FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Report on a Survey of Chemical Residues in Domestic and Imported Aquacultured Fish

A national survey conducted under the Coordinated Food Survey Plan with participation by food regulatory agencies in all Australian States and Territories

November 2005

Executive Summary

Food Standards Australia New Zealand (FSANZ) was the lead agency for a national coordinated survey of Chemical Residues in Aquacultured Fish. The survey's aim was to determine if residues of antimicrobials and other substances are present in both local and imported aquaculture product. Prior to the survey there had been reports from overseas regulators of unapproved antimicrobials being found in aquacultured fish.

All Australian States and Territories participated in this national survey and a total of 60 samples of local and imported aquacultured finfish were sampled from across Australia. Samples were collected from late April until early June 2005.

The analysis of samples has been completed for a range of over 50 substances and their metabolites including; nitrofurans, chloramphenicol, sulphonamides, tetracyclines, malachite green, penicillins, macrolides, trimethoprim, quinolones and PCBs.

Overall, the results were very good with no detections for 54 of the 56 chemicals tested for. However, trace levels of malachite green and leucomalachite green were detected in 10 samples; 3 fish grown in Australia and 7 Basa fish samples imported from Vietnam. The residues were at low levels ie all less than 0.14 mg/kg. The 3 positives out of 14 (21%) in domestically farmed fish were 1 Rainbow Trout sample produced in NSW and 2 Silver Perch samples produced in NSW and WA. The 7 positives out of 46 (15%) in imported fish were all Basa from Vietnam, which equates to a 39% non-compliance rate from this country.

In accordance with the agreed protocol for national surveys, the results from the survey were discussed at the Food Surveillance Network (the Network) meeting on 2 August 2005 where a number of actions were agreed by jurisdictions.

- Jurisdictions (Home States) with positive samples discussed their follow up actions to ensure as consistent an approach as possible. In those States or Territories where malachite green residues were detected in domestically farmed fish, further investigations have been conducted to determine the scope of malachite green usage in the industry, including taking additional samples.

- Full sampling details were provided to the Australian Quarantine and Inspection Service (AQIS) who provided advice back to the Network on regulatory options at the border. As of 26 September 2005, AQIS initiated random testing for malachite green in imported aquacultured fish www.aqis.gov.au/foodimport.

- FSANZ prepared a risk assessment incorporating a dietary exposure assessment and toxicology assessment. The risk assessment conducted by FSANZ concluded that the public health risk associated with low residues of malachite green chloride and leucomalachite green in aquacultured fish is very low.

This coordinated national survey identified a compliance issue with the presence of malachite / leucomalachite green being detected in both domestic and imported fish. The findings do not appear to raise public health and safety concerns and are being managed in an appropriate and timely manner.

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1. Introduction

Australians currently consume around 13 kg of fish per person per year, and total domestic consumption is about 200,000 tonnes per annum. Fish forms a significant component of the total diet, particularly for those consumers who eat fish in preference to other meats. Though total dietary consumption of fish is below other meats such as poultry, beef and sheep, it is the only primary meat product where a significant proportion of that consumed is imported into Australia (~50% of fish is imported as opposed to less than 1% of poultry, beef, sheep meat). [1]

The long-term trend is for per capita consumption of fish to increase in Australia. There is also a significant global trend for the proportion of aquacultured (farmed) fish consumed to increase as opposed to wild caught. [2]

Over recent years there have been reports from overseas regulatory agencies that some aquacultured food may contain residues of unapproved antimicrobials as well as higher than expected residues of some environmental contaminants. There have also been unsubstantiated allegations made by aquaculture producers about the use of unapproved antimicrobials in aquaculture production overseas. The antimicrobial substance malachite green has been detected in farmed salmon overseas and Australian agencies are aware of other fish species such as tilapia and catfish in which the presence of unapproved antimicrobial residues has been reported.

The Australia New Zealand Food Standards Code (the Code) does not contain any maximum residue limits (MRLs) for antimicrobials in fish. There are a number of maximum levels (MLs) for environmental contaminants, including a maximum limit for Polychlorinated Biphenyls (PCBs) in fish of 0.5mg/kg.

Domestically produced fish are subjected to some chemical residue analysis through the National Residue Survey (NRS) and the results from this work indicate high compliance for certain antibiotics and PCBs. (Attachment A)

2. Survey Objective

Given the allegations and regulatory findings reported internationally, a survey of chemical residues in aquacultured fish was conducted under the National Coordinated Food Survey Plan. This survey aimed to:

- address the perceived lack of data in relation to chemical residues in aquacultured fish available for human consumption in Australia by sampling certain types of imported farmed fish as well as domestic farmed fish; and
- indicate whether there is an issue with non-compliance with the Code regarding the levels of antibiotic residues present in some farmed fish;
- provide useful information on countries of origin, species and the substances involved that could assist in targeting future compliance testing or enforcement action;
- support advice provided to AQIS on imported food testing; and

[1] Apparent Consumption of Foodstuffs, Australia – ABS, 2000
http://www.abs.gov.au/Ausstats/abs@.nsf/0/123fcdbf086c4daaca2568a90013939a?OpenDocument#Links
[2] Aquaculture and the Environment, Yearbook 2003 – ABS, 2003
http://www.abs.gov.au/Ausstats/abs@.nsf/46d1bc47ac9d0c7bca256c470025ff87/2f9296e45b52b49bca256cae0015caac!OpenDocument

- address an international issue of concern that may affect the Australian food supply by determining whether there are health or safety implications of detectable residues in fish for the people of Australia or New Zealand.

3. National Coordinated Survey Under ISC

On 30 October 2003 the Food Regulation Standing Committee's Implementation Sub-Committee (ISC) agreed to the development of a 'Coordinated Food Survey Plan' (the Plan) for the Australian jurisdictions, food regulatory partners and New Zealand. This was in recognition that there were significant advantages in implementing agreed national survey priorities in a prospective and coordinated manner. ISC agreed to the conduct of a nationally coordinated survey of chemical residues in aquacultured fish to be undertaken during 2004-05.

4. Laboratory Selection

FSANZ prepared a tender specification and a competitive process was undertaken to identify a laboratory capable of testing for a number of chemical residues that may be present in domestic and imported aquacultured fish species. A suitably accredited laboratory was awarded the contract.

5. Sample Collection

A sampling plan was developed after an examination of the aquacultured fish available on the Australian market. The species and the country of origin were selected to best represent aquaculture fish available for sale.

Food regulatory agencies in all Australian States & Territories participated in the survey through collection of both imported and domestic fish species. Two sampling phases were outlined for fish sample collection to provide for an accurate indication of the fish species available for sale and their country of origin. Samples for Stage 1 and Stage 2 were collected from late April until early June 2005 (see Attachment B for full sample details).

Jurisdictions purchased, where possible, fish samples at the wholesale level and sampling officers were requested to target fish varieties from countries of origin where reports of fish contamination were frequent (see Attachment C for Initial Sampling Plan).

Fish samples were specified to be purchased raw, either frozen or fresh. Samples were transported frozen to the laboratory for analysis.

Table 1: Collection of Fish Samples by Country of Origin

Country of Origin	Stage 1 No. samples collected	Stage 2 No. samples collected	Total (Stages 1 and 2)
Taiwan	5	4	9
Vietnam	11	8	19
Australia	7	12	19
Myanmar (Burma)	5	1	6
Norway	3	-	3
China	2	-	2
Thailand	-	1	1
Philippines	-	1	1
Total	**33**	**27**	**60**

Table 2: Collection of Fish Samples by Species

Fish species	Stage 1 No. samples collected	Stage 2 No. samples collected	Total (Stages 1 and 2)
Basa	11	7	18
Tilapia	4	2	6
Barramundi	6	4	10
Salmon	4	-	4
Milkfish	1	2	3
Trout	6	6	12
Silver Perch	1	6	7
Total	**33**	**27**	**60**

6. Sample Preparation

Three (3) to five (5) fillets or whole fish were received as each sample. These were combined as a single sample and prepared by firstly chopping the fish into smaller pieces and then processing using a fish mincer. All samples were prepared according to the Code (Schedule 4) for commodities to which an MRL applies. In the case of whole fish, the whole commodity including bones and head (but removing the digestive tract) was prepared. For fish fillets, the whole fillet was prepared.

7. Analytical Tests Conducted

Fish samples were tested for a number of chemical residues, including nitrofuran metabolites, chloramphenicol, sulfonamides, tetracyclines, malachite green, penicillins (B-lactams), macrolides, quinolones (oxolinic acid), trimethoprim, and polychlorinated biphenyls (PCBs). Table 3 shows the list of the 56 antimicrobials that were tested for in the fish samples.

8. Method of Analysis

The laboratory that undertook sample analysis for this survey was a NATA accredited testing facility. Testing methods that were not currently validated were expected to be fully validated before the commencement of sample analysis and then submitted to NATA for review and accreditation.

Nine (9) tests were performed by Liquid Chromatography with Triple Quadrupole Spectrometry (LCMSMS) techniques and one (1) by Gas Chromatograph Mass Spectrometry (GCMS). All chemical residues tested for, excluding PCB congeners, were done so via LCMSMS instrumentation, which provides the greatest degree of confidence in the results at the lowest available levels of quantification. It also provides confirmation of the presence or absence of a more extensive range of antibiotics at the lowest levels of quantitation than other commonly used techniques. PCB congeners were tested for via GCMS techniques.

Table 3: Antibiotics Tested for in Fish Samples

Activity/Test	**LOQ * (mg/kg)**
PCBs (as congeners)	0.02
Malachite green and leucomalachite green	0.002
Tetracyclines	0.002
Nitrofuran metabolites	0.001
Chloramphenicol	0.0003
Sulfonamides	0.002
Penicillin (B-lactams)	0.01
Oxolinic Acid	0.002
Trimethoprim	0.002
Macrolides	0.002

PCB's (congener number)
2,4'-Dichlorobiphenyl (#008)
2,2',5-Trichlorobiphenyl (#018)
2,4,4'-Trichlorobiphenyl (#028)
2,2',3,5'-Tetrachlorobiphenyl (#044)
2,2',5,5'-Tetrachlorobiphenyl (#052)
2,3'4,4'-Tetrachlorobiphenyl (#066)
3,3'4,4'-Tetrachlorobiphenyl (#077)
2,2',4,5,5'-Pentachlorobiphenyl (#101)
2,3,3',4,4'-Pentachlorobiphenyl (#105)
2,3'4,4',5-Pentachlorobiphenyl (#118)
3,3'4,4',5-Pentachlorobiphenyl (#126)
2,2'3,3',4,4'-Hexachlorobiphenyl (#128)
2,2,3,4,4',5'-Hexachlorobiphenyl (#138)
2,2',4,4',5,5'-Hexachlorobiphenyl (#153)
3,3',4,4',5,5'-Hexachlorobiphenyl (#169)
2,2',3,3',4,4',5-Heptachlorobiphenyl (#170)
2,2'3,4,4',5,5'-Heptachlorobiphenyl (#180)
2,2',3,4',5,5',6-Heptachlorobiphenyl (#187)
2,2',3,3',4,4',5,6-Octachlorobiphenyl (#195)
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (#206)
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (#209)

Nitrofurans (as metabolites)
Aminohydantoin (AHD)
3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ)
3-amino-2-oxazolidinone (AOZ)
Semicarbazide (SC)

Tetracyclines
Chlorotetracycline
Doxycycline
Oxytetracycline
Tetracycline

Malachite Green
Malachite green
Leucomalachite green

Sulphonamides
Sulfachloropyridazine
Sulfadiazine
Sulfadimethoxine
Sulfadoxine
Sulfamerazine
Sulfamethazine (Sulfadimidine)
Sulfameter
Sulfamethoxazole
Sulfamethoxypyridazine
Sulfaquanidine
Sulfapyridine
Sulfaquinoxaline
Sulfathiazole
Sulfatroxazole
Sulfisoxazole
Sulfacetamide

Penicillin (B-lactams)
Amoxicillin
Ampicillin
Cloxacillin
Penicillin G

Chloramphenicol
Chloramphenicol

Quinolones
Oxolinic acid

Macrolides
Tylosin,
Erythromycin

Others
Trimethoprim

*LOQ = Level of quantification: The LOQ is the lowest concentration of a chemical that can be detected and quantified, with an acceptable degree of certainty, using a specified laboratory method and/or item of laboratory equipment.

9. Quality Assurance

Quality control procedures were applied throughout the duration of sample analysis to monitor the validity of test results, and various measures were used to ensure that the potential for cross-contamination was eliminated. All positive sample results were re-extracted and re-analysed for confirmation.

For analyses undertaken in this project, systematic quality control procedures including the use of control charts and check samples were implemented. The quality control samples run with each batch of samples may have included, but was not limited to: reagent blank, duplicate analysis, certified reference material/in-house reference material, matrix spikes, surrogate spikes, independent check standard, calibration verification (drift) standard.

10. Results

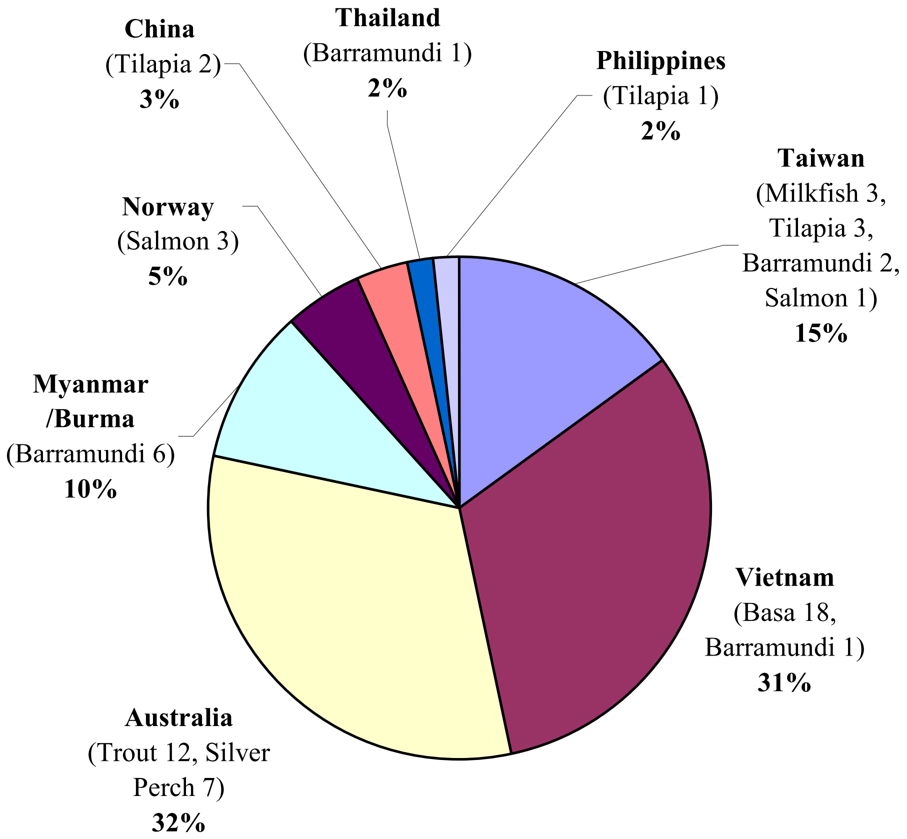
A total of 60 fish samples were analysed for this survey. Samples of fish species for both stages of this survey were collected from either the wholesaler or at retail. Locally aquacultured fish samples, as well as imported products, were targeted. Countries of origin in which fish samples were purchased included Australia, China, Myanmar/Burma, Norway, Philippines, Taiwan, Thailand and Vietnam.

Fish were sampled based on research of the market so that they were representative of the fish available to consumers in Australia.

The variety of fish samples that were collected for analyses included Barramundi, Salmon, Basa, Rainbow Trout, Tilapia, Milk Fish and Silver Perch. For a full list of fish sample collection details refer to Attachment B.

Graph 1 below represents the different species of fish collected for analysis and the countries in which these fish species originated.

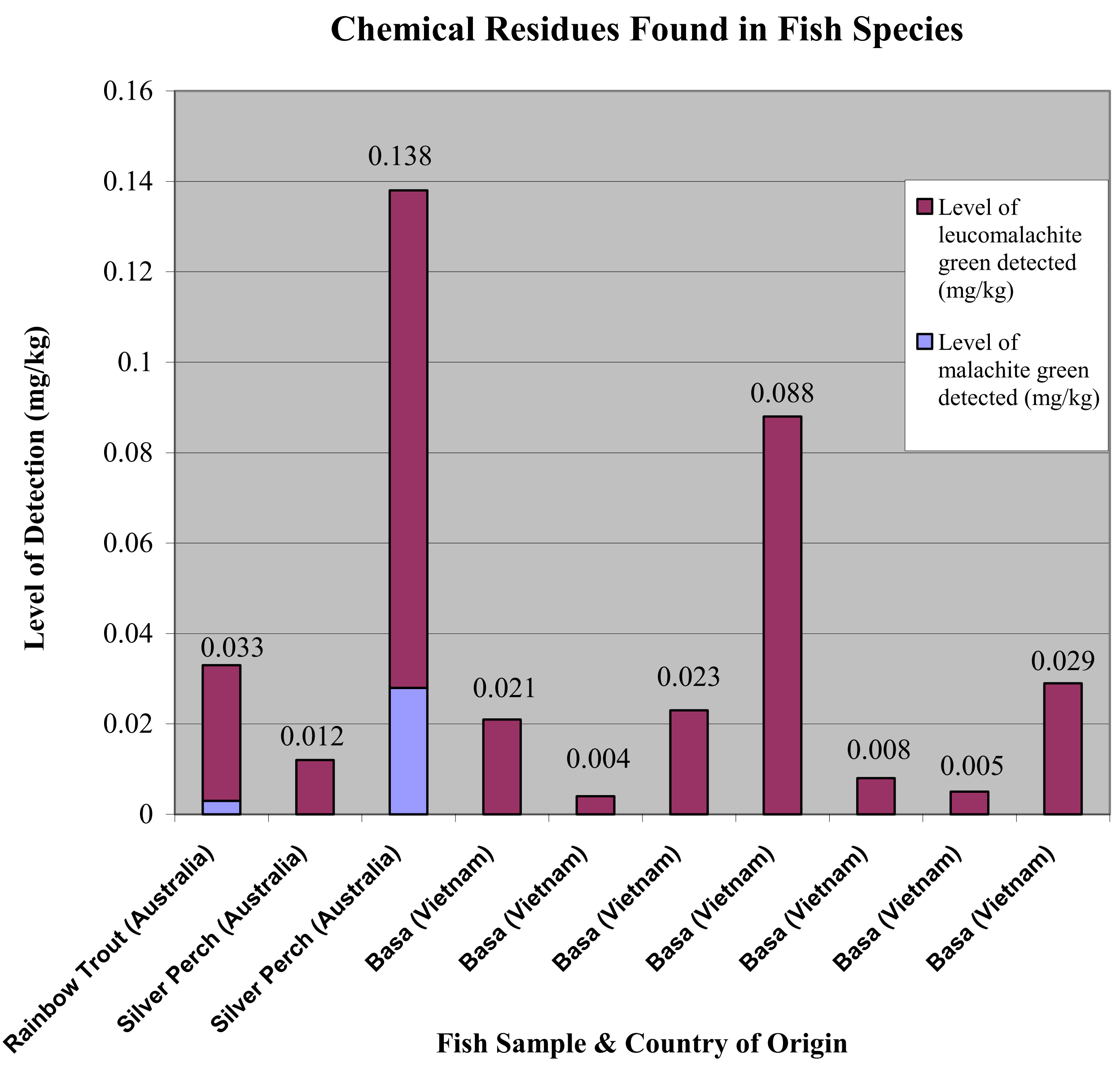
Graph 1: Total Number of Fish Samples Collected & Analysed by Country of Origin



Of the 56 antimicrobials tested, 54 were not detected in any sample. However, trace levels of leucomalachite green and in some cases malachite green were detected. The levels of malachite green and leucomalachite green recorded exceeded the specified level of quantification of 0.002mg/kg. There was no trace of any other antimicrobial listed in Table 3.

From a total of 60 fish samples, 10 contained trace levels of leucomalachite green. Two of these 10 samples also contained malachite green. Graph 2 below represents the levels of leucomalachite and malachite green detected in fish by species and country of origin.

Graph 2: Levels of Leucomalachite Green and/or Malachite Green Found in Fish Samples



Of the 10 samples that contained traces of malachite/leucomalachite green, three were domestically produced species, including one rainbow trout sample from NSW and two silver perch samples from NSW and WA. The sample of silver perch produced in WA was found to contain traces of leucomalachite green. The samples of rainbow trout and silver perch produced in NSW were found to contain traces of both malachite and leucomalachite green.

Seven imported aquacultured fish samples tested positive to chemical residues. All seven samples were Basa from Vietnam, all of which tested positive to leucomalachite green.

The highest level of antibiotic detected was 0.11 mg/kg of leucomalachite green, recorded in a domestically produced fish sample of Silver Perch. This sample also recorded levels of malachite green at 0.028 mg/kg. The overall value of malachite and leucomalachite green in this sample was therefore 0.138 mg/kg.

The Australian sample of rainbow trout originating from NSW also detected malachite green and leucomalachite green at levels of 0.003 mg/kg and 0.03 mg/kg respectively.

The highest level of detection recorded in imported fish samples was 0.88 mg/kg of leucomalachite green found in Basa from Vietnam.

Of the 60 fish samples collected for analysis, 19 of these were domestically produced and 41 were imported. Of the 19 domestic samples analysed, 3 tested positive to malachite green and/or leucomalachite green, representing a domestic non-compliance rate of ~16%. Of the 41 imported fish samples analysed, 7 tested positive to malachite green and/or leucomalachite green. This represents a non-compliance rate of ~17% for imported samples. The non-compliance rates for imported and Australian aquacultured fish samples was therefore similar.

When considered in conjunction with the results of the 2004 National Residue Survey (NRS) of Wildcaught Fish and Aquaculture (see Attachment A) it is evident that chemical contamination in fish in the Australian food supply is generally low.

Overall, the results of this survey demonstrated that antimicrobial chemicals are generally not present in aquacultured fish present in the Australian marketplace. Of the 60 different fish samples analysed for 56 chemicals, only malachite green and/or its metabolite, leucomalachite green, was detected in 10 samples. The residues of malachite/leucomalachite reported were at low levels i.e. all less than 0.14 mg/kg.

11. Assessment of the Health Risk Associated with Residues in Fish

Malachite green is a synthetic dye used to colour textiles and paper and is also used in the ornamental fish trade (e.g. goldfish) for treating fish parasites. It is not registered for use in aquaculture in many countries, including Australia, Canada, the European Union, Hong Kong, Indonesia, the United States and Vietnam. Leucomalachite green is formed from the metabolism of malachite green.

The survey results show that the residue levels for all of the antimicrobials, except for malachite green, were below the level of detection. The survey results also show that the residue levels for the PCB's were below the level of detection. For the vast majority of the chemicals examined in this survey, therefore, it can be stated without further analysis that there is no health risk from the consumption of aquacultured fish.

In the case of malachite green, the potential health risks associated with consumption of these fish have been assessed (see Attachment D). This assessment concluded that on the basis of the toxicity data and dietary intake data available to FSANZ, the health risk from the consumption of malachite green residues in fish is very low.

12. Follow-up Action

Overall, the survey revealed that antimicrobial chemicals are not present in aquacultured fish, with the exception of malachite green and leucomalachite green, which were detected at very low levels. This presence of malachite/leucomalachite green in some samples identified a compliance issue, which gave rise to a number of management actions.

- Jurisdictions with domestically farmed samples in which malachite green/leucomalachite green were detected all responded with further investigatory and follow up action.

- FSANZ has conducted a risk assessment to determine if the consumption of the antibiotic malachite green and leucomalachite green, at the levels detected in this survey, posed a threat to public health and safety. This risk assessment concluded that the public health risk associated with low residues of malachite green and leucomalachite green in aquacultured fish is very low. (See Attachment D)

- FSANZ also prepared a fact sheet on Malachite green in aquacultured fish for posting on their website to provide further information to the community.

- The Australian Quarantine Inspection Service (AQIS), who have responsibility for imported food under the Imported Food Control Act 1992 initiated random testing of relevant imported fish for malachite/leucomalachite green on 26 September 2005. (Imported Food Notice i.e. at: http://www.daff.gov.au/content/output.cfm?ObjectID=6A51B47A-495E-44C3-B66C19020AC71B1D

13. Conclusion

Of the 56 microbials tested, 54 were not detected in this survey. However, trace levels of malachite green and leucomalachite green were detected in 10 of the 60 fish samples collected. These positive detections in fish were found in 3 domestic samples and 7 imported samples from Vietnam.

In response to these positive detections, FSANZ conducted a risk assessment to determine if the consumption of the antibiotic malachite green and leucomalachite green, at the levels detected in this survey, posed a threat to public health and safety. This risk assessment concluded that the public health risk associated with low residues of malachite green and leucomalachite green in aquacultured fish is very low.

The lack of detections of any other chemical residues and the conclusion that the low level detections of malachite/leucomalachite green present a very low risk to public health and safety are important findings given there have been overseas reports of unapproved chemical residues being found in fish.

The compliance issue identified from this survey, in relation to malachite green being found in aquacultured fish species, has prompted the jurisdictions, and AQIS to take appropriate follow-up actions to assure this compliance issue is addressed.

14. Attachments

Attachment A: National Residue Survey Report on Wildcaught Fish and Aquaculture

Attachment B: Full List of Fish Samples Collected for Stages 1 & 2 of Sampling

Attachment C: Proposed Sampling Plan for Participating Jurisdictions

Attachment D: An Assessment of the Public Health Risk Associated with Low Residues of Malachite Green chloride and Leucomalachite green in Aquacultured Fish

Attachment A

National Residue Survey Report on Wildcaught fish and Aquaculture

4 August 2004
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National Residue Survey Report
1 July 2003 to 30 June 2004

Background

Report on the residue analysis of samples of wildcaught and aquaculture fish collected and analysed during the 2003/04 financial year are summarised in Table 1 and 2 below.

Wildcaught Fish

The present program for wildcaught fish shows good level of compliance with Australian Standards. It was designed to provide further information for specific target issues:

- Some importing countries may have more stringent standards than Australian Standards or in some instances Australia has no standard where importing countries do (e.g. cadmium in crustaceans);
- Markets such as the EU and Japan have, or are in the process of, implementing standards for dioxins and dioxin-like PCBs in one single stringent standard which may include PCBs.

In response to a request of information for the purpose of conducting a Global Assessment of Mercury and its Compounds by the United Nations Environmental Program (UNE), the NRS has made available a summary of the Australian data on mercury levels in fish, crustaceans and molluscs. This data, together with data provided by other Government and research institutions will be used to assess the status of mercury contamination in different areas of the globe. The Australian component will ensure that Australian interests are taken into consideration. This program will be extended in the future to cadmium and lead.

Focusing on POPs was initiated in 2003/04, aiming to provide a baseline of the status of Australian seafood, following endorsement of the Stockholm and Rotterdam Conventions by Australia. Information on the Stockholm and Rotterdam Conventions is summarised in Attachment 1.

Results

The report covers wildcaught species tested for trace elements (cadmium, copper, lead and mercury) and persistent organic pollutants including PCBs (measured in milligrams per kilogram - mg/kg). Dioxins and PCBs were also analysed using a screen test (CALUX), with the results reported using International toxic equivalents. This test reports the added toxic equivalents of dioxins and PCBs together.

Table 1: Results of the analysis of wildcaught fish for trace elements and persistent organic pollutants.
Samples collected during the 2003/04 financial years. LOR refers to the limit of quantification of the method.

No. Requested	***No. Tested***	***Residue***	***No. with Residues***	***Av Concn***	***Min Concn***	***Max Concn***	***MRL***	***LOR***
ABALONE ***(44 Black Lip, 2 Brown Lip, 13 Green Lip, 1 Tiger)***								
60	60	Cadmium	60	0.133	0.005	0.39	2	0.01
	60	Copper	60	1.509	0.27	15	No Limit	0.1
	60	Lead	60	0.006	0.005	0.03	2	0.01
	60	Mercury	60	0.005	0.005	0.02	0.5	0.01
EEL ***(5 Long Fin, 5 Short Fin)***								
10	10	Dioxins and PCBs (in toxic equivalents)	5	0.00000030	0.00000015	0.00000053	No Limit	0.0000001
	10	Cadmium	10	0.005	0.005	0.005	No Limit	0.01
	10	Copper	10	0.343	0.16	0.69	No Limit	0.1
	10	Lead	10	0.007	0.005	0.02	0.5	0.01
	10	Mercury	10	0.211	0.04	0.5	0.5	0.01
10	9	DDE (p,p')	4	0.015	0.01	0.022	1	0.02
	9	DDT	4	0.015	0.01	0.022	1	0.02
	9	PCB 1254 (Total Aroclor 1254)	1	0.033	0.033	0.033	0.5	0.03
LOBSTER ***(28 Southern Rock, 15 Tropical, 15 Western Rock)***								
60	58	Cadmium	58	0.010	0.005	0.09	No Limit	0.01
	58	Copper	58	2.821	0.45	5.8	No Limit	0.1
	58	Lead	58	0.005	0.005	0.02	No Limit	0.01
	58	Mercury	58	0.048	0.005	0.29	0.5	0.01
	30	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
MACKEREL								
10	9	Dioxins and PCBs (in Toxic Equivalents)	8	0.00000031	0.00000013	0.00000054	No Limit	0.0000001
	10	Cadmium	10	0.023	0.005	0.05	No Limit	0.01
	10	Copper	10	0.685	0.4	1.2	No Limit	0.1
	10	Lead	10	0.006	0.005	0.01	0.5	0.01
	10	Mercury	10	0.072	0.05	0.11	0.5	0.01
	10	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
PRAWN ***(15 Banana, 14 Brown Tiger, 15 Eastern King, 8 Endeavour, 7 King, 1 Red Spot King)***								
60	60	Cadmium	60	0.202	0.02	0.88	No Limit	0.01
	60	Copper	60	3.634	0.95	6.8	No Limit	0.1
	60	Lead	60	0.009	0.005	0.24	No Limit	0.01
	60	Mercury	60	0.040	0.005	0.11	0.5	0.01
	26	Persistent Organic	Nil					

		Pollutants (incl. PCBs in mg/kg)						
SCALLOP ***(15 Ballots, 15 Pectin Alba, 15 Saucer, 15 Southern)***								
60	60	Cadmium	60	0.534	0.12	1.1	2	0.01
	60	Copper	60	0.858	0.06	10	No Limit	0.1
	60	Lead	60	0.008	0.005	0.06	2	0.01
	60	Mercury	60	0.005	0.005	0.005	0.5	0.01
	30	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
SNAPPER ***(Pink)***								
10	11	Dioxins and PCBs (in Toxic Equivalents)	2	0.00000019	0.00000013	0.00000025	No Limit	0.0000001
	6	Cadmium	6	0.006	0.005	0.01	No Limit	0.01
	6	Copper	6	0.182	0.13	0.23	No Limit	0.1
	6	Lead	6	0.008	0.005	0.02	0.5	0.01
	6	Mercury	6	0.190	0.12	0.3	0.5	0.01
	6	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
TROUT ***(Coral)***								
10	3	Dioxins and PCBs (in Toxic Equivalents)	1	0.00000025	0.00000025	0.00000025	No Limit	0.0000001
	10	Cadmium	10	0.006	0.005	0.01	No Limit	0.01
	10	Copper	10	2.230	0.08	14	No Limit	0.1
	10	Lead	10	0.009	0.005	0.02	0.5	0.01
	10	Mercury	10	0.126	0.03	0.46	0.5	0.01
	10	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
TUNA ***(1 Albacore, 1 Big Eye, 8 Yellow Fin)***								
10	8	Dioxins and PCBs (in Toxic Equivalents)	1	0.00000044	0.00000044	0.00000044	No Limit	0.0000001
	10	Cadmium	10	0.007	0.005	0.02	No Limit	0.01
	10	Copper	10	1.515	0.22	4.6	No Limit	0.1
	10	Lead	10	0.012	0.005	0.043	0.5	0.01
	10	Mercury	10	0.343	0.18	0.76	1	0.01
	10	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					.
WHITING ***(3 King George, 4 Red Spot, 3 School, 1 Sand)***								
10	6	Dioxins and PCBs (in Toxic Equivalents)	1	0.00000014	0.00000014	0.00000014		0.0000001
	9	Cadmium	9	0.017	0.005	0.04	No Limit	0.01
	9	Copper	9	1.234	0.15	4.5	No Limit	0.1
	9	Lead	9	0.010	0.005	0.02	0.5	0.01
	9	Mercury	9	0.023	0.005	0.03	0.5	0.01

	7	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil

Aquaculture Program

Results

The results of the aquaculture residue analysis are summarised in Table 2 below. A significant proportion of the samples (10 out of 15) were collected to date: 4 out of 5 barramundi, most yabbies, and 2 out of 5 eels. The requested sample of kingfish and redclaw were also collected and analysed. One extra marron was collected and analysed.

Table 2: Results of the analysis of aquaculture products for antimicrobials, trace elements and persistent organic pollutants. Samples collected during the 2003/04 financial years. LOR refers to the limit of quantification of the method.

No. Requested	No. Tested	Residue	No. with Residues	Av Concn	Min Concn	Max Concn	MRL	LOR
BARRAMUNDI								
5	3	Antimicrobials	Nil					
	4	Dioxins and PCBs (in Toxic Equivalents)	3	0.000	0.00000013	0.00000063	No Limit	
	3	Copper	3	0.327	0.25	0.42	No Limit	0.1
	3	Lead	3	0.010	0.005	0.02	0.5	0.01
	3	Mercury	3	0.022	0.005	0.04	1	0.01
	3	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
MARRON								
1	2	Antimicrobials	Nil					
	1	Dioxins and PCBs (in Toxic Equivalents)	1	0.00000015	0.00000015	0.00000015	No Limit	0.0000001
	2	Copper	2	1.800	1.7	1.9	No Limit	0.1
	2	Lead	2	0.005	0.005	0.005	No Limit	0.01
	2	Mercury	2	0.040	0.04	0.04	0.5	0.01
	2	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
REDCLAW								
1	1	Antimicrobials	Nil					
	1	Dioxins and PCBs (in Toxic Equivalents)	Nil					
	1	Cadmium	1	0.030	0.03	0.03	No Limit	0.01
	1	Copper	1	13.000	13	13	No Limit	0.1
	1	Lead	1	0.005	0.005	0.005	No Limit	0.01
	1	Mercury	1	0.010	0.01	0.01	0.5	0.01
	1	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
								Cont.
YABBY								
2	1	Antimicrobials	Nil					
	2	Dioxins and PCBs (in Toxic	2	0.00000067	0.00000014	0.00000119	No Limit	0.0000001

			Equivalents)						
		1	Copper	1	5.700	5.7	5.7	No Limit	0.1
		1	Lead	1	0.005	0.005	0.005	No Limit	0.01
		1	Mercury	1	0.060	0.06	0.06	0.5	0.01
		1	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
EEL ***(Long Fin)***									
	5	2	Sulfadimidine (sulfamethazine)	1	0.120	0.12	0.12	0	0.05
		1	Dioxins and PCBs (in Toxic Equivalents)	1	0.00000030	0.0000003	0.0000003	No Limit	0.0000001
		2	Cadmium	2	0.025	0.01	0.04	No Limit	0.01
		2	Copper	2	4.350	4	4.7	No Limit	0.1
		2	Lead	2	0.015	0.01	0.02	0.5	0.01
		2	Mercury	2	0.055	0.03	0.08	0.5	0.01
		2	Persistent Organic Pollutants (incl. PCBs in mg/kg)	Nil					
KINGFISH ***(Yellowtail)***									
	1	1	Antimicrobials	Nil					
		1	Dioxins and PCBs (in Toxic Equivalents)	1	0.00000108	0.00000108	0.00000108	No Limit	0.0000001
		1	Copper	1	0.500	0.5	0.5	No Limit	0.1
		1	Lead	1	0.005	0.005	0.005	0.5	0.01
		1	Mercury	1	0.040	0.04	0.04	0.5	0.01
		1	PCB 1254 (Total Aroclor 1254)	1	0.010	0.01	0.01	0.5	0.03

Conclusions

The overall results of the survey of trace elements and persistent organic pollutants in Australian wildcaught fish and aquaculture products indicate low level of contaminants.

Australia has no standard for cadmium in crustaceans, but levels of cadmium are elevated in prawns and may affect exports to countries where a standard exists.

Persistent organic pollutants were detected in low levels in samples of wild caught eels, mackerel and tuna. Low levels of dioxins plus PCBs were found in aquaculture barramundi, marrons, yabbies, eels and kingfish. One sample of eel was positive for sulphonamide.

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Appendix 1

Stockholm Convention and Rotterdam Convention

Persistent Organic Pollutants (POPs)

Persistent Organic Pollutants (POPs) are pesticide and industrial chemicals that are toxic, persist in the environment and animals, bioaccumulate through the food chain, and pose a risk of causing adverse effects to human health and the environment even at low concentrations.

Many developed countries, including Australia, have taken strong measures to reduce and eliminate releases of POPs.

Stockholm Convention: negotiations and state of play

Multilateral negotiations on the Convention on POPs concluded in Johannesburg in December 2000, after a negotiating period of about three years. The Convention was adopted and opened for signature at a Diplomatic Conference held in Stockholm in May 2001.
Australia and over 150 other countries have signed the Stockholm Convention. At least 30 countries including Australia have ratified the Convention and the United States and a number of other countries have begun considering ratification.

Prior to entry into force of the Convention, governments are undertaking further work to prepare for decisions that have to be taken by the Conference of the Parties.

POPs included in the Stockholm Convention

The Stockholm Convention will initially cover control measures on twelve POPs - DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls, dioxins and furans - identified for international action because of their persistence, bioaccumulation, long-range dispersion and toxicity.

The initial twelve POPs	
aldrin[1]	toxaphene[1]
chlordane[1]	mirex[1]
DDT[1]	hexachlorobenzene (HCB)[1,2,3]
dieldrin[1]	polychlorinated biphenyls (PCBs)[2,3]
endrin[1]	polychlorinated dibenzo-p-dioxins (dioxins)[3]
heptachlor[1]	polychlorinated dibenzofurans (furans)[3]
[1]Pesticide chemical [2]Industrial chemical [3]By-product	

The Convention also includes provisions for further chemicals with similar toxic, persistent and bioaccumulative properties to be added to the twelve existing POPs after a science-based review and assessment process and a decision by the Conference of the Parties. Article 8 and its related annexes include a science-based process for assessing chemicals nominated for addition to the Convention.

The Rotterdam Convention on the Prior Informed Consent (PIC) Procedure for Certain Hazardous Chemicals and Pesticides in International Trade is an international procedure that:

- Helps participating countries learn more about the characteristics of certain potentially hazardous chemicals and certain severely hazardous pesticide formulations that may be exported to them;

- Initiates a decision making process on the future import of these chemicals and formulations by the countries themselves and facilitates the dissemination of these decisions to other countries; and
- Requires exporting countries to comply with the decisions.

The Convention covers industrial chemicals and pesticides (including in specific circumstances, severely hazardous pesticide formulations). The core of the Rotterdam Convention is information exchange.

Rotterdam Convention: negotiations and state of play

The growth in world trade in chemicals during the 1960s and 1970s led to increasing concerns about the environmental and health risks of using hazardous chemicals.

These concerns led to the development of the voluntary Prior Informed Consent (PIC) procedure in 1989, which was embodied in the Food and Agriculture Organization's International Code of Conduct on the Distribution and Use of Pesticides and the United Nations Environment Programme's London Guidelines for the Exchange of Information on Chemicals in International Trade.

This PIC procedure was voluntary and unanimously accepted by member countries of the Food and Agriculture Organization and the United Nations Environment Programme, and was supported by the leading chemical industry associations and a variety of non-governmental organisations.

Australia signed the Convention in July 1999, adding to the more than 70 signatories to the Convention. 50 ratifications are required for the Convention to enter into force. At least 40 countries have ratified the Convention and the United States and a number of other countries have begun considering ratification of the Rotterdam Convention.

Chemicals included in the Rotterdam Convention
At the time the Convention was adopted, 17 pesticides, 5 severely hazardous pesticide formulations and 5 industrial chemicals were included in Annex III of the Convention. Additionalchemicals have been added to Annex III of the Convention under the interim PIC arrangements and will need to be confirmed once the Convention has entered into force.

Chemicals currently subject to the Interim PIC Procedure (May 2002)

	CAS number		CAS number
Pesticides			
2,4,5-T	93-76-5	Ethylene dichloride*	107-06-2
Aldrin	309-00-2	Ethylene oxide*	75-21-8
Binapacryl*	485-31-4	Fluoroacetamide	640-19-7
Captafol	2425-06-1	HCH (mixed isomers)	608-73-1
Chlordane	57-74-9	Heptachlor	76-44-8
Chlordimeform	6164-98-3	Hexachlorobenzene	118-74-1
Chlorobenzilate	510-15-6	Lindane	58-89-9
DDT	50-29-3	Mercury compounds	
Dieldrin	60-57-1	Pentachlorophenol	87-86-5
Dinoseb & dinoseb salts	88-85-7	Toxaphene*	8001-35-2
Ethylene dibromide (EDB)	106-93-4		
Severely hazardous pesticide formulations			
Methamidophos soluble liquid formulations of the substance that exceed 600g active ingredient per litre	10265-92-6	Phosphamidon soluble liquid formulations of the substance that exceed 1000g active ingredient per litre	13171-21-6 (mixture, (E)& (Z) isomers) 23783-98-4 ((Z)-isomer) 297-99-4 ((E)-isomer)

Methyl-parathion emulsifiable concentrates with 19.5%, 40%, 50%, 60% active ingredient and dusts containing 1.5%, 2% and 3% active ingredient	298-00-0	Monocrotophos soluble liquid formulations of the substance that exceed 600g active ingredient per litre	6923-22-4
Parathion all formulations aerosols, dustable powder, emulsifiable concentrate, granules and wettable powders, excluding capsule suspensions	56-38-2		
Industrial chemicals			
Crocidolite	12001-28-4	Polychlorinated terphenyls (PCT)	61788-33-8
Polybrominated biphenyls (PBB)	36355-01-8 (hexa) 27858-07-7 (octa) 13654-09-6 (deca)	Tris (2,3-dibromopropyl) phosphate	126-72-7
Polychlorinated biphenyls (PCB)	1336-36-3		

* Chemicals added during the interim procedure

Further information about the Convention and interim arrangements cand be found on the following internet sites:

- Rotterdam Convention on the Prior Informed Consent (PIC)
- United Nations Environment Program (UNEP)

Attachment B: Full List of Fish Samples Collected for Stages 1 & 2 of Sampling

State	Phase of sampling.	Date Sampled	Fish species	Retail/ wholesale	Country of origin
ACT	1	21/04/05	Barramundi Fillets	Wholesale	Myanmar
NT	1	27/05/05	Salmon Herring	Wholesale	Taiwan
S.A.	1	3/05/05	Barramundi	Retail	Taiwan
S.A.	1	3/05/05	Basa	Retail	Vietnam
S.A.	1	3/05/05	Rainbow Trout	Retail	Australia
WA	1	11/05/05	Basa	Wholesale	Vietnam
WA	1	11/05/05	Tilapia	Wholesale	China
WA	1	11/05/05	Salmon	Wholesale	Norway
WA	1	11/05/05	Barramundi	Wholesale	Myanmar/ Burma
WA	1	11/05/05	Trout – Rainbow	Wholesale	Australia
QLD	1	09/06/2005	Basa	Retail	Vietnam
QLD	1	09/06/2005	Barramundi	Retail	Burma
QLD	1	09/06/2005	Basa	Retail	Vietnam
QLD	1	09/06/2005	Atlantic Salmon	Retail	Norway
QLD	1	09/06/2005	Milkfish	Wholesale	Taiwan
QLD	1	09/06/2005	Silver Perch	Retail	Australia
QLD	1	10/06/2005	Basa	Retail	Vietnam
VIC	1	16/6/05	Rainbow Trout	Wholesale	Australia
VIC	1	16/6/05	Basa	Wholesale	Vietnam
VIC	1	16/6/05	Basa	Wholesale	Vietnam
VIC	1	16/6/05	Rainbow Trout	Wholesale	Australia
VIC	1	16/6/05	Basa	Retail	Vietnam
VIC	1	16/6/05	Tilapia	Retail	Taiwan
VIC	1	16/6/05	Rainbow Trout	Retail	Australia
NSW	1	19/04/05	Basa	Wholesale	Vietnam
NSW	1	20/04/05	Basa	Retail	Vietnam
NSW	1	20/04/05	Tilapia	Retail	China

State	Phase of sampling.	Date Sampled	Fish species	Retail/ wholesale	Country of origin
NSW	1	20/04/05	Barramundi	Wholesale	Myanmar
NSW	1	20/04/05	Atlantic Salmon	Wholesale	Norway
NSW	1	20/04/05	Barramundi	Retail	Myanmar
NSW	1	21/04/05	Tilapia	Retail	Taiwan
TAS	1	31/05/05	Basa	Retail	Vietnam
TAS	1	31/05/05	Nile Perch – wild (omitted from survey)	Retail	Kenya
TAS	1	31/05/05	Rainbow Trout	Retail	Australia
ACT	2	29/04/05	Basa Fillets	Retail	Vietnam
S.A.	2	25/05/05	Freshwater trout	Retail	Australia
S.A.	2	25/05/05	Barramundi	Retail	Thailand
S.A.	2	25/05/05	Basa	Retail	Vietnam
WA	2	16/11/05	Silver Perch	Retail	Australia
QLD	2	22/06/2005	Barramundi	Retail	Myanmar
QLD	2	22/06/2005	Barramundi	Retail	Vietnam
QLD	2	22/06/2005	Silver Perch	Retail	Australia
QLD	2	22/06/2005	Silver Perch	Retail	Australia
QLD	2	22/06/2005	Barramundi	Retail	Taiwan
QLD	2	22/06/2005	Basa	Retail	Vietnam
VIC	2	28/6/05	Basa	Wholesale	Vietnam
VIC	2	28/6/05	Basa	Wholesale	Vietnam
VIC	2	28/6/05	Basa	Wholesale	Vietnam
VIC	2	28/6/05	Milkfish	Retail	Taiwan
VIC	2	28/6/05	Tilapia	Retail	Taiwan
VIC	2	28/6/05	Rainbow Trout	Retail	Australia
VIC	2	28/06/05	Rainbow Trout	Retail	Australia
NSW	2	27/04/05	Basa	Retail	Vietnam

State	Phase of sampling.	Date Sampled	Fish species	Retail/ wholesale	Country of origin
NSW	2	27/04/05	Milkfish	Retail	Taiwan
NSW	2	28/04/05	Tilapia	Retail	Philippines
NSW	2	22/04/05	Rainbow Trout	Wholesale	Australia
NSW	2	27/04/05	Rainbow Trout	Wholesale	Australia
NSW	2	22/04/05	Rainbow Trout	Wholesale	Australia
NSW	2	26/04/05	Silver Perch	Wholesale	Australia
NSW	2	27/04/05	Silver Perch	Wholesale	Australia
NSW	2	28/04/05	Silver Perch	Wholesale	Australia

Attachment C: Proposed Sampling Plan for Participating Jurisdictions

	Fish Species	*Other names*	Target Region of Origin (in order of preference)	Potential Analytes	Total approx sample No.	Phase 1: 18-20 April Jurisdictions:	No. samples per State/Territory	Phase 2: 25-27 April Jurisdictions:	No. samples per State/Territory
IMPORTED	Bssa	*Pacific Dory, Freshwater fillet, Shark Catfish*	Vietnam, Thailand, Myanmar/Burma, Indonesia, China (if applicable)	All proposed	14	NSW QLD VIC WA SA TAS	2* 1 2* 1 1 1	NSW QLD VIC ACT	1 2* 2* 1
	Tilapia	*Mouthbrooder*	Taiwan, Indonesia, Myanmar/Burma, Vietnam, Phillipines and China (if applicable)	All proposed	10	NSW QLD VIC SA	2* 1 1 1	NSW QLD VIC WA	1 2* 1 1
	Salmon	*Atlantic Salmon*	Europe, preferably Norway and Chile (not North America or Scotland)	All proposed	8	NSW WA SA TAS (local)	1 1 1 1	QLD VIC	2* 2*
	Barramundi	*Barra, Giant Perch, Silver Barramundi*	Taiwan, Thailand, Myanmar/Burma, Indonesia, Vietnam, India and China (if applicable)	All proposed	8	NSW SA ACT	2* 1 1	QLD VIC WA	1 2* 1
	Milkfish	*Salmon Herring*	Taiwan, Myanmar/Burma, Phillipines, Indonesia, and China (if applicable)	All proposed	6	QLD VIC WA SA	1 1 1 1	NSW NT	1 1
DOMESTIC	Tuna Southern Bluefin Salmon	*Bluefin, Tuna*	SA	NA	-	-	-	-	-
	Salmon	*Atlantic Salmon*	TAS, SA	NA	-	-	-	-	-
	Trout freshwater	*Brown trout, rainbow trout*	NZ, VIC, NSW, SA, WA	All proposed	8	VIC WA	3* 1	NSW SA	3* 1
	Barramundi	*Barra, Giant Perch, Silver Barramundi*	QLD, SA, NSW, NT, WA	NRS Data available	-	-	-	-	-
	Silver Perch (freshwater)	*Bibyan, Black perch, Grunter*	NSW, QLD,WA	All proposed	6	QLD	3*	NSW	3*

An Assessment of the Public Health Risk Associated with Low Residues of Malachite Green chloride and Leucomalachite green in Aquacultured Fish

Food Standards Australia New Zealand September 2005

Introduction

Malachite green has been previously used in other countries to treat fungal and protozoa infections on fish and fish eggs but it is not permitted in aquaculture in Australia. It is still used to treat ornamental fish and as an industrial dye for fabric and paper. Malachite green is relatively inexpensive, readily available, and highly efficacious. The chemical has been used routinely in some countries in aquaculture since the early 1930s and is considered by many in the fish industry as the most effective antifungal agent. Leucomalachite green is a metabolite of malachite green and can be found in fish as a result of the use of malachite green.

An analytical survey was coordinated by FSANZ on Chemical Residues in Aquacultured Fish, including malachite green and leucomalachite green, as part of the ISC Coordinated Survey Plan. A total of 60 domestic and imported aquacultured fish samples were tested. Ten fish (18.3%) tested positive for malachite green, and/or its metabolite leucomalachite green with levels between 0.003-0.028 mg/kg and 0.004-0.110 mg/kg, respectively. The limit of detection for the two substances is 0.002 mg/kg. There were 3 positives out of 14 (21.4%) in domestically farmed fish and 7 positives out of 46 (15.2%) in imported fish.

The Joint FAO/WHO Expert Committee on Food Additives and the International Agency for Research on Cancer have not evaluated the safety of malachite green or leucomalachite green.

Hazard identification and characterisation

Absorption, Distribution, Metabolism and Excretion
Malachite green is reduced in fish to leucomalachite green and is persistent in the latter form. In trout, malachite green is rapidly excreted, while leucomalachite green is stored in muscle tissue for a relative long time with a half-life of about 40 days (NTP, 2005).

Malachite green is reduced to leucomalachite green by intestinal bacteria from humans, rat, mouse, and monkey (Henderson, 1997).

In rats, the presence of *N*-demethylated and *N*-oxide malachite green and leucomalachite green metabolites, including primary arylamines, was detected in the liver of rats fed 100 or 600 mg malachite green /kg diet or similar concentrations of leucomalachite green for 28 days. This may indicate that these compounds are metabolised in a manner similar to carcinogenic aromatic amines (NTP, 2005).

No studies on the absorption, distribution, metabolism, or excretion of malachite green chloride or leucomalachite green in humans were found in a review of the literature (NTP, 2005).

Toxicity

Acute toxicity
For malachite green the following doses were acute lethal doses for 50% of the animals (LD_{50}) (in NTP, 2005):

Species	LD_{50}, mg/kg body weight
NMRI mice	50
Wistar rats	275
Female SD rats	520

Reproductive and developmental toxicity
The teratogenic effects of malachite green oxalate were studied in New Zealand white rabbits (Meyer and Jorgenson, 1983). The rabbits were administered 0, 5, 10 or 20 mg malachite green oxalate/kg body weight by gavage on day 6 through 18 of gestation.

This study is not considerate appropriate for studying potential teratogenic effects for the following reasons:

- There was significant maternal toxicity at all dose levels
  - During treatment with malachite green, the does showed a greatly reduced intake of dry food.
  - The maternal body weight was significantly decreased compared to the control group (+230 g, + 60 g, -30 g, - 60 g, respectively).
- Decreased body weight of progeny is considered to be related to maternal toxicity.
- The skeletal deviations are considered to be related to maternal toxicity

In conclusion, the study is not considered appropriate to examine the teratogenic potential of malachite green oxalate.

Genotoxicity
Malachite green
Malachite green chloride was not mutagenic in several strains of *Salmonella typhimurium* with or without S9 metabolic activation. Negative results were also obtained in two *in vivo* micronucleus tests, one that assessed induction of micronuclei in rat bone marrow erythrocytes after three intraperitoneal injections of malachite green chloride, and a second study that determined the level of micronuclei in circulating erythrocytes of male and female mice following 28 days of exposure to malachite green chloride via dosed feed (reviewed in NTP, 2005).

Leucomalachite green
A weak increase in the frequency of micronucleated normochromatic erythrocytes in peripheral blood was observed in female mice exposed to 290, 580 or 1160 mg/kg leucomalachite green in feed for 28 days (2.14 ± 0.26, 3.69 ± 0.33, 4.19 ± 0.50, 3.44 ± 0.53 micronucleated cells/ 1000 normochromatic erythrocytes for female mice treated with 0, 290, 580 and 1160 mg/kg, respectively). No significant increase was observed in the frequency of micronucleated polychromatic erythrocytes. Nonetheless, the National Toxicology Program concluded that the effects seen in the normochromatic erythrocytes were sufficient to conclude that the result of the micronucleus test with leucomalachite green was positive (NTP, 2004).

There were some limitations with this study in that leucomalachite green was only tested in female mice and there was not a dose response relationship.

Bone marrow was also examined for the induction of micronuclei in female Big Blue rats after 4, 16, and 32 weeks of exposure to 0, 9, 27, 91, 272, or 543 mg/kg leucomalachite green in feed. No significant increase in the frequency of micronuclei was observed for any of the doses or time points assayed. In these studies, analysis of the livers for *lacI* mutations 4, 16, and 32 weeks after exposure revealed that 21% were clonal in origin and that the majority of the independent mutations were base-pair substitutions involving GC to AT transitions similar to those found for control rats. These data suggest that leucomalachite green is not a mutagen in the liver of female rats and that the DNA adduct formed in the liver of rats fed leucomalachite green does cause a mutagenic event (reviewed in NTP, 2005).

In conclusion, the available evidence indicates that malachite green is not genotoxic. Leucomalachite green gave both a positive as well as a negative result in *in vivo* micronucleus tests, however analysis of mutations in rats liver indicated no relation to leucomalachite green DNA adducts. The overall conclusion is that leucomalachite green is unlikely to be genotoxic based on current data.

<u>Long-term studies in animals</u>
The National Toxicology Program (NTP) in the USA has recently performed a 2-year study on toxicity and carcinogenicity in rats and mice with both malachite green and leucomalachite green (NTP, 2005).

Malachite green
<u>Mice</u>
Groups of 48 female mice were fed diets containing 0, 100, 225, or 450 mg/kg malachite green chloride for 2 years (equivalent to average daily doses of approximately 0, 15, 33, and 67 mg malachite green chloride/kg body weight).

NTP concluded that under the conditions of this 2-year feeding study, there was no evidence of carcinogenic activity[1] of malachite green chloride in female B6C3F1 mice exposed to 100, 225, or 450 mg/kg.

Exposure to malachite green chloride in feed resulted in non-neoplastic lesions in the urinary bladder (inclusion body cytoplasmic; 7/47[2], 15/46, 34/45, 39/48, for the 0, 100, 225 and 450 mg/kg groups, respectively) of female mice.

<u>Rats</u>
Groups of 48 female rats were fed diets containing 0, 100, 300, or 600 mg/kg malachite green chloride for 2-years (equivalent to average daily doses of approximately 0, 7, 21, and 43 mg malachite green chloride/ kg bw).

NTP concluded that under the conditions of this 2-year feeding study, there was equivocal evidence of carcinogenic activity[3] of malachite green chloride in female F344/N rats based on the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) (0/46[2], 0/48, 3/47, 2/46) and marginal increase in hepatocellular adenoma (1/48, 1/48, 3/48, 4/48) and mammary gland carcinoma (2/48, 2/48, 1/48, 5/48) in exposed rats.

[1] No evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
[2] number of animals with lesions /number of animals analysed
[3] Equivocal evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.

Exposure to malachite green chloride in feed resulted in non-neoplastic lesions in the thyroid gland (follicle cyst, 0/46[2], 1/48, 1/47, 3/46) and liver (eosinophilic focus: 5/48, 10/48, 13/48, 14/48) of female rats.

Leucomalachite green
<u>Mice</u>
Groups of 48 female mice were fed diets containing 0, 91, 204, or 408 mg/kg malachite green chloride for 2 years (equivalent to average daily doses of approximately 0, 13, 31, and 63 mg leucomalachite green chloride/kg body weight).

Under the conditions of this 2-year feeding study, there was some evidence of carcinogenic activity[4] of leucomalachite green in female B6C3F1 mice based on an increase in hepatocellular adenoma or carcinoma (combined) (3/47[2], 6/48, 6/47, 11/47).

Exposure to leucomalachite green in feed resulted in non-neoplastic lesions in the urinary bladder (inclusion body intracytoplasmic; 14/46[2], 33/48, 44/47, 44/44) of female mice.

<u>Rats</u>
Groups of 48 male and female rats were fed diets containing 0, 91, 272, or 543 mg/kg leucomalachite green in feed for 2 years (equivalent to average daily doses of approximately 0, 5, 15, and 30 mg/ kg bw for males and 0, 6, 17, and 35 mg/kg bw for females).

Under the conditions of these 2-year feeding studies, there was equivocal evidence of carcinogenic activity of leucomalachite green in male F334/N rats based on an increase in interstitial cell adenoma of the testes (37/48[2], 42/47, 43/48, 45/47) and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) (0/47, 2/47, 1/48, 3/46) in exposed rats. There was equivocal evidence of carcinogenic activity of leucomalachite green in female F334/N rats based on marginally increased incidences of hepatocellular adenoma (0/46, 1/46, 2/47, 3/48) and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined) (1/48, 3/48, 0/48, 3/48) in exposed rats.

Exposure to leucomalachite green in feed resulted in non-neoplastic lesions in the thyroid gland (follicle cyst; 0/47[2], 0/47, 0/48, 3/46 for male rats and 0/46, 1/46, 0/47, 2/48 for female rats) and liver (eosinophilic focus: 3/48, 14/47, 19/48, 33/47 for male rats; cystic degeneration: 4/48, 18/47, 13/48, 19/47 for male rats; eosinophilic focus: 3/48, 12/48, 20/48, 16/48 for female rats; vacuolisation cytoplasmic: 5/48, 5/48, 17/48, 22/48 for female rats) of exposed rats.

In conclusion, long-term studies in rats and mice found treatment related liver toxicity. Leucomalachite green resulted in adverse effects at lower doses than malachite green. The National Toxicology Program concluded that there was 'equivocal' or 'some' evidence that malachite green or leucomalachite green might produce tumours in experimental animals at 5 mg/kg body weight per day and above.

[4] Some evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.

Dietary Exposure Assessment

A dietary exposure assessment was undertaken to estimate dietary exposure to malachite green and leucomalachite green for the whole Australian population (2 years and above). An exposure assessment was also carried out for children aged 2-6 years.

Estimated chronic exposures were calculated using the FSANZ dietary modelling computer program DIAMOND.

The exposure assessment was carried out using mean concentrations of malachite green and leucomalachite green based on the analytical data from the *Survey of Chemical Residues in Domestic and Imported Aquacultured Fish*, conducted by FSANZ. A lower bound mean (assuming not detected results = 0) and an upper bound mean (assuming not detected results = limit of reporting of 0.002 mg/kg) concentration level were derived for each substance. For example, for malachite green, there were two detected values of 0.003 mg/kg and 0.028 mg/kg, therefore, to derive the lower bound mean, the two detected samples were used as is and 59 samples were assigned a concentration of zero. Therefore the concentrations used in the exposure assessment for malachite green were a lower bound mean of 0.0005 mg/kg and an upper bound mean of 0.002 mg/kg. For leucomalachite green the concentrations used in the exposure assessment were a lower bound mean of 0.005 mg/kg and an upper bound mean of 0.007 mg/kg.

The 1995 Australian National Nutrition Survey (NNS) does not report consumption for all of the specific fish analysed in the residues survey. For the dietary exposure assessment, fish consumption figures included all types of fish, were derived from the NNS for the whole population and for children (survey population = 13 858 aged 2 years and above; 989 aged 2-6 years). The fish consumption data included fish consumed on its own and where fish was an ingredient in mixed foods.

Whilst it is understood that malachite green and leucomalachite green are primarily found in 'aquacultured' fish, and the analytical results were reported for species of fish, the model for malachite green was a 'worse case scenario' in that it was assumed that all fish were assigned the mean upper and lower bound concentrations derived, and likewise for leucomalachite green, as analytical results could not be assigned to specific fish species. This is a reasonable assumption in this case, as positive results were found in domestic and imported fish. The population dietary exposure estimates were derived from each individual person's fish consumption and potential dietary exposure.

Table 1: Estimated chronic dietary exposures to malachite green (lower bound-upper bound)

Country	Population group	Number of consumers of malachite green	Mean consumers (µg/day)	Mean consumers (µg/kg bw/day)*	95th percentile consumers (µg/day)	95th percentile consumers (µg/kg bw/day)*
Australia	Whole population (2 years+)	1627	0.1 - 0.2	0.002-0.003	0.3 - 0.7	0.005 - 0.010
	2-6 years	74	0.1 - 0.1	0.003 – 0.006	0.2 – 0.5	0.009 – 0.019

◊ Consumers only – This only includes the people who have consumed a food that contains malachite green.
* Based on each individual's exposure divided by his or her own body weight.

Table 2: Estimated chronic dietary exposures to leucomalachite green (lower bound-upper bound)

Country	Population group	Number of consumers of leucomalachite green	Mean consumers (µg/day)	Mean consumers (µg/kg bw/day)*	95th percentile consumers (µg/day)	95th percentile consumers (µg/kg bw/day)*
Australia	Whole population (2 years+)	1627	0.5 – 0.7	0.008 – 0.012	1.6 – 2.3	0.024 – 0.034
	2-6 years	74	0.3 – 0.4	0.015 – 0.021	1.2 – 1.6	0.046 – 0.065

◊ Consumers only – This only includes the people who have consumed a food that contains leucomalachite green.
* Based on each individual's exposure divided by their own body weight.

Estimated dietary exposures to malachite green and leucomalachite green are presented below in Table 1 and Table 2 respectively. Estimated mean exposures for consumers of malachite green and leucomalachite green are presented as well as estimated 95th percentile exposures, both in milligrams per day and milligrams per kilogram of body weight per day. The estimated exposures are presented as a range. The lower end of the range are the exposures based on the lower bound mean concentrations of the substances, and the upper end of the range are the exposures based on the upper bound mean concentrations.

Risk Characterisation

For both malachite green and leucomalachite green no assessment reports from international or other national agencies were available. There is only limited information on the potential toxicity of malachite green and leucomalachite green.

Recently, the National Toxicology Program in the USA has performed long-term studies in rats and mice with both malachite green and leucomalachite green. The results of the rodent studies found treatment-related liver toxicity, anaemia and thyroid abnormalities. Leucomalachite green resulted in adverse effects at lower doses than malachite green. The most sensitive non-neoplastic endpoint in rat studies was liver toxicity. These non-neoplastic lesions in the liver were found in rats at 5 mg leucomalachite green/kg body weight per day, the lowest dose tested.

No conclusion can be made regarding in vivo genotoxicity – one *in vivo* mouse micronucleus test was positive while an *in vivo* rat micronucleus test was negative.

The study authors concluded that there was 'equivocal' or 'some' evidence that malachite green or leucomalachite green might produce tumours in experimental animals at 5 mg/kg bw per day and above. For this purpose the incidences of adenomas and carcinomas was combined. This is considered a conservative approach, which assumes that adenomas will give rise to carcinomas. Furthermore, the study authors considered most of the results as 'equivocal' that is, showing a marginal increase of neoplasms that may be chemical-related. The liver tumours found in mice treated with leucomalachite green were considered to demonstrate 'some' evidence of carcinogenic activity.

The overall conclusion on carcinogenicity is that there is only limited evidence that malachite green and leucomalachite green could cause tumours in rodents. In relation to its relevance for human health the carcinogenicity data together with the genotoxicity suggests there is a very low risk.

Since the data available are limited no acceptable daily intake (ADI) could be established. To estimate what the potential exposure of leucomalachite green compared to observed effect levels after long-term exposure in rats, a margin of exposure was calculated for both mean and high consumer taking the non neoplastic lesions in the liver as the most sensitive endpoint (Table 3 and 4).

Table 3: Margin of exposure for Mean Consumers

Population group	Exposure to leucomalachite green (μg/kg bw/day)	Dose resulting in non-neoplastic lesions in live of rats (mg/kg bw/day)	Margin of exposure
Whole population (2 years +)	0.012 (Upper bound)	5	420,000
	0.008 (Lower bound)	5	625,000
2-6 years	0.021 (Upper bound)	5	238,000
	0.015 (Lower bound)	5	333,000

Table 4: Margin of exposure for High Consumers (95% dietary exposure level)

Population group	Exposure to leucomalachite green (μg/kg bw/day)	Dose resulting in non-neoplastic lesions in live of rats (mg/kg bw/day)	Margin of exposure
Whole population (2 years +)	0.034 (Upper bound)	5	147,000
	0.024 (Lower bound)	5	208,000
2-6 years	0.065 (Upper bound)	5	77, 000
	0.046 (Lower bound)	5	110,000

When the dietary exposure for high consumers of fish (upper bound) was compared to the dose shown to cause tumours in animal studies, there was an approximate 150,000-fold difference. At this level of dietary exposure, the risk of non-neoplastic liver lesions from exposure to leucomalachite green is likely to be extremely small.

The estimated dietary exposure for high level consumers is very conservative since consumers are highly unlikely to consume fish every day at a high level. Furthermore, for dietary exposure modelling it was assumed that all fish would contain leucomalachite green, while in reality malachite green and leucomalachite green has only been found in some aquacultured fish species. The 95th percentile consumption figure is therefore a highly conservative estimate of exposure. The mean exposure level is a more realistic estimate of long-term exposure and if this figure (upper bound) is used in the above comparison, then the margin between dietary exposure and the dose causing tumours in animals increases to 420,000.

Conclusion

The residues of malachite green and/or leucomalachite green found in fish are considered to arise from the illegal use of malachite green to treat protozoa and fungal infections on fish and fish eggs. Leucomalachite green, a metabolite of malachite green, may persist in fish tissues for long periods. The currently available data indicate evidence of very weak carcinogenic activity in rodents, which is unlikely to be relevant to humans at the estimated levels of intake. There is also a wide margin of exposure between the intake of leucomalachite green residues from fish for the high consumer (95^{th} percentile) and the dose at which it caused liver lesions in animals.

On the basis of information available to FSANZ, even with a worst-case scenario, the public health and safety risk from malachite green residues in fish is considered very low.

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