009	Not provided	Vietnam	-	Mitsubishi Corporation UK Plc Mid City Place 71 High Holborn London WCV1	-	Compliant
09N1284	Pangasius Fillets	Not provided	12/11/2009	12.09.2011	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1285	Pangasius Fillets	Not provided	24/11/2009	13.09.2011	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1286	Pangasius Fillet	Freshway	02/12/2009	22/09/11	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1287	Pangasius Fillets	Not provided	03/12/2009	13.04.11	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1288	Tilapia Fillets	Not provided	10/12/2009	05/072010	Ecuador	-	Tropical Aquaculture Europe SA 3 Rue du Fort Rheinsheim L-2419 Luxembourg	-	Compliant
09N1291	Pangasius Fillets	Not provided	12/01/2010	Not provided	Vietnam	-	Bloomsbury International Ltd 1 Knightsbridge London SW 1X	-	Compliant
09N1292	Tilapia	Not provided	13/01/2010	Not provided	China	-	J P & L Exotics 11 Ernest Road Hornchurch Essex RM11	-	Compliant
09N1294	Pangasius Fillet	Not provided	17/12/2009	Not provided	Vietnam	-	Kamal & Sons Ltd 94 Grange Road Small Heath Birmingham B10 9QS	-	Compliant
09N1295	8 Basa Fillets	Young's	08/01/2010	Not provided	Vietnam	-	Mitsubishi Corporation UK Plc Mid City Place 71 High Holborn London WC1V	-	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1299	Pangasius Goujon	Not provided	08/12/2009	29 03 11	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1300	Pangasius Fillets	Not provided	18/12/2009	01 10 2011	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1301	Pangasius Fillets	Not provided	05/01/2010	07 11 2011	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1302	Pangasius Belly Block	Not provided	29/12/2009	Not provided	Vietnam	-	Fastnet Fish Ltd Estate Road No 5 South Humberside Ind Estate Grimsby DN31 2TG	-	Compliant
09N1359	Whole Sea Bass	The Fishmonger	07/12/2009	12/02/2010	Turkey	Aldi Alphington Road Exeter EX4 8JD	-	-	Compliant
09N1360	Whole Sea Bream	The Fishmonger	07/12/2009	06/09/2010	Turkey	Aldi Alphington Road Exeter EX4 8JD	-	-	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1361	2 Sea bass fillets	Sainsbury's Taste the difference	07/12/2009	OCT 10	Farmed in Greece and Turkey	Sainsbury's Alphington Road Exeter EX2 8QN	-	-	Compliant
09N1362	2 Sea Bass Fillets	Tesco Finest	07/12/2009	OCT 2010	Farmed in Greece and Turkey	Tesco Exeter Vale Exeter Devon EX2 7EZ	-	-	Compliant
09N1363	Whole Sea Bass	The Fishmonger	06/12/2009	05.03.2010	Turkey	Aldi Whittlesey Road Peterborough Cambs PE2 8SQ	-	-	Compliant
09N1364	2 Aegean Sea Bass Fillets	Asda Extra Special	06/12/2009	JUL 2010	Greece/ Turkey	Asda Viersen Platz Rivergate Peterborough Cambs PE1 1 ET	-	-	Compliant
09N1365	2 White Rainbow Fillets Trout	Tesco	06/12/2009	SEP 10	Spain	Tesco Extra Serpentine Green Shopping Centre Hampton Peterborough Cambs PE7 8BE	-	-	Compliant
09N1367	2 Sea bass fillets	Sainsbury's Taste the difference	07/12/2009	OCT 10	Greece/ Turkey	Sainsbury's Silksworth Lane Sunderland SR3 1PD	-	-	Compliant
09N1368	Sea bass fillets	Sainsbury's Taste the difference	07/12/2009	09 Dec	Greece	Sainsbury's Silksworth Lane Sunderland SR3 1PD	-	-	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1369	Sea Bream	Sainsbury's The Fishmonger	07/12/2009	08-12-09	Greece	Sainsbury's Silksworth Lane Sunderland SR3 1PD	-	-	Compliant
09N1370	Basa Fillets	Sainsbury's The Fishmonger	07/12/2009	08-12-09	Vietnam	Sainsbury's Silksworth Lane Sunderland SR3 1PD	-	-	Compliant
09N1371	Sea Bass	Not provided	07/12/2009	08-12-09	Greece	Sainsbury's 1 Nash Way Kenton HA3 0JA	-	-	Compliant
09N1372	Basa Fillets	Not provided	07/12/2009	10/12/2009	Vietnam	Sainsbury's 1 Nash Way Kenton HA3 0JA	-	-	Compliant
09N1373	Pangasius Fillet	Lilly	07/12/2009	31.05.2011	Vietnam	Asda Park Royal 2 -20 Western Road NW10 7LW	-	-	Compliant
09N1374	River cobbler	Tesco Market value	07/12/2009	11 DEC	Vietnam	Tesco Brent Park Great Central Way NW10 0TL	-	-	Compliant
09N1375	River cobbler	Tesco Market value	11/01/2010	13 JAN	Vietnam	Tesco Warwick Road Carlisle CA1 2SB	-	Produced in the UK for Tesco Stores Ltd Cheshunt EN8 9SL	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1376	7 Icelandic Arctic Char Fillets	Tesco Finest	11/01/2010	21/10/10	Iceland	Tesco Warwick Road Carlisle CA1 2SB	-	-	Compliant
09N1377	2 Sea Bass Fillets	Tesco Finest	11/01/2010	NOV 2010	Greece/ Turkey	Tesco Warwick Road Carlisle CA1 2SB	-	-	Compliant
09N1380	Whole Sea Bass	The Fishmonger	10/01/2010	04.03.2010	Turkey	Aldi Meadowhall Sheffield S9 2YZ	-	-	Compliant
09N1381	Rainbow Trout	Tesco	11/01/2010	12 JAN	Eire	Tesco 50 Montgomery Terrace Road Sheffield S6 3BU	-	-	Compliant
09N1382	River cobbler	Tesco Market value	12/01/2010	16 JAN	Vietnam	Tesco Station Road Addlestone Surrey KT15 2AS	-	-	Compliant
09N1383	Sea Bass Fillets	Tesco Finest	12/01/2010	12 Jan	Turkey	Tesco Station Road Addlestone Surrey KT15 2AS	-	-	Compliant
09N1384	River Cobbler	Not provided	12/01/2010	13-01-10	Vietnam	Tesco Station Road Addlestone Surrey KT15 2AS	-	-	Compliant

Sample Number	Product as Described	Brand	Date of Purchase/ Collection	Best Before/ Use By Date	Country Of Origin As Declared	Retailer/ Wholesaler/ Importer/ Distributor Address	BIPs Samples Importer Address	Supplier/ Manufacturer Address From Packaging	Result
09N1388	Not provided	Nireus	09/12/2009	Not provided	Turkey	Leleu & Morris Stand D 10-11-12 Billingsgate Market London E14 5ST	-	-	Compliant
09N1389	Sea Bream	Not provided	09/12/2009	17/12/2009	Turkey	Leleu & Morris Stand D 10-11-12 Billingsgate Market London E14 5ST	-	-	Compliant
09N1390	Not provided	Catla	09/12/2009	Not provided	India	British Exotics Stand E2 & E3 Billingsgate Market London E14 5ST	-	-	Compliant
09N1395	Not provided	Paradise Products	13/01/2010	15.09.11	China	J & P Exotics Shop 5 Billingsgate Market E14 5ST	-	-	Compliant
09N1396	Catfish Whole Gutted	The Ultimate Taste	13/01/2010	28.09.2011	Indonesia	J & P Exotics Shop 5 Billingsgate Market E14 5ST	-	-	Compliant

ANALYTICAL METHODOLOGY

All fish samples were transported to the Food and Environment Research Agency (Fera) frozen. Single-piece samples (e.g. a whole fish) had testing aliquots cut from the bulk sample without homogenisation. For subsequent analyses these aliquots were taken from adjacent sites. Multiple-piece samples (e.g. fillets) were maintained whole. Test aliquots were taken from individual pieces and any subsequent related assay was taken from an adjacent site on that same piece.

Test samples were divided to give separate sub-samples for screening and confirmatory analysis. Screening and confirmation sub-samples were stored separately. All prepared sub-samples were stored frozen at approximately -20 °C prior to analysis.

Samples were analysed in accordance with the quality criteria defined in EU legislation^a including using ISO17025 accredited methods. Chloramphenicol (CAP) screening was performed using an optical biosensor. Tetracyclines screening was performed using a receptor-based assay, with any suspect “positive” (potentially non-compliant) samples reanalysed using High Performance Liquid Chromatography with Ultra-Violet detection (HPLC-UV). Antimicrobial screening was performed using a microbial inhibition test. Nitrofurans, dyes, florfenicol and quinolones were screened using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). All confirmatory testing was performed in duplicate, by LC-MS/MS. All screening and confirmation analyses were carried out at Fera. A brief description of the analytical methods is as follows:

Chloramphenicol (screening)

CAP is extracted from tissue by sonicating and homogenising in methanol/water. The extract is centrifuged, applied to a reverse phase (C18) Solid Phase Extraction (SPE) cartridge, washed and eluted with methanol. The eluate is reduced to dryness under nitrogen and reconstituted in buffer. Samples are analysed using an immunoassay performed in a biosensor. The detection principle employed is Surface Plasmon Resonance (SPR). The immunoassay configuration is competitive inhibition format. Extracted matrix standards (*i.e.* a known amount of analyte added to a known blank sample prior to extraction) are used for calibration, which gives implicit recovery correction.

Chloramphenicol (confirmation)

CAP is extracted from tissue by homogenising in acetonitrile. The extract is centrifuged and applied to a disposable cartridge packed with a specially cleaned diatomaceous earth and eluted with dichloromethane. The eluate is reduced to near dryness under nitrogen, reconstituted in hexane/ethyl acetate and loaded onto an amino (NH₂) SPE cartridge. The cartridge is washed with ethyl acetate and the analyte eluted with ethyl acetate/methanol. The eluate is reduced to dryness under nitrogen and reconstituted in water. Samples are analysed using LC-MS/MS. Identification of CAP residues in a sample is made *via* two LC-MS/MS transitions. Solvent standards (*i.e.* a known amount of analyte added to a known volume of blank solvent) are used for calibration.

^a Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

A deuterated internal standard d₅-CAP is used in the quantification of CAP residues, which gives implicit recovery correction.

Nitrofurans (screening and confirmation)

Nitrofuran metabolites are extracted from tissue with dilute hydrochloric acid. The metabolites are derivatised with 2-nitrobenzaldehyde, and the sample neutralised with alkaline buffer. The resulting extract, containing 2-nitrobenzaldehyde derivatives of nitrofuran metabolites, is applied to a reverse phase SPE cartridge. The analytes are eluted from the cartridge with ethyl acetate, which is evaporated to dryness and the residue reconstituted in methanol/water prior to analysis by LC-MS/MS. Extracted matrix standards are used for calibration and d₅-3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ), d₄-3-amino-2-oxazolidone (AOZ), 2,4,5-[¹³C₃]- 1-aminohydantoin (AHD) and 1,2-[¹⁵N₂¹³C]- semicarbazide (SEM) are used as internal standards, which gives implicit recovery correction for all analytes.

For confirmation of SEM residues, initial washing of the confirmation sub-sample is performed according to EU Reference Laboratory (RL) guidelines to remove 'free' SEM and leave tissue bound SEM for measurement. The sample pre-treatment involves washing with methanol, ethanol, and diethyl ether prior to hydrolysis and derivatisation. Identification of nitrofuran metabolite residues in a sample is made *via* two LC-MS/MS transitions.

Dyes (screening and confirmation)

Tissue is extracted by acetonitrile using homogenisation. The extract is acidified with formic acid and applied to a strong cation exchange SPE cartridge. Target analytes are eluted by passing acidified ammonium acetate solution through the column. Determination is by LC-MS/MS. Matrix matched standards (*i.e.* a known amount of analyte added to a known blank sample after extraction) are used for calibration. Deuterated standards d₅-malachite green and d₅-brilliant green are used as internal standards, which gives implicit recovery correction for those analytes. For all other analytes, a recovery correction is applied.

Tetracyclines (screening)

The sample is extracted with buffer solution and the extract applied to a competitive receptor-based assay in a dipstick format. Tetracycline molecules present in the sample extract interact with the receptor to form a stable non-active complex. In this form, the receptor is no longer able to bind the first capture line printed on the dipstick and no response is observed. However, if the receptor remains free of a tetracycline antibiotic, binding occurs at the test line, which is visible as a red line. Visual interpretation of the dipstick is made to determine the result.

Tetracyclines (repeat screen and confirmation)

'Suspect positive samples' from the rapid screening assay (above) are reanalysed by a separate HPLC method, as follows; sample is extracted by buffer and the extract applied to a chelating sepharose column previously loaded with copper ions. Tetracyclines are eluted with ethylene diamine tetra-acetic acid buffer (EDTA). Further sample clean-up is then performed using a polymer-based SPE column. Determination is made by HPLC-UV. Matrix matched standards are used for calibration and a recovery correction is applied.

For confirmation, determination is made using the same extraction method and a combination of HPLC-UV (quantitation) and LC-MS/MS (confirmation of

identity). Matrix matched standards are used for calibration and a recovery correction is applied. Identification of tetracyclines residues in a sample is made *via* two LC-MS/MS transitions.

Antimicrobial screen

Sample extracts are applied to an ampoule containing *Bacillus stearothermophilus var. calidolactis* in a solid agar medium. Incubation is then carried out at 64°C until known blank samples change colour from purple to yellow (usually after about 3 hours). At this point, any samples that have failed to change colour are suspected to contain anti-microbial substances. Samples that have tested “Screen Positive” are subjected to post-screening analysis. Post-screening is by the same method, in duplicate and with and without the addition of β -lactamase (a penicillinase enzyme). “Screen Positives” from post-screening are subjected to a group screen by an alternative screening method specific to an analyte group (e.g. sulphonamides). Any samples giving a “Screen Positive” result from the group screen are confirmed by LC-MS/MS.

Florfenicol (screening and confirmation)

Tissue is homogenised in water, hydrolysed with hydrochloric acid and a liquid/liquid partition conducted with ethyl acetate. After adjusting to an alkaline pH, the aqueous phase is adsorbed onto a column packed with diatomaceous earth. Florfenicol amine is eluted with dichloromethane, evaporated to dryness and determined by LC-MS/MS. Extracted matrix matched standards are used for calibration. A deuterated standard d_3 -florfenicol is used as an internal standard, which gives implicit recovery correction.

Quinolones (screening and confirmation)

Tissue is extracted into acetonitrile/formic acid; the supernatant is then taken to dryness, reconstituted and the extract applied to a solid phase extraction column. Fluoroquinolones and quinolones are eluted with ammonia in acetonitrile. Analysis is by LC-MS/MS. Matrix matched standards are used for calibration. Deuterated standards d_8 -ciprofloxacin, d_3 -danofloxacin, d_3 -difloxacin, d_5 -enrofloxacin, $^{13}C_3$ -flumequine, d_5 -nalidixic acid, d_5 -norfloxacin, d_5 -oxolinic acid and d_8 -sarafloxacin are used as internal standards, which gives implicit recovery correction for those analytes. For marbofloxacin, a recovery correction is applied.

Quality Assurance

All *screening batches* included known blank samples, along with other Quality Assurance samples and standards as follows:

Chloramphenicol

Screening batches included multi-level extracted matrix matched standards to produce a calibration curve. Additional extracted matrix matched standards at the Screening Target Concentration (STC^b; see table) were also included. Calibration based on extracted matrix matched standards gives implicit recovery correction.

^b The Screening Target Concentration (STC) is the concentration at which a screening test categorises the sample as suspect “positive” (potentially non-compliant) and triggers a confirmatory test (see http://ec.europa.eu/food/food/chemicalsafety/residues/Guideline_Validation_Screening_en.pdf.)

Nitrofurans

Screening batches included extracted matrix matched standards at two different concentrations. All results were recovery corrected against deuterated internal standards (see above).

Dyes

Screening batches included multi-level matrix matched standards to produce a calibration curve. For malachite green and brilliant green, results were recovery corrected against deuterated internal standards (see above). For the other analytes, results were recovery corrected against extracted matrix matched standards at the STC.

Tetracyclines

Screening batches included extracted matrix matched standards at the STC.

Antimicrobial screen

Screening batches included extracted matrix matched standards at the STC.

Florfenicol

Screening batches included multi-level extracted matrix matched standards to produce a calibration curve. Results were recovery corrected against deuterated internal standard (see above).

Quinolones

Screening batches included matrix matched standards at two different concentrations. Results were recovery corrected against deuterated internal standards (see above), with the exception of marbofloxacin which was recovery corrected against extracted matrix matched standards at the STC.

Table 4

Screening method	Analyte*	STC ($\mu\text{g kg}^{-1}$)
Chloramphenicol	Chloramphenicol	0.15
Nitrofurans	SEM, AHD, AMOZ, AOZ	1
Dyes	Brilliant Green, New Methylene Blue, Ethyl Violet, Rhodamine 6G, Crystal violet, Malachite green	2
	Victoria Pure Blue BO, Victoria Blue R, Victoria Blue B, Nile Blue A	5
	Azure B, Pararosaniline Base	10
	Methylene Blue	15
Tetracyclines	Chlortetracycline, Doxycycline, Oxytetracycline, Tetracycline	50
Antimicrobial screen	β -lactams (STC based on response to Penicillin G)	10
	Sulphonamides (STC based on response to a mixed standard of sulphamethazine, sulphamethoxypyridazine, sulphaquinoxaline, sulphadiazine and sulphadimethoxine; $100 \mu\text{g kg}^{-1}$ sulphonamides total)	100
Florfenicol	Florfenicol amine (STC expressed as florfenicol equivalents)	50
Quinolones	Sarafloxacin	15
	Ciprofloxacin, Danofloxacin, Enrofloxacin, Nalidixic acid, Norfloxacin, Oxolinic acid	50
	Marbofloxacin	75
	Flumequine	100
	Difloxacin	150

* Key to abbreviations: SEM, semicarbazide; AHD, 1-aminohydantoin; AMOZ, 3-amino-5-morpholinomethyl 2-oxazolidone; AOZ, 3-amino-2-oxazolidone.

Suspect “positive” (potentially non-compliant) samples from these initial screening analyses, *i.e.* samples with screening concentrations \geq STC, were subject to confirmatory tests.

All *confirmatory analyses* were conducted in duplicate using test portions taken from the retained sample and included known blank samples. Depending on the analyte, Quality Assurance samples and standards included multi-level matrix matched standards, solvent standards and/or extracted matrix matched standards as appropriate. Results were recovery corrected against deuterated internal standard where available (see above).

In the absence of internal standards, external calibrations were used and results were corrected for recovery.

Quality assurance was also demonstrated by regular participation in both UK and EU-wide proficiency schemes and collaborative studies.

Decision Limit (CC α)

All methods were validated at Fera according to the requirements of Commission Decision 2002/657/EC. This required the determination of a number of key parameters including "CC α " (decision limit). For confirmatory methods, CC α values are in Table 5.

Table 5

Confirmatory method	Analyte*	MRL/MRPL (μgkg^{-1})*	CCα (μgkg^{-1})
Chloramphenicol	Chloramphenicol	0.3	0.07
Nitrofurans metabolites	SEM	1	0.30
	AHD	1	0.32
	AMOZ	1	0.47
	AOZ	1	0.20
Dyes	Brilliant green		1.1
	New methylene blue		1.1
	Ethyl violet		1.3
	Rhodamine 6G		1.6
	Crystal violet		1.4
	Malachite green	2 (sum + Leucomalachite green)	1.2
	Victoria pure blue BO		3.4
	Victoria blue R		2.7
	Victoria blue B		4.2
	Nile blue A		2.2
	Azure B		6.2
	Pararosaniline base		6.2
	Methylene blue		14
Sulphonamides	Sulphadiazine	100 (total Sulphonamides)	121
	Sulphamethazine (sulphadimidine)		108
	Sulphamerazine		110
	Sulphamethizole		117
	Sulphathiazole		111
	Sulphisoxazole		124
	Sulphamethoxazole		123
	Sulphaquinoxaline		107
	Sulphadimethoxine		114
	Sulphadoxine		115
	Sulphamoxole		118
	Sulphamonomethoxine		113
	Sulphapyridine		113
	Sulphamethoxy pyridazine		112
	Sulphachloropyridazine		112
	Sulphanilamide		112
	Sulphaguanidine		123
Tetracyclines	Chlortetracycline	100	116
	Doxycycline		_**
	Oxytetracycline	100	112
	Tetracycline	100	113
Florfenicol	Florfenicol amine	1000	1158
Quinolones	Sarafloxacin	30 (<i>Salmonidae</i>)	42
	Ciprofloxacin		118
	Enrofloxacin	100 (Sum + Ciprofloxacin)	115
	Danofloxacin	100	120
	Nalidixic acid		20
	Norfloxacin		_**
	Oxolinic acid	100	115
	Marbofloxacin		6.5
	Flumequine	600	627
	Difloxacin	300	329

Key to abbreviations: SEM, semicarbazide; AHD, 1-aminohydantoin; AMOZ, 3-amino-5-morpholinomethyl 2-oxazolidone; AOZ, 3-amino-2-oxazolidone

**** Data not available.**