Technical Compilation of Heavy Metals, Pesticide Residues, Histamine and Drug Residues in Fish and Fish Products in Southeast Asia

Japanese Trust Fund II Project on Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products
2004 ~ 2008

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in collaboration with
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SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER

The Southeast Asian Fisheries Development Center (SEAFDEC) is a technical organization devoted to the accelerated development of fisheries in the region. The member countries of SEAFDEC are Japan, Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. SEAFDEC has four departments, namely, the Marine Fisheries Research Department (MFRD) in Singapore, the Training Department (TD) in Thailand, the Aquaculture Department (AQD) in Philippines and the Marine Fishery Resources Development and Management Department (MFRDMD) in Malaysia.

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Foreword

Fish safety and quality related issues on chemical or veterinary drugs are increasingly of concern as fish is an important source of protein for the people in Southeast Asia. In addition, fishery trade is important to Southeast Asia as some Southeast Asian countries are also amongst the top ten importers to the European Union, United States and Japan.

In response to increasing demands for pertinent and succinct scientific and technical information, the Marine Fisheries Research Department (MFRD) of the South East Asian Fisheries Development Center (SEAFDEC) in Singapore co-ordinated and conducted the Japanese Trust Fund II Project on "Chemical and Drug Residues in Fish and Fish Products in Southeast Asia". This project aimed to obtain a baseline level of the current situation of chemical and drug residues in seafood in Southeast Asia. Another component of this project was to build up technical capability in testing for chemical contaminants in Southeast Asia's fish inspection laboratories through on-site and regional training courses, which were well received by participants and had also served to promote the exchange of knowledge between participating laboratories.

This Technical Compilation, which is the end result of the Project, would be valuable to policy makers, technologists, scientists as well as extension and regulatory personnel in the fisheries sector. I would like to express my sincere gratitude and appreciation to the fisheries officers in Member Countries and the staff of MFRD for their effort in making this Technical Compilation possible.

In addition, we would like to thank the Government of Japan for funding this project under the Japanese Trust Fund Program. I am sure this compilation will provide useful information for the success of the fisheries trade and industry in providing safe seafood in the Southeast Asian region.

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Introduction

In view of the presence of chemical contaminants and drug residues in seafood products and the serious implications to trade and human health, there is a need to gain an understanding of current levels of such chemical and drug residues in fish and fish products, as well as build up the capacity for detection of these residues in the region. Only with this science-based knowledge and capability, can respective countries take appropriate actions to manage the safety of such fish and fish products. Thus this project was implemented and the information obtained would benefit policy makers, technologists, scientists as well as extension and regulatory personnel in the fisheries sector. In addition, this project allowed SEAFDEC to fulfill part of the recommendations and plan of actions from the Millennium Conference.

The objectives of this project were;

- To obtain an understanding of levels of chemical contaminants in fish and fish products in Southeast Asia.
- To transfer technology in chemical contaminant testing in the region through setting up of methodologies and human resource training.

This project was made up of four components as follows:

Component 1: Survey of Heavy Metals in Fish and Fish Products in Southeast Asia

There were two activities under this component, namely Activity 1.1 "Regional survey of heavy metals in fish and fish products" and Activity 1.2 "On-site training on heavy metals".

In Activity 1.1, regional surveys on heavy metals (arsenic, cadmium, lead and total mercury) in fish and fish products was conducted in SEAFDEC member countries. Various participating regional laboratories conducted a survey each in their respective countries. Fish and fish products that are of economical and social importance to the participating countries were targeted. Fish and fish products samples were sent to the local/collaborating laboratories for analysis. A total of 49 fish and fish products were surveyed. The results of the survey were deposited in the database of the Fish and Fish Products Safety Information Network. In addition, to ensure the accuracy and comparability of the different methods used by member countries, member countries participated in the Inter-laboratory Proficiency Testing for the test methods.

In Activity 1.2, MFRD assisted in the upgrading of regional laboratory personnel skills in heavy metals analysis using Atomic Absorption Spectrometry (AAS) through a series of on-site trainings. On-site training sessions were conducted in Indonesia, Myanmar and Vietnam with a total of 36 trainees.

Component 2: Survey of Pesticide Residues in Fish and Fish Products in Southeast Asia

There were two activities under this component, namely Activity 2.1 "On-site training on pesticide residues analysis" and Activity 2.2 "Regional survey of pesticide residues in fish and fish products".

Activity 2.1 served to upgrade the regional laboratory staff in conducting pesticide residues analysis using Gas Chromatography Mass Spectrometry (GC-MS) and to facilitate the execution of the survey under Activity 2.2. The training were carried out in two ways. Firstly, MFRD with the assistance of an expert from Japan conducted a regional training course in pesticide residues analysis at MFRD for SEAFDEC member countries. Subsequently, an on-site training was conducted in Malaysia for 8 provincial officers.

In Activity 2.2, regional surveys on the pesticide residues (organochlorines) in fish and fish products were conducted in SEAFDEC member countries. Various participating regional laboratories conducted a survey each in their respective countries. Fish and fish products that are of economical and social importance to the participating countries were targeted. Samples of fish and fish products were sent to their local/collaborating laboratories for analysis. Dried products from both marine and freshwater species, as well as fish and fish products that are dried or from inland and estuarine waters and rivers were also targeted. A total of 35 fish and fish products were surveyed. The results of the survey were deposited in the database of the Fish and Fish Products Safety Information Network.

Component 3: Survey of Histamine Levels in Fish and Fish Products in Southeast Asia

There were two activities under this component,

namely, Activity 3.1 "Regional survey of histamine in fish and fish products" and Activity 3.2 "On-site training on histamine analysis".

In Activity 3.1, regional surveys on the naturally occurring toxin, histamine, in fish and fish products was conducted in SEAFDEC member countries. Various participating regional laboratories conducted a survey each in their respective countries. Fish and fish products from scombroid species were targeted. In the Mid-term Review Meeting held in 2007, the Meeting agreed to include the analysis of fermented fish and fish products such as prawn paste and fish sauce in the survey but it would be up to each country to decide on the type of fermented fish and fish products given that the country worked within the allocated budget. A total of 28 fish and fish products were surveyed. The results of the activities were deposited in the database of the Fish and Fish Products Safety Information Network.

In Activity 3.2, MFRD with the assistance of a trainer from Thailand's Department of Fisheries (DOF) conducted 2 on-site training courses for a total of 26 participants on histamine analysis using fluorometric and High Performance Liquid Chromatography (HPLC) methods. The objective was to upgrade the technical capability in histamine testing in the region and facilitate the execution of Activity 3.1.

Component 4: Survey on Drug Residues in Fish and Fish Products in Southeast Asia

There were two activities under this component namely, Activity 4.1 Regional survey of chloramphenicol and nitrofuran in fish and fish products" and Activity 4.2 "Regional training course in seafood safety for laboratory personnel".

In Activity 4.1, survey on the levels of chloramphenicol and nitrofuran was conducted on fish and fish products from the region. Six participating regional laboratories conducted a survey each in their respective countries. At the Mid-term Review Meeting held in 2007, the meeting recommended that Malachite Green and leuco-Malachite Green should be included in the survey. Hence, the title of Activity 4.1 was changed to "Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products". The Meeting also agreed to obtain the samples from processing plants. In view of the different analytical methods used in the survey, participating countries participated in the inter-laboratory proficiency testing under FAPAS Round 02109 for chloramphenicol in prawn sample in March 2008. Those member countries surveying malachite green were encouraged to participate in FAPAS Round 02107 for leuco-malachite green and malachite green in fish muscle sample in January 2008. This would ensure the countries produce comparable and accurate results even though they were using different methods. A total of 15 fish and fish products were surveyed. The results of the activities were deposited in the database of the Fish and Fish Products Safety Information Network.

The Activity 4.2 was initiated after the 1st Planning Meeting for Japanese Trust Fund IV Projects held between 14 to 16 December 2004 in Singapore. The Meeting agreed that there was a need to train the laboratory personnel on the analysis of antibiotics especially in the detection of prohibited drugs such as chloramphenicol and nitrofuran used in aquaculture farms. The training was essential for those analysis that required sophisticated instrument such as the Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS) for detection and determination. Through this regional training course, member countries shared their experience and their methods.. As decided at the Mid-term Review Meeting, Vietnam National Agriculture, Forestry and Fisheries Quality Assurance Department (NAFIQAD) and MFRD co-organized this training for 8 participants. An expert from NAFIQAD was invited to be the trainer for the 3 methods namely Chloramphenicol, Nitrofuran and Malachite Green using Liquid Chromatography Mass Spectrometry (LC-MS-MS) method.

New Activities 5 and 6 (2007 and 2008)

In 2007, MFRD held the JTF II Mid-Term Review Meeting in Singapore under a new Activity 5. The change of the project title as well as the scope of the project to exclude the aquaculture component were announced and the progress of all activities were updated at the meeting. In 2008, 2 new activities, namely, Activity 5 "Publication of Report" and Activity 6 "End-of-Activity Seminar" were conducted. The final results and reports of all SEAFEC member countries were published and distributed under Activity 5. In Activity 6, MFRD held the End-of-Activity Seminar on 22 to 24 October 2008 in Singapore. All key project leaders attended this 3-day meeting and reported on the project since the implementation in 2004. The challenges faced in the implementation of the project were identified and the future plans were deliberated.

This publication is the final output of the 5-year programme and summarises the results of the regional surveys conducted by the participating member countries. The publication is a definitive work highlighting to the importing countries the region's commitment to ensure safety of fish and fish products from the region.

Transfer of Technology in Chemical Contaminants Testing in the Region through Setting up of Methodologies and Human Resource Training

1. Introduction

At the 1st planning meeting, Marine Fisheries Research Department (MFRD) informed the Meeting that there would be four training activities under this project. The Activities were: Activity 1.2 "On-site training on heavy metal analysis", Activity 2.1 "On-site training on pesticide residues analysis", Activity 3.2 "On-site training on histamine analysis" and Activity 4.2 "Regional training course in seafood safety for laboratory personnel". These training activities aimed to transfer technology in chemical contaminants testing in the region through setting up of methodologies and upgrading the technical capability in testing in the region. Member countries identified the training schedule and the countries that required training. The Meeting agreed that the country hosting the training would appoint an officer to assist MFRD's trainer and ensure that all requirements including equipment, glassware and columns were available and adequate.

2. Training Courses conducted from 2005 to 2007:

Activity 1.2: On-site training on heavy metals

Activity 1.2 "On-site training on heavy metals" was successfully conducted in Indonesia from 1 to 8 July 2005 for a total of 12 participants from provincial laboratories in Indonesia, Myanmar from 7 to 11 November 2005 for a total of 13 participants from provincial laboratories in Myanmar and Ho Chi Minh, Vietnam from 28 August to 1 September 2006 for a total of 11 participants from Vietnam provincial laboratories. Two MFRD staff were the trainers for this course. The methods used for the training were MFRD's in-house methods. Using the Atomic Absorption Spectrometry.

Activity 2.1: On-site training on pesticide residues analysis

MFRD together with a Japanese expert, Mr. Kazuki Maruyama, successfully conducted the training on pesticide residues analysis in MFRD from 6 to 15 June 2005 for a total of 8 participants from Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam. MERD conducted on-site training in Malaysia from 27 November to 1 December 2006 for 12 Malaysia provincial officers. The multi-residues method used for the training was MFRD's in-house method. Using the Gas Chromatography Mass Spectrometry (GC-MS).

Activity 3.2: On-site training on histamine analysis

Activity 3.2 "On-site training on histamine analysis" was successfully conducted in Jakarta, Indonesia from 16 to 20 May 2005 for a total of 14 participants from Indonesia provincial laboratories and Philippines from 25 to 29 September 2006 for 12 participants from Philippines governmental and private laboratories. An officer from Thailand's DOF led the on-site training on the fluorometric method for histamine analysis and 2 MFRD staff led the HPLC method of histamine analysis.

Activity 4.2: Regional training course in seafood safety for laboratory personnel

Activity 4.2 "Regional training course in seafood safety for laboratory personnel" was successfully conducted in Thailand Fish Inspection and Quality Control Division (FIQD) from 15 to 19 August 2005 for 16 participants from Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam. A Japanese expert from Japan Food Research Laboratory (Tama branch), Ms Sasakura Naoko, was the advisor for the training course. The main trainers for this training were MFRD and FIQD staff. The training covered three methods namely, Chloramphenicol Analysis by ELISA, Chloramphenicol Analysis by LC/MS-MS and Nitrofuran Metabolites Analysis by LC/MS-MS.

The 2nd workshop was successfully conducted in Singapore from 2 to 5 October 2007. The Workshop was jointly conducted by MFRD and the National Agriculture, Forestry and Fisheries Quality Assurance Department (NAFIQAD). A total of 8 participants from Brunei, Cambodia, Laos, Indonesia, Malaysia, Philippines, Thailand and Vietnam attended the Workshop. Mr Nguyen Anh Dung from Vietnam shared his valuable experience and covered methods on Chloramphenicol, Nitrofuran and Malachite Green Analysis using Liquid-Chromatography Mass-Spectrometer-Mass Spectrometry (LC-MS/MS) during the Workshop.

3. Review of Training Program:

In view of the increased testing capability in member countries, it is envisaged that participating countries, especially those countries exporting to European Union, would host the training courses in their countries. This could also facilitate the sharing of their experiences in ensuring the reliability of their analyses, which would be beneficial to the other member countries.

Due to the stringent export requirements, the host countries could recommend experts from importing countries to conduct the on-site trainings. This could better help the exporting member countries to identify the technical problems and meet their requirements.

Regional Survey of Heavy Metals in Fish and Fish Products

- Cambodia
- Indonesia
- Malaysia
- Myanmar
- Singapore
- Thailand
- Vietnam

Cambodia

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1. Introduction

In order to ensure that Iridescent shark-catfish and fish products in Cambodia are free from contamination by chemicals such as heavy metals (mercury, cadmium and lead) in fish farms, Cambodia carried out the survey under the Japanese Trust Fund II project to examine heavy metals contamination. The fish species targeted for the survey was *Pangasianodon hypophthalmus* cultured in the ponds of Kandal province. The survey started in March 2006 and was completed in May 2008.

2. Objectives And Goals

The objective of the survey was to obtain information on heavy metals (mercury, cadmium and lead) contamination in Iridescent shark-catfish.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Fish samples of Iridescent shark-catfish, *Pangasianodon hypophthalmus*, were collected from the fish farm in Kandal province since 2006. The sampling times were as follows:

Species surveyed	Year	Month	Number of samples collect
Iridescent shark-catfish (*Pangasianodon hypophthalmus)*	2006	March	6
		June	7
		December	10
	2007	August	9
		December	5
	2008	April	7

Each sample weighed at least 500 g/fish. The physical parameters were recorded. The samples were packed in plastic bags filled with ice and sent to the laboratory. The samples were stored at -18°C for 1 to 2 days before transporting to Vietnam for analysis. Only the edible part of the fish was used for analysis.

b. Method of Analysis

All samples were analyzed at National Agriculture, Forestry and Fisheries Quality Assurance Department-Branch 4 (NAFIQAD-BRANCH 4) in Ho Chi Minh City with certificate No: 105

SN	Analyte	Test Method	Method References
1.	Mercury (Hg)	Atomic Absorption Spectrophotometry (AAS-Hydride)	AOAC No. 974.14-1990 and 971.21-1990
2.	Cadmium (Cd)	Atomic Absorption Spectrophotometry (AAS-Graphite Furnace)	NMKL No 139.1991
3.	Lead (Pb)	Atomic Absorption Spectrophotometry (AAS-Graphite Furnace)	NMKL No 139.1991

AOAC: Association of Official Analytical Chemists

c. Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is as shown:

Analyte	LOD (µg/kg)
Mercury	10
Lead	5
Cadmium	1

d. National Regulatory Limits

The maximum levels for heavy metals is set based on commission regulation (EC) No 1881/2006 of 19 December.

Analyte	Maximum levels (mg/kg wet weight)
Mercury	0.50
Lead	0.30
Cadmium	0.050

Chemical standard specification maximum permitted level in fish & fish products. Enforced by EU, USA, Canada and Japan

SN.	Type of Chemical Hazardous	Product Types	EU	USA	Canada	Japan
1.	Contaminants Mercury (Hg)	All Fish & Shell Fish Products	1.0 ppm	1.0 ppm	0.5 ppm	0.4 (Total) 0.3 (Organic)
2.	Lead (Pb)		0.4-1.0 ppm	1.5-1.7 ppm	0.5 ppm	Not Set
3.	Cadmium (Cd)		0.1-1.0 ppm	3-4.0 ppm	–	Not Set

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Cambodia did not participate in any Inter-laboratory Proficiency Testing Program.

b. Survey Results and Discussion

Thirty-five fish samples were analyzed for Mercury (Hg), Lead (Pb) and Cadmium (Cd). Mercury (Hg) was detected in 15 samples but the level was not quantifiable. Lead (Pb) and Cadmium (Cd) were not detected.

These results indicated Catfish *P. hypophthalmus* cultured in the ponds of Kandal province are safe for direct consumption and processing.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2006 fish farm in Kandal province	Total mercury	Iridescent shark-catfish	*Pangasianodon hypophthalmus*	15	0.011	0.047	0.026	Detected but not quantifiable
2007 fish farm in Kandal province				13	0.006	0.024	0.017	Detected but not quantifiable
2008 fish farm in Kandal province				7	0.016	0.027	0.021	Detected but not quantifiable

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2006 fish farm in Kandal province	Lead	Iridescent shark-catfish	*Pangasianodon hypophthalmus*	15	0	0.004	0.002	Not Detected
2007 fish farm in Kandal province				13	0	0.004	0.002	Not Detected
2008 fish farm in Kandal province				7	0	0.004	0.002	Not Detected

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2006 fish farm in Kandal province	Cadmium	Iridescent shark-catfish	*Pangasianodon hypophthalmus*	15	0.005	0.009	0.007	Detected but not quantifiable
2007 fish farm in Kandal province				13	0	0	0	Not Detected
2008 fish farm in Kandal province				7	0	0	0	Not Detected

c. Corrective Actions

The results above indicated that cultured catfish *P. hypophthalmus* are safe for Cambodian consumption and export.

5. Problems and Challenges Encountered

The main problem encountered was that Cambodia's National Laboratory could not analyse heavy metals (Mercury, Lead and Cadmium) and the fish samples had to be sent to Vietnam for analysis.

6. Recommendations and Suggestions for Future Follow up Action

Due to limited budget, collected data is not sufficient to reflect the actual situation of chemical contaminants in aquacultured fish in Cambodia. There is a need to train laboratory personal on sampling and analysis of heavy metals.

Indonesia

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1. Introduction

Indonesia is an archipelago and comprises a total of 17,500 islands. Indonesia has a coastline length of about 81,000km and is located on the crossroads between two oceans, the Indian and the Pacific. Indonesia is also a bridge between two continents, Asia and Australia. The total production of Indonesian fish and fish products over the period from 2000 to 2005 has increased steadily from 4,875,649 tonnes (2000) to 5,452,651 tonnes (2005), by volume, with an average increase of 3.51% annually.

Indonesia was involved in the Japanese Trust Fund II Project to participate in the regional survey on chemical contaminants such as heavy metals (Mercury, Cadmium and Lead) in fish and fish products.

Under the Indonesia Food Law No. 7/1996 and Fisheries Law No. 31/204 as well as Government Regulation No. 28/2004 pertaining to Food Quality, Safety and Nutrition; it was clearly stipulated that all food products including fish and fish products put in the market place intended for human consumption shall comply with the prevailing laws and regulations, to ensure that the products are safe and shall not pose any threats to human health.

With the above laws and regulations, it is imperative to formulate the Ministry of Marine Affairs and Fisheries Decree, and clearly define the important requirements to guarantee quality and safety of fish products derived from fishing, collecting vessel, landing sites, auction centers, processing unit and distribution facilities. Three latest Indonesian Regulations and Decrees of Ministry of Marine Affairs and Fisheries are effectively implemented from 1st August 2007. These Regulations and Decrees focused on (1) The Control of Quality Assurance and Food Safety System of Fisheries Products; (2) Monitoring of Drug Residues, Chemicals, Biological Substances and Contaminants in Aquaculture; and (3) Requirements for Quality Assurance and Safety of Fishery Products During Production, Processing and Distribution. In addition, one guideline on the Inspection and Monitoring Program for Fisheries Products is described in the Decree of Director General of Fisheries Products Processing and Marketing.

These Regulations and Decrees are parallel with the activities under the Japanese Trust Fund II Project such as regional surveys on chemical contaminants. These activities are useful and valuable to implement in Indonesia in order to support our national residues monitoring program to produce good and safe Indonesian fish products.

In order to ensure that Indonesian fish and fish products are free of chemical contaminants such as Mercury (Hg), Cadmium (Cd) and Lead (Pb), it is mandatory to survey and monitor the fish and fish product. If chemical contaminants of heavy metal obtained higher than the maximum residue limit (MRL) or minimum required performance limit (MRPL), the corrective actions would be taken according to the procedure stipulated in the regulation.

2. Objectives and Goals

Through participation of the research and analysis of Hg, Cd and Pb in fish and fish products in Indonesia, we expect to be able to:

- Obtain an understanding on the level of heavy metals contamination in fish and fish products in Indonesia;
- Set up and implement the monitoring program on heavy metals contamination in fish and fish products;
- Strengthen the fish inspection and quality control system, including the improvement of laboratory personal skill in conducting fish inspection and quality analysis;
- Improve and facilitate the analysis of chemical contamination in fish and fish products in Provincial Laboratories for Fish Inspection and Quality Control; and

- Supply fish and fish products that comply with international market standard and food safety requirements.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling method was conducted according to the National Sampling Plan and the monitoring program of each Provincial Laboratory for Fish Inspection and Quality Control (PL-FIQC). During 2005 to 2008, sampling of raw material for heavy metal analysis was carried out in 6 provinces which are representatives for producing fish and fish products, namely:

(1) Jakarta
(2) East Java
(3) South Sulawesi
(4) North Sulawesi
(5) Bali, and
(6) Maluku

All the raw material collected as samples were marine fish products especially *Scombridae* and deep sea fish species such as tuna, skipjack tuna, swordfish, marlin, red snapper, grouper, etc. In most cases, raw materials were taken from the fish landing places and some fish processing plants. After the sample collection, fish and fish products were frozen at -18ºC prior laboratory analysis.

Frozen fish collected from some processing plants were usually analyzed directly for heavy metals such as Hg, Pb and Cd. The period of sampling was monthly and quarterly in 2005 and 2006 and every two months in 2007 and 2008.

b. Method of Analysis

The Atomic Absorption Spectrometry (AAS) method was used for analyzing heavy metals.

c. Limit of Detection and Limit of Quantification

Latest methods validation results for heavy metals conducted by National Center for Fish Inspection and Quality Control (NCQC) are: Hg (LOD = 0.05 ppm), Pb (LOD = 0.04 ppm) and Cd (LOD = 0.07 ppm).

d. National Regulatory Limits

Indonesia latest National Standard Limits (2007) of Heavy Metal are as:

- Hg = 1.0 ppm for predator fish such as tuna, swordfish marlin, bivalve mollusk and crustacean. For other fish and fish products, the limit was stipulated as 0.5 ppm.
- Pb = 0.4 ppm for predator fish such as tuna, swordfish marlin; 1.5 ppm for bivalve mollusk; 0.5 ppm for crustacean. For other fish and fish products, the limit was stipulated as 0.3 ppm.
- Cd = 0.5 ppm for predator fish such as tuna, swordfish marlin; 1.0 ppm for bivalve mollusk and crustacean. For other fish and fish products, the limit was stipulated as 0.1 ppm.

4. Results and Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Inter-laboratory proficiency testing are usually conducted routinely by the NCQC to all the Provincial Laboratory for Fish Inspection and Quality Control (PL-FIQC) in Indonesia. The focus for proficiency testing is usually microbiology (*E-coli, Salmonella* etc.) and chemical testing such as heavy metals.

NCQC also participated in the international proficiency testing program for heavy metals (Hg, Cd and Pb) conducted by Asia Pacific Laboratory Accreditation Cooperation (APLAC) in 2003.

b. Survey Results and Discussion

The older version of Indonesia National Standard was used for Year 2005 and 2006. The maximum limits for all fish and fish products for Hg, Pb and Cd are 0.5, 0.3 and 2.0 ppm respectively.

Survey of heavy metals (Hg, Cd and Pb) during the year of 2005 was focused on 5 locations namely Jakarta, East Java (Surabaya), Bali, South Sulawesi (Makassar) and North Sulawesi (Bitung) on the following fish species were surveyed, tuna, marlin, swordfish, skipjack, grouper, red snapper and canned tuna. The total samples taken were 104 samples.

Survey of Mercury (Hg) was conducted monthly from April to September 2005 and it was found that from 96 samples, 6 samples analyzed such as swordfish, marlin and tuna had Mercury (Hg) higher than standard (Indonesia National Standard), that is 0.5 ppm, and the results were between 0.52 and 0.6 ppm. None of the 96 samples analyzed for Cadmium (Cd) was higher than CAC and EU standards (0.5 ppm) and Indonesia National Standard (2.0 ppm) for tuna, frigate mackerel, grouper, skipjack tuna, swordfish and canned tuna. Lead (Pb) were found in 8 samples with values between 0.32 to 0.42 ppm, they were higher than the Indonesia National Standard of 0.3 ppm.

In 2006, the survey of the heavy metals was conducted quarterly in March, June and September. From the 49 samples analyzed, no sample was found to be higher than the maximum limit stipulated in the Indonesia National Standard and the CAC and EU standards for Hg and Cd. For lead (Pb), 4 samples were found higher than Indonesia National Standard of 0.3ppm.

In 2007, Indonesia continued to participate in the survey on heavy metals and focused on 45 samples in 5 locations namely Jakarta, Bali, South Sulawesi, North Sulawesi and Maluku Provinces for the fish species such as tuna, marlin, swordfish, skipjack tuna, grouper, red snapper, canned tuna, etc. These locations have been chosen as they produce mostly scombroid fish. The sampling was conducted on April, June and August 2007. For Mercury (Hg), none of the 46 samples collected was higher than the Indonesia National Standard of 1.0 ppm. For Cadmium (Cd) and Lead (Pb), no sample was found to be higher than the Indonesia National Standard, CAC and EU standards. Comparing with the survey results of heavy metals in 2005 and 2006, the violation in 2007 has decreased.

In 2008, the survey focused on 4 locations namely Jakarta, Bali, South Sulawesi (Makassar) and North Sulawesi (Bitung). The sampling time was only two times on February and April 2008. The results of all sample analysed showed that the heavy metals (Hg, Cd and Pb) were lower than the national and international standards.

Table 1. Data Monitoring Of Heavy Metals Conducted in Jakarta, East Java, Bali, South Sulawesi and North Sulawesi in 2005.

No.	Location	Time of Sampling	Species / Products	Heavy Metals (ppm)		
				Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
1.	Jakarta	April 2005	1. Tuna	0.13	0.01	0.23
			2. Canned tuna	0.27	0.02	0.22
			3. Red snapper	0.08	0.04	0.09
		May 2005	1. Tuna	0.24	0.03	0.18
			2. Skipjack tuna	0.21	0.02	0.35
			3. Red snapper	0.18	0.02	0.16
			4. Grouper	0.17	ND	0.35
		June 2005	1. Tuna	0.42	0.02	0.32
			2. Red snapper	0.16	0.03	0.21
			3. Frigate mackerel	0.20	0.02	0.14
		July 2005	1. Tuna	0.54	0.01	0.18
			2. Red snapper	0.10	0.02	0.14
			3. Frigate mackerel	0.20	0.01	0.12
		August 2005	1. Canned tuna	0.32	0.02	0.16
			2. Skipjack tuna	0.28	0.02	0.20
			3. Grouper	0.14	0.01	0.02
			4. Red snapper	0.12	0.01	0.14
		Sep. 2005	1. Sword fish	0.60	0.02	0.22
			2. Marlin	0.52	0.03	0.30
			3. Baby tuna	0.41	0.02	0.18
2.	East Java	April 2005	1. Tuna	0.35	ND	0.42
			2. Canned tuna	0.05	ND	0.21
			3. Red snapper	0.12	ND	0.10
		May 2005	1. Tuna	0.40	0.04	0.24
			2. Skipjack tuna	0.24	0.02	0.25
			3. Red snapper	0.12	ND	0.10
			4. Grouper	0.12	ND	0.03
		June 2005	1. Tuna	0.38	0.18	0.20
			2. Red snapper	0.12	0.01	0.12
			3. Frigate mackerel	0.08	0.18	0.40
		July 2005	1. Tuna	0.38	0.02	0.18
			2. Red snapper	0.12	0.02	0.14
			3. Frigate mackerel	0.08	0.21	0.26
		August 2005	1. Canned tuna	0.10	0.02	0.24
			2. Skipjack tuna	0.20	0.03	0.22
			3. Grouper	0.08	0.02	0.02
			4. Red snapper	0.12	0.02	0.20
		Sep. 2005	1. Sword fish	0.48	0.03	0.36
			2. Marlin	0.44	0.02	0.28
			3. Baby tuna	0.22	ND	0.10

3.	Bali	April 2005	1. Tuna	0.35	0.01	0.07
			2. Canned tuna	0.48	0.07	0.02
			3. Red snapper	ND	0.01	0.06
		May 2005	1. Tuna	0.38	0.02	0.08
			2. Skipjack tuna	0.24	0.02	0.10
			3. Red snapper	ND	0.08	0.04
			4. Grouper	0.06	0.11	0.05
		June 2005	1. Tuna	0.21	0.01	0.10
			2. Red snapper	0.08	0.01	0.08
			3. Frigate mackerel	0.07	0.24	0.38
		July 2005	1. Tuna	0.24	0.03	0.10
			2. Red snapper	0.14	ND	0.08
			3. Frigate mackerel	0.10	0.01	0.38
		August 2005	1. Canned tuna	0.42	0.12	0.03
			2. Skipjack tuna	0.22	0.02	0.14
			3. Grouper	0.08	0.06	0.02
			4. Red snapper	ND	ND	0.04
4.	South Sulawesi	April 2005	1. Tuna	0.22	0.04	0.03
			2. Canned tuna	0.12	0.02	0.08
			3. Red snapper	0.28	0.02	0.12
		May 2005	1. Tuna	0.42	0.03	0.14
			2. Skipjack tuna	0.15	0.10	0.05
			3. Red snapper	0.14	0.04	0.08
			4. Grouper	0.29	0.02	0.06
		June 2005	1. Tuna	0.52	0.08	0.04
			2. Red snapper	0.12	0.06	0.02
			3. Frigate mackerel	0.05	0.03	0.08
		July 2005	1. Tuna	0.52	0.02	0.10
			2. Red snapper	0.31	0.06	0.06
			3. Frigate mackerel	0.04	0.02	0.06
		August 2005	1. Canned tuna	0.48	0.10	0.02
			2. Skipjack tuna	0.18	0.04	0.01
			3. Grouper	0.19	ND	ND
			4. Red snapper	0.08	0.06	0.04
		Sep. 2005	1. Sword fish	0.58	0.14	0.08
			2. Marlin	0.44	0.08	0.06

5.	North Sulawesi	April 2005	1. Tuna	0.13	0.04	0.12
			2. Canned tuna	0.08	0.02	0.08
			3. Red snapper	0.09	0.01	0.12
		May 2005	1. Tuna	0.16	0.02	0.08
			2. Skipjack tuna	0.08	0.02	0.06
			3. Red snapper	0.14	0.04	0.09
			4. Grouper	0.22	0.07	0.12
		June 2005	1. Tuna	0.28	0.02	0.12
			2. Red snapper	0.08	0.04	0.08
			3. Frigate mackerel	0.20	0.01	0.12
		July 2005	1. Tuna	0.46	0.04	0.08
			2. Red snapper	0.13	0.01	0.04
			3. Frigate mackerel	0.24	0.02	0.14
		August 2005	1. Canned tuna	0.18	0.02	0.01
			2. Skipjack tuna	0.10	0.04	0.10
			3. Grouper	0.20	0.05	0.08
			4. Red snapper	0.18	0.02	0.06
		Sep. 2005	1. Sword fish	0.48	0.02	0.04
			2. Marlin	0.32	0.04	0.08
			3. Baby tuna	0.20	0.08	0.02

Table 2. Data Monitoring Of Heavy Metals Conducted in Jakarta, East Java, Bali, South Sulawesi and North Sulawesi in 2006.

No.	Location	Time of Sampling	Species / Products	Heavy Metals (ppm)		
				Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
1.	Jakarta	March 2006	1. Tuna	0.42	0.02	0.12
			2. Swordfish	0.48	0.04	0.10
			3. Red snapper	0.04	0.02	ND
			4. Frigate mackerel	0.14	0.03	0.10
		June 2006	1. Marlin	0.36	0.28	0.08
			2. Tuna	0.14	0.04	0.02
			3. Red snapper	0.02	ND	ND
		Sep. 2006	1. Swordfish	0.30	0.14	0.20
			2. Red snapper	0.06	ND	ND
			3. Grouper	0.08	0.04	0.06
2.	East- Java	March 2006	1. Tuna	0.28	ND	0.02
			2. Red snapper	0.12	0.10	ND
			3. Barred Spanish	ND	0.06	ND
			4. Grouper	0.12	0.03	ND
		June 2006	1. Tuna	0.22	ND	0.02
			2. Grouper	0.10	ND	0.01
			3. Marlin	0.48	0.04	0.14
		Sep. 2006	1. Tuna	0.08	0.06	0.02
			2. Canned Tuna	0.04	ND	0.01
			3. Swordfish	0.36	0.10	0.28

3.	Bali	March 2006	1. Swordfish	0.10	0.02	ND
			2. Marlin	0.21	0.05	0.09
			3. Yellowfin Tuna	0.06	0.05	0.06
			4. Striped Marlin	0.18	0.07	0.12
		June 2006	1. Butterfish	0.02	0.02	0.10
			2. Marlin	0.12	0.03	0.18
			3. Bluefin Tuna	0.04	0.03	0.35
		Sep. 2006	1. Striped Marlin	0.02	0.02	0.50
			2. Black Marlin	0.02	0.02	0.46
			3. Bluefin Tuna	0.02	0.03	0.36
4.	South Sulawesi	March 2006	1. Tuna	0.06	0.02	ND
			2. Red Snapper	0.02	0.03	0.15
			3. Grouper	0.01	ND	0.09
			4. Baramundi	0.02	0.02	ND
		June 2006	1. Tuna	0.04	0.01	0.08
			2. Leather Jacket	0.02	0.01	ND
			3. Baramundi	0.02	0.02	ND
		September 2006	1. Tuna	0.04	0.08	0.04
			2. Grouper	0.06	0.09	0.09
			3. Red Snapper	0.02	ND	0.01
5	North Sulawesi	March 2006	1. Fresh Tuna	0.24	0.04	0.16
			2. Canned Tuna	0.02	0.04	0.05
			3. Skipjack	0.06	0.10	0.02
			4. Marlin	0.34	0.06	0.14
		June 2006	1. Marlin	0.46	0.08	0.12
			2. Fresh Tuna	0.30	0.10	0.08
			3. Skipjack	0.08	0.06	0.02
		Sep. 2006	1. Fresh Tuna	0.10	0.04	0.12
			2. Skipjack	0.10	0.02	0.01
			3. Canned Tuna	-	-	-

Table 3. Data Monitoring Of Heavy Metals Conducted in Jakarta, Bali, South Sulawesi, North Sulawesi and Maluku in 2007.

No.	Location	Time of Sampling	Species / Products	Heavy Metals (ppm)		
				Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
1.	Jakarta	April 2007	1. Marlin	0.30	0.04	0.12
			2. Tuna	0.28	0.03	0.14
			3. Spanish- mackerel	0.11	0.02	0.13
		June 2007	1. Red snapper	0.11	0.03	0.11
			2. Spanish-mackerel	0.20	0.02	0.18
			3. Tuna	0.26	0.02	0.21
		August 2007	1. Marlin	0.31	0.03	0.13
			2. Red snapper	0.22	0.03	0.12
			3. Oil-fish	0.35	0.03	0.12
2.	Bali	April 2007	1. Marlin	0.82	0.03	0.07
			2. Yellow-fin tuna	0.12	0.02	0.12
			3. Blue-fin tuna	0.46	0.01	0.04
		June 2007	1. Sailfish	0.16	0.02	0.13
			2. Blue-fin tuna	0.45	0.01	0.07
			3. Marlin	0.82	0.03	0.08
			4. Yellow-fin tuna	0.25	0.07	0.04
		August 2007	1. Striped marlin	0.17	0.06	0.17
			2. King fish	0.21	0.03	0.08
			3. Yellow-fin tuna	0.26	0.04	0.05
3.	South Sulawesi	April 2007	1. Yellow-fin tuna	0.25	0.01	0.07
			2. Baramundi	0.18	0.04	0.02
			3. Red snapper	0.20	0.02	0.04
		June 2007	1. Yellow-fin tuna	0.28	0.02	0.06
			2. Grouper	0.24	0.02	0.14
			3. Red snapper	0.16	0.08	0.04
		August 2007	1. Yellow-fin tuna	0.22	0.02	0.10
			2. Red snapper	0.18	0.02	0.08
			3. Leather Jacket	0.07	0.24	0.18
4.	North Sulawesi	April 2007	1. Yellow-fin tuna	0.38	0.02	0.04
			2. Canned tuna	0.14	0.02	0.08
			3. Red snapper	0.22	0.03	0.04
		June 2007	1. Yellow-fin tuna	0.32	0.03	0.11
			2. canned tuna	0.14	0.08	0.06
			3. Swordfish	0.44	0.04	0.10
			4. Skipjack tuna	-	-	-
		August 2007	1. Yellow-fin tuna	0.22	0.06	0.06
			2. Red snapper	0.18	0.04	0.04
			3. Marlin	0.34	0.03	0.06

5.	Maluku	April 2007	1. Skipjack tuna	0.13	0.04	0.12
			2. Marlin	0.38	0.02	0.08
			3. Skipjack tuna	0.19	0.04	0.06
		June 2007	1. Skipjack tuna	0.16	0.06	0.04
			2. Yellow-fin tuna	0.28	0.02	0.06
			3. Marlin	0.34	0.06	0.08
		August 2007	1. Skipjack tuna	0.18	0.02	0.12
			2. Marlin	0.28	0.04	0.08
			3. Yellow-fin tuna	0.20	0.01	0.12

Table 4. Data Monitoring Of Heavy Metals Conducted in Jakarta, Bali, South Sulawesi and North Sulawesi in 2008.

No.	Location	Time of Sampling	Species / Products	Heavy Metals (ppm)		
				Mercury (Hg)	Cadmium (Cd)	Lead (Pb)
1.	Jakarta	February	1. Frozen tuna*	0.20	0.01	0.20
			2. Fresh loin tuna*	0.30	0.01	0.12
		April	1. Frozen tuna*	0.18	0.02	0.10
			2. Frozen loin tuna*	0.26	0.02	0.12
			3. Red snapper*	0.10	0.01	0.10
2.	Bali	February	1. Tuna *(BE)*	0.50	0.01	0.14
			2. Tuna *(YF)*	0.16	0.01	0.04
			3. Skipjack	0.08	0.04	0.18
		April	1. Tuna *(YF)*	0.43	0.03	0.11
			2. Skipjack	0.20	0.05	0.14
			3. Tuna *(BE)*	0.18	0.03	0.10
3.	South Sulawesi	February	1. *Lutjanus* sp.	0.12	0.05	0.11
			2. Canned tuna	0.25	0.04	0.09
			3. *P. monodon*	0.04	0.02	0.03
		April	1. Tuna (BE)	0.21	0.04	0.10
			2. *P. vannamei*	0.22	0.10	ND
			3. *P. monodon*	0.10	0.01	ND
4.	North Sulawesi	February	1. Fresh Tuna loin*	0.20	0.10	0.08
			2. Fresh Tuna	0.26	0.04	0.12
			3. Canned Tuna*	0.10	0.01	0.04
		April	1. Frozen Tuna*	0.30	0.04	0.02
			2. Canned Tuna*	0.12	0.06	0.08
			3. Skipjack tuna	0.10	0.01	0.02

Note
ND = Not Detected
Method of Analysis: Atomic Absorption Spectrometer (AAS)
Limit of Detection (LOD): Hg = 0.52ppb**;** Cd = 0.11ppb; Pb = 0.19ppb
* Sample taken from fish processing plant

c. Corrective Actions

Corrective actions will be taken when the results of monitoring does not comply with the standard or higher than the standard. The corrective action process for non-compliant results is as follows:

1) Carry out the investigation such as traceability to the fishing ground areas.
2) Verification of sampling and analytical methods.
3) Perform repeat sampling.
4) Exclusion of products with higher than the maximum residue limit allowed from establishment or continue processing as products not for human consumption.
5) Intensively control chemical contaminants (Hg, Cd and Pb) in fish and fish products during processing or during fishing.

Moreover, in order to ensure the safety of fish and fish products, Indonesia Government undertakes continuous actions such as: (1) Surveillance of stakeholders to ensure their compliance with the rules and regulations in the standards/procedures and (2) Obtain data and information periodically on the level of chemical contaminants such as heavy metal in fish and fish products.

5. Problems and Challenges Encountered

Problem faced during the heavy metals monitoring and surveys are as follow:

1) Analysts are not properly qualified to perform the heavy metals (Hg, Cd and Lead) analysis using Atomic Absorption Spectrometer (AAS).
2) Lack of networking system to harmonize the standard and methods of analysis for heavy metals among ASEAN countries.
3) Lack of knowledge and skill of fishermen regarding the presence of heavy metals in fish and fish products.
4) Stringent requirements set by importing countries for monitoring program such as heavy metal in fish and fish products.

6. Recommendations and Suggestions for Future Follow up Action

1) Need training program for methods validation and verification especially in the determination of the limit of detection and minimum residue performance limit for heavy metals (Hg, Cd and Pb).
2) Need training to improve the methods of analysis for heavy metals.
3) Need to establish networking system to develop the methods of analysis for heavy metals among ASEAN countries.
4) Need to establish harmonizing standard especially for maximum level of each content of heavy metals in order to fulfill the requirements of importing countries such as EU.
5) Need to improve the accuracy of testing results by participating in proficiency testing program especially for heavy metals among ASEAN countries

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1. Introduction

This study was divided into two components of the Japanese Trust Fund (JTF) IV and JTF II. JTF IV was carried out from the year 2005 to 2006, under Component I Activity CI-1: Regional survey of heavy metals in fish and fish product and in the environment, The survey was divided into two areas namely species identification of marine, freshwater and brackish aquacultured fish and marine and freshwater captured fish. Marine, freshwater and brackish aquacultured fish samples comprised of seabass (barramundi), tilapia, catfish, cockles, green mussel and black tiger prawn. Marine and freshwater captured fish samples were Spanish mackerel, grouper, red snapper, prawns and squid.

However, in 2007, the program previously conducted under JTF IV was moved to JTF II, which excluded aquaculture products. The emphasis was on Component I, Activity CI-1: Regional survey of heavy metals in fish and fish products. The survey focused on fish samples collected from processing establishments.

The survey provided information on the level of heavy metals concentration in seafood consumed by people whether it is for domestic use and/or for export. The survey carried out complemented with our on-going Sanitary and Phytosanitary Monitoring Program (SPS), which has the overall objective to ensure safe fish and fish products for human consumption.

Presently, fish and fish products from Malaysia exported to Europe constitute about 30% of the fish production. The Department of Fisheries (DOF) as one of the Competent Authority (CA) should ensure that fish and fish product exported to EU complies with the requirements of importing country and the European Commision (EC) Regulation 1881/2006 – setting maximum levels for certain contaminants in foodstuffs.

2. Objective and Goals

The objective of this study is to determine the level of heavy metals namely Cadmium (Cd), Lead (Pb) and total Mercury (Hg). All the data generated will be deposited collected into the Fish and Fish Products Safety Information Network.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Site

From 10 to 13 May 2005, 160 samples were collected throughout Johor. The samples were collected from east and west Johor especially from Tg. Sedili and Pontian. Spanish mackerel (*Scomberomorus sp*.), grouper (*Epinephelus sp*.), red snapper (*Lutjanus sp*.), shrimp (*Metapeneaus sp*.), cultured white shrimp (*Peneaus vannamei*), squid (*Loligo sp*.) and seabass (*Lates calcarifer*) were collected from the east and west coast of Johor, while catfish (*Clarias batrachus*) and tilapia (*Oreochromis niloticus*) were collected from Kota Tinggi. Green mussel (*Perna viridis*) were harvested only from aquaculture farms in Masai and Pantai Lido, covering an area from the west and east coasts of Johor. The black tiger prawn (*Peneaus monodon*) were sampled from Desaru of east coast of Johor and cockles (*Anadara granosa*) were collected only from Pontian, Johor. The second sampling was carried out from 4 to 8 September 2006, of which 152 samples were collected from the same venues as in the earlier sampling.

Eight samples per species were randomly taken from each location of sampling plan. All the samples were stored at -18ºC in a cold room for four days before being transported to the laboratory for analysis.

In the laboratory, the length and weight of the samples were measured.

From 5 to 6 June 2007, 72 samples of fish products were collected from processing establishments in the states of Perak and Penang. The second sampling was carried out on 28 August 2007, of which 32 samples were collected from other processing establishments in Perak.

Samples comprised of fishcakes, fishballs, frozen shrimps (*P.vannamei*), surimi kintokidai A (from big eye snapper), surimi (from threadfin bream), frozen octopus (*Octopus doflein*), frozen squid (*Sepia inermis*), frozen black tiger prawn (*P. monodon*) and frozen cuttlefish.

Eight samples per species were randomly taken from each location of sampling plan. The samples were kept in cooler boxes filled with ice to maintain the temperature before being transported to the laboratory on the same day.

b. Method of Analysis

Sample Preparation

Frozen samples were thawed at room temperature. Each sample was homogenized using the Seward stomacher 80 Biomaster at 300 rpm for 120 seconds. The homogenized sample was formed into a composite pie and 20g sample was stored in a 50 ml plastic bottle and kept in a freezer for further analysis.

Detection of Heavy Metals

i) Detection of Total Mercury (Hg)

Initially, one layer of additive M was applied onto the surface of a sample boat and about 20-40 mg of sample was placed onto the middle of sample boat before being covered by additive M. This was followed by a layer of additive B and covered again with additive M (Method 2: for a solid or powder sample). The sample was applied to the MA 2000 instrument for detection of total mercury content.

The specifications of MA 2000 instrument are presented in Table 1.

Table 1. The specifications of MA 2000 for total mercury analysis.

Item	Description
1. Method	Gold-amalgam collection method with thermal decomposition by a ceramic tube heater
2. Carrier Gas	Purified dry air
3. Detection Method	Non-dispersion double-beam cold atomic absorption spectrometry
4. Detection Limit	0.002 ng (weight of sample)
5. Measuring Range	0 – 1,000 ng
6. Wavelength	253.7 nm

ii) Detection of Cadmium (Cd) and Lead (Pb)

Digestion of samples

About 400 mg of sample was prepared into HF100 teflon liner. 4 ml nitric acid (HNO_3) (Merck, Damstard, Germany, 65%) and 2 ml of hydrogen peroxide (H_2O_2) (Merck, Damstard, Germany, 30%) was added into the liner and digested using the USEPA procedure of 3015, 3051 and 3052 wet digestion method (PAAR001H). The digestion was performed using Anton Paar 3000, 16 rotors HF100 teflon vessels at a frequency of 2.45 GHz at 1500 W under operating pressure at 40 bars (580 psi) and maximum temperature of 240°C for 45 minutes. A p/T-sensor was used to monitor the digestion reaction till completion. The sample was topped up to 25 ml prior analysis.

Detection of Lead (Pb) and Cadmium (Cd)Using Atomic Absorption Spectrometry method

Lead (Pb) and cadmium (Cd) were measured by Atomic Absorption Spectrometry (AAS) Perkin Elmer model AA800. The diluted samples were analyzed using an in-house furnace method that was developed by Perkin Elmer, PE TS04. The analytical conditions for the AAS are presented in Table 2.

Analysis was performed under room temperature. All the containers and the apparatus were placed in 10% purified acid nitric.

Table 2. Analytical conditions for AA800.

Item	Description
Argon gas	
- Flow rate	0.9 – 1.5 liters/min
- Operating pressure	50 psig
Air compressor	
- Flow rate	10-28 liters/min
- Operating pressure	60 psig
Lamp/current	Cd & Pb – Hollow cathode lamp/12 mA
Wavelength	Cd – 228.8 nm Pb – 283.3 nm

c. Limit of Detection and Limit of Quantification

Element	LOD (mg/kg)	LOQ (mg/kg)
Year 2005 & 2006		
Cd	0.0039	0.039
Pb	0.0018	0.018
Total Hg	0.00002	0.0002
Year 2007		
Cd	0.0048	0.048
Pb	0.0006	0.006
Total Hg	0.00002	0.0002

d. National Regulatory Limits

Country	Element	Regulatory Limits (mg/kg)
Malaysia	Cd	1.0
	Pb	2.0
	Hg	0.5 (1.0 for predator)
EC. Reg. 1881/2006	Cd	0.05 (excluding fish species in 3.2.6 & 3.2.7)*
	Pb	0.3
	Hg	0.5 (excluding fish species in 3.3.2)*

* Refer to Appendix 1

4. Results and Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of Participation	Program Name	Analyte Tested	Reported Results (ppb)	True Value (ppb)	z-Score	Remarks
2004	ASEAN-SEAFDEC Regional Inter-Laboratory Proficiency Testing	Total As	10.1740	10.20	-0.018	–
		Total Pb	0.5066	0.319	0.197	–
		Total Hg	3.1299	3.37	-0.234	–
		Total Cd	18.6340	19.40	-0.509	–
2007	FAPAS Proficiency Test 0786 Metallic Contaminants in canned Fish	Total As	780.16	1124	-1.900	–
		Cd	55.50	52.4	0.3	–
		Pb	–	–	–	z-score not issued
		Total Hg	597.05	704	-0.9	–

b. Survey Results and Discussion

Physical Parameters of Samples Collected in 2005 & 2006

Table 3. Physical parameters of samples collected from east coast of Johor.

Type of system/fish	Location	Weight (g) Average (range)	Length (cm) Average (range)
Aquaculture			
Catfish	Kota Tinggi/Desaru	380 ± 39.5 (343 – 458)	40.3 ± 1.9 (36.5 – 42.1)
Mussel	Masai/Pasir Gudang	45.3 ± 13.4 (22 – 85.4)	8.9 ± 1.0 (7.2 – 40)
Seabass	Tg. Sedili	450.8 ± 61.0 (319 – 578)	30.5 ± 2.3 (24.1 – 33.5)
Tilapia	Kota Tinggi/Desaru	491.3 ± 19.7 (478 – 536)	22.2 ± 2.0 (20.5 – 26.7)
Tiger prawn	Desaru	27.8 ± 7.6 (17.5 – 38)	N.A*
Shrimp	Desaru	27.5 ± 5.0 (17 – 36)	N.A
Marine captured			
Grouper	Tg. Sedili/Mersing	206.5 ± 206.3 (55 – 706)	21.7 ± 6.0 (15.2 – 35.7)
Red snapper	Tg. Sedili	240.9 ± 276.9 (47 – 903)	20.8 ± 8.7 (13 – 39.5)
Squid	Tg. Sedili/Mersing	97.7 ± 33.5 (57 – 175)	21.5 ± 2.8 (18 – 26)
Spanish Mackerel	Tg. Sedili/Mersing	1131.5 ± 147.2 (1013 – 1407)	49.5 ± 1.7 (48 – 52)
Shrimp	Tg. Sedili	14.6 ± 0.6 (13.6 – 15.4)	4.8 ± 1.2 (3.3 – 6.5)

*N.A – Not Available

Table 4. Physical parameters of samples collected from west coast of Johor.

Type of system/fish Aquaculture	Location	Weight (g) Average (range)	Length (cm) Average (range)
Catfish	Senai	258.6 ± 205.3 (98 – 767)	29.6 ± 6.3 (22 – 44.2)
Mussel	Pantai Lido	37.6 ± 26.5 (14.8 – 124)	7.8 ± 0.8 (5.6 – 9.0)
Seabass	Pendas	409.9 ± 96.7 (191 – 535)	29.2 ± 2.5 (23 – 32.4)
Tilapia	Senai	454.1 ± 42.5 (408 – 526)	21.1 ± 0.6 (20.5 – 22.4)
Cockle	Pontian	7.5 ± 1.3 (5.9 – 9.5)	2.7 ± 0.2 (2.4 – 3.3)
Shrimp	Desaru	11.4 ± 1.6 (8.1 – 13.9)	N.A*
Marine captured			
Grouper	Pontian	696 ± 395.5 (390 – 1509)	35.3 ± 5.9 (30.5 – 46.2)
Red snapper	Pontian	895.6 ± 449.1 (330 – 1608)	40 ± 4.8 (32 – 47.2)
Squid	Pontian	105.9 ± 55.2 (55 – 260)	29.1 ± 12.3 (14 – 54)
Spanish Mackerel	Pontian	1131.5 ± 147.2 (1013 – 1407)	16.5 ± 6.3 (11.4 – 31.2)
Shrimp	Pantai Lido	14.6 ± 0.6 (13.6 – 15.4)	N.A*

* N.A – Not Available

Analytical Results For Heavy Metals for Samples collected in 2005 and 2006

Table 5. Results of Cd, Pb and Hg in marine/freshwater/brackishwater aquacultured fish and marine captured fish from east coast of Johor in 2005 and 2006.

Type of system/fish	Sampling Location	Analyte	Fish sample Analysed		No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
			Common name	Scientific name						
Aquacultured										
Catfish	Kota Tinggi / Desaru	Cd	Ikan keli	*Clarias batrachus*	16	ND	0.03	0.01		
		Pb				0.04	0.88	0.16		
		Hg				0.005	0.04	0.02		
Green mussel	Masai / Pasir Gudang	Cd	Kupang	*Perna viridis*	16	0.04	0.12	0.07		
		Pb				0.01	0.32	0.09		
		Hg				0.005	0.02	0.01		
Seabass	Tg. Sedili	Cd	Siakap	*Lates calcarifer*	16	ND	0.02	0.01		
		Pb				ND	0.52	0.19		
		Hg				0.01	0.18	0.12		
Tilapia	Kota Tinggi / Desaru	Cd	Tilapia	*Oreochromis niloticus*	16	ND	0.02	0.02		
		Pb				ND	0.18	0.10		
		Hg				0.01	0.02	0.01		

Cd = 97.6
Pb = 102.8
Hg = 99.8

Note : ND – less than LOD.

Table 5. Results of Cd, Pb and Hg in marine/freshwater/brackishwater aquacultured fish and marine captured fish from east coast of Johor in 2005 and 2006 (cont'd).

Type of system/fish	Sampling Location	Analyte	Fish sample Analysed		No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
			Common name	Scientific name						
Aquacultured									Cd = 97.6 Pb = 102.8 Hg = 99.8	
Black tiger prawn	Desaru	Cd	Udang harimau	*Penaeus monodon*	16	ND	0.07	0.01		
		Pb				ND	0.40	0.16		
		Hg				0.02	0.03	0.02		
White shrimp	Desaru	Cd	Udang putih	*Penaeus vannamei*	8	0.01	0.10	0.04		
		Pb				ND	0.18	0.09		
		Hg				0.02	0.03	0.02		
Captured Fish										
Grouper	Tg. Sedili/ Mersing	Cd	Kerapu	*Epinephelus sp.*	16	ND	0.06	0.02		
		Pb				0.01	0.14	0.05		
		Hg				0.05	0.27	0.14		
Red snapper	Tg. Sedili	Cd	Ikan merah	*Lutjanus sp.*	16	ND	0.01	0.01		
		Pb				ND	0.27	0.08		
		Hg				0.02	0.18	0.07		

Note : ND – less than LOD.

Table 5. Results of Cd, Pb and Hg in marine/freshwater/brackishwater aquacultured fish and marine captured fish from east coast of Johor in 2005 and 2006 (cont'd).

Type of system/fish	Sampling Location	Analyte	Fish sample Analysed		No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
			Common name	Scientific name						
Captured Fish										
Squid	Tg. Sedili/ Mersing	Cd	Sotong	*Loligo sp.*	16	0.02	0.21	0.10		
		Pb				ND	0.31	0.06		
		Hg				0.01	0.03	0.02		
Spanish mackerel	Tg. Sedili/ Mersing	Cd	Tenggiri	*Scomberomorus sp.*	16	ND	0.01	0.01	Cd = 97.6 Pb = 102.8 Hg = 99.8	
		Pb				ND	0.15	0.06		
		Hg				0.04	0.22	0.12		
Shrimp	Tg. Sedili	Cd	Udang laut	*Metapeneaus sp.*	8	ND	0.04	0.02		
		Pb				ND	0.14	0.05		
		Hg				0.02	0.26	0.06		
Total number of samples =					160					

Note : ND – less than LOD.

Table 6. Results of Cd, Pb and Hg in marine/freshwater/brackishwater aquacultured fish and marine captured fish from west coast of Johor in 2005 and 2006.

Type of system/fish	Sampling Location	Analyte	Fish sample Analysed		No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
			Common name	Scientific name						
Aquacultured										
Catfish	Senai	Cd	Ikan keli	*Clarias batrachus*	16	ND	0.06	0.01		
		Pb				ND	0.95	0.24		
		Hg				0.004	0.05	0.02		
Green mussel	Pantai Lido	Cd	Kupang	*Perna veridis*	16	0.01	0.18	0.03		
		Pb				0.01	0.24	0.09		
		Hg				0.005	0.03	0.02		
Seabass	Pendas	Cd	Siakap	*Lates calcarifer*	16	ND	0.06	0.02		
		Pb				ND	0.43	0.11		
		Hg				0.04	0.11	0.07		
Tilapia	Senai	Cd	Tilapia	*Oreochromis niloticus*	8	ND	ND	ND		
		Pb				ND	0.18	0.15		
		Hg				0.005	0.01	0.01		
									Cd = 97.6 Pb = 102.8 Hg = 99.8	

Note : ND – less than LOD.

Table 6. Results of Cd, Pb and Hg in marine/freshwater/brackishwater aquacultured fish and marine captured fish from west coast of Johor in 2005 and 2006 (cont'd).

Type of system/fish	Sampling Location	Analyte	Fish sample Analysed		No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
			Common name	Scientific name						
Aquacultured										
Cockles	Pontian	Cd	Kerang	*Anadara granosa*	16	0.06	0.81	0.33		
		Pb				0.09	0.58	0.28		
		Hg				0.01	0.06	0.02		
White shrimp	Pontian	Cd	Udang putih	*Penaeus vannamei*	8	ND	0.02	0.01		
		Pb				ND	0.09	0.05		
		Hg				0.004	0.03	0.02		
Captured Fish										
Grouper	Pontian	Cd	Kerapu	*Epinephelus sp.*	8	ND	0.02	0.01		
		Pb				ND	0.07	0.03		
		Hg				0.05	0.28	0.13		
Red snapper	Pontian	Cd	Ikan merah	*Lutjanus sp.*	16	ND	0.01	0.005		
		Pb				ND	0.12	0.06		
		Hg				0.04	0.32	0.12		

Cd = 97.6
Pb = 102.8
Hg = 99.8

Note : ND – less than LOD.

Table 6. Results of Cd, Pb and Hg in marine/freshwater/brackishwater aquacultured fish and marine captured fish from west coast of Johor in 2005 and 2006 (cont'd).

Type of system/fish	Sampling Location	Analyte	Fish sample Analysed		No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
			Common name	Scientific name						
Captured Fish										
Squid	Pontian	Cd	Sotong	*Loligo* sp.	16	0.03	0.30	0.08	Cd = 97.6 Pb = 102.8 Hg = 99.8	
		Pb				ND	0.33	0.07		
		Hg				0.02	0.07	0.05		
Spanish mackerel	Pontian	Cd	Tenggiri	*Scomberomorus sp.*	16	ND	0.08	0.02		
		Pb				ND	0.74	0.11		
		Hg				0.02	0.14	0.05		
Shrimp	Pontian	Cd	Udang laut	*Metapeneaus sp.*	16	ND	0.03	0.01		
		Pb				ND	0.23	0.08		
		Hg				0.01	0.04	0.02		
Total number of samples =					152					

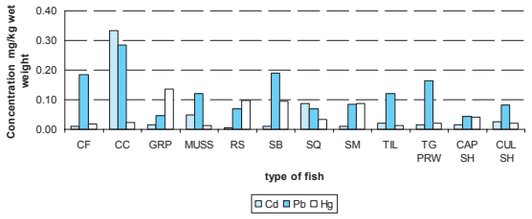
Note : ND – less than LOD.

Discussion

Mean Concentration of Heavy Metals in various fishes in Johor

The mean concentration of Cadmium (Cd), Lead (Pb) and Mercury (Hg) of all the species of fish, green mussel and prawn in Johor was much lower than the standard of Commission Regulation (EC)[1] No. 1881/2006; Food Act of Malaysia[2] (1983) (amendment, 1993) and Malaysia Food Regulation (1985).

Graph 1. Mean Concentrations of Cd, Pb and Hg in various species of fish in Johor.



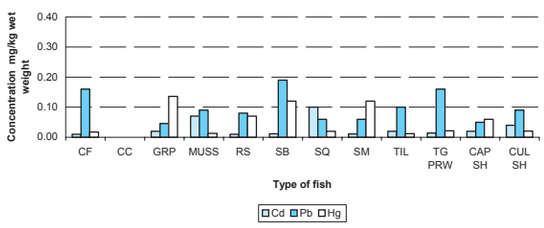
CF - Catfish; CC – Cockles; GRP – Grouper; MUSS – Mussel; RS – Red Snapper; SB – Seabass; SQ – Squid; SM – Spanish Mackerel; TIL – Tilapia; TG PRW – Tiger Prawn; CAP SH – Captured Shrimp; CUL SH – Cultured Shrimp

Graph 1 shows the mean concentration of Cd and Pb were less than 0.20 ppm except for cockles. The highest concentrations of Cd and Pb were found in cockles collected from Pontian, west coast of Johor in 2005, with a concentration of 0.81 ppm for Cd and 0.58 ppm for Pb. The mean concentration of Hg for all samples was less than 0.20 ppm. The highest concentration of Hg was detected in red snapper from Pontian, west coast of Johor in 2005 at a concentration of 0.32 ppm.

Mean Concentration of Heavy Metals in various fishes from east coast of Johor

The mean concentration of Cadmium, Lead and Mercury of all species of fish, green mussel and prawn from east coast of Johor was also lower than the regulatory standards.

Graph 2. Mean Concentrations of Cd, Pb and Hg from east coast of Johor.



CF - Catfish; CC – Cockles; GRP – Grouper; MUSS – Mussel; RS – Red Snapper; SB – Seabass; SQ – Squid; SM – Spanish Mackerel; TIL – Tilapia; TG PRW – Tiger Prawn; CAP SH – Captured Shrimp; CUL SH – Cultured Shrimp

Marine, brackish water and freshwater Aquacultured Fish

Graph 2 shows the mean concentrations of Cd, Pb and Hg in various species of aquacultured fish from east coast of Johor were less than 0.20 ppm. The concentration of Cd was found to be highest in mussel from Masai at 0.12 ppm in 2005. For Pb, it was found that catfish from Kota Tinggi in 2005 had the highest concentration at 0.88 ppm. The highest concentration of Hg was observed in seabass from Tg. Sedili in 2005 at 0.18 ppm. Details are shown in Table 5.

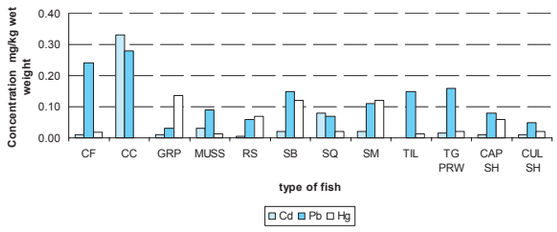
This site is considered to be not suitable for cockles culture due to high water current and soil profile (sandy area), therefore there is no reported data on cockle from this area.

Marine Captured Fish

Graph 2 also shows the mean concentrations of Cd, Pb and Hg in various species of marine captured fish from east coast of Johor were less than 0.20 ppm. The concentration of Cd was found to be highest in squid from Mersing at 0.21 ppm in 2006. In addition, the concentration of Pb was found in squid from Tg. Sedili in 2005 at 0.31 ppm. The highest concentration of Hg was observed in grouper from Pontian in 2005 at 0.27 ppm. Details are shown in Table 5.

Mean concentration of heavy metals in various fishes from west coast of Johor

Graph 3. Mean Concentrations of Cd, Pb and Hg from west coast of Johor.



CF - Catfish; CC – Cockles; GRP – Grouper; MUSS – Mussel; RS – Red Snapper; SB - Seabass; SQ – Squid; SM – Spanish Mackerel; TIL – Tilapia; TG PRW – Tiger Prawn; CAP SH – Captured Shrimp; CUL SH – Cultured Shrimp

Marine, brackish water, freshwater Aquacultured Fish

Graph 3 shows the mean concentrations of Cd, Pb and Hg in various species of aquacultured fish from west coast of Johor were lower than 0.20 ppm except for Cd and Pb in cockles and also for Pb in catfish. The highest concentrations of Cd and Pb in cockles were found from Air Baloi, Pontian at 0.81 ppm and 0.58 ppm respectively. The highest concentration of Cd from cockles collected in Desaru showed lower concentration

at 0.19 ppm as compared to those collected in Pontian. Meanwhile, the concentration of Pb in catfish was found to be highest in Senai at 0.95 ppm. The concentration of Hg was observed at 0.11 ppm. Details are shown in Table 6.

Marine Captured Fish

Graph 3 shows the mean concentrations of Cd, Pb and Hg in various species of marine captured fish from west coast of Johor were also lower than 0.20 ppm. The highest concentration of Cd was found in squid collected from Pontian in 2005 at 0.30 ppm. The concentration of Pb was found to be highest in Spanish Mackerel from Pontian at 0.74 ppm. In addition, the highest concentration of Hg was found in red snapper collected from Pontian at 0.32 ppm. Details are shown in Table 6.

Analytical Results For Heavy Metals for Samples collected in 2007

Table 7. Mean concentrations of Cd, Pb and Hg in the various fish products.

Fish Product	Analyte	No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
Fishcake	Cd	16	ND	0.02	0.008		
	Pb		ND	0.24	0.09		
	Hg		0.018	0.022	0.02		
Fishball	Cd	16	ND	ND	ND		
	Pb		ND	0.25	0.05		
	Hg		0.01	0.03	0.02		
Frozen shrimps (*Penaeus vannamei*)	Cd	8	ND	ND	ND		
	Pb		ND	0.11	0.06		
	Hg		0.01	0.02	0.01		
Surimi Kintokidai A (big eye snapper)	Cd	16	ND	0.08	0.08		
	Pb		ND	0.38*	0.32		
	Hg		0.02	0.05	0.03		
Surimi (threadfin bream)	Cd	8	ND	0.005	0.005		
	Pb		ND	0.06	0.04		
	Hg		0.03	0.04	0.03		
Frozen Octopus (*Octopus dofleini*)	Cd	8	0.03	0.47	0.18	Cd = 99.5	
	Pb		0.01	0.44	0.18	Pb =101.1	ND for 14 samples
	Hg		0.01	0.02	0.02	Hg =100.4	
Frozen squid (*Loligo sp.*)	Cd	8	0.12	0.45	0.26		
	Pb		ND	0.64	0.31		
	Hg		0.007	0.02	0.01		
Frozen black tiger prawn (*Penaeus monodon*)	Cd	8	0.002	0.03	0.01		
	Pb		ND	ND	ND		
	Hg		0.01	0.02	0.02		
Frozen cuttlefish (*Sepia inermis*)	Cd	8	0.01	0.07	0.04		
	Pb		ND	ND	ND		
	Hg		0.007	0.01	0.01		
Total number of samples =		96					

Note : ND – less than LOD., * reanalyzed

Table 8. Mean concentrations of Cd, Pb and Hg in the various fish products from Hutan Melintang, Perak.

Fish Product	Analyte	No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
Fishcake	Cd	8	0.004	0.01	0.007		
	Pb		ND	0.02	0.07		
	Hg		0.019	0.02	0.02		
Fishball	Cd	8	ND	ND	ND		
	Pb		ND	0.07	0.05		
	Hg		0.017	0.03	0.02		
Surimi (Kintokidai A; big eye snapper)	Cd	8	ND	ND	ND		
	Pb		ND	ND	ND		
	Hg		0.03	0.04	0.03		
Frozen shrimp (*Penaeus vannamei*)	Cd	8	ND	ND	ND		
	Pb		ND	0.06	0.06		
	Hg		0.01	0.02	0.01		
Frozen Octopus (*Octopus dofleini)*	Cd	8	0.03	0.47	0.18	Cd = 99.5 Pb =101.1 Hg =100.4	
	Pb		0.01	0.44	0.18		
	Hg		0.01	0.02	0.02		
Frozen squid (*Loligo sp.*)	Cd	8	0.12	0.45	0.26		
	Pb		ND	0.64	0.31		
	Hg		0.007	0.02	0.01		
Frozen black tiger prawn (*Penaeus monodon*)	Cd	8	0.002	0.03	0.01		
	Pb		ND	ND	ND		
	Hg		0.01	0.02	0.02		
Frozen cuttlefish (*Sepia inermis*)	Cd	8	0.01	0.07	0.04		
	Pb		ND	ND	ND		
	Hg		0.007	0.01	0.01		
Surimi (threadfin bream)	Cd	8	ND	0.005	0.005		
	Pb		ND	0.06	0.04		
	Hg		0.03	0.04	0.03		
Total number of samples =		72					

Note : ND – less than LOD.

Table 9. Mean concentrations of Cd, Pb and Hg in the various fish products from Bukit Mertajam, Penang.

Fish Product	Analyte	No. of Samples	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
Fishcake	Cd	8	ND	0.02	0.01		
	Pb		0.02	0.24	0.11		
	Hg		0.02	0.022	0.02		
Fishball	Cd	8	ND	ND	ND	Cd = 99.5	
	Pb		0.02	0.25	0.06	Pb =101.1	
	Hg		0.01	0.02	0.02	Hg =100.4	
Surimi Kintokidai A (big eye snapper)	Cd	8	ND	0.08	0.08		
	Pb		ND	0.15	0.12		
	Hg		0.02	0.03	0.03		
Total number of samples =		24					

Note : ND – less than LOD.

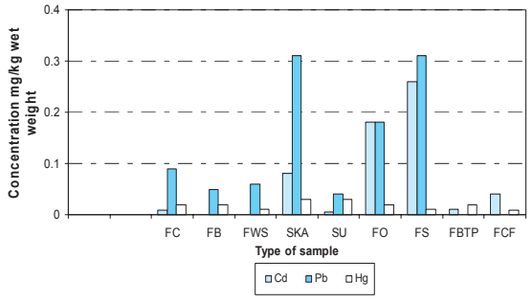
Discussion

Mean Concentrations of Heavy Metals in various Fish Products

All samples of fish products showed the mean concentration of Cadmium, Lead and Mercury were much lower than the standard of Commission Regulation (EC) No. 1881/2006, Food Act of Malaysia, 1983 (amendment 1993) and Food Regulation, 1985.

The mean concentrations of heavy metals in Table 7, was actually the overall result from Tables 8 and 9. However, some of the samples were collected from different states, where frozen shrimp, octopus, squid, black tiger prawn and cuttlefish can only be found in Perak. In Penang, only samples of fishcake, fishball and surimi kintokidai A were collected.

Graph 4. Mean Concentrations of Cd, Pb and Hg in various fish products.



FC - Fishcake; FB – Fishball; FWS – Frozen Shrimp (*P.vannamei*); SKA – Surimi Kintokidai A (big eye snapper); SU – Surimi (threadfin bream); FO – Frozen Octopus; FS – Frozen Squid; FBTP – Frozen Black Tiger Prawn; FCF – Frozen Cuttlefish

Graph 4 shows the mean concentration of Cd in fish products was less than 0.20 ppm except for frozen squid. Meanwhile, the concentration of Pb in surimi kintokidai A (big eye snapper) and frozen squid were higher than 0.30 ppm. For Hg, the concentration was observed to be lower than 0.1 ppm.

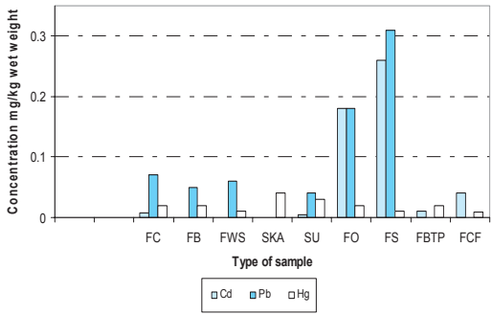
Surimi kintokidai A showed concentrations of Cd and Pb exceeding the standard limits set by EC standard 1881/2006. However, only 1 sample out of 16 samples was analyzed to be more than this standard with their concentration of 0.08 for Cd and 0.37 ppm for Pb. However the rest of the samples had very low levels and not detected. Cd and Pb were not detected in the samples collected from processing establishments from Perak .

Frozen octopus and squids collected from Perak showed a higher concentration but not exceeding the EC standard for Cd, Pb and Hg. The highest concentration of Cd and Pb in frozen octopus was 0.47 and 0.44 ppm respectively. Frozen squid also had the highest concentration of Cd and Pb of 0.45 and 0.64 ppm. It is lower than the EC standard for Cd and Pb with their concentration of 0.5 and 1.0 ppm for crustaceans and cephalopods.

All the samples collected for Hg analysis showed that the concentration did not exceed the EC and National standards.

Mean concentration of heavy metals in various fish products from Perak

Graph 5. Mean Concentrations of Cd, Pb and Hg in various fish products from Perak.

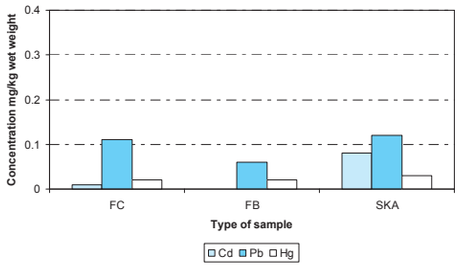


FC - Fishcake; FB – Fishball; FWS – Frozen Shrimp (*P.vannamei*); SKA – Surimi Kintokidai A (big eye snapper); SU – Surimi (threadfin bream); FO – Frozen Octopus; FS – Frozen Squid; FBTP – Frozen Black Tiger Prawn; FCF – Frozen Cuttlefish

Graph 5 shows the mean concentrations of Cd, Pb and Hg in the various fish products in Perak as stated in Table 8. The concentration of heavy metals in these products were less than 0.1 ppm except for frozen octopus and frozen squid.

Mean concentration of heavy metals in various fish products from Penang

Graph 6. Mean Concentrations of Cd, Pb and Hg in various fish products from Penang.



FC - Fishcake; FB – Fishball; SKA – Surimi Kintokidai A (big eye snapper);

Graph 6 also shows the mean concentrations of Cd, Pb and Hg in the various fish products in Penang which were stated in Table 9. The concentration of heavy metals in these products were less than 0.1 ppm for Cd and Hg and 0.2 ppm for Pb respectively.

Findings

The mean concentrations of heavy metals for all samples such as in fish and fish products were found to be not exceeding the food safety standard under the national standards. This implies that fishes from Johor east and west coasts and the fish products collected from Perak and Penang are within food safety limits for heavy metals contaminants. However, a more comprehensive study needs to be carried out to ensure seafood safety in Malaysia for heavy metals and other chemical contaminants.

c. Corrective Actions

Only one of the surimi kintokidai A sample from Perak exceeded the Pb level set by EC standard. The sample extraction and analysis was conducted again. No heavy metals was detected for this sample.

5. Problems and challenges encountered

- Method validation
- Proficiency testing
- Accreditation

6. Recommendations and Suggestions for Future Follow up Action

- The JTF programs should be continued among the ASEAN country to collect data which can support regional trade opportunities and benefits.
- The proficiency testing (PT) should be extended to another scope of analysis such as antibiotics, pesticides and histamine.
- Other elements such as Copper (Cu) and Arsenic (As) should be included in the survey.
- This survey should also cover canned products.
- SEAFDEC should consider the possibility of training for methyl Mercury and inorganic Arsenic for member countries because of the possibility that these analytes might be part of the requirement for food safety for products exported to EU country in the next few years.

Appendix 1

COM.REG. 1881/2006 – setting maximum levels for certain contaminants in foodstuff

	Foodstuff	Maximum level (mg/ kg wet weight)	Performance criteria for sampling and for methods of analysis
3.1 LEAD (Pb)	3.1.5. Muscle meat of fish ([1])([2])	0,30	Reg 333/2007
	3.1.6. Crustacean, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (Nephropidae and Palinuridae) ([3])	0,50	
	3.1.7. Bivalve molluscs ([3])	1,50	
	3.1.8. Cephalopods (without viscera) ([3])	1,00	
3.2 CADMIUM (Cd)	3.2.5. muscle meat of fish ([1])([2]), excluding fish species in 3.2.6 and 3.2.7	0,05	Reg 333/2007
	3.2.6. Muscle meat of the following fish ([1]) ([2]): [1] **anchovy** (*Engraulis* species) [2] **bonito** (*Sarda sarda*) [3] **common two-banded seabream** (*Diplodus vulgaris*) [4] **eel** (*Anguilla anguilla*) [5] **grey mullet** (*Mugil labrosus labrosus*) [6] **horse mackerel or scad** (*Trachurus trachurus*) [7] **louvar or luvar** (*Luvarus imperialis*) [8] **sardine** (*Sardina pilchardus*) [9] **sardinops** (*Sardinops* species) [10] **tuna** (*Thunnus, Euthynnys* species, *Katsuwonus pelamis*) [11] **wedge sole** (*Dicologoglossa cuneata*)	0,10	
	3.2.7. Muscle meat of swordfish (*Xiphias gladius*) ([1]) ([2])	0,30	
	3.2.8. Crustaceans, excluding brown meat crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae* and *Palinuridae*) ([3])	0,50	
	3.2.9. Bivalve molluscs ([3])	1,00	
	3.2.10. Cephalopods (without viscera) ([3])	1,00	

3.3 MERCURY (Hg)	3.3.1. Fishery products ([3]) and muscle meat of fish ([1]) ([2]), excluding species listed in 3.3.2. The maximum level applies to crustaceans, excluding the brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae* and *Palinuridae*)	0,50	Reg 333/2007
	3.3.2. Muscle meat of the following fish ([1]) ([2]) [1] **anglerfish** (*Lophius* spp.) / Baudroie [2] **atlantic catfish** (*Anarhichas lupus*) / Loup [3] **bonito** (*Sarda sarda*) [4] **eel** (*Anguilla* spp.) /anguille [5] **emperor, orange roughly, rosy soldierfish** (Hoplostethus atlanticus) [6] **grenadier** (*Coryphaenoides rupestris*) [7] **halibut** (**Hippoglossus hippoglossus**) /flétan [8] **marlin** (*Makaira* spp.) [9] **megrim** (*Lepidorhombus species*) [10] **mullet** (*Mullus species*) [11] **pike** (*Esox lucius*)/ Brochet [12] **plain bonito** (*Orcynopsis unicolor*) / palomète [13] **poor cod** (*Tricopterus minutes*) [14] **Portuguese dogfish** (*Centroscymnes coelolepis*) [15] **rays** (*Raja* spp.) [16] **redfish** (*Sebastes marinus, S. mentella, S. viviparus*) [17] **sail fish** (*Istiophorus platypterus*) [18} **scabbard fish** (*Lepidopus caudatus*, *Aphanopus carbo*) [19] **sea bream, Pandora** (*Pagellus species*) [20] **shark** (all species) [21] **snake mackerel** or butterfish (*Lepidocybium flavobrunneum*, *Ruvettus pretiosus*, *Gempylus serpens*) [22] **sturgeon** (*Acipenser* spp.) / esturgeon [23] **swordfish** (*Xiphias gladius*) / espadon [24] **tuna** (*Thunnus* spp., *Euthynnus* species, *Katsuwonus pelamis*)	1,00	
3.4 TIN (inorganic) (Sn)	Canned foods other than beverages	200	Reg 333/2007 LOD<5 mg/kg LOQ<10 mg/kg

Reg 333/2007: for HM, Detection limit (LOD): 1/10 maximum level
Quantification limit (LOQ): 1/5 maximum level

Myanmar

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1. Introduction

Myanmar is currently exporting seafood and is desirous of exporting to European Union (EU), Australia, Japan, U.S.A, Canada, Middle East, ASEAN Countries and China. The international market is becoming more demanding on food safety and quality standards. The role of the department is to ensure the wholesomeness and public health safety of fish and fish products for human consumption as well as to meet health and sanitary requirements of importing countries.

2. Objectives And Goals

- To determine the heavy metals in fish and fish products in Myanmar.
- To obtain the basline level of heavy metals in fish and fish products in Myanmar
- To set up the monitoring programme on heavy metals in fish and fish products in Myanmar
- To provide data for Fish and Fish Products Safety Information Network for seafood safety programme

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling Method: Random sampling (n =7)

1st & 2nd Quarters

Location: Yangon Division (Kyauk Tan & Twentee Aquaculture zone)
Ayeyarwaddy Division (Nyaung Done Aquaculture zone Minzarni processing plant)
Tanintharyi Division (United Myeik, Pyi Phyo Tun & Vantage Processing Plant in Myeik)
Rakhine State (Thantwe Marine Processing Plant)

Species & Sampling Size: 1st Quarter (Oct 2006 Jan 2007)

Black Tiger Shrimp (31/35 size x1kg)
Snake Head (0.5-1.0 kg x 7)
Striped Catfish (2-3 kg x7)
Squid (Total 1 kg)
Cuttle Fish (Total 1-kg)

Species & Sampling Size: 2nd Quarter (Dec 2007 - April 2008)

Rohu (2-3 kg x 7)
Grouper (0.5-1.0 kg x 7)
Sea bass (0.5-1.0 kg x7)
Squid (Total 1 kg)
Cuttlefish (Total 1 kg)
Giant Freshwater Prawn (Total 1 kg)

Number of Samples: 1st Quarter = 105
2nd Quarter =126
Total number = 231

Sample Preparation: Edible portions of fish and fish muscle tissue was used for testing.

b. Method of Analysis

Method References
(1) Total Mercury
AOAC Official Method 977.15
GBC AA Hydride System HG 3000 Operation and Services Manual
ISO/IEC 17025 Accredited Methods, MFRD

(2) Total Cadmium
AOAC Official Method 973.34
GBC Graphite System 3000 Methods Manual for Atomic Absorption Spectrometry
ISO / IEC 17025 Accredited Methods, MFRD

(3) Total Lead
AOAC Official Method 973.34
GBC Graphite System 3000 Methods Manual for Atomic Absorption Spectrometry
ISO / IEC17025 Accredited Methods, MFRD

Brand of Instrument
GBC 932 Double Beam Atomic Absorption Spectrometer (Australia)
GBC System 3000 Automated Graphite Furnace System
GBC HG 3000 Auto Hydride and Vapour Generator

c. Limit of Detection and Limit of Quantification
Limit of Detection (LOD)
(i) Total Mercury = 0.199 ppb
(ii) Total Cadmium = 0.497 ppb
(iii) Total Lead = 0.763 ppb

Limit of Quantification (LOQ)
(i) Total Mercury = 0.099 ppm
(ii) Total Cadmium = 0.0404 ppm
(iii) Total Lead = 3.235 ppm

d. National Regulatory Limits
There is no national regulatory limits in Myanmar. The Department of Fisheries (DOF) is complying and adopting mainly the European Union (EU) Standards and the importing countries' regulations.

Table 1. Maximum Permitted Level in Fish &Fish Products, Enforced by EU, USA, Canada and Japan.

Type of Chemical Hazards	Product Types	EU	USA	Canada	Japan
Mercury (Hg)	All Fish & Shell Fish Products	1.0 ppm	1.0 ppm	0.5 ppm	0.4 (total Hg) 0.3 (methyl Hg)
Lead (Pb)		0.4 - 1.0 ppm	1.5 - 1.7 ppm	0.5 ppm	not set
Cadmium (Cd)		0.1 - 1.0 ppm	3.0 - 4.0 ppm	0.5 ppm	not set

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results	True value	z-score	Remarks
2004	Regional Inter-Laboratory Proficiency Testing For SEAFDEC Key Laboratories Metallic Contaminants Series, Round 0401 Ref no: 07	Total mercury	2.05 ppm	3.37 ppm	-1.288	Passed

b. Survey Results and Discussion

Table 2. Result of Total Mercury in Fish and Fish Products Surveyed in 2006.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2006 (Oct) Kyauk Tan Aquaculture Zone, Yangon Division	Total Mercury (Hg)	Black Tiger Shrimp	*Penaeus monodon*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)
2006 (Nov) Central Fish Market (wholesale market) Kyi Myin Daing Township Yangon, Division		Snake Head	*Channa striata*	7	Not Detected	Detected but not quantifiable	Detected but not quantifiable	Captured (Fresh/ Chilled)
2006 (Nov) Nyaung Done Aquaculture Zone, Ayeyarwaddy Division		Yellowtail Cat Fish	*Pangasius pangasius*	7	Not Detected	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)
2006 (Nov) Vantage Processing Plant in Myeik Tanin - Tharyi Division		Squid	*Loligo duvauceli*	7	0.0597	0.0935	0.0766	Captured (Frozen)
2006 (Nov) Vantage Processing Plant in Myeik, Tanin - Tharyi Division		Cuttlefish	*Sepia pharaonis*	7	0.0540	0.0794	0.0667	Captured (Frozen)

Table 3. Result of Total Cadmium in Fish and Fish Products Surveyed in 2006.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2006 (Dec) Kyauk Tan Aquaculture Zone, Yangon Division	Total Cadmium (Cd)	Black Tiger Shrimp	*Penaeus monodon*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)
2006 (Dec) Central Fish Market (wholesale market) - Kyi Myin Daing Township Yangon , Division		Snake Head	*Channa striata*	7	Not Detected	Detected but not quantifiable	Detected but not quantifiable	Captured (Fresh/ Chilled)
2006 (Dec) Nyaung Done Aquaculture zone, Ayeyarwaddy Division		Yellowtail Cat Fish	*Pangasius pangasius*	7	Not Detected	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)
2006 (Dec) Vantage Processing Plant in Myeik, Tanin - Tharyi Division		Squid	*Loligo duvauceli*	7	0.0597	0.0935	0.0766	Captured (Frozen)
2006 (Dec) Vantage Processing Plant in Myeik, Tanin - Tharyi Division		Cuttlefish	*Sepia pharaonis*	7	0.0540	0.0794	0.0667	Captured (Frozen)

Table 4. Result of Total Lead in Fish and Fish Products Surveyed in 2006.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2006 (Dec) Kyauk Tan Aquaculture Zone, Yangon, Division	Total Lead (Pb)	Black Tiger Shrimp	*Penaeus monodon*	7	Not Detected	0.0143	0.0076	Aquacultured (Fresh/ Chilled)
2006 (Dec) Central Fish Market (wholesale market) - Kyi Myin Daing Township Yangon, Division		Snake Head	*Channa striata*	7	0.0322	0.0815	0.0517	Captured (Fresh/ Chilled)
2006 (Dec) Nyaung Done Aquaculture Zone, Ayeyarwaddy Division		Yellowtail Cat Fish	*Pangasius pangasius*	7	0.0033	0.0543	0.0365	Aquacultured (Fresh/ Chilled)
2006 (Dec) Vantage Processing Plant in Myeik, Tanin - Tharyi Division		Squid	*Loligo duvauceli*	7	0.0738	0.1126	0.0937	Captured (Frozen)
2006 (Dec) Vantage Processing Plant in Myeik, Tanin - Tharyi Division		Cuttlefish	*Sepia pharaonis*	7	0.0831	0.1058	0.0956	Captured (Frozen)

Table 5. Result of Total Mercury in Fish and Fish Products Surveyed in 2007.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2007 (Dec) Twente Aquaculture Zone, Yangon Division	Total Mercury (Hg)	Rohu	*Labeo rohita*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)
2007 (Dec) Tanintharyi Division (Pyi Phyo Tun processing plant)		Grouper	*Epinephelus tauvina*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2007 (Dec) Ayeyarwaddy Division (Min Zar Ni processing plant)		Sea bass	*Lates calcarifer*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Rakhin State (Thantwe Marine processing plant)		Squid	*Loligo duvauceli*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Rakhin State (Thantwe Marine processing plant)		Cuttlefish	*Sepia pharaonis*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Pantanaw Aquaculture Zone, Ayeyarwaddy Division		Giant Fresh-water Prawn	*Macrobrachium rosenbergii*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)

Table 6. Result of Total Cadmium in Fish and Fish Products Surveyed in 2007-2008.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2007 (Dec) Twente Aquaculture Zone, Yangon Division	Total Cadmium (Cd)	Rohu	*Labeo rohita*	7	Detected but not quantifiable	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2007 (Dec) Tanintharyi Division (Pyi Phyo Tun processing plant)		Grouper	*Epinephelus tauvina*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2007 (Dec) Ayeyarwaddy Division (Min Zar Ni processing Plant)		Sea bass	*Lates calcarifer*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Rakhin State (Thantwe Marine processing plant)		Squid	*Loligo duvauceli*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Rakhin State (Thantwe Marine processing plant)		Cuttlefish	*Sepia pharaonis*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Pantanaw Aquaculture zone, Ayeyarwaddy Division		Giant Fresh-water Prawn	*Macrobrachium rosenbergii*	7	Not Detected	Detected but not quantifiable	Not Detected	Aquacultured (Fresh/ Chilled)

Table 7. Result of Total Lead in Fish and Fish Products Surveyed in 2008.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Remarks
		Common name	Scientific name					
2008 (Jan) Twente Aquaculture Zone,Yangon Division	Total Lead (Pb)	Rohu	*Labeo rohita*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Aquacultured (Fresh/ Chilled)
2008 (Jan) Tanintharyi Division (Pyi Phyo Tun processing plant)		Grouper	*Epinephelus tauvina*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Jan) Ayeyarwaddy Division. Min Zar Ni processing plant)		Seabass	*Lates calcarifer*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Rakhin State (Thantwe Marine processing plant)		Squid	*Loligo duvauceli*	7	Not Detected	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Rakhin State (Thantwe Marine processing plant)		Cuttle Fish	*Sepia pharaonis*	7	Detected but not quantifiable	Detected but not quantifiable	Detected but not quantifiable	Captured (Frozen)
2008 (Mar) Pantanaw Aquaculture zone, Ayeyarwaddy Division		Giant Fresh-water Prawn	*Macrobrachium rosenbergii*	7	Not Detected	Detected but not quantifiable	Not Detected	Aquacultured (Fresh/ Chilled)

c. Corrective Actions

- Verification of sampling and analytical methods.
- Carry out investigation studies such as traceability to the fishing grounds.
- Intensive monitoring of contaminants such as heavy metals in fish and fish products.

5. Problems and Challenges Encountered

- The budget is limited.
- Standard reference materials were unavailable for method validation.

6. Recommendations and Suggestions for Future Follow up Action

- Need a short training course for all activities.
- Survey of chemical contaminant such as heavy metals under the Japanese Trust Fund (JTF) project is beneficial in supporting Myanmar Fisheries Monitoring Programme and reducing the number of RASFF notifications from EU due to high contents of heavy metals.
- Participate in proficiency testing for SEAFDEC Key Laboratories within the allocated budget.
- To organise Inter-laboratory Proficiency Testing for SEAFDEC Key Laboratories at appropriate time.

Singapore

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This survey was conducted by The Marine Fisheries Research Department (MFRD) Chemistry Laboratory of the Fish Quality Management and Technology Branch as part of the Japanese Trust Fund II Program on the Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products.

1. Introduction

In 2007, Singapore's per capita consumption of fish is 19.5 kg. Though highly nutritious, fish pose potential health concern, as it could be contaminated with environmentally persistent chemicals. Fish is able to accumulate large amounts of toxic contaminants from their living environment. One group of contaminants accumulated by aquatic organisms is heavy metals such as Mercury, Arsenic, Cadmium and Lead. They are cumulative poisons, which are not detoxified by metabolic activities.

The United States Environmental Protection Agency (USEPA) has divided metals into two categories, namely hazardous and non-hazardous. Hazardous metals includes Mercury, Cadmium and Lead. Inorganic Arsenic, though known to be toxic to human beings, is not included in this list because its concentration in the environment is low.

The heavy metals are usually released to the atmosphere from both natural and human activities with the majority being terrestrial sources. Most of the toxic action of heavy metals involve binding to the metabolically amino-, sulphydryl-, carboxyl-, phenolic- or phosphoryl- groups. Toxicity is mainly determined by its solubility, stability and biological reactivity.

Mercury poisoning can result in loss of vision, hearing and intellectual abilities and nervous disorder and the degree of poisoning is dependent on many factors such as the dietary level and the age of the fish, microbial activity, salinity, pH and redox potential.

Cadmium, on the other hand, is a highly toxic metal because of the absence of homeostatic control for the metal in the human body. Ingestion of small amounts of cadmium would cause symptoms of nausea and headache while long-term exposure to the metal could cause renal damage and bone brittleness. Cadmium poisoning also leads to kidney malfunction and a drop in the phosphate level of the blood serum.

Lead is another metal that can affect the nervous system and the intellectual development in children. It is a general protoplasmic poison, which is cumulative and slow-acting due to its relatively low solubility in water and in cells. It inhibits the normal functioning of many enzymes. It also obstructs the utilization of oxygen and glucose for life sustaining energy production. Higher level of lead in the blood can cause kidney dysfunction and brain damage.

Arsenic and its compounds are widely distributed in nature primarily in two oxidation states, arsenite (trivalent) and arsenate (pentavalent). Inorganic arsenic is toxic to the liver and causes necrosis and cirrhosis. It also affects the bone marrow and cellular elements of blood.

2. Objectives And Goals

The objective of this survey is to ascertain the levels of heavy metals, namely total Arsenic, Mercury, Lead and Cadmium, in edible portions in commonly consumed fresh fish and those that are used in delicacies in Singapore. This would give an indication of the extent of consumers' exposure to the named heavy metals from consumption of these fishes.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Six species of fish and shellfish were used in this

study. The species selected for analysis were based on their popularity among local consumers, habitat and feeding habits. All samples were obtained fresh from different locations, mainly from three wet-markets in Singapore. These wet-markets were Zhujiao market located in Upper Serangoon Road, Geylang Serai market in Jalan Pasar Baru and Chinatown market situated near New Bridge Road. Fishes were randomly selected and purchased. The fishes collected were of "market" size. The fishes were packed in ice and transported in an insulated container back to the laboratory. Upon arrival at the laboratory, the specimens were identified. Sea cucumbers could not be identified as the samples were bought in the processed form where most physical features had been largely destroyed.

The total length, standard length and body weight of the samples were measured. Only the edible portions were sampled. For finfish, the samples used were right skin-off fillets except for Spanish mackerel where skin-on fillets were used. Whole squids with their eyes, viscera and softshell removed were used. As for mud crabs and blood cockles, only the muscles were studied. For shellfish, a composite sample was prepared from a pool of at least 1 kg of the shellfish. In composite samples, only samples of similar size were used. After sampling, a mill was used to mince and blend the tissue to obtain a homogeneous sample.

Seven samples of each type of species (n = 7) were used for analysis. Each sample was analysed in duplicates. Two portions of the minced tissue were weighed. These were dried at 102°C overnight for the determination of moisture. The dried samples were used directly for analysis.

b. Method of Analysis

The instrument used for all analysis is Perkin Elmer 3300 AAS, FIAS 100, HGEA-600 and AS-60.

c. Limit of Detection and Limit of Quantification

All values are in dry weight basis.
Mercury:
Limit of detection $_{sample}$: 0.026 ug/g
Limit of quantitation $_{sample}$: 0.088ug/g

Arsenic:
Limit of detection $_{sample}$: 0.340 ug/g
Limit of quantitation $_{sample}$: 1.133 ug/g

Cadmium:
Limit of detection $_{sample}$: 0.091 ug/g
Limit of quantitation $_{sample}$: 0.305 ug/g

Lead:
Limit of detection $_{sample}$: 0.189 ug/g
Limit of quantitation $_{sample}$: 0.632 ug/g

d. National Regulatory Limits

Country	Cadmium	Lead	Arsenic	Mercury
Singapore	• 1 ppm in molluscs	• 2 ppm in fish, crustaceans, molluscs and in canned fish	• 1 ppm in fish, crustaceans, molluscs and in canned fish	• 0.5 ppm in fish and fish products
	Reference: Sale of Food Act, Chapter 283, Section 56 (1), Food Regulations, 2005 Revised Edition			

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results (ppb)	True value	z-score	Remarks
Jun 2004	FAPAS	Total As and Total Hg in canned fish	As: 937.9 Hg: 74.63	As: 1750 Hg: 74.63	As: **-3.2** Hg: -0.4	Passed for Hg, results rectified for As.
Nov 2004	FAPAS	Total As, Cd and Pb in canned fish	As: 597.35 Cd: 13.63 Pb: 30.75	As: 505 Cd: 12.9 Pb: 8.51	As: 1.0 Cd: 0.3 Pb: **11.9**	Passed for As and Cd, results rectified for Pb.
May 2005	FAPAS	Total As and Total Hg in canned fish	As: 850.4 Hg: 380.92	As: 1030 Hg: 397	As: -1.1 Hg: -0.2	Passed
Apr 2005	Canadian Food Inspection Agency (CFIA) Quality Assurance Program	Total Hg in Tuna	4 samples: 1) 0.306 2) 0.529 3) 0.249 4) 0.409	Not given	1) –0.908 2) –1.046 3) –0.838 4) –1.371	Passed
Mar 2006	FAPAS	Total As, Cd and Pb in canned fish	As: 479.64 Cd: 9.7427 Pb: ND	As: 499 Cd: 13.7 Pb: 9.13	As: -0.2 Cd: -1.3 Pb: -	Passed
Oct 2006	FAPAS	Total As, Total Hg, Cd and Pb in canned fish	As: 321.92 Hg: 14.28 Cd: 9.7674 Pb: 33.77	As: 344 Hg: 19.9 Cd: 2.59 Pb: not set	As: -0.3 Hg: -1.3 Cd: **12.6** Pb: -	Passed for As and Hg, results rectified for Cd.
Dec 2007	Hong Kong Government Laboratory Proficiency Testing Programme on Heavy Metals in Food	Total As, Cd and Pb in dried shrimp powder All in mg/Kg	As: 53.221 Cd: 0.145 Pb: 1.608	As: 60 Cd: 0.187 Pb: 1.20	As: -1.3 Cd: -1.0 Pb: 0.99	Passed
Jun 2007	FAPAS	Total As, Total Hg, Cd and Pb in canned fish	As: 749.88 Hg: 747.63 Cd: 49.23 Pb: ND	As: 1124 Hg: 704 Cd: 52.4 Pb: not set	As: **-2.1** Hg: 0.4 Cd: -0.3 Pb: -	Passed

b. Survey Results and Discussion

Table 1. Results for Total Mercury.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific name						
2004	Total Mercury	Sea cucumber	-	n=7	ND	ND	ND	97.4%	*ND-not detected
		Blood cockle	*Anadara granosa*	n=7	0.012	0.019	0.014		
		Mitre squid	*Loligo chinensis*	n=7	0.008	0.029	0.018		
		Mud crab	*Scylla serrata*	n=7	0.018	0.069	0.053		
		Longtail tuna	*Thunnus tonggol*	n=7	0.014	0.117	0.069		
		Barred Spanish mackerel	*Scomberomorus commerson*	n=7	0.037	0.162	0.081		

Table 2. Results for Total Arsenic.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific name						
2004	Total Arsenic	Sea cucumber	-	n=7	ND	0.034	ND	104.8%	*ND-not detected
		Blood cockle	*Anadara granosa*	n=7	0.432	1.116	0.741		
		Barred Spanish mackerel	*Scomberomorus commerson*	n=7	0.707	1.354	1.014		
		Longtail tuna	*Thunnus tonggol*	n=7	0.685	2.083	1.068		
		Mud crab	*Scylla serrata*	n=7	0.737	4.566	2.167		
		Mitre squid	*Loligo chinensis*	n=7	0.785	4.691	2.859		

Note: The method used was for the detection of total arsenic. Only approximately 10-20% of the arsenic in seafood is present in an inorganic form, which is toxic.

Table 3. Results for Cadmium.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific name						
2004	Cadmium	Longtail tuna	*Thunnus tonggol*	n=7	ND	ND	ND	100.8%	*ND-not detected
		Barred Spanish mackerel	*Scomberomorus commerson*	n=7	ND	ND	ND		
		Sea cucumber	-	n=6	ND	0.044	0.010		
		Mitre squid	*Loligo chinensis*	n=7	0.096	0.246	0.166		
		Blood cockle	*Anadara granosa*	n=7	0.136	0.794	0.542		

Table 4. Results for Lead.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific name						
2004	Lead	Barred Spanish mackerel	*Scomberomorus commerson*	n=7	ND	ND	ND	94.0%	*ND-not detected
		Longtail tuna	*Thunnus tonggol*	n=7	ND	ND	ND		
		Mitre squid	*Loligo chinensis*	n=7	ND	ND	ND		
		Sea cucumber	-	n=7	ND	0.207	0.041		
		Blood cockle	*Anadara granosa*	n=7	0.096	0.336	0.212		

c. Corrective Actions

Not applicable.

5. Problems and Challenges Encountered

In this survey, only six types of species were analysed and a total of forty-two fish samples were collected. Thus, the samples collected may not be truly representative of the catches, which landed in Singapore.

6. Recommendations and Suggestions for Future Follow up Action

The use of quicker digestion methods, for example, use of microwave digestion, could be explored to reduce sample preparation time.

Thailand

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1. Introduction

Heavy metals are individual metals and metal compounds that negatively affect people's health. In very small amounts, many of these metals are necessary to support life. However, in larger amounts, they become toxic. They may build up in biological systems and become a significant health hazard. Therefore the maximum levels for some heavy metals such as Cadmium, Lead and Mercury in food products are set in many countries. To manage the safety of food products, the science-based knowledge of these heavy metals should be known.

2. Objectives And Goals

To obtain an understanding of the level of heavy metals (Cadmium, Lead and total Mercury) in fish and fish products.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Frozen cuttlefish (*Sepia* spp.*),* frozen baby octopus (*Octopus* spp.), frozen squid (*Loligo* spp.), canned skipjack (*Katsuwonus pelamis*), canned albacore (*Thunnus alalunga*), canned yellowfin (*Thunnus albacares*), frozen vannamei shrimp (*Penaeus vannamei*), frozen nile tilapia (*Tilapia nilotica*), canned sardinella (*Sadinella gibbosa*), frozen baby clam (*Paphia undulata*), frozen green mussel (*Perna viridis*), frozen salmon (*Salmon oncorhynchusketa*), frozen ribbon fish (*Trichiurus affinis*) and frozen imitation crabmeat (*Nemipterus* spp.) were collected from the factories in Samutsakorn province. After sampling, frozen samples were packed in plastic bags and sent to the laboratory. Samples were kept at -18°C if they were not analyzed immediately.

b. Method of Analysis

The samples were analyzed for Cadmium, Lead and total Mercury at the Samutsakorn Fish Inspection and Research Center. About 300g of the edible part of sample was blended and analyzed according to the method as in Appendix 1. Graphite furnace AAS (Brand: Perkin Elmer) was used for Cadmium and Lead while the cold vapor mercury analyzer (Brand: Perkin Elmer) was used for total Mercury.

c. Limit of Detection and Limit of Quantification

Limit of Detection of method is 0.01 ppm for Cadmium, Lead and total Mercury.

Limit of Quantification of method is 0.04 ppm for Cadmium and 0.05 ppm for Lead and total Mercury.

d. National Regulatory Limits

The regulatory limit of each contaminant is dependent on the type of product and the importing country. Information is available at the website: **www.fisheries.go.th**.

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results (ppm)	True value (ppm)	z-score	Remarks
2007	FAPAS	Cadmium	0.048	0.052	- 0.4	Passed
	APLAC	Cadmium	0.187	0.187	0.01	Passed
	APLAC	Lead	1.110	1.195	- 0.20	Passed
	FAPAS	Total mercury	0.051	0.053	- 0.2	Passed
	FAPAS	Total mercury	0.524	0.704	- 1.5	Passed

b. Survey Results and Discussion

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2005 Samutsakorn province	Cadmium	Frozen cuttlefish	*Sepia* spp.	24	0.02 Detected but not quantifiable	0.90	0.36	103
		Frozen octopus	*Octopus* spp.	24	0.04	0.94	0.31	104
		Frozen squid	*Loligo* spp.	24	0.04	0.54	0.18	103
	Total Mercury	Frozen cuttlefish	*Sepia* spp.	24	Not detected	0.05	0.02 Detected but not quantifiable	97
		Frozen octopus	*Octopus* spp.	24	Not detected	0.08	0.02 Detected but not quantifiable	96
		Frozen squid	*Loligo* spp.	24	Not detected	0.04 Detected but not quantifiable	0.02 Detected but not quantifiable	97
	Lead	Frozen cuttlefish	*Sepia* spp.	24	Not detected	0.14	0.03 Detected but not quantifiable	94
		Frozen octopus	*Octopus* spp.	24	Not detected	0.09	0.02 Detected but not quantifiable	92
		Frozen squid	*Loligo* spp.	24	Not detected	0.11	0.02 Detected but not quantifiable	93

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2006 Samutsakorn province	Cadmium	Canned skipjack	*Katsuwonus pelamis*	21	Not detected	0.06	0.02 Detected but not quantifiable	104
		Canned albacore	*Thunnus alalunga*	21	Not detected	0.03 Detected but not quantifiable	0.01 Detected but not quantifiable	102
		Canned yellowfin	*Thunnus albacares*	20	Not detected	0.02 Detected but not quantifiable	0.01 Detected but not quantifiable	105
		Frozen vannamei shrimp	*Penaeus vannamei*	21	Not detected	0.02 Detected but not quantifiable	Not detected	106
	Total Mercury	Canned skipjack	*Katsuwonus pelamis*	21	0.01 Detected but not quantifiable	0.26	0.08	97
		Canned albacore	*Thunnus alalunga*	21	0.12	0.54	0.28	99
		Canned yellowfin	*Thunnus albacares*	20	0.02 Detected but not quantifiable	0.17	0.07	95
		Frozen vannamei shrimp	*Penaeus vannamei*	21	Not detected	0.02 Detected but not quantifiable	0.01 Detected but not quantifiable	96
	Lead	Canned skipjack	*Katsuwonus pelamis*	21	Not detected	0.09	0.02 Detected but not quantifiable	94
		Canned albacore	*Thunnus alalunga*	21	Not detected	0.09	0.01 Detected but not quantifiable	93
		Canned yellowfin	*Thunnus albacares*	20	Not detected	0.07	0.02 Detected but not quantifiable	92
		Frozen vannamei shrimp	*Penaeus vannamei*	21	Not detected	0.06 Detected but not quantifiable	Not detected	92

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2007 Samutsakorn province	Cadmium	Frozen nile tilapia	*Tilapia nilotica*	22	Not detected	0.01 Detected but not quantifiable	Not detected	96
		Canned sardinella	*Sardinella gibbosa*	23	0.01 Detected but not quantifiable	0.10	0.03 Detected but not quantifiable	100
		Frozen baby clam	*Paphia undulata*	25	0.02 Detected but not quantifiable	0.35	0.12	96
		Frozen green mussel	*Perna viridis*	26	0.02 Detected but not quantifiable	0.79	0.10	94
	Total Mercury	Frozen nile tilapia	*Tilapia nilotica*	22	Not detected	0.07	0.01 Detected but not quantifiable	88
		Canned sardinella	*Sardinella gibbosa*	23	Not detected	0.04 Detected but not quantifiable	0.02 Detected but not quantifiable	94
		Frozen baby clam	*Paphia undulata*	25	Not detected	0.06	0.01 Detected but not quantifiable	94
		Frozen green mussel	*Perna viridis*	26	Not detected	0.07	0.03 Detected but not quantifiable	98
	Lead	Frozen nile tilapia	*Tilapia nilotica*	22	Not detected	0.03 Detected but not quantifiable	Not detected	92
		Canned sardinella	*Sardinella gibbosa*	23	Not detected	0.24	0.04 Detected but not quantifiable	88
		Frozen baby clam	*Paphia undulata*	25	Not detected	0.33	0.14	89
		Frozen green mussel	*Perna viridis*	26	Not detected	0.31	0.10	85

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of results (ppm) – wet weight basis	Average value of results (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2008 Samutsakorn province	Cadmium	Frozen salmon	*Oncorhynchus keta*	14	Not detected	0.05	0.01 Detected but not quantifiable	98
		Frozen ribbon fish	*Trichiurus sp*.	13	Not detected	0.05	0.02 Detected but not quantifiable	98
		Frozen imitation crab meat	*Nemipterus* spp.	29	Not detected	0.03 Detected but not quantifiable	0.01 Detected but not quantifiable	97
	Total Mercury	Frozen salmon	*Oncorhynchus keta*	14	Not detected	0.05	0.02 Detected but not quantifiable	88
		Frozen ribbon fish	*Trichiurus* spp.	13	0.01 Detected but not quantifiable	0.06	0.03 Detected but not quantifiable	89
		Frozen imitation crab meat	*Nemipterus* spp.	29	Not detected	0.09	0.03 Detected but not quantifiable	88
	Lead	Frozen salmon	*Oncorhynchus keta*	14	Not detected	0.05	0.01 Detected but not quantifiable	91
		Frozen ribbon fish	*Trichiurus sp*.	13	Not detected	0.14	0.03 Detected but not quantifiable	86
		Frozen imitation crab meat	*Nemipterus* spp.	29	Not detected	0.17	0.02 Detected but not quantifiable	89

c. Corrective Actions (if applicable)

No corrective action was taken as no results were found to exceed the regulations limits. However, in case of exceeding the importing country regulation, that lot of product will be rejected for export. The processor will be required to identify any other products that were produced from the same lot of raw material and provide a corrective action plan. A follow-up at plant may be deemed necessary in some cases. If the processor could not bring its system to comply with the safety requirement, the processor will be suspended for export. The processor will be withdrawn from the Department of Fisheries list of approved fishery establishment when the offence is repeated.

5. Problems and Challenges Encountered

The equipment is sometimes out of order.

6. Recommendations and Suggestions for Future Follow up Action

The fund should be given at the beginning of the year because it is easier to plan and conduct the activity.

Appendix 1

Method of Analysis

Weigh 1 g of sample in a 50 ml polypropylene screw-cap centrifuge tube
↓
Add 5 ml of conc. nitric acid
↓
Cap centrifuge tube
↓
Heat in water bath at 95 °C for 2 hrs
(Use fumehood for safety reasons)
↓
Cool to room temperature
↓
Transfer solution to 50 ml volumetric flask and dilute to mark with deionized water
↓
Measure Cadmium at 228.8 nm and Lead at 283.3 nm using graphite furnace AAS
Measure total Mercury at 253.7 nm using mercury analyzer

Calculations

Heavy metal (ppm) = C x 0.05 / W

C = Concentration of heavy metal from calibration curve (ug/L)
W= Sample weight (g)

References :

1. Official Methods of Analysis of AOAC International 18th edition. 2005. Chapter 9. Metals and other elements. 9.1.08. AOAC Official method 999.10. p. 16-19

2. Official Methods of Analysis of AOAC International 18th edition. 2005. Chapter 9. Metals and other elements. 9.2.24. AOAC Official method 974.14. p. 37.

Vietnam

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1. Introduction

Specific requirements on chemical residues in fish and fish products were set up to meet the strict standards of food safety and hygiene of importing countries.

For this reason, survey and study activities organized by SEAFDEC and funded by JTF IV were significant in providing an overview on the residue levels of chemicals and contaminants in fish and fish products in the South-East Asian region. Member countries would determine appropriate policies to control quality and safety of fish and fish products for meeting importing countries' requirements and protecting domestic consumers' health.

In Vietnam, as the national competent authority responsible for controlling quality, hygiene and safety of fish and fish product since 1997, the National Fisheries Quality Assurance and Veterinary Directorate – NAFIQAVED (now renamed as the National Agriculture, Forestry and Fisheries Quality Assurance Department-NAFIQAD) has been carrying out the Monitoring Program for certain harmful substances and residues in aquaculture animals. The authority had also actively participated in "Research and Analysis of chemical residues and contamination in fish and fish products and in their environment such as fishing ground and aquaculture field" as well as other activities organized by SEAFDEC.

2. Objectives And Goals

Survey of heavy metals was carried out to provide information on residue levels of contaminants in fish and fish products as well as their environment in South-East Asia. The results of the survey were deposited in the database of the Fish and Fish Products Safety Information Network.

3. Survey Methodologies

- NAFIQAVED (now NAFIQAD) was the Lead for implementing all activities in 2006 to 2008. NAFIQAVED Branches took samples and analyzed them as required by the activity.

- Activities carried out by NAFIQAD include the following:
  a) Activities using SEAFDEC's budget
  b) Activities using budget from the Monitoring Program for certain harmful substances and residues in aquaculture animals and certification activities for exported fishery consignments

- NAFIQAD had signed contracts with its Branches for the implementation of activities, and then is responsible for data analysis and treatment and making report.

- NAFIQAD branches carry out the procedures of sampling, sample preservation, dispatch of samples to laboratories and analysis, in compliance with the Residues Monitoring Program Manual and Quality Manual of NAFIQAD.

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Species

- Black Tiger Shrimps: *Penaeus monodon* (semi and final products have been taken from processing plant and the market)
- Bigeye tuna: *Thunnus albacares*
- Tra Catfish: *Pangasius hypophthalmus* (semi and final products have been taken from processing plant and the market)
- Clam: *Meretrix lyrata*
- Octopus: *Octopus* spp.
- Squids: squid (*Loligo edulis*), cuttlefish (*Sepia* spp.)

- Marlin fish: *Makaira indica*
- Swordfish: *Xiphias gladius*
- Ribbon fish: *Trichiurus lepturus*
- Mackerels: *Acanthocybium solandri*

b. Method of Analysis

Method: AOAC 999.10.1999
Equipment: AAS Perkin Elmer
Testing method had been accredited with ISO 17025 by BoA – VILAS.

c. Limit of Detection and Limit of Quantification

Limit of Detection (LOD):
- Hg: 10 ppb
- Pb: 5 ppb
- Cd: 1 ppb

d. National Regulatory Limits

MRLs required:
- Vietnam: Pb: 0.5 mg/kg, Cd: 1 mg/kg, Hg: 0.5 mg/kg
- EU: Hg, Cd: 0.5 mg/kg; Pb: 0.2 mg/kg
- USA: Cd (crustaceans: 3 ppm, bivalve mollusk: 4.0 ppm), Pb (crustaceans: 1.5 ppm, bivalve mollusk: 1.7 ppm)

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Remarks
2007	Canned fish/FAPAS/2007	Cd, Hg	Passed
2007	NAFIQAVED Proficiency Test	Cd	Passed

Year of analysis & Sampling location	Analyte	Fish sample analysed		Average value of results (ppm)
		Common name	Scientific name	
2006	Cd	Black Tiger Shrimp	*Penaneus monodon*	0.003
		Tuna	*Thunnus albacares*	0.030
		Tra/Basa catfish	*Pangasius* spp.	0.001
		Clams	*Meretrix lyrata*	0.993
		Octopus/ squid	*Octopus* spp./ *Loligo edulis*	0.134
		Others	-	0.090
2006	Pb	Black Tiger Shrimp	*Penaneus monodon*	0.077
		Tuna	*Thunnus albacares*	0.100
		Tra/Basa catfish	*Pangasius* spp.	0.115
		Clams	*Meretrix lyrata*	0.015
		Octopus/ squid	*Octopus* spp./ *Loligo edulis*	0.025
		Others	-	0.030
2006	Hg	Black Tiger Shrimp	*Penaneus monodon*	ND
		Tuna	*Thunnus albacares*	ND
		Tra/Basa catfish	*Pangasius* spp.	ND
		Clams	*Meretrix lyrata*	ND
		Octopus/squid	*Octopus* spp./ *Loligo edulis*	ND
		Others	-	ND

Year of analysis & Sampling location	Analyte	Fish sample analysed		Average value of results (ppm)
		Common name	Scientific name	
2007	Cd	Black Tiger Shrimp	*Penaneus monodon*	0.001
		Tuna	*Thunnus albacares*	0.001
		Tra/Basa catfish	*Pangasius* spp.	0.001
		Clams	*Meretrix lyrata*	3.335
		Octopus/ squid	*Octopus* spp./ *Loligo edulis*	1.200
		Others	-	0.351
2007	Pb	Black Tiger Shrimp	*Penaneus monodon*	0.051
		Tuna	*Thunnus albacares*	1.009
		Tra/Basa catfish	*Pangasius* spp.	0.089
		Clams	*Meretrix lyrata*	0.010
		Octopus/ squid	*Octopus* spp./ *Loligo edulis*	0.010
		Others	-	0.010
2007	Hg	Black Tiger Shrimp	*Penaneus monodon*	ND
		Tuna	*Thunnus albacares*	ND
		Tra/Basa catfish	*Pangasius* spp.	ND
		Clams	*Meretrix lyrata*	ND
		Octopus/ squid	*Octopus* spp./ *Loligo edulis*	ND
		Others	-	ND

Year of analysis & Sampling location	Analyte	Fish sample analysed		Average value of results (ppm)
		Common name	Scientific name	
2008	Cd	Tra/Basa catfish	*Pangasius* spp.	ND
		Tuna	*Thunnus albacares*	0.023
		Clams	*Meretrix lyrata*	0.010-0.063
		Mackerel	*Acanthocybium solandri*	0.166
		Ribbon fish	*Trichiurus lepturus*	0.142
		Marlin fish	*Makaira indica*	0.946
		Swordfish	*Xiphias gladius*	0.790
		Octopus	*Octopus* spp.	1.132-1.396
		Squid	*Loligo edulis*	0.210-0.621
2008	Pb	Tra/Basa catfish	*Pangasius* spp.	0-0.010
		Tuna	*Thunnus albacares*	ND
		Clams	*Meretrix lyrata*	0-0.005
		Mackerel	*Acanthocybium solandri*	ND
		Ribbon fish	*Trichiurus lepturus*	ND
		Marlin fish	*Makaira indica*	ND
		Swordfish	*Xiphias gladius*	ND
		Octopus	*Octopus* spp.	ND
		Squid	*Loligo edulis*	ND
2008	Hg	Tra/Basa catfish	*Pangasius* spp.	ND
		Tuna	*Thunnus albacares*	ND
		Clams	*Meretrix lyrata*	ND
		Mackerel	*Acanthocybium solandri*	ND
		Ribbon fish	*Trichiurus lepturus*	ND
		Marlin fish	*Makaira indica*	ND
		Swordfish	*Xiphias gladius*	ND
		Octopus	*Octopus* spp.	ND
		Squid	*Loligo edulis*	ND

Note: ND - Not detected

c. Corrective Actions

- Carry out investigation such as traceability studies to the fishing ground areas.
- Verification of sampling and analytical methods.
- Perform repeat sampling.
- Intensive control of chemical contaminants in fish and fish products during fishing and processing.

5. Problems and Challenges Encountered

Heavy metals are some of the parameters that can reflect the contamination level in fish due to the environment. Analysis results showed that heavy metals (Pb, Cd, Hg) are mostly detected in fish. This also meant that the heavy metals contamination caused by industrial wastes, is one of the criterias to be controlled. In spite of some samples exceeding the MRLs, it could be concluded that the contamination of heavy metals is under good control in Vietnam with reference to control results achieved since 1999.

6. Recommendations and Suggestions for Future Follow up Action

- Due to limited budget for each activity, collected data are not representative enough to reflect the real impact of chemical contaminants and antibiotics residues on fish.

- It would be highly appreciated if the Project could focus on training in new analytical methods, specifically in multi-residues analysis methods in order to improve testing capabilities in fish quality and safety control in the South-East Asia region.

Regional Survey of Pesticide Residues in Fish and Fish Products

- Cambodia
- Malaysia
- Myanmar
- Philippines
- Singapore
- Thailand
- Vietnam

Cambodia

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1. Introduction

Cambodia has small-scale aquaculture. Some species of fresh water fish with economic value were selected to be cultured in cage in Tonle Sap Great Lake. They were *Micronema micronema* in 2006 and *Oxyeleotris marmorata* in 2007-2008. Most fish species are easily contaminated by the pesticide residues present in the water runoff from agricultural fields or water discharged from nearby industrial areas. The contaminants can affect the health of consumers who consume fish as food everyday. This survey under the Japanese Trust Fund II Project was started in March 2006 and completed in May 2008.

2. Objectives And Goals

Survey of pesticide residues was carried out to provide information on the contamination levels in aquacultured fish.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Fish samples of Trey kes (*Micronema micronema)* were collected from the fishing lots store in Kompong Chnang province in 2006 according to the sampling schedule as follows:

Sampling in 2006

- 2 specimens on 17 March
- 2 specimens on 12 June
- 4 specimens on 11 December

Each selected specimen had a body weight of 300-450g.

Fish samples of Marbled Sleeper (*Oxyeleotris marmorata)* were also collected from pond in Kompong Thom Province in 2007 and 2008 as shown:

Sampling in 2007

- 4 specimens on 10 July
- 4 specimens in August

Sampling in 2008

- 4 specimens on 27 March

Each specimen had a weight of 450-650 g and the physical parameters of the specimen was measured and recorded. After sampling, samples were frozen and packed in a plastic bag. The bags were placed in an icebox amd sent to the Inland Fisheries Research and Development Institute (IFReDI) Laboratory. The samples were kept in a freezer at -18^{o}C for a few days until transportation to the National Agriculture, Forestry and Fisheries Quality Assurance Department (NAFIQAD)-Branch 4 in Ho Chi Minh City. 8 compounds (Lindane, HCB, Heptachlor, Aldrin, Dieldrin, Endrin, DDT, Chordane) were analysed. Only the edible part of the fish was used for analysis.

b. Method of Analysis

All samples analyzed in laboratories of the National Agriculture, Forestry and Fisheries Quality Assurance Department (NAFIQAD) -Branch 4 in Ho Chi Minh City have certificate number: 105.
Instrument used: GC-ECD 6890N, Agilent.

Analytical method used: Manual of Chemical analysis method AOAC 983.21.1995 (chapter 10, 12-13)

Weigh 20 ± 0.1 g of sample
↓
Mix well with 40 g of Na_2SO_4
↓
Add 100 ml of Petroleum Ether
↓
Extract the Petroleum Ether layer
↓
Repeat extraction 2 times with 100 ml and 70 ml of Petroleum Ether
↓
Evaporate on a rotary evaporator at 40°C
↓
Load on Florisil column
↓
Elute with 200 ml of Petroleum Ether and Diethylether mixture (85:15)
↓
Dry at 40°C with N_2 gas
↓
Resolve with 10 ml iso-octane for analysis

c. Limit of Detection and Limit of Quantification

Limit of Detection (LOD) are as follows:

- Aldrine, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene and Lindane: 2 µg/kg
- Chordane: 5 µg/kg
- DDT: 10 µg/kg.

d. National Regulatory Limits

Based on Commission Regulation (EC) No. 1881/2006 of 19 December, Setting Maximum Levels for Certain Contaminants in Foodstuff.

Hexachlorobenzene	2.0 µg/kg
Lindane	2.0 µg/kg
Heptachlor	2.0 µg/kg
Aldrin	2.0 µg/kg
Dieldrin	2.0 µg/kg
Endrin	2.0 µg/kg
Chlordane	5.0 µg/kg
DDT	5.0 µg/kg

Maximum permitted levels of hazardous chemicals in fish & fish products enforced by Canada, EU and FDA

Contaminants	Product Type	Action Level
Aldrin/ Dieldrin	All Fish	0.3 ppm
Benzene Hexachloride, BHC (HCH)	Frog legs	0.3 ppm
Chlordane	All Fish	0.3 ppm
Chlordecone	All Fish/ Crab meat	0.3 ppm
DDT, DDD, DDE	All Fish	5.0 ppm
Diquat	All Fish	0.1 ppm
Fluridone	Fin Fish and Crayfish	0.5 ppm
Glyphosate	Fin Fish/ Shell fish	0.25 ppm
Heptachlor/ Heptachor Epoxide	All Fish	0.3 ppm
Mirex	All Fish	0.1 ppm
Polychlorinated Biphenyls (PCB's)	All Fish	0.2 ppm
Simazine	Fin Fish	12 ppm
2,4-D	All Fish	1.0 ppm

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Cambodia did not participate in any inter-laboratory proficiency testing.

b. Survey Results and Discussion

After 3 years of pesticide residues monitoring in fish and fish products, the results showed that though some samples contained pesticide residues, the level of contamination does not exceed the MRLs.

These results indicated that fish species *Mircronema micronema* and *Oxyeleotris marmorata* cultured in ponds and cages around the Tonle Sap Lake in Kompong Chnang and Kompong Thom Provinces are safe for consumption and export.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of result (ppm)	Average value of result (ppm)	Average Recovery (%)	Remarks
		Common name	Scientific name						
2006, Tonle Sap River in Kompong Chnang Province	Lindane, HCB, Heptachlor, Aldrin, Dieldrin, Endrin, DDT and Chlordane	Trey kes	*Micronema micronema*	8	-	-	-	-	Not detected
2007, Pond in Kompong Thom Province		Marbled sleeper	*Oxyeleotris marmorata*	8	-	-	-	-	Not detected
2008, Tonle Sap Lake in Kompong Chnang Province		Marbled sleeper	*Oxyeleotris marmorata*	3	-	-	-	-	Not detected

Results for *Micronema micronema*

No.	Pesticide Name	Maximum value of results based on wet weight (µg/kg)
1	Lindane	0.7
2	HCB	4.5
3	Heptachlor	1.0
4	Aldrin	1.5
5	Dieldrin	2.5
6	Endrin	5.0
7	DDT	1.5
8	Chlordane	1.0

c. Corrective Actions (if applicable)

The results indicated that the *Oxyeleotris marmorata* and *Micronema micronema* cultured in the Tonle Sap Great Lake are safe for consumption in Cambodia and for export.

5. Problems and Challenges Encountered

As Cambodia's National Laboratory does not have the facilities to analyze the 8 targetted pesticide residues, the fish samples must be transported to the laboratory in Ho Chi Minh City, Vietnam, which is very expensive.

6. Recommendations and Suggestions for Future Follow up Action

Due to limited budget for each activity, the amount of collected data does not reflect the real impact of chemical and contaminants on the fish natural in Tonle Sap Great Lake and aquacultured fish.

MALAYSIA

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1. Introduction

The Department of Fisheries of Malaysia started the Sanitary and Phyto Sanitary Programme 10 years ago and has been analyzing pesticide residues in fish samples. Malaysia started the JTF II Regional Survey of Pesticide Residues in Fish and Fish Products in Year 2007. Environmental contamination has become a major issue, causing problems in many countries all over the world. Rapid development in the region has also created various developing sectors such as agriculture, manufacturing, transportation, and mining, contributing a great amount of contamination in eco-systems, including the maritime or oceanic ecosystems.

Issues on maritime eco-system contamination is given high priority and has been taken seriously by the government due to the diversification of its activities in the ecosystem. Inland eco-system activities indirectly impact maritime eco-system. Among some of the contaminants are chlorinated hydrocarbon and petroleum hydrocarbon. These two components are produced by various activities in the agriculture, mining, industrial and transportation sectors. Coasts along the Straits of Melaka are one of the areas, which receive the flow of contaminants from the various anthropogenic activities.

2. Objectives And Goals

The objective of this study was to determine the level of pesticide residues in fish and fish products. Data collected was deposited in the database of the Fish and Fish Products Safety Information Network. This survey was undertaken as part of Malaysia's commitment and responsibilities towards exporting safe fish products internationally, especially in meeting importers' requirements and EU's standards and regulations.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Site

In June 2007, 40 samples of fish and fish products were taken from fish processing establishments at three different locations. Two establishments are from the state of Perak and another from the state of Penang. The second sampling was done in August 2007. Sixteen samples were collected from one of the processing establishment in Perak.

The fish and fish products samples comprised of surimi (threadfin bream), surimi kintokidai A (big eye snapper), fishball, frozen shrimp (*Penaeus vannamei*), frozen black tiger prawn (*Penaeus monodon*) and frozen squid (*Loligo* spp.).

Eight replicates of each sample were randomly taken from each species and were kept in cooling boxes filled with ice. The boxes were transported to the laboratory immediately. Samples were stored at a temperature of -18 to -20ºC before the analysis was carried out.

b. Method of Analysis

Method of analysis for this survey is based on UNEP/ IAEA/OIC, 1995 (Analysis, Persistence and Bioaccumulation of Chlorinated Compound in Marine Environmental Material).

Solvents, Reagents and Standards:

- Sodium sulphate (Na_2SO_4) – Anhydrous
- n-hexane (C_6H_{14})
- Dichloromethane (CH_2Cl_2)
- Methanol
- Florisil
- Teflon boiling stone
- 16 standards of organochlorine (prepared from stock solutions in sealed glass ampoules)
- 1 internal standard (with 2 compounds)

Glassware and Apparatus:

- Soxhlet extractor apparatus
- Nitrogen evaporator and apparatus
- GC-MS QP2010 plus (Shimadzu)
- Glass jars, test tubes, volumetric flasks, beakers and measuring cyclinders (cleaned with detergent and solvent before use)
- Cellulose extraction thimble
- Aluminium foil
- Stainless steel knives
- Spatulas, forceps, glass pasteur pipettes
- Ultrasonic bath
- Marker pens, labels and log book
- Analytical balance
- Insulated plastic box for transporting samples
- Ice and dry ice
- Deep freezer, -18 to -20^{o}C for sample preservation (frost-free)

Preparation of Samples:

Frozen samples were kept at room temperature. Reagent water was used to rinse the samples, if necessary, to remove extraneous material. This is to ensure that the targeted parts were contaminant-free. Samples were then homogenized using a Waring laboratory blender. 1g of these samples were used for moisture analysis (dry weight basis).

Sample Extraction:

Samples were extracted using a Soxhlet extractor. Before the samples were added in, a layer of ±10 g of sodium sulfate was placed in the thimble to absorb moisture from the samples. Approximately 20 g of samples (wet weight), was filled into the middle of the thimble. Approximately 3 g of sodium sulfate was then added in to cover the surface of the samples. The thimbles were then placed in a glass jar. Internal standard (IS) was added to all samples prior to extraction. The IS consisted of 2,4,5,6-tetrachloro m-xylene and decachlorobiphenyl. The amount of IS added for every 10 g of samples based on wet weight was 50 µL of 20 ppm IS. 130 ml of n-hexane was added with teflon boiling stones before connecting the soxhlet apparatus to a condenser and chiller. The glass jar was heated to 150ºC and then cooled through the condenser, with water circulation until the temperature dropped to 17ºC. The Soxhlet extraction process would take about 2 hours and 35 minutes.

Concentration of Samples:

The extracts were concentrated with nitrogen gas. Florisil column (SPE method) was used to clean up the extract and remove matrix interferences. A 50 ml of dichloromethane and 50 ml of n-hexane mixture was added to remove pesticide residues from the extract. The organic compound from the mixtures was eluted into a clean test-tube. This organic compound was then concentrated again to a final volume of 1ml. This organic compound was transferred to a 1.5 ml vial and dried using nitrogen gas. Another 1ml of n-hexane was added to the vial and the sample extract was analyzed using the GC-MS.

Analyze sample using GC-MS:

The specific GC-MS analytical conditions will be dependent on the analysis requirements, for example, the required precision, accuracy, and detection limits.

GC-MS operating condition for pesticide analysis:

Injector :

Temperature	:	250˚C
Injection Mode	:	Splitless
Injection Volume	:	2 µl
Syringe Capacity	:	10 µl
Linear Velocity	:	36.8 cm/sec
Pressure	:	65 kPa
Column Flow	:	1.00 ml/min
Purge Flow	:	3.0 ml/min

Oven Temperature Program:

Rate	Temperature (˚C)	Hold Time (min)
-	80.0	2.00
10.00	320.0	8.00
-	-	-

Run Time : 34 min
Column : 30 m, 0.25 µm film, 0.25 mm internal diameter, Restek Rtx - 5MS
Carrier Gas : Helium

Detector (MS-QP2010 plus):

Ion Source Temperature	:	200.00 ˚C
Interface Temperature	:	230.00 ˚C
Solvent Cut Time	:	2.00 min

Auto sampler (AOC-20i+s):

No. of rinses with solvent (Pre-run) : 3
No. of rinses with solvent (Post-run) : 4
No. of rinses with sample : 2

Calibration:

Calibration is done prior to the actual running of the analysis. The four standard concentration solutions used for calibration are 1000 ppb, 500 ppb, 100 ppb and 50 ppb. The calibration curve was then plotted based on the data from these four standards. This calibration was done to ensure that mid-level sample readings remain within the calibration curve.

a. Limit of Detection and Limit of Quantification

Limit of Detection: 0.025 ppb
Limit of Quantification : 0.25 ppb

b. National Regulatory Limits

EU and Malaysia's Maximum Residue Limits for Pesticide Residues.

Parameters	Concentration (ppm)	
	EU	MALAYSIA
Aldrin	0.3	0.01
Dieldrin	0.3	0.01
Chlordane	0.3	0.01
DDT	5.0	0.01
DDD	5.0	0.01
DDE	5.0	0.01
Heptachlor	0.3	0.01
Heptachlor Epoxide	0.3	0.01

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Malaysia did not participate in any inter-laboratory proficiency testing.

b. Survey Results and Discussion

Analytical results of 16 chlorinated pesticides for all samples.
Unit used is ug/kg (ppb).

Analytcal Results of 16 Chlorinated Pesticides in Fish and Fish Products for Year 2007

Fish product	No. of samples	α-BHC	β-BHC	γ-BHC	δ-BHC	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE	γ-CHLORDANE	α-CHLORDANE	DIELDRIN	2,4' -DDE	4,4' -DDE	2,4'-DDD	4,4'-DDD	2,4'-DDT	4,4'-DDT
Fishball	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Frozen shrimp	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Surimi kintokidai A	16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Surimi threadfin bream	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Frozen squid	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Frozen black tiger prawn	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note :
ND: Not Detected
LOD = 0.025ppb
Unit of measurement: ug/kg (ppb)
Percentage recovery: 80 - 120 %

The findings of this survey indicated that the level of chlorinated pesticide residues was relatively low and was under the Maximum Residue Level (MRL) set by the EU. However, there is still a need to continue monitoring the level of pesticide residues to ensure that the fish and fish products are free from contamination of chlorinated organic compounds, which can be persistent in marine environment for a long period of time.

5. Problem and Challenges Encountered

- Most of the processing plants were reluctant to cooperate in the study. The processing plants did not allow staff to enter the operational area for sampling.
- There was a lack of trained staff, especially in analytical chemistry, to carry out analysis.

6. Recommendations and Suggestions for Future Follow up Action

- For any further survey, it is recommended that the focus of the survey be extended to aquaculture farms and inland capture fisheries such as fish ponds, rivers, lakes, ex-mining pools and paddy fields, instead of confining to processing plants.
- The analytes should not be limited to chlorinated pesticides, PCBs and PAHs should also be included.
- SEAFDEC can provide or coordinate inter-laboratory proficiency testing in chlorinated pesticides among member countries.
- Training for laboratory staff should be continuously carried out to update their knowledge and skills on new techniques and technologies.

Myanmar

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1. Introduction

In order to ensure that fish and fish products from Myanmar are free from pesticide residues, a survey on pesticide residues, such as DDT, Aldrin & Dieldrin, was conducted in Myanmar under the JTF II Project from Year 2006 to 2008. Most of the organochlorine compounds, such as endrin, dieldrin, aldrin, heptachlor and chlordane, are banned in Myanmar since 1993 by the Myanmar Agriculture Law. Compounds, such as DDT and DDE, are restricted for Malaria prevention. Japan International Corporation Agency (JICA), with the support of the Department of Fisheries (DOF), had laid down the rural development plan in Myanmar and initiated the project of Rice-Fish or Rice-Prawn in Paddy fields. The plan of action was to increase the individual income of a villager from consumption of fish. Through the survey, pesticide residues in fishes from paddy fields can be monitored.

2. Objectives And Goals

- To determine and obtain an understanding of the level of pesticide residues in fish and fish products in Myanmar.
- To collect data and deposit the results in the database of the Fish and Fish Products Safety Information Network.
- To set up the monitoring program on pesticide residues in fish and fish products in Myanmar.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling Method
Random sampling
n=7

Location
- Yangon Division (Twente & Khayoung Aquaculture Zone, Central Fish Market)
- Tanintharyi Division (United Myeik Processing Plant)
- Ayeyarwaddy Division (Nyaung Done Aquaculture Zone and May Yu Processing Plant)
- Bago Division (Fish Market)

Species & Sampling Size for 1st quarter (Dec 2006-Feb 2007)
Rohu, 2-3kg x 7
Giant Freshwater Prawn, 20/25 size x 7
Tilapia, 0.25-0.5 kg x 7
Cat Fish, 0.25-0.5 kg x 7

Species & Sampling Size for 2nd quarter (Dec 2007-Apr 2008)
Marigal, 2-3 kg x 7
Snake Head, 0.25-0.50 kg x 7
Common Carp, 1.5-2.0 kg x 7
Tarpian, 0.5-1.0 kg x 7
Boal, 2-3 kg x 7
Indian Cat Fish, 0.2-0.3 kg x 7
Climbing Perch, 0.2-0.3 kg x 7

Number of Samples
1st Quarter = 84
2nd Quarter =147
Samples = 231

Sample Preparation
Sample used edible portions and fish muscle tissue for testing

b. Method of Analysis

Method Reference

Training on Pesticide Residues Analysis (Trust Fund II Project), 2005, MFRD –SEAFDEC

Brand of Instrument

Shimadzu Gas Chromatograph, GC 17-A, Ver-3, Japan
ECD, TCD, FID & FTD Detectors

Capillary columns (DB-1-30N-STD 0.25mm x 30m)
Different columns (DB-5 & DB-17)
ECD-17cell unit with heater

c. Limit of Detection and Limit of Quantification

Limit of Detection (LOD):
(i) DDT = 0.008 ppm
(ii) Aldrin = 0.006 ppm
(iii) Dieldtin = 0.009 ppm

Limit of Quantification (LOQ):
(i) DDT = 0.05 ppm
(ii) Aldrin = 0.04 ppm
(iii) Dieldtin = 0.05 ppm

d. National Regulatory Limits

There is no national regulatory limits in Myanmar. The Department of Fisheries (DOF) is complying and adopting mainly the European Union (EU) Standards and to the importing countries' regulations.

Table 1. Maximum Permitted Level in Fish &Fish Products, Enforced by EU, USA, Canada and Japan.

Contaminants	Product type	Action Level
DDT / DDE / DDD	All Fish Products	5.0 ppm
Aldrin /Dieldrin	All Fish Products	0.3 ppm
Polychlorinated Biphenyl (PCB)	All Fish Products	2.0 ppm
BHC (HCH)	Dried Cod	0.3 ppm
Chlordane	All Fish Products	0.3 ppm
Heptachlor/ Heptachlor Epoxide	All Fish	0.3 ppm
Mirex	All Fish	0.1 ppm

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Myanmar did not participate in inter-laboratory proficiency testing.

b. Survey Results and Discussion

Table 2. Results of DDT in Fish and Fish Products Surveyed in First Quarter.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
2006 (Dec) Twente Aquaculture Zone, Yangon Division	DDT	Rohu	*Labeo rohita*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2006 (Dec) United Myeik Processing Plant in Myeik, Tanintharyi Division		Giant Fresh Water Prawn	*Macrobrachium rosenbergii*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2006 (Dec) Khayoung Aquaculture Zone, Yangon Division		Tilapia	*Oreochromis niloticus*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2006 (Dec) May Yu Processing Plant in Yangon, Ayeyarwaddy Division		Cat Fish	*Clarias batrachus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)

Table 3. Results of Aldrin in Fish and Fish Products Surveyed in First Quarter.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
2007 (Jan) Twente Aquaculture Zone, Yangon Division	Aldrin	Rohu	*Labeo rohita*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2007 (Jan) United Myeik Processing Plant in Myeik, Tanintharyi Division		Giant Fresh Water Prawn	*Macrobra-chium rosenbergii*	7	Not Detected	Not Detected	Not Detected	Captured (Fresh/ Chilled)
2007 (Jan) Khayoung Aquaculture Zone, Yangon Division		Tilapia	*Oreochromis niloticus*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2007 (Jan) May Yu Processing Plant in Yangon, Ayeyarwaddy Division		Cat Fish	*Clarias batrachus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)

Table 4. Results of Dieldrin in Fish and Fish Products Surveyed in First Quarter.

Year of Analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
2007 (Feb) Twente Aquaculture zone,Yangon Division	Dieldrin	Rohu	*Labeo rohita*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2007 (Feb) United Myeik Processing Plant in Myeik , Tanintharyi Division		Giant Fresh Water Prawn	*Macrobrachium rosenbergii*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2007 (Feb) Khayoung Aquaculture zone, Yangon Division		Tilapia	*Oreochromis niloticus*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2007 (Feb) May Yu Processing Plant in Yangon , Ayeyarwaddy Division		Cat Fish	*Clarias batrachus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)

Table 5. Results of DDT in Fish and Fish Products Surveyed in 2nd Quarter.

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
2007 (Dec) Nyaung Done Aquaculture Zone, Ayeyarwaddy Division (Mya Yu Processing Plant)	DDT	Mrigal	*Cirrhinus mrigala*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Frozen)
2007 (Dec) Bago Fish Market, Bago Division		Snake Head	*Channa striata*	7	Not Detected	Not Detected	Not Detected	Captured (Fresh/ Chilled)
2008 (Jan) Nyaung Done Aquaculture Zone, Ayeyarwaddy Division (Mya Yu Processing Plant)		Common Carp	*Cyprinus carpio*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Frozen)
2008 (Jan) Central Fishmarket – Kyimyindaing (Wholesale Market) Yangon, Division		Tarpon	*Puntius chola*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2008 (March) Ayeyarwaddy Division (Mya Yu Processing Plant)		Boal	*Wallago attu*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2008 (March) Yangon Division (Mya Yu Processing Plant)		Indian Cat Fish	*Clarias batrachus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2008 (March) Yangon Division (Mya Yu Processing Plant)		Climbing Perch	*Anabas testudineus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)

Table 6. Results of Aldrin in Fish and Fish Products Surveyed in 2nd Quarter.

Year of Analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
2008 (April) Ayeyarwaddy Division (Mya Yu Processing Plant)	Aldrin	Boal	*Wallago attu*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2008 (April) Yangon Division (Mya Yu Processing Plant)		Indian Cat Fish	*Clarias batrachus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2008 (April) Yangon Division (Mya Yu Processing Plant)		Climbing Perch	*Anabas testudineus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)

Table 7. Results of Dieldrin in Fish and Fish Products Surveyed in 2nd Quarter.

Year of Analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
2008 (Feb) Nyaung Done Aquaculture Zone, Ayeyarwaddy Division (Mya Yu Processing Plant)	Dieldrin	Mrigal	*Cirrhinus mrigala*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Frozen)
2008(Feb) Bago Fish Market, Bago Division		Snake Head	*Channa striata*	7	Not Detected	Not Detected	Not Detected	Captured (Fresh/ Chilled)
2008 (Feb) Nyaung Done Aquaculture Zone, Ayeyarwaddy Division (Mya Yu Processing Plant)		Common Carp	*Cyprinus carpio*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Frozen)
2008 (Feb) Central Fishmarket – Kyimyindaing (Wholesale Market) Yangon , Division		Tarpon	*Puntius chola*	7	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
2008 (April) Ayeyarwaddy Division (Mya Yu Processing Plant)		Boal	*Wallago attu*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2008 (April) Yangon Division (Mya Yu Processing Plant)		Indian Cat Fish	*Clarias batrachus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)
2008 (April) Yangon Division (Mya Yu Processing Plant)		Climbing Perch	*Anabas testudineus*	7	Not Detected	Not Detected	Not Detected	Captured (Frozen)

c. Corrective Actions

- Verification of sampling and analytical methods.
- Carry out investigation studies such as traceability to the fishing grounds.
- Intensive monitoring of contaminants such as heavy metals in fish and fish products.

5. Problems and Challenges Encountered

- The budget is limited.
- Standard reference materials were unavailable for method validation.

6. Recommendations and Suggestions for Future Follow up Action

- Need on-site training courses.
- Survey of chemical contaminant such as pesticide residues under the Japanese Trust Fund (JTF) project is beneficial in supporting Myanmar Fisheries Monitoring Programme and reducing the number of RASFF notifications from EU due to high contents of pesticide residues.
- Participate in proficiency testing.

Philippines

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1. Introduction

Successful national food control systems are necessary to protect the health and safety of domestic consumers. They are also essential for ensuring the quality and safety of aquacultured products entering the international trade and making sure that the product conforms to the international requirements.

Trading partners are taking much interest in the way food is produced, processed and marketed and these partners are relying heavily on the governments to take greater responsibility in food quality and safety assurance. They require supplementary efforts and proactive behavior from the authorities and private sector in an increasingly competitive environment in establishing food safety control structures or its equivalent.

The Philippines has to keep up with the dynamic trade requirements, notably with the technical requirements and SPS measures to gain a better access to foreign markets. Trade partners have become more demanding and the corresponding technical requirements tend to become more complex and stringent.

One of the positive responses to manage the aforementioned stringent requirements on fish trade is through the Philippines' participation in this Japanese Trust Fund (JTF) II "Research for the Safety of Fisheries Products in the Southeast Asia: Chemical and Drug Residues in Fish and Fish Products in Southeast Asia" Project, specifically on Regional survey of pesticide residues in fish and fish products and Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.

Food Safety Management

At the Central Office, the food safety management for Fish Inspection and Quality Assurance Services is directly under the Office of the Director and is composed of five units: the Fish Product Testing Laboratory (FPTL), the Marine Biotoxin Laboratory (MBL), the Administrative Support and Product Certification Unit (ASPCU), the HACCP-based Fish Inspection Unit (HFIU) and the Fish Health Management and Quality Assurance Section (FHMQAS).

The FPTL and MBL are in charge of providing services to other units in their respective fields. The HFIU is responsible for the inspection of accredited fish processing plants based on 94/356/EEC, 91/493/EEC and to ensure consistent compliance with EU requirements as per 91/493/EEC, the new European Food Law, and other relevant national rules and regulations on the export of fishery and aquaculture products. The APSCU is responsible for the issuance of product health certificates for fishery and aquaculture products for export to the EU and other markets. To ensure the safety and quality of fishery and aquaculture products for export, the FIQAS implements a HACCP-based inspection program. It coordinates with the FHMQAS, FPTL and the BFAR Regional Offices on the effective implementation of the Quality Assurance Program. The FIQAS supervises the overall inspection of fish processing plants and certification of fishery and aquaculture products for export in coordination with the BFAR Regional Offices. The FHMQAS spearheads the registration of aquaculture farms, residues and health monitoring, as well as provision of diagnostic services, technical and advisory assistance to the aquaculture industry. Its monitoring program includes residues and disease surveillance and the reporting system, aquatic animal health certification, implementation of quarantine procedures, assessment of the health status of stocks of selected fish and the management of other aquatic resource farms in the Philippines.

At the Regional Offices, food safety management is under the responsibility of BFAR regional directors located in 15 regional offices. Each has a Fish Health Unit, linked to the central FHMQAS, and a Fishery Inspection unit, linked to the HFIU and central laboratories.

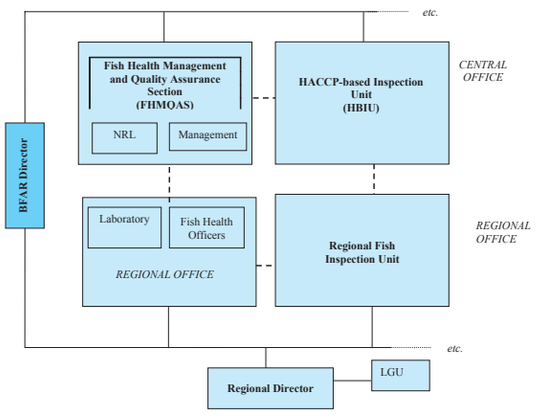
Central Fish Health Management and Quality Assurance Laboratory

The residues monitoring is implemented by the FHMQAS in coordination with the 15 BFAR Regional Offices. The regional directors have full responsibility over their area of jurisdiction. However, matters of policy-determining nature still lie under the jurisdiction of the Bureau Director. The organizational structure and coordination between regional and central offices are presented in Figure 1.

Role of National and Regional Fish Health Officers are as follows:

- Register aquaculture farms.
- Monitor the hygiene of production.
- Disseminate information and educate the aquaculture chain operators on the need of aquatic animal feeds, veterinary drugs and products registration prior to marketing and usage.
- Conduct surveillance and monitoring of aquatic animal feeds, veterinary drugs and products in their areas of responsibility.
- Recommend regulatory actions for any violations on policies and guidelines on registration, manufacturing, distribution and usage of aquatic animal feeds.
- Assist in planning, directing and supervising national programs on aquatic feeds, veterinary drugs and products control.
- Assist in the registration, evaluation and inspection of establishments engaged in manufacturing, distribution and sale of aquatic animal feeds, drugs, products premixes and water solubles, whenever necessary.

Figure 1. Organizational structure for residues monitoring and coordination between regional and central departments.



Coordination of the Activities of Central and Regional Departments

The FHMQAS develops and updates standardized routine procedures and guidelines for the operation of the regional fish health laboratories. It also supervises the activities and sets directions for the operation of such laboratories. The FHMQAS also provides technical guidance and instructions to the 44 BFAR-designated Regional Fish Health Officers on the execution of diagnostic activities and provide technical assistance on fish health-related and residues monitoring problems. It also provides specialized training on sanitary and phyto-sanitary programs of the government to fishery biologists, extension workers and fish farmers. The section sets national programs for the surveillance and monitoring of aquatic animal feeds, veterinary drugs, products and their control as well as formulates standardized monitoring procedures on field inspections and reporting, to ensure high feed quality and compliance to the regulations of the government. The FHMQAS also supervises the implementation and monitoring activities of the Fish Health Officers deputized as Aquatic Animal Feeds, Veterinary Drugs and Products Control Officers (AAFVDAPCO) nationwide. To harmonize activities, the FHMQAS coordinates with the other sector on the results of monitoring aquaculture farms supplying raw materials to the fish processing plants. Regional Fish Health Officers regularly coordinate with the officers of the Central FHMQAS in the implementation of the official program, including residues monitoring. Reports are also submitted to the Central FHMQAS for consolidation and analysis.

2. Objectives And Goals

Objectives:

- To increase export of aquacultured products like shrimp and milkfish, and marine products like lobster, crab and grouper.
- To develop monitoring and surveillance mechanism for pesticide residues for food safety and quality assurance of aquacultured and marine fish products.
- To develop sampling method and establish baseline information on the level of pesticides in fish and fish products.

Goals:

- To ensure food safety and quality of fish and fish product for export.
- To obtain baseline information of the extent of pesticide contamination.
- To develop capability on the analysis of harmful substances through attending trainings held by SEAFDEC-MFRD.

- To be able to establish a good network and increase communication with the laboratories in the region.
- To adopt methods used by recognized laboratories, for example, SEAFDEC-MFRD.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling

The sampling for official samples took place at random timings, unknown to the farms, markets and processing plants. Sampling was carried out at variable intervals over the year at farms, markets and receiving areas of processing plants. The processing plants, farms and markets sampling were defined using as much targeting criteria as possible, making sampling homogeneous.

All accredited establishments and registered farms that export products to the EU and other countries were under the scope of the monitoring plan. The number of sampling was defined using the regional production statistics as a minimal level, as well as taking into account the risks and results of past monitoring plans.

All available information of targeted samples were taken into consideration, for example, the use of presently unknown substances, sudden appearance of diseases in particular regions and indications of fraudulent activities.

Collection of samples from farms, auction areas/ markets and processing establishments

Samples were taken from farms, processing plants or purchased from the markets in a random manner. Identification and traceability of the samples back to the source was ensured. Important information like the source was taken. Information of the source was only available up to the provincial level for samples taken from the markets. Measures to prevent contamination of samples were taken during transportation of the samples to the laboratory. Suitable sampling tools, plastic bags and transportation boxes were provided for the collection of samples.

Each sample was equivalent to 1 kg of raw material. Every sample was packed separately into a disposable plastic sample container to prevent contamination and labelled accordingly with a unique number on the container. Each sample container was closed, sealed properly and chilled with ice during transportation to the laboratory. The samples could be kept in a refrigerator for several days. Plastic containers were placed in plastic bags to avoid contact with other samples. Every sample was separately packed in a plastic bag and frozen.

A report was produced after each sampling with at least the following information in it:

- Address of the authority that took the sample.
- Name and surname for identification.
- Sampling date, name and address of the exporter or the person in charge of the animals or the animal products.
- Remarks.

Sending of samples

Preliminary sample preparation was conducted in the regional laboratories. Duplicates of homogenised samples were prepared and frozen for immediate submission. One sample may be subjected for analysis of several analytes. Samples sent to FHMQAL were packed separately in clean sample containers to prevent contamination, coded for easy identification, and accompanied with sampling information for traceability purposes. Samples were chilled with ice bags and stored in clean durable containers to prevent any damage and contamination during transportation.

b. Method of Analysis

The samples were sent to recognized private and government laboratories, for example, National Pesticide Analytical Laboratory (NPAL), Societe Generalle Surveillance (SGS) and SENTROTEK.

The Central FHMQAL is responsible for the analysis of residues in foodstuffs of aquatic animal origin. It is located at the BFAR Central Office in Quezon City. The FHMQAL is under the supervision of the chief of the FHMQAS and has five personnel, one in-charge of field operations (veterinarian), one in-charge of the laboratory (veterinarian), a permanent analyst (chemist), two temporary analysts (chemist), and one administrative support for receiving samples and releasing results. Chemists are involved in the analysis of residues.

FHMQAS issued formal instructions to the Fish Health Officers on how to carry out sampling and to ensure the traceability of the samples. It had also implemented quality checks (temperature and

visual inspection) on samples received in order to confirm their suitability for analysis for this study. The FMHQAL either performed the analysis in its own facilities or in collaboration with a third-party laboratory i.e. SGS,Phils.

A multi-residue gas liquid chromatogaphy method was used. Both NPAL and SGS analysed α-HCH, β-HCH, δ-HCH, HCB, Lindane, Heptachlor, Aldrin, 2,4-DDE, 4,4-DDE, Dieldrin, 2,4-DDD, 4,4-DDD Endrin, 2,4-DDT and 4,4-DDT. SENTROTEK can analyze Aldrin, BHC, DDD, DDE, DDT, Dieldrin, Endosulfan, Endrin, Heptachlor and Methoxyxchlor.

c. Limit of Detection and Limit of Quantification

The limit of detection is 0.01 ppm.

d. National Regulatory Limits

Several organochlorine and organophosphate compounds are banned. The standards used in aquaculture are the lowest ones set by the *Codex Alimentarius*, the United States Food Development Authority (US-FDA) and the European Union (EU). Food-business operators placing products on export markets must comply with these values and they are monitored by the Competent Authority (CA). Details of the decision limit for EU market are as follows:

ORGANOCHLORINE COMPOUNDS	
COMPOUND or MARKER RESIDUE	DECISION LIMIT [µg/kg]
a-BHC	200 µg/kg
β-BHC	100 µg/kg
Aldrin	200 µg/kg
Dieldrin	200 µg/kg
Chlordane	50 µg/kg
DDT	400 µg/kg
DDD	400 µg/kg
DDE	400 µg/kg
Endrin	50 µg/kg
Endosulfan1	100 µg/kg
Endosulfan 11	100 µg/kg
Heptachlor	200 µg/kg
Heptachlor Epoxide	200 µg/kg
Lindane	20 µg/kg
Methoxychlor	10 µg/kg

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Philippines did not participate in inter-laboratory proficiency testing.

b. Survey Results and Discussion

Table 1. Summary of results of analysis for pesticides in fish products from 2005-2007.

Year of analysis & Sampling location	Analyte	Sample analysed		No. of samples analysed	Results	Remarks
		Common name	Scientific name			
2005, Aquaculture farms	Organochlorines	Shrimp	*Penaeus monodon*	5	ND	NPAL
2005, Aquaculture farms	Organochlorines	Milkfish	*Chanos chanos*	9	ND	NPAL
2006, Aquaculture farms	Organochlorines	Shrimp	*Penaeus monodon*	5	ND	SGS
2006, Aquaculture farms	Organochlorines	Milkfish	*Chanos chanos*	14	ND	SGS
2007, Wild caught	Organochlorines	Mudcrab	*Scylla serrata*	5	ND	SENTROTEK
2007, Wild caught	Organochlorines	Lobster	*Panulirus sp.*	5	ND	SENTROTEK

Laboratory : National Pesticide Analytical Laboratory (NPAL)
Laboratory : Societe Generalle Surveillance (SGS)
Laboratory : SENTROTEK
Method of analysis : Gas Liquid Chromatogaphy
LOD : 0.01 ppm
ND : Not detected

Pesticides analyzed: Multi-residues method for α-HCH, β-HCH, δ-HCH, HCB, Lindane, Heptachlor, Aldrin, 2,4-DDE, 4,4-DDE, Dieldrin, 2,4-DDD, 4,4-DDD Endrin, 2,4-DDT, 4,4-DDT.

Method of analysis: Multi-residues method for Aldrin, BHC, DDD, DDE, DDT, Dieldrin, Endosulfan, Endrin, Heptachlor, Methoxychlor

In 2005, a total of 14 fresh frozen shrimp (*Penaeus monodon) and* milkfish (*Chanos chanos*) samples were collected from the aquaculture farms from August to December. The samples were analyzed for pesticides residues using Gas Liquid Chromatogaphy with the multi-residues method for α-HCH, β-HCH, δ-HCH, HCB, Lindane, Heptachlor, Aldrin, 2,4-DDE, 4,4-DDE, Dieldrin, 2,4-DDD, 4,4-DDD, Endrin, 2,4-DDT and 4,4-DDT.

In 2006, a total of 19 fresh frozen shrimp samples were taken from aquaculture farms. All samples were analyzed using Gas Liquid Chromatogaphy for pesticides with the multi-residues method for α-HCH, β-HCH, δ-HCH, HCB, Lindane, Heptachlor, Aldrin, 2,4-DDE, 4,4-DDE, Dieldrin, 2,4-DDD, 4,4-DDD Endrin, 2,4-DDT and 4,4-DDT. All samples also showed negative results.

In 2007, marine fish samples were taken from public markets from March to November for the analysis of pesticides residues. The analytes were Aldrin, BHC, DDD, DDE, DDT, Dieldrin, Endosulfan, Endrin, Heptachlor, and Methoxychlor. A total of 10 Samples of fresh mudcrab and lobster were collected and analyzed. The results showed that pesticides were not detected with a detection limit of 0.01 ppm. The summary of analysis is presented in Table 1 above.

a. Corrective Actions

Presence of residues of banned chemicals in shrimp/fish meat and in harvested animals are subjected to the following corrective actions:

The farm owner is immediately informed of the presence of residues of banned chemicals in shrimp/fish samples obtained from his farm, so that he can take appropriate measures to withdraw his products from export. For the next 12 months, the farm is subjected to more stringent checks for the residues in question. The results are also immediately provided to the concerned auction market owners and exporters who have to recall the products if the products are no longer under their control. No health certificates are issued in cases where banned chemicals are tested positive.

The presence of residues of banned chemicals in shrimp/fish meat is also reported immediately to the Local Government Units concerned, with

the recommendation of the proper action and application of National Laws and Local Ordinances pertaining to pollution and consumer protection. An investigation is carried out to determine the reasons for the presence of residues and the extent of the problem.

In cases of positive findings, the registration of the farm concerned will be suspended and the information will be given to the auction markets and processing plants the farm supplies to. The suspension will be lifted after three successive negative findings.

5. Problems and Challenges Encountered

- Delayed release of funds caused problems in the implementation of the project according to schedule.
- Formal official control on the designated laboratory analyzing official samples must be strengthened.

6. Recommendations and Suggestions for Future Follow up Action

- SEAFDEC-MFRD must continue leading the coordination and networking among the participating countries.
- To continue activities on the harmonization of protocol procedures on the analysis of pesticides using sensitive equipment like the LC-MS-MS.
- To include activities on the preparation of IEC materials on food safety and quality.
- Financial Assistance in the purchase of equipment and technical support for the implementation of the food safety program.

Singapore

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Preamble

This survey was conducted by the Marine Fisheries Research Department (MFRD) Chemistry Laboratory of the Fish Quality Management and Technology Branch as part of the Japanese Trust Fund II Program on the Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products.

1. Introduction

The Marine Fisheries Research Department (MFRD) Chemistry Laboratory of the Fish Quality Management and Technology Branch is the responsible laboratory for conducting the analysis of heavy metals in fish and fish products in this Japanese Trust Fund II Program on the Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products.

In July 2002, MFRD received a report from Food and Agriculture Organization of the United Nations (FAO) on the traditional fish drying in coastal villages of Bangladesh: Practices, shortcomings and scope for improvements. It had been reported that organochlorine insecticides were widely used in order to control blowfly and beetle infestation during the sun-drying of fish. Typically, the insecticides were applied three times during the process, and reapplied during storage if further infestation was found. Thus MFRD decided to investigate the level of organochlorine pesticide residues in the different types of dried salted fish commonly found in Singapore under this JTF II program.

2. Objectives And Goals

The objective of this activity is to investigate on the level of 15 types of organo-chlorine pesticide residues contamination in dried fish commonly available in Singapore.

Table1. Pesticide analysed for this activity.

S/N	Compounds	Chemical Name
1	α-HCH	alpha-Hexachlorocyclohexane
2	β-HCH	beta-Hexachlorocyclohexane
3	γ-HCH	gamma-Hexachlorocyclohexane
4	δ-HCH	delta-Hexachlorocyclohexane
5	HCB	Hexachlorobenzene
6	Heptachlor	Heptachlor
7	o,p'-DDE	ortho-para- Dichlorodiphenyldichloroethylene
8	p,p'-DDE	para-para- Dichlorodiphenyldichloroethylene
9	o,p'-DDD	ortho-para-Dichlorodiphenyldichloroethane
10	p,p'-DDD	para-para-Dichlorodiphenyldichloroethane
11	o,p'-DDT	ortho-para-Dichlorodiphenyltrichloroethane
12	p,p'-DDT	para-para-Dichlorodiphenyltrichloroethane
13	Aldrin	Aldrin
14	Dieldrin	Dieldrin
15	Endrin	Endrin

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Seven species of dried and dried salted fish were used in this survey. The species selected for analysis were based on their popularity among local consumers. All dried samples were obtained from eight different shops in Pasir Panjang Wholesale Market. The dried fishes were randomly selected and purchased. At least six to ten pieces of fish for each species were collected and were of consumable or "market" size.

The dried fishes were transported at atmospheric temperature. Upon arrival at the laboratory, the samples were identified. The common and scientific names of the fish studied are shown in Table 2. The samples were stored at –10°C until sampled.

Table 2. The common, scientific and indigenous names of the seven fish species surveyed.

Common Names	Scientific Name	Indigenous Name	Denoted by
Spanish Mackerel	*Scomberomorus commerson* (Lacepede)	Tenggiri batang	SM
Snakeskin Gouramy	*Trichogaster pectoralis*	Ikan Sepat Sepat, Ikan Sepat Siam	IS
Mei Ren Yu	-	-	MR
Sand Whiting	*Sillago sihama*	-	SW
Yellow-banded Scad	*Selaroides leptolepis* (Valenciennes)	Selar Kuning	KG
Threadfin	*Polynemus indus* Shaw	Ikan Kurau	KR
Indian Mackerel	*Rastrelliger kanagurta* (Cuvier)	Kembong	IM

Note: A two-letter short form (last column) is assigned to each species.

b. Method of Analysis

The fish samples were sampled and analysed by the Fish Quality Management & Technology Branch. The total length, standard length and body weight of the samples were measured and recorded. Seven samples of each type of species (n = 7) were used for analysis. Only the edible portions were sampled. The sampling procedures were as follows;

i. Clean and scale fish.
ii. Fillet fish to obtain all flesh and skin.
iii. Mince sample rapidly and thoroughly with chopper.
iv. Remove unground material from blade of chopper and mix thoroughly with ground material and mince thoroughly again.
v. Make mince into a burger and divide into equal quarters.
vi. Remove the first quadrant of minced meat for testing.

If necessary, repeat i to vi until sufficient sample weight is obtained for the test.

The in-house method used is a multi-residues method (MRM) that allows the simultaneous detection and quantification of the 15 organochlorine pesticides. The sample extraction procedure and analytical instrumentation are as follows;

Sample Extraction

10 g of sample, to which 100 ml of acetonitrile and 2 spatulas of Celite 545 were added, was homogenized. It was then filtered using the Kiriyama filtration apparatus under vacuum. The filtrate was collected in a round-bottomed flask (RBF) and concentrated to about 10 ml on the rotary evaporator. The spiked sample was treated last.

The extract was then diluted with 150 ml of distilled water and transferred to a 300 ml separatory flask

(F1), which contained approximately 5 g of sodium chloride. 50 ml of n-hexane was used to rinse the RBF and was transferred to the separatory flask. The mixture was manually shaken for 1 minute until all the sodium chloride had dissolved. It was then left to stand for phase separation.

An additional 50 ml of n-hexane was used to rinse the round-bottomed flask, and transferred to a 200 ml separatory flask (F2). The bottom layer of F1 was eluted to F2, which was then shaken manually for 1 minute and left to stand for phase separation. The bottom aqueous layer of F2 was discarded, and the organic top layer was transferred and combined with that in F1. F2 was rinsed twice with 5 ml of n-hexane. The organic phase in F1 was then filtered through a filter funnel plugged with cotton wool at its base and 70% filled with anhydrous sodium sulphate. The filtrate was collected in a new 200 ml RBF. F1, the filter funnel and tip were rinsed with n-hexane.

After liquid-liquid extraction, the extract was evaporated to about 1 to 2 ml and dried with nitrogen gas. As the fatty tissue could not be dried with nitrogen gas, it would remain on the RBF walls as small oil droplets. The following would only be done if there were visible oil droplets (*). If not, 5 ml of n-hexane was added into the RBF.

(*) 20 ml of n-hexane was added to the extract, and transferred into a 125 ml separatory flask. The RBF was rinsed twice with 20 ml of acetonitrile saturated with n-hexane (20 ml twice), and its contents transferred into the separatory flask. The mixture was shaken for 1 minute and the phases were allowed to separate. The bottom acetonitrile layer was eluted into the same RBF. The partitioning and collection was repeated for another two times using 20 ml of acetonitrile saturated with n-hexane. The sample was then concentrated to about 1 to 2 ml and dried with nitrogen gas before it was dissolved in 5 ml of n-hexane and transferred into the chromatographic tube.

To prepare the chromatographic tube, the base of a 20 mm ID tube was plugged with cotton wool and wetted with n-hexane. A suspension of 12 g of florisil and n-hexane was then transferred into the tube. Anhydrous sodium sulphate was then added until a 1cm thick layer above the florisil bed was formed. n-Hexane was used to wash the tube, and the solvent was eluted out until it was 1cm above the ring of anhydrous sodium sulphate.

The extract was then transferred into the tube, and the RBF was rinsed twice with 2 ml of n-hexane. The tube wall was also rinsed with n-hexane. The sample was eluted out of the tube at a rate of 1 drop/second, and collected in a new 200 ml RBF. When 1cm of solution remained above the florisil bed, a separatory funnel containing 120 ml of diethyl ether: n-hexane mixture was attached to the tube. The elution was allowed to continue till completion.

The sample was then concentrated to about 1 to 2 ml on the evaporator and dried completely with nitrogen gas. 5 ml of n-hexane was pipetted into the RBF. The solution was transferred to a small bijou bottle, and nitrogen gas was blown into the airspace above the solution before it was capped, sealed with parafilm, and stored in a 5ºC refrigerator.

Analytical Instrumentation

A fused silica capillary SPB-5 (Supelco, 0.25 mmID, 30 m long, and 0.25 μm film thickness) was used. The Shimadzu Gas Chromatograph Mass Spectrometer (GC/MS) GC17A/QP5050A was operated under the following conditions: helium constant flow 1.0 ml/min, injection temperature 250ºC, detector temperature 230ºC, ionization voltage 70eV, EI ionization method, and injection volume 1μl (splitless). The oven temperature was programmed in the following manner: 80ºC (2min), then 10ºC/min ramp to 320ºC (held for 8min). The MS was operated in the selective ion monitoring (SIM) mode. The retention times and monitor ions were as shown in Table 3.

Table 3. Monitor and determination ions of the 15 organochlorine compounds.

Compounds	Molecular weight/g	m/z ratio of Determination ion	m/z ratio of Identification ion
α-HCH	290.8	219	217
β-HCH	290.8	219	217
γ-HCH	290.8	219	217
δ-HCH	290.8	219	217
HCB	284.8	284	286
Heptachlor	372.5	272	274
o,p'-DDE	318.0	318	316
p,p'-DDE	318.0	318	316
o,p'-DDD	320.0	235	237
p,p'-DDD	320.0	235	237
o,p'-DDT	354.5	235	237
p,p'-DDT	354.5	235	237
Aldrin	364.9	263	265
Dieldrin	380.9	263	265
Endrin	380.9	263	265

c. Limit of Detection and Limit of Quantification

The limit of quantification of all the 15 organochlorine pesticides are 0.005ppm.

d. National Regulatory Limits

Singapore Guidelines extracted from The Sale of Food Act, Chapter 283, Section 56, Food Regulations, 2005 Revised Edition.

Substance	Type of Food	Maximum Residues Limit (ppm)
Aldrin	Fish (edible portion)	0.2
DDT (including DDD and DDE)	Fat of Meat	5
Dieldrin	Fat of Meat	0.2
Endrin	Fat of Meat	0.1
Heptachlor (including its epoxide)	Fat of Meat	0.2
Lindane (γ-HCH)	Fish	1

*extracted only for organochlorines

Where it is not provided in these Regulations, the pesticide residues contained in any food shall not exceed the limits as recommended by the Codex Alimentarius Commission.

EU Guidelines extracted from Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC, 5058/VI/98, 3 November 2004.

Crop Group	Substance	MRL	Directive			Remarks
Meat and edible offal	Aldrin (see Dieldrin)					
Meat and edible offal	DDT	1	93	57	EEC	
Meat and edible offal	Dieldrin	0.2	93	57	EEC	Singly or combined with Aldrin, expressed as Dieldrin
Meat and edible offal	Endrin	0.05	93	57	EEC	
Meat and edible offal	HCH	0.3	93	57	EEC	the MRL for EC is the sum of 0.2 for alpha & 0.1 for beta isomers
Meat and edible offal	Heptachlor	0.2	93	57	EEC	
Meat and edible offal	Lindane	0.02	02	66	EC	Based on monitoring data

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results (ug/kg)	True value (ug/kg)	z-score	Remarks
2007 (August-September)	FAPAS® Proficiency Test 0555 Pesticide Residues in Minced Chicken	(ß-HCH)	N.A.	57.3	N.A.	Not analysed.
		(γ-HCH)	36.39	59.7	-1.8	Passed. Within z±2
		p,p'-DDE	23.49	39.2	-1.8	
		p,p'-DDD	30.52	40.4	-1.1	
		o,p'-DDT	17.82	27.4	-1.6	
		p,p'-DDT	44.91	72.7	-1.7	

b. Survey Results and Discussion

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Spanish Mackerel (Tenggiri Batang) (Dried)	*Scomberomorus commerson* (Lacepede)	7	N.D (2)	<LOQ (5)	<LOQ	106.04	52.83
	β-HCH				N.D/ (4)	0.0204 (1)	<LOQ	115.44	
	γ-HCH				N.D (2)	<LOQ (5)	<LOQ	108.57	
	δ-HCH				N.D (6)	<LOQ (1)	<LOQ	105.97	
	HCB				N.D (2)	<LOQ (5)	<LOQ	71.06	
	Heptachlor				N.D. (1)	0.0104 (1)	<LOQ	87.61	
	o,p'-DDE				N.D (7)	N.D	N.D	127.73	
	p,p'-DDE				N.D (3)	0.0212 (1)	<LOQ	152.68	
	o,p'-DDD				N.D (6)	0.0396 (1)	<LOQ	127.41	
	p,p'-DDD				N.D (3)	0.1201 (1)	<LOQ	144.57	
	o,p'-DDT				N.D (4)	0.0923 (1)	<LOQ	137.67	
	p,p'-DDT				N.D (2)	0.2252 (1)	<LOQ	163.28	
	Aldrin				N.D (7)	N.D	N.D	89.53	
	Dieldrin				N.D (7)	N.D	N.D	79.13	
	Endrin				0.0057	0.0079	0.0066 (4)	111.63	

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Snakeskin Gouramy (Ikan Sepat) (Dried)	*Trichogaster pectoralis*	7	N.D (5)	0.0079 (1)	<LOQ	114.91	45.14
	β-HCH				N.D (7)	N.D	N.D	114.81	
	γ-HCH				N.D (6)	0.1829 (1)	<LOQ	108.82	
	δ-HCH				N.D (6)	0.2260 (1)	<LOQ	114.11	
	HCB				N.D (6)	<LOQ (1)	<LOQ	104.68	
	Heptachlor				N.D (3)	0.0700 (1)	<LOQ	112.48	
	o,p'-DDE				N.D (7)	N.D	N.D	115.34	
	p,p'-DDE				N.D (7)	N.D	N.D	120.65	
	o,p'-DDD				N.D (7)	N.D	N.D	123.33	
	p,p'-DDD				N.D (7)	N.D	N.D	127.83	
	o,p'-DDT				N.D (5)	<LOQ (2)	<LOQ	112.39	
	p,p'-DDT				N.D (5)	0.0087 (1)	<LOQ	120.49	
	Aldrin				N.D (7)	N.D	N.D	109.17	
	Dieldrin				0.0086	0.0126	0.0100 (3)	99.79	
	Endrin				N.D (2)	<LOQ (5)	<LOQ	117.76	
2005, Singapore	α-HCH	Mei Ren Yu (Dried)		7	N.D (7)	N.D	N.D	118.22	20.99
	β-HCH				0.0066	0.0259	0.0162 (2)	74.94	
	γ-HCH				N.D (5)	<LOQ (2)	<LOQ	105.95	
	δ-HCH				N.D (7)	N.D	N.D	109.43	
	HCB				N.D (2)	<LOQ (5)	<LOQ	75.52	
	Heptachlor				N.D (7)	N.D	N.D	94.43	
	o,p'-DDE				N.D (3)	<LOQ (4)	<LOQ	122.35	
	p,p'-DDE				N.D (7)	N.D	N.D	127.31	
	o,p'-DDD				N.D (7)	N.D	N.D	140.33	
	p,p'-DDD				N.D (7)	N.D	N.D	151.08	
	o,p'-DDT				N.D (7)	N.D	N.D	136.39	
	p,p'-DDT				N.D (7)	N.D	N.D	147.92	
	Aldrin				N.D (2)	<LOQ (5)	<LOQ	90.77	
	Dieldrin				N.D (2)	0.0059 (1)	<LOQ	90.08	
	Endrin				N.D (7)	N.D	N.D	105.90	

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Sand Whiting (Dried)	*Sillago sihama*	7	N.D (7)	N.D	N.D	70.09	25.44
	β-HCH				N.D (6)	0.0096 (1)	<LOQ	53.94	
	γ-HCH				N.D (5)	0.0110 (1)	<LOQ	61.16	
	δ-HCH				N.D (7)	N.D	N.D	65.79	
	HCB				N.D (7)	N.D	N.D	43.63	
	Heptachlor				N.D (7)	N.D	N.D	41.62	
	o,p'-DDE				N.D (7)	N.D	N.D	78.69	
	p,p'-DDE				N.D (7)	N.D	N.D	85.64	
	o,p'-DDD				N.D (7)	N.D	N.D	83.97	
	p,p'-DDD				N.D (7)	N.D	N.D	89.85	
	o,p'-DDT				N.D (7)	N.D	N.D	72.36	
	p,p'-DDT				N.D (7)	N.D	N.D	78.66	
	Aldrin				N.D (7)	N.D	N.D	45.61	
	Dieldrin				N.D (7)	N.D	N.D	51.76	
	Endrin				N.D (6)	<LOQ (1)	<LOQ	61.94	
2005, Singapore	α-HCH	Yellow-banded Scad (Selar Kuning) (Dried)	*Selaroides leptolepis* (Valenciennes)	7	N.D (7)	N.D	N.D	65.80	47.78
	β-HCH				N.D (5)	0.0241 (1)	<LOQ	64.05	
	γ-HCH				N.D (2)	0.0121 (1)	<LOQ	66.71	
	δ-HCH				N.D (6)	<LOQ (1)	<LOQ	71.06	
	HCB				N.D (7)	N.D	N.D	42.48	
	Heptachlor				N.D (3)	<LOQ (4)	<LOQ	53.44	
	o,p'-DDE				N.D (7)	N.D	N.D	61.80	
	p,p'-DDE				N.D (7)	N.D	N.D	71.18	
	o,p'-DDD				N.D (7)	N.D	N.D	71.11	
	p,p'-DDD				N.D (7)	N.D	N.D	74.04	
	o,p'-DDT				N.D (7)	N.D	N.D	61.69	
	p,p'-DDT				N.D (5)	<LOQ (2)	<LOQ	61.28	
	Aldrin				N.D (7)	N.D	N.D	51.45	
	Dieldrin				N.D (7)	N.D	N.D	70.85	
	Endrin				N.D (7)	N.D	N.D	59.27	

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Threadfin (Ikan Kurau) (Dried)	*Polynemus indus* Shaw	7	N.D (7)	N.D	N.D	77.52	43.07
	β-HCH				0.0086	0.0122	0.0107 (7)	56.70	
	γ-HCH				N.D (7)	N.D	N.D	72.14	
	δ-HCH				N.D (7)	N.D	N.D	84.05	
	HCB				N.D (7)	N.D	N.D	46.18	
	Heptachlor				N.D (7)	N.D	N.D	62.06	
	o,p'-DDE				N.D (7)	N.D	N.D	64.25	
	p,p'-DDE				N.D (7)	N.D	N.D	70.53	
	o,p'-DDD				N.D (7)	N.D	N.D	68.06	
	p,p'-DDD				N.D (7)	N.D	N.D	69.49	
	o,p'-DDT				N.D (6)	0.0196 (1)	<LOQ	48.81	
	p,p'-DDT				N.D (7)	N.D	N.D	63.50	
	Aldrin				N.D (7)	N.D	N.D	60.00	
	Dieldrin				N.D (6)	0.0298 (1)	<LOQ	65.27	
	Endrin				Not tested				
2003, Singapore	α-HCH	Indian Mackerel (Kembong) (Dried)	*Rastrelliger kanagurta*	7	N.D (7)	N.D	N.D	44.85	49.42
	β-HCH				N.D (7)	N.D	N.D	49.12	
	γ-HCH				0.0241	0.0546	0.0413 (4)	47.61	
	δ-HCH				N.D (7)	N.D	N.D	51.46	
	HCB				N.D (7)	N.D	N.D	31.37	
	Heptachlor				N.D (6)	<LOQ (1)	<LOQ	39.25	
	o,p'-DDE				N.D (7)	N.D	N.D	54.36	
	p,p'-DDE				N.D (7)	N.D	N.D	56.28	
	o,p'-DDD				N.D (3)	<LOQ (4)	<LOQ	56.29	
	p,p'-DDD				N.D (7)	N.D	N.D	59.85	
	o,p'-DDT				N.D (7)	N.D	N.D	62.18	
	p,p'-DDT				N.D (7)	N.D	N.D	69.62	
	Aldrin				N.D (7)	N.D	N.D	41.54	
	Dieldrin				N.D (6)	<LOQ	N.D	42.35	
	Endrin				0.0102	0.0522	0.0209 (6)	90.42	

The above results were reported in wet weight basis. All results obtained were within Singapore regulatory's limit of 0.1ppm.

c. Corrective Actions

No regulatory action was taken as the results were within Singapore's regulatory limits.

5. Problems and Challenges Encountered

In this preliminary survey, only seven types of species were analysed and a total of 49 samples were collected. Due to the small number of samples collected, it may not be truly representative of the fishes available in Singapore.

6. Recommendations and Suggestions for Future Follow up Action

It would be good to replace the extraction method with a more rapid method.

Thailand

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1. Introduction

Organochlorine pesticides were used widely from the 1940s to the 1960s for agricultural pest control and for malaria programs. Since the 1960s their use has been reduced greatly due to their health and environmental effects and persistence. Fish and shellfish accumulate pesticides from the environment in which they live in, but the extent of accumulation depends on such factors as geographical location, fish species feeding patterns, solubility and lipophilicity of the pesticides. These pesticides may accumulate in fish at levels that can cause illness. Therefore the maximum levels for some organochlorine pesticides in fish products are set in many countries. To manage the safety of fish and fish products, the residues level should be known.

2. Objectives And Goals

To set up the laboratory analysis method for organochlorine pesticides and obtain an understanding of levels of organochlorine pesticides in fish and fish products.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Frozen vannamei shrimp (*Penaeus vannamei*), frozen nile tilapia (*Tilapia nilotica*) and dried yellowstripe trevally (*Selaroides leptolepis*) from the factories in Samutsakorn province were collected. After sampling, samples were packed in plastic bag and sent to laboratory. The samples if not analyzed immediately were kept at –18°C.

b. Method of Analysis

About 300 g of the edible part of the sample was blended and extracted according to the method as attached in Appendix 1 at Samutsakorn Fish Inspection and Research Center and injected into Hewlett Packard Gas Chromatograph-Electron Capture Detector (GC-ECD) at the Central Laboratory (Thailand) Co. Ltd.

c. Limit of Detection and Limit of Quantification

Limit of Detection is 0.01 ppm.
Limit of Quantification is 0.05 ppm.

d. National Regulatory Limits

The regulatory limits of each organochlorine pesticides depend on products and importing countries. For Thailand's regulatory limits of pesticide residues in fish and fish products:

- Aldrin, Dieldrin, Heptachlor - 0.02 ppm
- Chlordane, Endrin - 0.05 ppm
- DDT - 1 ppm

4. Results And Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Thailand did not participate in any Inter-Laboratory Proficiency Testing Program.

b. Survey Results and Discussion

Nineteen samples of frozen vannamei shrimp (*Penaeus vannamei*), 18 samples of frozen nile

tilapia (*Tilapia nilotica*) and 11 samples of dried yellowstripe trevally (*Selaroides leptolepis*) were collected from the factories in Samutsakorn province. The samples were analysed for the following pesticide residues and no pesticide residues was detected in any samples.

S/N	Compounds	Chemical Name
1	α-HCH	alpha-Hexachlorocyclohexane
2	γ-HCH	gamma-Hexachlorocyclohexane
3	δ-HCH	delta-Hexachlorocyclohexane
4	HCB	Hexachlorobenzene
5	Heptachlor	Heptachlor
6	2,4'-DDE	ortho-para- Dichlorodiphenyldichloroethylene
7	4,4'-DDE	para-para- Dichlorodiphenyldichloroethylene
8	2,4'-DDD	ortho-para-Dichlorodiphenyldichloroethane
9	4,4'-DDD	para-para-Dichlorodiphenyldichloroethane
10	2,4'-DDT	ortho-para-Dichlorodiphenyltrichloroethane
11	4,4'-DDT	para-para-Dichlorodiphenyltrichloroethane
12	Aldrin	Aldrin
13	Dieldrin	Dieldrin
14	Endrin	Endrin

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of result (ppm) – wet weight basis	Average value of result (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2008 Samutsakorn province	Aldrin	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	85
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	91
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	46
	4,4′-DDE	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	87
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	93
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	48
	2,4′-DDE	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	80
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	89
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	46
	4,4′-DDD	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	88
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	101
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	50
	2,4′-DDD	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	90
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	97
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	50
	4,4′-DDT	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	69
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	88
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	49

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of result (ppm) – wet weight basis	Average value of result (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2008 Samutsakorn province	2,4′-DDT	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	74
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	86
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	48
	Dieldrin	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	88
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	94
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	50
	Endrin	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	108
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	126
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	75
	α-HCH	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	81
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	94
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	51
	γ-HCH	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	78
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	91
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	49
	δ-HCH	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	88
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	99
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	51

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm) – wet weight basis	Max. value of result (ppm) – wet weight basis	Average value of result (ppm) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2008 Samutsakorn province	Heptachlor	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	81
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	96
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	51
	HCB	Frozen vannamei shrimp	*Penaeus vannamei*	19	Not detected	Not detected	Not detected	78
		Frozen nile tilapia	*Tilapia nilotica*	18	Not detected	Not detected	Not detected	91
		Dried yellowstripe trevally	*Selaroides leptolepis*	11	Not detected	Not detected	Not detected	49

a. Corrective Actions

No correction action was taken as no pesticide residues were detected. In cases of exceeding the importing country regulation, that lot of product will be rejected for export. The processor will be required to identify any other products that were produced from the same lot of raw material and provide a corrective action plan. A follow-up at plant may be deemed necessary in some cases. If the processor could not bring its system to comply with the safety requirement, the processor will be suspended for export. The processor will be withdrawn from the Department of Fisheries list of approved fishery establishment when the offence is repeated.

5. Problems and Challenges Encountered

Equipment

The existing GC-ECD in the laboratory is old and less sensitive. Hence, the laboratory had to use the GC-ECD at Central Laboratory (Thailand) Co. Ltd. for the analysis. This resulted in the high expenditure.

Method

The method taught in the regional training course uses a lot of solvent, has many steps and takes a long time to complete the extraction. So another method was adopted in order to reduce extraction time and solvent usage.

This method gave good recovery for testing of fresh samples. The average recovery is 89%. For dried samples, however, the recovery was poor at 51% on average. This method thus needs to be improved further.

6. Recommendations and Suggestions for Future Follow up Action

- Organophosphate should be surveyed as there is little problem with organochlorine pesticide residues now due to the ban of organochlorine pesticides for agriculture use in Thailand.
- The fund should be given at the beginning of the year for easier planning and conducting of activities.

Method of Analysis

Weigh 2 g sample in 50 ml polypropylene screw cap centrifuge tube
↓
Add about 2 spoonful of sodium sulfate
↓
Mix with stirring rod
↓
Add 20 ml petroleum ether
↓
Homogenize for 1 min
↓
Decant petroleum ether extracted through about 2 spoonful of sodium sulfate
(which was placed on whatman no. 1 filter paper in funnel) into evaporation flask
↓
Extract another 3 times by using 10 ml petroleum ether
↓
Evaporate with vacuum rotary evaporator at room temperature until nearly dry
↓
Add about 1ml petroleum ether
↓
Load into Florisil SPE
(which was pre-conditioned with 5ml petroleum ether)
↓
Elute with 10 ml of 6% diethyl ether in petroleum ether
Follow by 10 ml of 15% diethyl ether in petroleum ether
↓
Evaporate with vacuum rotary evaporator at room temperature
↓
Dissolve the residues with 2 ml n-hexane
↓
Inject into GC-ECD

Calculation

Organochlorine pesticides (ppm) = C x 2 / W where,
C = concentration of organochlorine pesticides from calibration curve (ug/ml)
W = sample weight (g)

Reference

Official Methods of Analysis of AOAC International 17th edition 2000. Chapter 10. Pesticide and industrial chemical residues. 10.2.01. AOAC Official method 983.21. p. 12E-13.

VIETNAM

Ngo Hong Phong
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National Agro-Forestry and Fisheries Quality Assurance Department-NAFIQAD
Ministry of Agriculture and Rural Development

1. Introduction

The control of residues of harmful substances, especially pesticides, is important because these residues affect consumers' health seriously, affecting the respiration system, central nerve system and may result in death. The more important fact is that there is no way to eliminate these substances in the processing stage when fish products are already contaminated.

The increasing import of fish and fish products into high-income countries requires strict standards on hygiene and safety, resulting in specific requirements on chemical residues in fish and fish products being set up.

For this reason, pesticide residues survey and studies activities organized by SEAFDEC and funded by JTF II are significant as the activities provide an overview of residue levels of pesticides in fish and fish products in South-East Asia. Member countries would then determine appropriate policies to control quality and safety of fish and fish products for meeting importing countries' requirements and protecting domestic consumers' health.

In Vietnam, as the national competent authority responsible for controlling quality, hygiene and safety of fish and fish product since 1997, the National Agro-Forestry and Fisheries Quality Assurance Department-NAFIQAD (known as the National Fisheries Quality Assurance and Veterinary Directorate- NAFIQAVED before February 23, 2008) has been carrying out the Monitoring Program for certain harmful substances and residues present in aquaculture animals. NAFIQAD also actively participated in the "Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products" as well as other activities organized by SEAFDEC.

2. Objectives And Goals

Surveys of pesticides are carried out to provide information on the level of residues of contaminants in farmed fish and fish products. The data obtained was deposited into the database of the Fish and Fish Products Safety Information Network. The network's website: www.fishsafetyinfo.com contains general information on fishery hygiene and safety in SEAFDEC's member countries.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

- NAFIQAD branches took samples and analyzed samples as required by the activity.
- Activities carried out by NAFIQAD including the following:
  a) Activities using SEAFDEC's budget;
  b) Activities using budget from the Monitoring Program for certain harmful substances and residues in aquaculture animals and certification activities for exported fishery consignments.
- NAFIQAD has signed contracts with its Branches for the implementation of activities, and then is responsible for data analysis and treatment and making report.
- NAFIQAD branches carry out the procedures of sampling, sample preservation, dispatch of samples to laboratories and analysis, in compliance with the Residues Monitoring Program Manual and Quality Manual of NAFIQAD.
- In 2007, the targetted samples were changed. Wild fish and processed products, not aquaculture fish, were sampled.

- Species sampled in 2006, 2007 and 2008:

2006	2007	2008
• Black Tiger Shrimps: *Penaeus monodon* • Clam: *Meretrix lyrata* • Basa Cat Fish: *Pangasius bocourti* • Tra Cat Fish: *Pangasius hypophthalmus* • Tilapia: *Oreochromis* spp.	• Black Tiger Shrimps: *Penaeus monodon* (semi and final products were taken from processing plant and the market) • Clam: *Meretrix lyrata* • Fillet Cat Fish: *Pangasius hypophthalmus* (semi and final products were taken from processing plant and the market)	• Black Tiger Shrimps: *Penaeus monodon* (semi and final products were taken from processing plant and the market) • Clam: *Meretrix lyrata* • Fillet Cat Fish: *Pangasius hypophthalmus* (semi and final products were taken from processing plant and the market)

b. Method of Analysis

Method: AOAC 983.21.1995

Equipment: GC-ECD Agilent

Testing method has been accredited with ISO 17025 by BoA – VILAS

c. Limit of Detection Limit of Quantification

Limit of Detection (LOD):

- Aldrin, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Lindane: 2 ppb
- Chlordane: 5 ppb
- DDT: 10 ppb

d. National Regulatory Limits

MRLs required:

- Vietnam

  Aldrin: 0.2 mg/kg, Dieldrin: 0.2 mg/kg, Endrin: 0.05 mg/kg, Lindane: 2 mg/kg, DDT: 1 mg/kg, Heptachlor: 0.2 mg/kg, Hexachlorobenzene: 0.2 mg/kg, Chlordane: 0.05 mg/kg.

- EU

  Aldrin: 200 ppb, Dieldrin: 200 ppb, Endrin: 50 ppb, Heptachlor: 200 ppb, Lindane: 1000 ppb, DDT: 1000 ppb

- USA

  Aldrin and Dieldrin: 0.3 ppm, Heptachlor: 0.3 ppm, DDT: 5 ppm

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Remarks
2005	Fish meal/ FAPAS	Organo-chlorinated pesticides	Passed

b. Survey Results and Discussion

No.	Sample Name	SEAFDEC's budget			Vietnam's budget			Total		
		No. of samples	Analysis results (ppb)	No. of samples exceeding MRL	No. of samples	Analysis results (ppb)	No. of samples exceeding MRL	No. of samples	Analysis results (ppb)	No. of samples exceeding MRL
2006										
1	Black Tiger Shrimp	10	ND	0	41	ND	0	51	ND	0
2	Clam	8	ND	0	43	ND	0	51	ND	0
3	Tra/Basa Cat Fish	16	ND	0	-	-	-	16	ND	0
4	Tilapia	-	-	-	3	ND	0	3	ND	0
2007										
1	Black Tiger Shrimp	5	ND	0	407	ND	0	412	ND	0
2	Clam	15	ND	0	45	ND	0	60	ND	0
3	Tra/Basa Cat Fish	20	ND	0	294	ND	0	314	ND	0
4	Tilapia	-	-	-	54	ND	0	54	ND	0
5	Others	-	-	-	56	ND	0	56	ND	0
2008										
1	Black Tiger Shrimp	5	ND	0	-	-	-	5	ND	0
2	Clam	5	ND	0	-	-	-	5	ND	0
3	Tra/Basa Cat Fish	5	ND	0	-	-	-	5	ND	0

Note: The average recovery for Black Tiger Shrimp, Clam, and Tra/Basa catfish analysed in 2008 was 80%, 85% and 78% respectively.

ND: Not detected

Contamination levels of organochlorinated substances in fish reflect how the use of pesticides in agriculture affects the environment and aquaculture fish. Analysis results showed that there was no pesticide residues detected in tested samples. In addition, from 1999 to 2007, there was no sample detected to have exceeded MRLs for pesticides in Vietnam. This signified that pesticides used in agriculture have not much impact on Vietnam aquaculture / capture environment.

5. Problems and Challenges Encountered

Due to limited budget for each activity, collected data may not be enough to reflect the real impact of chemical contaminants and antibiotics residues on aquaculture fish.

6. Recommendations and Suggestions for Future Follow up Action

It would be highly appreciated if the Project could focus on updating and training on new analytical methods, specifically multi-residues analysis method, in order to improve the testing capabilities on fish quality and safety in Southeast Asia.

Regional Survey of Histamine in Fish and Fish Products

- Cambodia
- Indonesia
- Myanmar
- Philippines
- Thailand
- Vietnam

Cambodia

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1. Introduction

Cambodia has rich resources of fresh water fish. The Mekong, Tonle Sap, and Basac Rivers and their many tributaries, a number of lakes, and a vast area of floodplain are a rich source of fish for the population. Among the water resources, the Great Lake, which is the largest lake in Southeast Asia, plays an important role in the country's fish wealth. The lake ranges in surface area from 3,000 to 10,000 sq. km. It is estimated that almost 60% of the country's freshwater fish is produced in the Great Lake region.

Cambodia has small-scale aquaculture. Some species of fresh water fish with economic value such as *Pangasianodon hypophthalmus, Micronema micronema, Pangasius bocourti* have been selected to be cultured in the ponds and cages along Mekong River and Tonle Sap River.

Cambodian Fisheries Administration, especially in the Laboratory of Inland Fisheries Research and Development Institute (IFReDI), did not have equipment for chemical testing under Japanese Trust Fund II Project. For all projects involving chemical analysis, laboratories in neighbour countries were rented for testing. Cambodia started participating in the regional survey of Histamine in Fish and Fish Products under Japanese Trust Fund II only from 2006 to 2008. Fortunately, from 2007, Cambodia has the National Laboratory for Drug Qualities Control, which can test for Histamine. The cost of analysis is however very expensive.

2. Objectives And Goals

- To examine the histamine levels in fish and fish products from Kendal province around Phnom Penh and in frozen marine fish from Kompong Som Province, Cambodia.
- To provide the results of these 3 years survey to the fisheries industry such as exporting fish factories, processing factories and others sectors related to food safety control.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

In 2006, a total of 12 samples of *Pangasius bocourti* (Basa-catfish), each with a weight of 500 to 700 g, were collected from a pond culture 12 km from Phnom Penh in the Kandal Province.

In 2007, a total of 14 samples of *Epinephelus coioides* and Indo-Pacific mackerel were taken. Each sample had a weight of 150 to 200 g. Samples were collected from marine fish and marine fish processing plants in the coastal areas of Kompong Som Province.

Year 2008, a total of 4 Indo-Pacific mackerel sized between 500 to 700 g were collected from Cambodian capital Phnom Penh market and a frozen factory in Kompong Som Province.

All samples were packed in plastic bags and kept at 18^{o}C for 1 to 2 days before transportation to the analytical laboratories.

b. Method of Analysis

In 2006, 12 samples were analyzed in laboratories of the National Agriculture, Forestry and Fisheries Quality Assurance Department (NAFIQAD) -Branch 4 in Ho Chi Minh City have certificate number: 105, using the method 05.2CL4/ST3.52 (HPLC-FLD). The equipment for analysis used was Hitachi Z 8200.

In 2007 and 2008, a total of 18 samples were analyzed at Cambodian National Laboratory for Drug Quality Control in Phnom Penh.

According to the certificate number 1685 and 1686-07 of the National Laboratory For Drug Quality Control, the method used was 05.2CL4/ ST3.52 (HPLC-FLD).

c. Limit of Detection and Limit of Quantification

- For analysis of fresh water fish (12 samples of *Pangasius borcouti*)

  Limit of detection (LOD) = 1.0 mg/kg

- For analysis of Marine fish (18 samples of *Epinephelus coioides* and Indo-Pacific mackerel)

  Limit of quantification (LOQ) = 1.2 mg/kg

d. National Regulatory Limits

Standard specification of Histamine, maximum permitted level in fish and fish products enforced by Cambodia, EU, USA, Canada and Japan

Type of products	Cambodia	EU	USA	Canada	Japan
Fish paste and fish sauce	<100 ppm	200 ppm	50 ppm	200 ppm	200 ppm
Fish products					100 ppm

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Cambodia did not participate in any inter-laboratory proficiency testing program.

b. Survey Results and Discussion

The results of the three years survey showed that histamine was detected in some specimens of fresh water and marine fish products. The level of contamination did not exceed the Cambodian National Standard and the standard enforced by USA, Canada and Japan.

Year of analysis & Sampling location	Fish sample analyzed		No. of samples analyzed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Remarks
	Common name	Scientific name						
2006, pond culture 12 km from Phnom Penh, Kandal Province	Basa-catfish	*Pangasius bocourti*	12	-	-	-	-	Detected but not quantifiable
2007, From fishing vessels, markets and processing plants at Kompong Som Province (Marine fish coastal areas)	Grouper Indo-Pacific mackerel	*Epinephelus coioides* *Scomberomorus guttatus*	14	5.3224	8.1208	6.8236	-	Detected but not quantifiable
2008, fishing vessels, marketsand processing plants at Kompong Som Province (Marine fish coastal areas)	Indo-Pacific mackerel	*Scomberomorus guttatus*	4	5.647	9.234	7.923	-	Detected but not quantifiable

c. Corrective Actions

These results indicated that the three targeted species were of good quality and were safe for exporting and domestic use.

5. Problems and Challenges Encountered

- In 2006, the cost of analyzing the samples was every expensive due to the need to transport the samples to Vietnam.
- Cambodian Fisheries Administration had limited budget for inter-laboratory proficiency test each year.

6. Recommendations and Suggestions for Future Follow up Action

- This activity should be continued until year 2010 if the budget allows. Budget for implementing all activities should be released earlier.

Indonesia

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1. Introduction

In the recent years, international trade for fish and fish products has been hampered by various tariff and non-tariff barriers particularly on the standards of quality, hygiene and safety of products. Therefore, efforts to improve the quality and safety of the products as well as to ensure the compliance with international standards are highly imperative. Such efforts will enhance market access and competitiveness in the international market and more importantly, it will also help to boost and stabilize the production and marketing of fish products.

Total production of Indonesian fish and fish products over the period of 2000 to 2005 has increased steadily, from 4,875,649 tonnes in year 2000 to 5,452,651 tonnes in year 2005, by volume, with an average increase of 3.51% annually.

Indonesia has been involved in the Japanese Trust Fund II Project to participate in the regional survey on chemical contaminants such as histamine in fish and fish products especially in scombroidae fish species.

Under the Indonesia Food Law No. 7/1996 and Fisheries Law No. 31/204 as well as Government Regulation No. 28/2004 pertaining to Food Quality, Safety and Nutrition; it was clearly stipulated that all food products including fish and fish products put in the market place intended for human consumption shall comply with the prevailing laws and regulations, to ensure that the products are safe and shall not pose any threats to human health.

With the above laws and regulations, it is imperative to formulate the Ministry of Marine Affairs and Fisheries Decree, and clearly define the important requirements to guarantee quality and safety of fish products derived from fishing, collecting vessel, landing sites, auction centers, processing unit and distribution facilities. Three latest Indonesian Regulations and Decrees of Ministry of Marine Affairs and Fisheries are effectively implemented from 1st August 2007. These Regulations and Decrees focused on (1) The Control of Quality Assurance and Food Safety System of Fisheries Products; (2) Monitoring of Drug Residues, Chemicals, Biological Substances and Contaminants in Aquaculture; and (3) Requirements for Quality Assurance and Safety of Fishery Products During Production, Processing and Distribution. In addition, one guideline on the Inspection and Monitoring Program for Fisheries Products is described in the Decree of Director General of Fisheries Products Processing and Marketing.

These Regulations and Decrees are parallel with the activities under the Japanese Trust Fund II Project such as regional surveys on chemical contaminants. These activities are useful and valuable to implement in Indonesia in order to support our national residues monitoring program to produce good and safe Indonesian fish products.

In order to ensure that Indonesian fish and fish products have a low chemical contamination or low level of histamine content, it is mandatory to survey and monitor the fish and fish product. If the concentration of histamine obtained is higher than the maximum residue limit (MRL) the corrective actions would be taken according to the procedure stipulated in the regulation.

2. Objectives and Goals

Through the participation of the research and analysis of histamine in fish and fish products in Indonesia, we expect to be able to:

- Obtain an understanding on the level of histamine content in fish and fish products in Indonesia;
- Set up and implement the monitoring program on histamine in fish and fish products;
- Strengthen the fish inspection and quality control system, including the improvement of laboratory personal skill in conducting fish inspection and quality analysis;
- Improve and facilitate the analysis of chemical

contamination in fish and fish products in Provincial Laboratories for Fish Inspection and Quality Control; and

- Supply fish and fish products that comply with international market standard and food safety requirements.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling method was conducted according to the National Sampling Plan and the monitoring program of each Provincial Laboratory for Fish Inspection and Quality Control (PL-FIQC). During 2005 to 2008, sampling of raw material for histamine analysis was carried out at 6 provinces which are representatives for producing fish and fish products, namely:

(1) Jakarta
(2) East Java
(3) South Sulawesi
(4) North Sulawesi
(5) Bali, and
(6) Maluku

All the raw material collected as samples were marine fish products especially scombroidae and deep sea fish species such as tuna, skipjack tuna, swordfish, marlin, etc. In most cases, raw materials were taken from the fish landing places and some fish processing plants. Frozen fish collected from some processing plants were usually analyzed directly for histamine. The period of sampling was quarterly in 2005 and 2006; every two months in 2007 and 2008.

After sampling, the samples were handled according to their characteristic. For fresh samples, they were handled carefully, quickly and maintained in a cool condition so that the sample characteristics would not change. The temperatures were also recorded. Samples were analyzed as soon as possible. If the analysis should be delayed, samples were kept at –18°C. For samples from locations that are far away, pre-preparation of samples was done in the mini laboratory inside the vehicle for sampling. Pre-extraction with methanol was done prior to laboratory testing. 10 g of sample was weighed into a beaker and 50 ml of methanol was added. The sample mixture was blended and histamine was extracted upon arrival at the laboratory. Nine replicates (n=9) were used for analysis.

b. Method of Analysis

The Spectro-fluorometer method was used for analysing histamine.

c. Limit of Detection and Limit of Quantification

Limit of Detection for Histamine : 2.50 ppm

d. National Regulatory Limits

National Standard Limit of histamine for fish and fish products is 50 ppm.

4. Results and Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Inter-laboratory proficiency testing are usually conducted routinely by the NCQC to all the Provincial Laboratory for Fish Inspection and Quality Control (PL-FIQC) in Indonesia. The focus for proficiency testing is usually microbiology (*E-coli, Salmonella*, etc.) and chemical testing such as heavy metals. Indonesia did not participate in any inter-labratory testing for histamine.

b. Survey Results and Discussion

Survey on histamine in fish and fish products in 2005 and 2006 was focused on the 5 locations namely Jakarta, East Java, Bali, South Sulawesi and North Sulawesi. The sampled species were tuna, marlin, swordfish, red snapper, canned tuna, etc., and the total samples collected were 104 samples. The sampling was conducted on July, October and December 2005. No sample out of the 104 samples analysed was found to contain histamine higher than the Indonesia National Standard of 50 ppm. This means that all sample analyzed complied with Indonesian and international requirements. There is only 1 exception in 2006 for Marlin. The low histamine content especially for 5 samples taken from North Sulawesi might be due to the short landing time or one day fishing from the small fishing vessel for tuna catch.

In 2007, the survey of histamine also focused on the 5 locations namely Jakarta, Bali, South Sulawesi, North Sulawesi and Maluku Provinces and the fish species sampled were tuna, marlin, swordfish, skipjack tuna, red snapper, canned tuna, etc., and the total of sample was 54 samples. Maluku Province was chosen to replace East Java as it produces mostly scombroidae fish species. The results of

histamine content analyzed in 2007 shows that no sample was found to contain histamine higher than the Indonesia National Standard of 50 ppm. This meant that all sample analyzed complied with the Indonesian and international requirements. Some samples taken from Jakarta, Bali, South Sulawesi and North Sulawesi showed low content of histamine as the samples collected were of sashimi grade and is usually exported to Japan.

In 2008, the survey of histamine focused on 4 locations which were Jakarta, Bali, South Sulawesi (Makassar) and North Sulawesi (Bitung) with 2 sampling conducted on February and April 2008 and the raw material was mainly tuna. The results of all samples analyzed shows that the histamine content were lower than the national and international standards.

Table 1. Data Monitoring Histamine Conducted in Jakarta, East Java, Bali, South Sulawesi and North Sulawesi in 2005.

No.	Location	Time of Sampling	Species / Products	Temperature Records During Sampling (°C)	Histamine (ppm)
1.	Jakarta	July 2005	1. Tuna	4.8	30.20
			2. Marlin	5.0	22.00
			3. Canned tuna	Not recorded	18.10
		October 2005	1. Tuna	4.4	24.10
			2. Marlin	4.8	34.40
			3. Swordfish	5.0	8.80
		December 2005	1. Tuna	4.4	16.40
			2. Marlin	5.0	14.20
			3. Canned tuna	Not recorded	18.20
			4. Baby tuna	4.0	22.0
2.	East Java	July 2005	1. Tuna	4.8	38.20
			2. Marlin	5.4	40.20
			3. Canned tuna	Not recorded	8.40
		October 2005	1. Tuna	4.4	22.20
			2. Marlin	4.8	18.10
			3. Swordfish	5.2	20.00
		December 2005	1. Tuna	4.0	16.0
			2. Marlin	5.4	32.40
			3. Baby tuna	4.2	18.40
3.	Bali	July 2005	1. Tuna	4.4	22.0
			2. Marlin	5.0	20.20
			3. Black marlin	4.4	12.30
		October 2005	1. Tuna	4.4	20.80
			2. Marlin	4.8	18.30
			3. Swordfish	4.4	12.00
		December 2005	1. Tuna	4.4	20.00
			2. Marlin	5.0	18.40
			3. Baby tuna	4.8	28.10

4.	South Sulawesi	July 2005	1. Tuna	5.0	24.80
			2. Marlin	5.2	20.20
			3. Black marlin	4.0	14.00
		October 2005	1. Tuna	4.8	30.20
			2. Marlin	5.0	28.10
			3. Swordfish	5.2	32.30
		December 2005	1. Tuna	4.4	22.00
			2. Marlin	4.4	18.10
			3. Baby tuna	4.8	28.20
5.	North Sulawesi	July 2005	1. Tuna	4.0	10.80
			2. Canned tuna	Not recorded	10.10
		October 2005	1. Tuna	4.2	16.40
			2. Marlin	4.4	12.30
			3. Red snapper	5.6	8.10
		December 2005	1. Tuna	4.4	20.30
			2. Canned tuna	Not recorded	16.10
			3. Baby tuna	4.0	12.10

Table 2. Data Monitoring Histamine Conducted in Jakarta, Bali, South Sulawesi and North Sulawesi in 2006.

No.	Location	Time of Sampling	Species / Products	Temperature Records During Sampling (°C)	Histamine (ppm)
1	Jakarta	March 2006	1. Tuna	5.0	28.40
			2. Marlin	5.0	20.60
			3. Swordfish	5.0	22.20
			4. Red Snapper	4.0	11.40
		June 2006	1. Tuna	5.0	40.60
			2. Marlin	5.4	44.30
			3. Swordfish	4.4	18.70
			4. Red Snapper	4.4	10.26
		September 2006	1. Tuna	4.8	34.80
			2. Grouper	4.4	18.40
			3. Swordfish	4.4	24.70
			4. Marlin	5.6	50.20
2.	Bali	March 2006	1. Bluefin tuna	4.4	19.40
			2. Swordfish	4.0	11.50
			3. Marlin	4.4	21.60
			4. Butterfish	4.0	6.70
		June 2006	1. Yellowfin tuna	4.4	24.20
			2. Swordfish	4.0	8.60
			3. Striped marlin	4.0	18.90
			4. Butterfish	4.0	7.20
		September 2006	1. Bluefin tuna	4.4	11.30
			2. Swordfish	4.0	5.40
			3. Black marlin	4.0	12.90
			4. Butterfish	4.0	10.20

3.	South Sulawesi	March 2006	1. Tuna	4.4	16.30
			2. Skipjack tuna	4.0	9.20
			3. Grouper	4.0	6.20
			4. Lether jacket	4.2	5.70
		June 2006	1. Tuna	4.4	14.90
			2. Grouper	4.4	8.60
			3. Lether jacket	4.4	6.40
			4. Baramundi	4.0	4.80
		September 2006	1. Skipjack tuna	4.4	10.60
			2. Tuna	4.4	14.20
			3. Snapper	4.0	6.30
			4. Grouper	4.0	6.80
4	North Sulawesi	March 2006	1. Bluefin tuna	4.4	10.40
			2. Canned tuna	Not recorded	7.60
			3. Marlin	4.8	12.80
			4. Skipjack tuna	4.0	10.80
		June 2006	1. Skipjack tuna	4.8	12.20
			2. Yellowfin tuna	4.4	10.20
			3. Canned tuna	Not recorded	9.40
			4. Bluefin tuna	4.4	9.80
		September 2006	1. Skipjack tuna	4.0	8.90
			2. Canned tuna	Not recorded	10.30
			3. Frozen tuna	4.4	14.20
			4. Marlin	5.0	17.10

Table 3. Data Monitoring Histamine Conducted in Jakarta, East Java, Bali, South Sulawesi, North Sulawesi and Maluku in 2007.

No.	Location	Time of Sampling	Species / Products	Temperature Records During Sampling (°C)	Histamine (ppm)
1.	Jakarta	April 2007	1. Blue-fin tuna	3.8	3.85
			2. Marlin	4.4	18.68
			3. Oil-fish	4.4	2.79
			4. Black marlin	4.4	8.86
		June 2007	1. Bluefin tuna	4.0	14.22
			2. Marlin	4.8	18.60
			3. Swordfish	4.8	6.40
			4. Spanish mackerel	4.4	4.21
		August 2007	1. Bluefin tuna	4.0	3.25
			2. Marlin	4.4	3.68
			3. Spanish mackerel	4.4	2.88
			4. Baby tuna	4.4	12.00

2.	Bali	April 2007	1. Yellowfin tuna	4.0	5.60
			2. Marlin	4.8	13.30
			3. Bluefin tuna	4.0	8.10
		June 2007	1. Yellowfin tuna	4.0	21.60
			2. Marlin	4.0	16.10
			3. Swordfish	4.8	22.00
			4. Bluefin tuna	4.4	15.60
		August 2007	1. Bluefin tuna	4.4	12.90
			2. Marlin	4.4	12.40
			3. Striped marlin	6.0	47.70
			4. Yellowfin tuna	4.4	11.40
3.	South Sulawesi	April 2007	1. Baramundi	4.4	8.10
			2. Bluefin tuna	5.0	20.20
			3. Marlin	4.0	14.60
		June 2007	1. Red snapper	4.4	6.10
			2. Swordfish	4.8	18.60
			3. Marlin	4.8	26.0
		August 2007	1. Bluefin tuna	4.4	12.0
			2. Marlin	4.8	16.40
			3. Baby tuna	4.8	18.10
4.	North Sulawesi	April 2007	1. Bluefin tuna	4.0	3.52
			2. Marlin	5.0	18.69
			3. Yellowfin tuna	4.4	12.67
			4. Black marlin	4.4	14.0
			5. Skipjack tuna	4.0	3.77
		June 2007	1. Yellowfin tuna	3.8	6.11
			2. Marlin	4.8	14.69
			3. Canned tuna	Not recorded	8.73
			4. Skipjack tuna	4.0	5.09
			5. Yellowfin tuna	4.0	6.55
		August 2007	1. Yellowfin tuna	4.4	12.96
			2. Marlin	4.8	9.52
			3. Canned tuna	Not recorded	2.48
			4. Skipjack tuna	4.8	8.84
5.	Maluku	April-May 2007	1. Yellowfin tuna	4.0	10.40
			2. Skipjack tuna	4.0	6.10
		June-July 2007	1. Yellowfin tuna	4.4	16.40
			2. Marlin	5.0	22.30
			3. Skipjack tuna	4.4	8.10
		August-September 2007	1. Yellowfin tuna	4.8	8.30
			2. Canned tuna	Not recorded	12.20
			3. Skipjack tuna	4.4	10.10

Table 4. Data Monitoring Histamine Conducted in Jakarta, Bali, South Sulawesi and North Sulawesi in 2008.

No.	Location	Time of Sampling	Species / Products	Temperature Records During Sampling (°C)	Histamine (ppm)
1.	Jakarta	February 2008	1. Loin tuna *	4.0	10.00
			2. Black marlin*	4.0	20.00
			3. Fresh tuna	4.0	14.00
		April 2008	1. Loin tuna *	4.4	12.00
			2. Marlin*	4.4	18.00
			3. Swordfish*	4.4	6.00
2.	Bali	February 2008	1. Skipjack tuna	4.4	14.20
			2. Fresh tuna	4.4	12.80
			3. Loin tuna*	4.4	11.05
		April 2008	1. Frozen *YF* tuna*	4.4	16.55
			2. Swordfish*	4.4	14.00
			3. Fresh tuna	4.4	10.40
3.	South Sulawesi	February 2008	1. Black marlin	4.4	18.00
			2. Swordfish	5.6	24.00
		April 2008	1. Frozen tuna*	4.8	20.00
			2. Black marlin	4.0	18.00
			3. Swordfish	4.4	16.00
4.	North Sulawesi	February 2008	1. Fresh tuna	4.2	6.95
			2. Fresh loin tuna*	4.4	5.50
			3. Black marlin	4.0	16.00
		April 2008	1. Canned tuna*	Not recorded	16.85
			2. Fresh loin tuna*	4.4	14.00
			3. Fresh tuna	4.0	8.00

Note
Method of Analysis: Spectrofluorometric
Limit of Detection (LOD) of Histamine: 2.50 ppm
* Sample taken from fish processing plant

c. Corrective Actions

The corrective action will be taken when the results of monitoring does not comply with the standard or higher than the standard. The corrective action process for non-compliant results is as follows:

- Carry out the investigation such as traceability to the fishing ground areas.
- Verification of sampling and analysis methods.
- Perform repeat sampling.
- Exclusion of products with higher than the maximum residue limit allowed from establishment or continue processing as products not for human consumption.
- Intensively control the histamine content in fish and fish products during processing or during fishing.

Moreover, in order to ensure the safety of fish and fish products, Indonesia Government undertakes continuous actions such as: (1) Surveillance of stakeholders to ensure their compliance with the rules and regulations in the standards/procedures and (2) Obtain data and information periodically on the level of histamine in fish and fish products.

5. Problems and Challenges Encountered

Problems faced during the histamine monitoring and surveys are as follow:

1) Analysts are not properly qualified to perform histamine analysis using the High Performance Liquid Chromatography (HPLC), and they are still using the Spectrofluorometer.
2) Lack of networking system to harmonize the standard and methods of analysis for histamine among ASEAN countries.
3) Lack of awareness of quality and safety issues amongst fishermen and fish handlers who subject temperature abuse to fish and fish products during landing and in fishing port and results in the increase of histamine content.
4) Lack of suitable infrastructure for preservation, storage and distribution system which caused the increase in histamine content in fish and fish products.
5) Stringent requirements set by importing countries for monitoring program such as histamine in fish and fish products.

6. Recommendations and Suggestions for Future Follow up in Action

1) Need training program for methods validation and verification especially in the determination of the limit of detection and minimum residue performance limit for histamine.
2) Need training to improve the methods of analysis for histamine.
3) Need to establish networking system to develop the method of analysis for histamine among ASEAN countries.
4) Need to establish harmonizing standard especially for maximum level of histamine in order to fulfill the requirements of importing countries such as EU.
5) Need to improve the accuracy of testing results by participating in proficiency testing program especially for histamine among ASEAN countries.

MALAYSIA

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1. Introduction

Histamine is a chemical compound formed in fish muscle after the fish dies, especially for pelagic fish. The formation of histamine in fish muscles is caused by the activity of the histidine *dikarboxilase* enzyme produced by *Proteus morganiii*, a bacteria in the gill and stomach of fish. Fish species with high histidine level in its muscles, specially those from the scrombroid group like tuna and mackerel, will tend to have higher histamine content than in pelagic fish. Non scrombroid group like anchovies, sardines and herring may also have high histamine content.

Histamine formation caused by bacteria can be controlled through good handling practices and fish must be kept at a low temperature as soon as possible after capture. If fish handling practices are poor, histamine content in fish muscles will increase and exceed the safety limit for consumption, leading to histamine poisoning.

Histamine is stable at high temperatures and the accumulation of histamine beyond the safety limit could pose a threat to the consumer. The symptoms of histamine poisoning include itchness of the skin. Unsafe levels of histamine may also be harmful to the digestion and nervous system. Cases of histamine poisoning caused by fish were reported in Japan, United States of America and United Kingdom in the late 1970s. In Malaysia, cases of histamine poisoning had been reported and most of the cases were due to consumption of low quality fish that might be subjected to poor handling.

The Department of Fisheries of Malaysia carried out histamine monitoring in Malaysia for fish landing at main fish landing jetties and complexes. It started from 2001 in a program known as Sanitary and Phytosanitary (SPS) Marine Program. Histamine monitoring under this program is done in Peninsular Malaysia and will be extended to Sabah and Sarawak from 2009 onwards.

Due to the importance of histamine monitoring, the first sample of anchovies was taken from Pahang in 2007. States with many anchovies landing will be included in the next histamine monitoring plan. There is also a plan to monitor fish and fish products, especially those sold at the hypermarkets and shopping complexes, which are likely to have a content of histamine.

2. Objectives And Goals

- To carry out monitoring program to check on the content of histamine in fish for official control and generation of useful database for management control;
- Department of Fisheries Malaysia (DoFM) has implemented the SPS monitoring program in accordance with the WTO requirements to ensure that fish produced in the country is safe to eat and is of good quality; and
- To enhance the on-going SPS program through training and increasing monitoring activities with the JTF program being proposed for 2007/ 08,

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

For the year of 2007, samples for histamine analysis were carried out at an accredited laboratory due to a technical problem faced by the Department of Fishery's histamine laboratory.

Fish samples were collected from the major fish landing jetties/complexes throughout Peninsular Malaysia. The fish species for the sampling of histamine analysis is based on the dominant pelagic fish species landed at the main landing jetties/complexes in the state. There were 18

landing jetties/complexes in 11 states chosen as the sampling locations for histamine monitoring program in 2007.

For histamine sampling, 9 samples were collected at the point of sampling location. One sample may consist of 1 to 3 pieces of fish depending on fish size. The amount of fish muscles needed for the histamine analysis is about 200 g for each sample.

Total number of samples for histamine analysis in 2007 is 468, including samples of anchovies. Temperature of samples were maintained at 0-4^{o}C.

b. Method of Analysis

The method of analysis of histamine in fish is based on Journal of Chromatography A, 809 (1998), 241-245 using HPLC FD.

Sample Preparation

Reagents:

Histamine dihydrochloride, 99% purity
Trichloroacetic acid
Hydrochloric acid
Sodium hydroxide
Borate buffer (pH 9.5) [Dissolve 2.47 g boric acid (Mw = 61.83 g/mol) with 90 mL water in a beaker. Adjust the pH with 10 M NaOH to 9.5. Transfer the solution into a 100 mL volumetric flask and dilute to volume with water].
Ethyl acetate
Methanol
Derivatizing solution [Weigh 50 mg of OPA into a 5-mL volumetric flask. Add 0.2 mL of MeOH into the vial, followed by 50 µL of 2-mercaptoethanol. (Make up to 5ml with buffer solution). [Dilute the content to volume with borate buffer. Keep this solution in the refrigerator and use within a day].
Potassium dihydrogen phosphate
Sodium chloride

Apparatus and Glassware:

Volumetric flasks, pipettes, centrifuge tubes
Sartorius 0.45 micron RC-membrane filter
Whatman filter paper No. 1
Blender
Centrifuge

Standards Preparation:

Histamine Stock Solution: 1000 ppm
Weigh 166 mg of histamine dihydrochloric into a 100 mL volumetric flask. Dissolve the contents and make up to mark with 0.1 M solution of HCl.

Standard Solutions for Calibration:

Histamine Standard: 10 ppm
Pipette 1.0 mL of the stock solution into a 100 mL volumetric flask and make up to mark with 0.1 M solution of HCl.

Preparation of Samples:

(i) Extraction:

TCA procedure: Homogenize 5 g of sample with 10 ml of TCA extracting solution and centrifuge for 15 minutes at 13 rpm (r=0.3m). The aqueous layer was recovered and the organic layer was discarded; the procedure was repeated twice on the sample. The collected aqueous fractions were filtered into a 50 ml flask and then made to volume with 10% TCA.

(ii) Derivatization:

2.0 ml of solution, prepared according to the above method, or standard histamine solutions are transferred into a 20 ml test tube and 1 ml of 1M NaOH. After 5 minutes, 1 ml of derivatizing solution is added. Allow the solution to stand for 10 minutes, and then add 0.5 g NaCl. The solution is extracted with 3 ml of ethyl acetate twice. The aqueous and organic layers are separated by centrifugation. The organic layer is evaporated under N_2 to 100 µl and reconstituted to 2 ml with mobile phase.

Analysis by HPLC:

HPLC system:
Column: Spherisorb C18, 5 micron, 4.6 x 250 mm
Mobile phase: Eluent A (20mM KH_2PO_4 at pH 3): MeOH=50:50
Flow rate: 1.0 mL/min
Detector: Fluor LC305, Ex:216 nm, Em:416 nm
HPLC settings: Range:5, rise time:10, lamp flash rate:100, PMT voltage:500
Injection volume: 20 µL

Calibration:

Calibration is done prior to actual running of the analysis. Standard concentration solutions use for calibration are 0.1 ppm, 0.5 ppm, 1.0 ppm, 5.0 ppm and 10.0 ppm. Calibration curve is then plotted based on the data from these standards.

c. Limit of Detection and Limit of Quantification

Limit of detection and limit of quantification in the analysis are 0.1 ppm and 10 ppm respectively.

d. National Regulatory Limits

There is no regulatory limit for histamine in Malaysia. For the histamine monitoring purposes under the SPS Marine program, the safety limit used is 50 ppm and it is based on the USFDA standard. Fish with histamine content exceeding this limit may cause histamine poisoning to the consumers. Compared to the European Union (EU) standard, histamine limit for the fish products from fish species associated with a high amount of histidine is between 100 – 200 mg/kg (100-200 ppm).

4. Results and Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Malaysia did not participate in any inter-laboratory proficiency testing for Histamine.

b. Survey Results and Discussion

Based on the results of histamine analysis in 2007, all of the samples with histamine detected were within the standard used by the Department of Fisheries Malaysia, the competent authority for fish monitoring in Malaysia. The limit of histamine detected in fish samples are between not detected to 347 ppm. Only 3 landing jetties/complexes at Tanjung Sedeli, Johor; Sg. Besar and Sg. Buloh, Selangor had samples of slightly higher content of histamine in fish. The highest limit detected was at 347 ppm.

Fish samples from the northern states (Perlis, Kedah, Penang and Perak) comprised of Japanese threadfin beam fish (kerisi), mackerel (kembung) and a mixture of a few pelagic species. The histamine content detected for fish samples from the northern states is between 10 - 35 ppm.

Fish samples from Selangor, Melaka, Negeri Sembilan are mainly Dorab wolf-herring (Parang), Narrow-barred Spanish Mackerel (Tenggiri), mackerel (kembung) which are dominant species in this area and also a mixture of few other species. The highest histamine content was detected in the samples from Selangor (Sungai Buloh jetty and Sungai Besar landing jetty) in March 2007 . The average content of histamine for the 9 samples from each of the landing jetty range from not detected to 347 ppm. The other sampling locations reported histamine content of under 77 ppm.

For the southern state (Johor), fish species were taken from a mixture of few pelagic species. 32 samples were taken from two landing jetties, Pontian Besar and Tanjung Sedeli in March, April, July and August with histamine content between 9 ppm - 260 ppm.

The east coast states (Pahang and Terengganu) contributed nearly 50% (225 samples out of 468 samples) of the total histamine samples in 2007. Dominant species from these two states are Shortfin scad (Selayang), Hardtail Scad (Cencaru), Big Eye Scad (Lolong), Round Herring (Tamban), Yellow-striped trevally (Selar kuning), anchovies and also a mixture of a few pelagic fish species. There are also 18 samples of anchovies collected for histamine analysis from Kuantan landing complex in Pahang. The highest histamine content for east coast states was reported at 101 ppm in Kemanaman and Terengganu which was collected in June 2007. Samples from the other sampling locations had low histamine levels.

Table 1. Names of species analysed.

No.	Common Name	Scientific Name
1.	Hardtail scad	*Megalaspis cordyla*
2.	Rainbow sardine/Round herring	*Sardinella fimbriata*
3.	Yellow-striped trevally	*Selaroides leptolepis*
4.	Big eye scad	*Selar crumenophthalmus*
5.	Shortfin scad	*Decapterus macrosoma*
6.	Narrow-barred spanish mackerel	*Scomberomorus commerson*
7.	Dorab wolf-herring	*Chirocentrus dorab*
8.	Swordfish	*Platybelone orgalus platyura*
9.	Mackerel	*Rastrelliger* spp.
10.	Japanese threadfin bream	*Nemipterus* spp.
11.	Indian Anchovy	*Stolephorus indicus*

Table 2. Results of Analysis in 2007.

State	Sampling Location	Month	Common Name	No. of Samples	Average Results (ppm)
Perlis	Kuala Perlis	March	Mix species	9	25 – 35
		August	Mix species	9	10 – 18
Kedah	Kuala Kedah	February	Mix species	9	12 – 24
		June	Japanese threadfin bream (Kerisi)	9	18 – 28
			Mackerel (Kembung)	9	22 – 31
Penang	Teluk Bahang	April	Mix species	9	19 – 31
Perak	Hutan Melintang	March	Japanese threadfin bream (Kerisi)	9	15 – 22
	Pangkor		Mix species	9	25 – 35
Selangor	Sg. Besar	March	Mix species	9	ND – 347
	Sg. Buloh		Mix species	9	ND – 269
	Sg. Buloh	June	Mix species	9	16 – 26
	Sg. Besar		Mackerel (Kembung)	9	19 – 27
	Sg. Buloh	September	Mix species	9	8 – 14
	Sg. Besar		Mackerel (Kembung)	9	8 – 15
N. Sembilan	Port Dickson	May	Dorab wolf-herring (Parang)	8	ND – 11
			Narrow-barred spanish mackerel (Tenggiri)	9	9 – 56
			Swordfish (Todak)	9	23 – 77
Melaka	Kuala Sungai Baru	March	Dorab wolf-herring (Parang)	9	7 – 23
			Narrow-barred spanish mackerel (Tenggiri)	9	ND – 10
		June	Dorab wolf-herring (Parang)	9	17 – 24
			Narrow-barred spanish mackerel (Tenggiri)	9	17 – 25
		September	Dorab wolf-herring (Parang)	9	8 – 14
			Narrow-barred spanish mackerel (Tenggiri)	9	8 – 14
Johor	Pontian Besar	March	Mix species	18	10 – 110
	Tanjung Sedeli	April	Mix species	9	9 -112
		July	Mix species	5	99 – 260
	Pontian Besar	August	Mix species	5	6 – 31
Pahang	Rompin	May	Shortfin scad (Selayang)	9	8 – 16
			Hardtail scad (Cencaru)	9	7 – 13
			Big eye scad (Lolong)	9	7 – 12
	Kuantan		Hardtail scad (Cencaru)	9	7 – 14
	Kuantan	June	Rainbow sardine, Round herring (Tamban)	9	29 – 36
	Rompin		Rainbow sardine, Round herring (Tamban)	9	27 – 37
	Kuantan	September	Rainbow sardine, Round herring (Tamban)	9	17 – 28
	Rompin		Yellow-striped trevally (Selar kuning)	9	17 – 23
	Kuantan		Anchovies (Ikan bilis)	9	35 – 44

State	Sampling Location	Month	Common Name	No. of Sample	Average Result (ppm)
Pahang	Kuantan	September	Mix species	9	9 – 20
	Rompin		Mix species	9	10 – 18
	Kuantan	October	Rainbow sardine/Round herring (Tamban)	9	17 – 28
	Kuantan	November	Shortfin scad (Selayang)	9	9 – 20
			Anchovies (Ikan bilis)	9	36 – 44
	Rompin		Rainbow sardine/Round herring (Tamban)	9	10 – 18
			Anchovies (Ikan bilis)	9	31 – 42
Terengganu	Dungun	June	Mix species	9	ND – 28
	Kuala Terengganu		Mix species	9	30 – 91
	Marang		Mix species	9	7 – 80
	Kemaman		Mix species	9	35 – 101
	Dungun	July	Mix species	9	17 – 28
	Kemaman		Mix species	9	18 – 24
	Kuala Terengganu		Big eye scad (Lolong)	9	14 – 19
	Chendering		Rainbow sardine/Round herring (Tamban)	9	17 – 26
	Kuala Besut		Mix species	9	17 – 24

Recovery of analysis = 80%

5. Problems and Challenges Encountered

- Lack of technical support staff.
- Insufficient information pertaining to fish preservation technique which might affect the fish quality.
- Implementation of good handling practices to assure fish safety and quality.
- Lack of regulatory enforcement.
- Implementation of good hygiene practices on fishing vessel.

6. Recommendations and Suggestions for Future Follow Up Action

- More allocations should be provided to cover for more samples during testing.
- Sampling points should be increased to cover more areas so as to have a more representative sampling distribution for the whole country.
- The frequency of the sampling should be increased depending on the amount of dominant species landed at the sampling locations.

Myanmar

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1. Introduction

The Department of Fisheries (DOF) under the Ministry of Livestock and Fisheries is the government agency responsible for the management of quality and safety of fish and fish products. The department also ensures that requirements of importing countries' rules and regulations are met. A low level of histamine in seafood is a pre-requisite for quality and safety of export products. The recent rejection cases notified to the DOF from the Rapid Alert System for Food and Feed (RASFF) were in March and May 2008 due to high levels of histamine found in dried anchovies exported to UK. The level of histamine detected was more than EU's criteria of 200 ppm. Presently, our laboratory thoroughly check and re-check samples of scombroid and non-scombroid species, especially fish sauce that is produced from anchovies and exported to Thailand.

2. Objectives And Goals

- To determine the level of histamine in fish and fish products made from scombroid species in Myanmar.
- To collect data for the database of the Fish and Fish Products Safety Information Network.
- To use the data for management planning of measures to improve post-harvest handling practices.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

The method of sampling was conducted according to the sampling plan and the monitoring program of each divisional Fishery Office and the Regional Laboratory for Fish Inspection & Quality Control Section (key laboratory). Between 2006-2008, sampling of raw materials was carried out at 5 states and divisions, which are representatives for producing fish and fish products. They are:

(1) Tanintharyi Division
(2) Yangon Division
(3) Rakhine State
(4) Ayeyarwady Division
(5) Mon State

All the samples collected were marine fish products, especially scombroidae such as Spanish Mackerel, Sardine, dried Anchovy, Hilsa, dried Lotia, fish paste, shrimp paste etc. In most cases, raw materials were taken from wholesale fish markets, fish processing plants and canning factories. After the sample collection, the fish and fish products, except canned and dried products, were frozen at –18°C prior laboratory analysis. Some frozen fish from processing plants were usually analyzed directly for histamine.

Sampling locations

Yangon Division (Kyauk Tan Aquaculture Zone)

Tanintharyi Division (Myanmar Garming Processing Plant, Twin Brother Processing Plant)

Yangon Division (Myanmar Makro Canning Factory)

Rakhine State (Lin Aung Processing Plant, Bayintnaung Wholesale Market)

Ayeyarwaddy Division (May Yu Processing Plant, Hypar Pon Fish Market)

Mon state (Bayintnaung Wholesale Market)

Species & Sampling Size for 1st Quarter (Nov 2006-Jan 2007)

Spanish Mackerel (2-3 kg x 1)

Canned Sardine (1 can x 7)

Fresh Sardine (0.10 - 0.25 kg x 7)

Dried Anchovy (Total 1 kg)

Species & Sampling Size for 2nd Quarter (Dec 2007-April 2008)

Spanish Mackerel (2-3 kg x 7)

Fresh Hilsa (0.25 – 0.50 kg x 7)

Dried Anchovy (Total 1 kg)

Dried Lotia (Total 1kg)

Canned Hilsa (1 can x7)

Fish paste (Total 1 kg)

Shrimp paste (Total 1 kg)

Number of Samples

1st Quarter = 36 Samples

2nd Quarter = 63 Samples

Total = 99 Samples

Random sampling with n=9

Sample Preparation
Only the edible portions and fish muscle tissue were used for testing.

b. Method of Analysis

Method of Analysis
Modified Kawabata's Method / Colorimetric 301-D

Method Reference
Training Course in Seafood Safety Management (JSPP 21) 2003 Singapore, MFRD-SEAFDEC

Brand of Instrument
Optima Spectrophotometer (Japan)
Wavelength: 330-1000 nm

c. Limit of Detection and Limit of Quantification

Limit of Detection (LOD) of histamine (Hm) = 5 ppm

Limit of Quantification (LOQ) of histamine (Hm) = 10 ppm

d. National Regulatory Limits

There are no national regulatory limits in Myanmar. DOF of Myanmar complies and adopts the EU Standards and that of the importing countries.

Maximum Permitted Level in Fish & Fish Products enforced by EU, USA, Canada and Japan

SN	Type of Chemical Hazardous	Product Types	EU	USA	Canada	Japan
1.	Histamine (Hm)	Fish paste and fish sauce			200 ppm	200 ppm
2.	Histamine (Hm)	Scombroid fish Products (eg. Tuna, Spanish Mackerel)	200 ppm	50 ppm		100 ppm

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Myanmar did not participate in any inter-laboratory proficiency testing.

b. Survey Results and Discussion

Table 1. Results of analysis for histamine in fish and fish products conducted in 1st quarter (Nov 2006-Jan 2007).

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
Nov 2006, Myanmar Garming Processing Plant in Myeik, Tanintharyi Division	Histamine (Hm)	Spanish Mackerel	*Somberomorus commerson*	9	32.37	44.87	39.26	Captured (Frozen)
Nov 2006, Myanmar Makro Canning Factory, Thaketa, Yangon Division		Sardine (Canned)	*Sardinella gibbosa*	9	20.63	24.54	22.61	Captured (Canned)
Dec 2006, Lin Aung Processing Plant in Rakhine State		Sardine	*Sardinella gibbosa*	9	22.22	30.97	27.63	Captured (Frozen)
Jan 2007, Bayint Naung Wholesale Market for Domestic, Rakhine State		Anchovy (Dried)	*Stolephorus indicus*	9	75.48	142.93	111.36	Captured (Dried)

Table 2. Results of analysis for histamine in fish and fish products conducted in 2nd Quarter (Dec 2007-April 2008).

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max value of results (ppm)	Average value of results (ppm)	Remarks
		Common name	Scientific name					
Dec 2007, Tanintharyi Division (Twin Brother Processing Plant)	Histamine (Hm)	Spanish Mackerel	*Somberomorus commerson*	9	9.82	39.10	22.28	Captured (Frozen)
Dec 2007, Ayeyarwaddy Division (May Yu Processing Plant)		Hilsa	*Tenualosa ilisha*	9	12.12	45.18	30.74	Captured (Frozen)
Jan 2008, Rakhine State (Bayintnaung Wholesale Market)		Anchovy (Dried)	*Stolephorus indicus*	9	12.54	55.31	33.07	Captured (Dried)
Feb 2008, Mon State (Bayintnaung Wholesale Market)		Lotia (Dried)	*Harpodon nehereus*	9	10.97	37.13	19.17	Captured (Dried)
Mar 2008, Yangon Division (Myanmar – Makro Canning Factory Thaketa)		Canned Hilsa	*Tenualosa ilisha*	9	20.20	24. 16	22.03	Captured (Canned)
April 2008, Hypar Pon Fish Market, Ayeyarwaddy Division		Fish paste	_	9	34.52	66.72	51.07	Traditional Product (Fermented)
April 2008, Hypar Pon Fish Market, Ayeyarwaddy Division		Shrimp paste	_	9	24.37	52.72	37.05	Traditional Product (Fermented)

a. Corrective Actions

- Verification of sampling and analytical methods.
- Carry out investigation studies such as traceability measures at the landing site, fishing ground areas and use of product flow diagram from processing plant.
- Intensive monitoring of fish handling.

5. Problems and Challenges Encountered

- The budget is limited
- Problems and constraints faced during project implementation, especially when purchasing chemicals and samples.

6. Recommendations and Suggestions for Future Follow up Action

- There is a need for on-site training in Myanmar.
- This activity gave fruitful outcomes, which are able to support the Myanmar Fisheries Monitoring Programme and reduce the number of RASFF notifications from EU due to high content of Histamine.
- Participation in inter-laboratory proficiency testing will be beneficial.

Philippines

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1. Introduction

The Philippine export of fish and fishery products is a 5 million dollars earner industry. The sustainability of the country's export performance is however being threatened by barriers to trade such as stricter product standards imposed by its trading partners. The European market has become more difficult to penetrate than the United States and Japanese markets due to stringent EU regulations covering issues like traceability, environmental contaminants, and antibiotic residues and histamine. Such issues caused considerable economic losses following product rejection from many Asian countries including the Philippines in the past few years.

The implementation of the project is an initial effort to address the problem in the fishery export industry and aims to develop a science-based data for effective food control system and to identify gaps with regards to food safety and quality issues.

2. Objectives And Goals

- To develop science-based data for food control system which focuses on consumer's health protection.
- To identify gaps in policies and regulations with regards to food safety and quality issues.
- To harmonize food standards with other ASEAN countries.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Quarterly collections of samples were conducted on the following sources:

- Fresh samples from 2 fish landing sites namely General Santos and Zamboanga cities and 3 wet markets in Metro Manila i.e. Malabon, Navotas and Muñoz Markets.

- Canned samples packed in oil/brine/tomato sauce were collected from 3 establishments located at General Santos and Zamboanga Cities.

- Dried anchovies and fermented products from Metro Manila markets. The species of fishes collected were blue marlin, skipjack tuna, yellowfin tuna or long tail tuna, sardines, dried anchovies, dried mackerel and fermented products such as salted anchovies and anchovy sauce.

b. Method of Analysis

Seven samples (n=7) per product type were sampled and immediately kept chilled in ice until the laboratory was reached. Samples were wrapped in PE bags and stored at -30°C until analysis.

Nine samples (n=9) for canned, fermented and dried samples were taken and kept at ambient temperature for one week.

Prior to analysis, physical parameters such as total length, standard length and body weight were taken for fresh fish samples. All the skinned off-fillet, including dark meat but excluding meat from the belly flaps, were prepared and thoroughly minced using a homogenizer.

For canned tuna samples, the products were drained to remove water, brine or similar medium. The samples were then homogenized and blended for 1 minute until homogeneity was obtained.

The method of analysis used is the AOAC, Fluorometric method using a TD-700 fluorometer.

c. Limit of Detection and Limit of Quantification

The LOD and LOQ of the method were:
LOD = 2.67 ppm
LOQ = 8.9 ppm

d. National Regulatory Limits

Fresh/frozen products
USA = 50 ppm

Canada = 100 ppm
EU = 100 ppm

Philippine National Standard = 200 ppm

Dried/fermented products
EU = 200 ppm
Philippine National Standard = 200 ppm

4. Results And Discussion

a. Survey Results and Discussion

This study provided valuable information for evaluating the levels of histamine in fish and fish products available in local markets and fishery establishments.

Table 1. Physical parameters of samples

Common Name	Scientific Name	Code	Sample size (n)	Mean body weight (g)	Mean total length (cm)	Mean standard length (cm)
Yellowfin tuna	*Thunnus albacares*	YT	18	990 ± 55	45.44 ± 9.09	36.7 ± 7.34
Sardines	*Sardinella longiceps*	SA	5	64.25 ± 12.85	17.9 ± 3.58	14.88 ± 2.97
Mackerel *(Dried)*	*Scomberomorus* spp.	MA	12	461.0 ± 36.9	40.9 ± 1.3	37.0 ± 1.1
Anchovies *(Dried)*	*Stolephorus commersonii*	AN	5	1.25 ± 0.25	3.5 ± 0.7	2.8 ± 0.56

The mean total length of finfish ranged from 3.5 cm to 45.44 cm, the mean standard length ranged from 2.8 cm to 37.0 cm and the mean body weight ranged from 1.25 g to 990 g.

Table 2. List of species analysed and results.

Year of analysis & Sampling location	Fish sample analyzed		No. of samples analyzed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Remarks
	Common Name	Scientific Name						
Zamboanga (2005)	Blue marlin	*Makaira nigricans*	Fresh/ Frozen 2	ND	ND	ND	81 - 90	Passed
Zamboanga (2005)	Yellowfin tuna	*Thunnus albacares*	Fresh/ Frozen 4	ND	ND	ND	81 - 90	Passed
General Santos City (2006)	Skipjack tuna	*Katsuwonus pelamis*	Canned 5	ND	ND	ND	81 - 112	Passed
General Santos City (2006)	Yellowfin tuna	*Thunnus albacares*	Fresh/ Frozen 5	ND	ND	ND	87 - 100	Passed
Metro Manila (2006)	Anchovies	*Stolephorus commersonii*	Dried 5	ND	ND	ND	73.5 - 84	Passed
Zamboanga (2006)	Sardines	*Sardinella longiceps*	Fresh/ Frozen 5	4.82	Detected but not quantifiable	Detected but not quantifiable	78 – 103	Passed
General Santos City (2007)	Skipjack tuna	*Katsuwonus pelamis*	Canned 36	3.20	14.5	11.68	81-90	Passed
General Santos City (2007)	Yellowfin tuna	*Thunnus albacares*	Canned 82	ND	Detected but not quantifiable	Detected but not quantifiable	78-101	Passed
Zamboanga (2007)	Sardines	*Sardinella longiceps*	Canned 282	ND	Detected but not quantifiable	Detected but not quantifiable	85-94	Passed
Metro Manila (2008)	Anchovies	*Stolephorus ronquilloi*	Salted 9	25.71	62.71	44.69	94.25	Passed
Metro Manila (2008)	Anchovies	*Stolephorus commersonii*	Sauce 9	27.27	49.77	42.20	105	Passed
Metro Manila (2008)	Mackerel	*Scomberomorus* spp.	Dried 12	95	153.5	120.5	110.22	Passed
Metro Manila (2008)	Yellowfin tuna	*Thunnus albacares*	Fresh/ Chilled 9	ND	112.69	61.15	126.75	Min. Value Passed Max. Value Failed
Pangasinan (2008)	Milkfish	*Chanos chanos*	Marinated 3	ND	ND	ND	95	Passed

Note:
ND = Not Detected
LOD = 2.67ppm
LOQ = 8.9 ppm

In general, samples analyzed had histamine levels below 100 ppm for fresh frozen products and below 200 ppm for dried and fermented products and were therefore safe for human consumption. All canned products for export met the regulatory limits for histamine, indicating good quality products.

Three samples of fresh chilled tuna loins were however found to exceed the level for histamine. The exporter concerned was advised to take some corrective measures in order to avoid the formation of histamine in their products.

b. Corrective Actions

The exporter of the samples of fresh chilled tuna loins that did not meet the standard requirement for histamine was advised by the inspector to take precautionary measures to preclude the same findings in the future. The measures were to closely maintain the temperature of the raw material during processing. This could be done by applying ice on the product throughout the supply chain, from the time it was caught until it reaches the fish processing plant.

5. Problems and Challenges Encountered

- There was a delay in remittance of funds for Year 2005.
- There was a delay in the processing of procurement request.
- There was a need to replace the existing fluorometer as it had broken down.

6. Recommendations and Suggestions for Future Follow up Action

- There is a need to purchase Certified Reference Material (CRM) for comparison of test results.
- Inter-laboratory proficiency testing should be carried out and supported to ensure reliability of data.
- There is a need to strengthen manpower skills on histamine analysis using high performance liquid chromatography (HPLC).

Thailand

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1. Introduction

Scombrotoxin formation, as a result of time and temperature abuse of certain species of fish, can cause scombroid poisoning. Certain bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with free histidine, a naturally occurring chemical that is present in larger quantities in some fish than in others. Histamine –forming bacteria are capable of growing and producing histamine over a wide temperature range. Growth is more rapid at a high-abuse temperature of 21.1°C than at a moderate abuse temperature of 7.2°C. Growth is particularly rapid at temperatures near 32.2°C. Histamine formation is more commonly the result of spoilage due to storage at high temperatures rather than spoilage due to long term, relatively low temperature storage.

Once the enzyme histidine decarboxylase has been formed, it can continue to produce histamine in the fish even if the bacteria are not active. The enzyme can be active at or near refrigeration temperatures. The enzyme is likely to remain stable while in the frozen state and may be reactivated very rapidly after thawing. Freezing may inactivate the enzyme-forming bacteria. Cooking can inactivate both the enzyme and the bacteria. However, once histamine is formed, it cannot be eliminated by heat, including retorting, and freezing. After cooking, recontamination of the fish with the enzyme-forming bacteria will cause additional histamine to form.

The canned tuna is the first priority of export for Thailand and dried anchovies are a favourite among consumers of traditional products. The survey of histamine in the raw material of tuna, canned products and dried anchovies is important for assessing the level of safety for consumption

2. Objectives And Goals

- To survey the histamine level in the raw material of skipjack and in canned products from processing plants in 2005.
- To survey the histamine level in raw material of skipjack and dried anchovies from processing plants in 2006.
- To survey the histamine level in canned products (skipjack, sardine and mackerel) and tuna fish sauce from processing plants in 2007.
- To survey the histamine level in canned products (skipjack and mackerel) and tuna fish sauce from processing plants in 2008.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Year 2005
The central dorsal portion of imported frozen Skipjack (*Katsuwonus pelamis*) were collected during February to December. These samples originated from the Western Pacific Ocean and Indian Ocean. Canned products from the processing plant at Songkhla Province were also collected during the same period. A total number of 297 samples were collected.

The total number of samples for frozen Skipjack was 198. Eighteen replicates were used for each sample (n=18). 2-3 samples of 500g each from each body weight category (0-1.4, 1.5-1.8, 1.9-2.4, 2.5-3.4, 3.5-4.5, 4.6-6.0 and 6.1-9.0 kg) were taken. The samples were kept in a freezer at -18 °C overnight and analysed for histamine the next day.

A total of 99 samples of canned products were collected. Nine replicates were used for each sample (n=9). The samples were kept at room temperature until analysis for histamine.

Year 2006
The central dorsal portions of imported frozen Skipjack (*Katsuwonus pelamis*) were collected

during February to December. These samples originated from the Western Pacific Ocean and Indian Ocean. Dried anchovies (*Stolephorus* spp.) from processing plants were also collected during the same period. A total of 83 samples were collected.

A total of 33 frozen Skipjack samples were collected, using 3 replicates for each lot (n=3/ lot). The samples were kept in a freezer at -18 ^{o}C overnight and analysed for histamine the next day.

A total of 50 dried anchovies samples were collected, using 5 replicates for each lot of 1 kg (n=5, sampling 1 kg/lot). There were 520-2,580 pieces of dried anchovies in each kg collected and the tandard length of the dried anchovies range between 3.75-7.00 cm. The samples were kept at room temperature until analysis for histamine.

Year 2007
Canned products of Skipjack (*Katsuwonus pelamis*), Sardine (*Sardinella gibbosa*) and Mackerel (*Decapterus maruadi*) and Tuna fish sauce from processing plants in Songkhla Province were collected from April to October. The total number of samples collected was 144 samples. Nine replicates were used (n=9) for each sample for analysis. The samples were kept at room temperature until analysis for histamine.

Year 2008
Canned products of Skipjack (*Katsuwonus pelamis*), Mackerel (*Decapterus maruadi*) and Tuna fish sauce from processing plants in Songkhla Province were collected from April to May. A total of 81 samples were collected. Nine replicates (n=9) were used for analysis. The samples were kept at room temperature until analysis for histamine.

b. Method of Analysis

Purpose
Determination of histamine level using fluorometric method.

Scope and application
Quantitative test of histamine in fish and fish products.

Reference
AOAC, 2005.

Principle of the method
The analyte wass extracted with methanol. The extract was passed through an ion-exchange column. O-Phthaldialdehyde solution was then added to the elute to form fluorescent histamine derivatives. The fluorescence intensity of derivatives was measured using a fluorometer and the level of histamine was quantified with an external standard.

Sample preparation
Whole fish should be analyzed immediately after grinding, however, if this is possible, the samples should be frozen quickly to ensure there is no further decomposition.

For products that are packed in water, brine or sauce, the products were drained for 1 minute. The samples were then blended until they were homogenous.

Apparatus
- Electronic balance
- Water bath
- Fluorometer : Turner Quantech, Excitation wavelength 360 nm and Emission wavelength 450 nm
- Homogenizer
- Beaker
- Paper filter No. 1
- Dispenser
- Volumetric flask grade A (10, 25, 100, 500 and 1,000 ml)
- Volumetric Pipette grade A (1, 2, 3, 4, 5, 10 and 20 ml)
- Erlenmeyer flask
- Chromatographic tube

Reagents
- Ion – exchange resin – Bio Rad AG 1-x8, 50-100 mesh
  The resin was convert to – OH form by adding 15 ml of NaOH for every gram of resin to a beaker. The mixture was swirled and left to stand about 30 minutes. The liquid was decanted liquid. The process was repeated with additional base. The resin was then washed throughly with H_2O.

  The resin was packed into a chromatography tube to a height of 8 cm while maintaining water above the resin bed at all times. The column was washed with 10 ml of water each time before adding an extract.

- 85% Phosphoric acid (85% H_3PO_4)

- 3.57N Phophoric acid
  12.18 ml of 85% H_3PO_4 was diluted in a 100 ml volumetric flask.

- 0.1% O-Phthaldialdehyde (OPT) solution
  0.1 g of OPT was dissolved in 100 ml methanol. The solution was stored in a refrigerator. This solution had to be prepared fresh weekly.

- Hydrochloric acid (HCl)

- 1.0N Hydrochloric acid (1.0N HCl)
  8.33 ml of concentrated HCl was diluted into a 100 ml volumetric flask with deionized water.

- 0.1N Hydrochloric acid (0.1N HCl)
  8.33 ml of concentraed HCl was diluted into a 1,000 ml volumetric flask with deionized water

- Sodium Hydroxide (NaOH)

- 1N Sodium Hydroxide (1N NaOH)

- 2N Sodium Hydroxide (2N NaOH)
  8 g of NaOH was dissolved in deionized water and diluted to 100 ml in a volumetric flask.

- Histamine dihydrochloride

- Histamine standard stock solutions (1000 ppm)
  0.0423 g of standard histamine dihydrochloride was dissolved with 0.1N HCl and diluted to 25 ml in a volumetric flask. The solution was stored in a refrigerator. The solution had to be prepared fresh weekly.

  For standard working solutions, pipette 5 ml of 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 ug/g for standard histamine. Histamine contents are 0.05, 0.25, 0.50, 0.75, 1.0, 1.25 and 1.50 ug respectively. Prepare fresh daily.)

Procedure of testing

- Sample extraction

(i) 5.000 $\pm$ 0.020 g of sample was weighted into a 50 g beaker. 20 ml of methanol was added and the mixture was blended for 2-3 minutes. The blended mixture was then transferred to a 50 ml volumetric flask.

(ii) The flask was placed for 15 minutes in a hot water bath at 60 $\pm$ 5 ^{o}C.

(iii) The contents were then cooled to room temperature and diluted to volume with methanol.

(iv) The contents were filtered and the filtrate was stored in a refrigerator until it is to be analyzed.

- Purification

(i) Impurities from the extract were removed by passing 4 or 5 ml of water through the ion exchange column. This solution was then discarded.

(ii) 1 ml of the extract was pipetted into the column and 5 ml of water was added. The column flow was initiated immediately and the eluate was collected into a 50 ml volumetric flask which contains 5 ml of 1N HCl.

(iii) Flow rate was controlled at > 3 ml/min by adjusting the height of column relative to the tubing outlet. When the liquid level is approximately 2 mm above the top of the resin, 5 ml of water was added, followed by increasingly large volumes of water until a total of 35 ml was eluted . The flow was stopped and the collected eluate was top up to 50 ml with water and mixed throughly.

- Derivatization and fluorometric measure

(i) 5 ml of sample solution and 10 ml of 0.1N HCl were pipetted into a Erlenmeyer flask. 3 ml of 1.0N NaOH was added and mixed. Within 5 minutes, 1 ml of 0.1% OPT solution was pipetted and the solution was mixed immediately. After exactly 4 minutes, 3 ml of 3.57N H_3PO_4 was added and the solution was mixed immediately. It is important to mix thoroughly after each addition and at least once during the OPT reaction.

(ii) Blank and standard solutions were prepared by pipetting into the Erlenmeyer flask containing 5 ml of 0.1 N HCl, 5 ml of 0.01, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 ug/ml histamine working standards. The above procedure [(i) and (ii)] were followed.

(iii) Within 1.5 hour, fluorescence intensity(I) using excitation wavelength of 360 nm and emission wavelength of 450 nm were recorded.

(iv) If fluorescence intensity of sample is greater than the fluorescence intensity of histamine standard (0.30 ppm). The eluate must be diluted with 0.1N HCl after that preparation of the fluorophore.

- Calculation

(i) The plot of I (measured by meter deflection or recorder response and corrected for blank) against histamine/5 ml of test solution un ug should be a straight line passing through the intercept.

(ii) Results were calculated as follows:
Histamine (ppm) = (50/5) x (50/W) x (Cs-Crb) x (E/D)
Cs = Histamine solution of sample from calibration curve
Crb = Histamine solution of reagent blank from calibration curve
W = Sample weight (g)
D = Solution volum for dilution (ml)
E = Total solution volume after dilution (ml)

c. Limit of Detection and Limit of Quantification

Fish and fish product
Limit of Detection= 3.00 ppm,
Limit of Quantification = 5.00 ppm

Tuna fish sauce
Limit of Detection= 35.00 ppm,
Limit of Quantification = 50.00 ppm

d. National Regulatory Limits

Thailand	100 ppm
Australia and New Zealand	200 ppm
China	100 ppm
Canada	100 ppm
EU	m = 100 ppm, M = 200 ppm (n=9,c=2)
Russia	100 ppm
USA	50 ppm

Remarks:
n = number of units comprising the sample
c = number of sample units giving values > m or between m and M.

Results are satisfactory if:
1) mean value does not exceed m.
2) 2 samples have values more than m but less than M.
3) No samples have a value exceeding M.

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results	True value	z-score	Remarks
2005	CFIA	Histamine	7.81 (tissue) 2.02 (tissue) 14.52 (sauce) 21.06 (sauce)	7.44 2.34 14.54 20.54	0.31 -0.49 0.07 0.13	Passed
2007	FAPAS	Histamine	32.24 (canned fish)	38.1	-1.7	Passed

b. Survey Results and Discussion

Year of analysis & Sampling location	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Remarks
	Common name	Scientific name						
2005 & Factory of Songkhla	Skipjack	*Katsuwonus pelamis*	198	0.53 (ND)	2.65 (ND)	1.2491 (ND)	95.80%	Frozen
	Skipjack	*Katsuwonus pelamis*	99	0.83 (ND)	35.41	7.8455	88.50%	Canned
2006 & Factory of Songkhla	Skipjack	*Katsuwonus pelamis*	33	0 (ND)	6.16	1.1760 (ND)	85.00%	Frozen
	Anchovies	*Stolephorus* spp.	50	1.77 (ND)	154.87	36.7660	88.00%	Dried
2007 & Factory of Songkhla	Skipjack	*Katsuwonus pelamis*	27	0 (ND)	4.65 (<LOQ)	1.7430 (ND)	98.96%	Canned
	Long tail Tuna	*Thunnus tonggol*	9	3.79 (<LOQ)	5.88	4.6744 (<LOQ)	90.10%	
	Sardine	*Sardinella gibbosa*	36	0 (ND)	4.33 (<LOQ)	1.9450 (ND)	92.52%	Canned
	Mackerel	*Decapterus sp.*	36	0 (ND)	2.27 (<LOQ)	0.8781 (ND)	95.00%	Canned
	Tuna sauce	–	36	71.31	136.55	103.3070	110.00%	Fermented
	Skipjack	*Katsuwonus pelamis*	27	0.42 (ND)	6.76	3.1048 (<LOQ)	87.56%	Canned
2008 & Factory of Songkhla	Mackerel	*Decapterus sp.*	27	1.1 (ND)	8.13	3.5041 (<LOQ)	85.16%	Canned
	Tuna sauce	–	27	83.03	144.04	106.2130	90.16%	Fermented

c. Corrective Actions

The histamine results were in the acceptable range, therefore no corrective actions were taken.

5. Problems and Challenges Encountered

Nil.

6. Recommendations and Suggestions for Future Follow up Action

MFRD should arrange for proficiency testing in histamine for ASEAN member.

Vietnam

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1. Introduction

The control of potential chemical risks, including residues of antibiotics, veterinary drugs, heavy metals, pesticides and growth promoters, in fish and fish products has become very important because it may seriously affect consumer's health.

The increase in export of fish and fish products to high-income countries requires strict standards on hygiene and safety. Specific requirements on chemical residues in fish and fish products are consequently set up.

For this reason, surveys and studies activities organized by SEAFDEC and funded by JTF IV were significant, as they contribute to providing an overview on residue levels of chemicals and contaminants in aquaculture fish in South-East Asia. Member countries would then determine appropriate policies to ensure quality and safety of fish and fish products, meet importing countries' requirements and protect the health of domestic consumers.

In Vietnam, as the competent national authority responsible for ensuring quality, hygiene and safety of fish and fish products since 1997, the National Fisheries Quality Assurance and Veterinary Directorate – NAFIQAVED (now renamed as the National Agriculture, Forestry and Fisheries Quality Assurance Department-NAFIQAD) has been carrying out the Monitoring Program for certain harmful substances and residues in aquaculture animals. NAFIQAD had also actively participated in the survey titled "Research and Analysis of Chemical Residues and Contaminants in fish and fish products" as well as other activities organized by SEAFDEC.

2. Objectives And Goals

Surveys of histamine are carried out to provide information on the level of histamine present in fish and fish products. The data obtained was deposited into the database of the Fish and Fish Products Safety Information Network. The network's website: www.fishsafetyinfo.com contains general information on fishery hygiene and safety in SEAFDEC's member countries.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

- NAFIQAD led to implement all activities in 2006 to 2008.
- NAFIQAD branches took samples and analyzed samples as required by the activity.
- Activities carried out by NAFIQAD including the following:
  (i) Activities using SEAFDEC's budget;
  (ii) Activities using budget from the Monitoring Program for certain harmful substances and residues in aquaculture animals and certification activities for exported fishery consignments.
- NAFIQAD has signed contracts with its Branches for the implementation of activities, and then is responsible for data analysis and treatment and making report.
- NAFIQAD branches carry out the procedures of sampling, sample preservation, dispatch of samples to laboratories and analysis, in compliance with the Residues Monitoring Program Manual and Quality Manual of NAFIQAD.
- Species studied:
  Anchovy (*Encrasicholina heteroloba*)
  Skipjack Tuna (*Sarda orientali*)
  Bigeye Tuna (*Thunnus albacares*)
  Sardine (*Sardinella gibbosa*)

b. Method of Analysis

Method: HPLC – Fluorescence Detector (MFRD method)

Equipment: HPLC Water 2695

c. Limit of Detection and Limit of Quantification

Limit of detection (LOD): 1 ppb

Limit of quantification (LOQ): 3 ppb

d. National Regulatory Limits

MRL of Vietnam: 20 mg/100g

MRLs of other markets:

EU: n = 9, c = 2, m = 100 mg/kg, M = 200 mg/kg

n = number of units comprising the sample

c = number of sample units giving values >m or between m and M.

Results are satisfactory if:

1) Mean value does not exceed m.
2) 2 samples have values more than m but less than M.
3) No samples have a value exceeding M.

USA : 50 mg/kg

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results	True value	z-score	Remarks
2005-2006	Fapas Proficiency Test 2720 Histamine in Canned Fish	Histamine	29.08	28.4	0.3	Passed

b. Survey Results and Discussion

Table 1. Results of histamine levels in samples analysed in 2006.

No	Sample	SEAFDEC's budget			Vietnam's budget			Total		
		No. of samples	Analysis results (ppm)	No. of samples exceeding MRL	No. of samples	Analysis results (ppm)	No. of samples exceeding MRL	No. of samples	Analysis results (ppm)	No. of samples exceeding MRL
1	Dried anchovy	18	2-50	0	49	2-185	0	67	2-185	0
2	Frozen tuna	18	5-87	0	701	2-120	0	719	2-120	0
3	Canned tuna	18	5-48	0	782	5-75	0	796	5-75	0
4	Canned Sardines	9	3-54	0	1	1-45	0	10	1-54	0
5	Others	-	-	-	1143	2-283	12	1143	2-283	12

Table 2. Results of histamine levels in samples analysed in 2007.

No	Sample	SEAFDEC's budget			Vietnam budget			Total		
		No. of samples	Analysis results (ppm)	No. of samples exceeding MRL	No. of samples	Analysis results (ppm)	No. of samples exceeding MRL	No. of samples	Analysis results (ppm)	No. of samples exceeding MRL
1	Dried anchovy	27	5-48	0	138	5-135	9	165	5-135	9
2	Frozen tuna	27	5-49	0	3,116	5-152	10	3,193	5-152	10
3	Canned tuna	27	5-48	0	2,307	5-141	8	2,334	5-141	8
4	Canned Sardines	-	-	-	112	ND	0	112	ND	0
5	Fish sauce	-	-	-	44	25-100	0	44	25-100	0
6	Others	-	-	-	1,143	5-40	0	1,143	5-40	0

Table 3. Results of histamine levels in samples analysed in 2008.

No	Sample	SEAFDEC's budget			Vietnam budget			Total		
		No. of samples	Analysis results (ppm)	No. of samples exceeding MRL	No. of samples	Analysis results (ppm)	No. of samples exceeding MRL	No. of samples	Analysis results (ppm)	No. of samples exceeding MRL
1	Dried anchovy	27	10-53	0	-	-	-	165	5-135	9
2	Frozen tuna	27	ND-89	0	-	-	-	3,193	5-152	10
3	Canned tuna	27	ND-68	0	-	-	-	2,334	5-141	8

c. Corrective Actions (if applicable)

Nil.

5. Problems and Challenges Encountered

Nil.

6. Recommendations and Suggestions for Future Follow up Action

Nil.

Regional Survey of Chloramphenicol, Nitrofuran, Malachite Green and Leuco-Malachite Green in Fish and Fish Products

- Indonesia
- Malaysia
- Myanmar
- Philippines
- Thailand
- Vietnam

Indonesia

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1. Introduction

The total production of Indonesian fish and fish products over the period of 2000 to 2005 has increased steadily from 4,875,649 tonnes in year 2000 to 5,452,651 tonnes in year 2005, by volume, with an average increase of 3.51 % annually. The total production from aquaculture in year 2005 was about 238,656 tonnes of which the black tiger shrimp (*Penaeus monodon*) was 134,682 tonnes and *Penaeus vannamei* shrimp was 103,874 tonnes.

In recent years, international trade for fish and fish products has been hampered by various tariff and non-tariff barriers particularly on the standards of quality, hygiene and safety of products. Therefore, efforts to improve the quality and safety of the products as well as to ensure the compliance with international standards are highly imperative. Such efforts will enhance market access and competitiveness in the international market and more importantly, it will also help to boost and stabilize the production and marketing of fish products.

Under the Indonesia Food Law No. 7/1996 and Fisheries Law No. 31/204 as well as Government Regulation No. 28/2004 pertaining to Food Quality, Safety and Nutrition; it was clearly stipulated that all food products including fish and fish products put in the market place intended for human consumption shall comply with the prevailing laws and regulations, to ensure that the products are safe and shall not pose any threats to human health.

With the above laws and regulation, it is imperative to formulate Ministry of Marine Affairs and Fisheries Decree, and clearly define the important requirements to guarantee quality and safety of fish products derived from fishing, collecting vessel, landing sites, auction centers, processing unit and distribution facilities. The 4 newest Indonesian Regulations and Decrees of Ministry of Marine Affairs and Fisheries are effectively implemented from 1st August 2007. These Regulations and Decrees focused on, (1) The Control of Quality Assurance and Food Safety System of Fisheries Products; (2) Monitoring of Drug Residues, Chemicals, Biological Substances and Contaminants in Aquaculture; (3) Requirements for Quality Assurance and Safety of Fishery Products During Production, Processing and Distribution; and (4) Good Aquaculture Practices. In addition, two guidelines for monitoring are described in:

1) Decree of Director General of Fisheries Products Processing and Marketing covering the Inspection and Monitoring Program for Fisheries Products,
2) Decree of Director General of Fisheries Aquaculture covering the Guidelines on the Implementation of Residue Monitoring of Fish Drugs, Chemical, Biological Agents and Contaminants in Aquaculture.

Both decrees are parallel with the activities under the Japanese Trust Fund II Project such as regional surveys on drug residues (chlorampenicol and nitrofurans). These activities are useful and valuable to implement in Indonesia in order to support our national residues monitoring program to produce good and safe of Indonesia fish products.

In order to ensure that Indonesian aquaculture products are free from drug residues contamination such as chloramphenicol (CAP) and nitrofurans (NF), it is mandatory to survey and monitor the aquaculture products. If the drug residues obtained is higher than the maximum residue limit (MRL) the corrective actions would be taken according to the procedure stipulated in the regulation.

2. Objectives and Goals

Through the participation of the research and analysis of drug residues in aquaculture products of Indonesia, we expect to be able to:

- Obtain an understanding of the level of drug residues in aquaculture products in Indonesia;
- Set up and implement the monitoring program on drug residues in aquaculture products;
- Strengthen the fish inspection and quality control system, including improvement of laboratory personal skills in conducting fish inspection and quality analysis;
- Improve and facilitate the analysis of chemical contaminant and drug residues in aquaculture products in Provincial Laboratories for Fish Inspection and Quality Control; and
- Supply fish and fish products including aquaculture products that comply with the international market standard and food safety requirements.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling method was conducted according to the National Sampling Plan and the monitoring program of each Provincial Laboratory for Fish Inspection and Quality Control (PL-FIQC). From 2005 to 2008, the sampling of raw materials for drug residues analysis was carried out at 8 provinces which are representatives of shrimp producing areas. The sampling locations were:

(1) North Sumatera
(2) South Sumatera
(3) Lampung
(4) West Java
(5) Jakarta
(6) East Java
(7) East Kalimantan, and
(8) South Sulawesi

All the samples collected were aquacultured products such as shrimp *Penaeus monodon* and *Penaeus vannamei.* In most cases, the samples were collected from farms but some other frozen shrimp samples were also collected from some processing plants. After sample collection from the farms, the samples were frozen at -18ºC prior laboratory analysis. Frozen shrimp samples collected from some processing plants were usually analysed for drug residues (CAP and NF) directly. The period of sampling was quarterly in 2005 and 2006; every two and three months in 2007 and 2008.

b. Method of Analysis

The High Performance Liquid Chromatography (HPLC) method was used to analyse drug residues and the Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method was used for analyzing NFs in some samples.

c. Limit of Detection and Limit of Quantification

Limit of detection (LOD) :
CAP : 0.12 ppb
Nitrofurans : 1 ppb

Minimum Required Performance Limit (MRPL):
MRPL for CAP : 0.3 ppb
MRPL for Nitrofuran : 1 ppb

Note:
Latest method validation results for drug residues analysis methods conducted by NCQC are as follow:

Limit of Detection (LOD):
CAP: 0.12 ppb
Nitrofurans:

Antibiotic	Name of Metabolite	LOD (ppb)
Furaltadon	AMOZ	0.93
Nitrofurazon	SEM	0.81
Nitrofurantoin	AHD	0.61
Furazolidon	AOZ	0.23

d. National Regulatory Limits

The setting of the standard limits for chlorampenicol and nitrofurans are under progress. Hence, the standard limits are currently based on the European Union (EU) regulation or importing countries standard.

4. Results and Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Inter-laboratory proficiency testing are usually conducted routinely by the NCQC to all the Provincial Laboratory for Fish Inspection and Quality Control (PL-FIQC) in Indonesia. The focus for proficiency testing is usually microbiology (*E-coli, Salmonella* etc.) and chemical testing such as heavy metals. Indonesia did not participate in any interlaboratory proficiency testing for drug residues.

b. Survey Results and Discussion

Survey of drug residues in 2005 only focused on chloramphenicol (CAP). Five sampling locations or provinces were targeted in, Lampung, North Sumatera (Medan), East Java (Surabaya), West Java and South Sulawesi. The samples collected from these locations were aquacultured shrimp (*Penaeus vannamei* and *Penaeus monodon*). The sampling period was every 3 monthly and 3 times a year, that is, on July, October and December 2005. The total number of samples were 48 samples and the results showed that all sample was negative (not detected).

In 2006, the survey of drug residues focused on chloramphenicol (CAP) and nitrofurans (NF). The sampling locations were different from the survey conducted in 2005. The sampling locations in 2006 were, Lampung, South Sumatera, Jakarta, East Java (Surabaya), South Sulawesi and East Kalimantan. All samples collected from these locations were aquacultured shrimp (*Penaeus vannamei* and *Penaeus monodon*). The sampling period was every 2 months and 4 times a year, that is, May, July, September and December 2006. From the 177 samples analyzed, 7 samples showed positive results for chlorampenicol and the results were between 0.17 to 2.50 ppb.

Survey of drug residues in 2007 was carried out 6 locations, namely, North Sumatera (Medan), Lampung, Jakarta, West Java (Cirebon), East Java (Surabaya) and South Sulawesi (Makassar). However, the survey of nitrofurans was conducted only for samples collected from East Java. All samples collected from these locations were aquacultured shrimp (*Penaeus vannamei* and *Penaeus monodon*). The sampling period was quarterly, on April, June and August 2007. For the sampling at East Java, the samples were collected three times with the first sampling conducted on March to April; second sampling on June to July and the third sampling on August to September 2007. The results showed that from the 63 samples analysed for CAP and 13 samples analysed for nitrofurans, most of the samples were not detected of both residues. One sample collected from Lampung (*Penaeus monodon*) was found to contain CAP (0.31 ppb) higher than the MRPL stipulated at 0.3 ppb. For NF, the result from one sample (1.15 ppb) was found to be higher than the stipulated MRPL at 0.1 ppb.

Comparing with the survey results of drug residues obtained in 2005 and 2006, the violation in shrimp products decreased in 2007. This might due to the new revision of the regulation issued in the beginning of 2007 and since it's implementation, the shrimp farmers pay more attention to the issues of food quality and drugs residues. The shrimp farmers are also more aware in issues such as prohibition for using of some chemicals or antibiotics in aquaculture farms. Moreover, according to the Indonesian regulation, all aquaculture farms must be evaluated for Good Aquaculture Practices certification. These requirements are very stringent and hence the monitoring of veterinary drugs residues in farms must be done continuously.

Survey of drug residues in 2008 was focused on 6 locations, namely North Sumatera (Medan), Lampung, Jakarta, West Java (Cirebon), East Java (Surabaya), and South Sulawesi. All samples collected from these locations are aquacultured shrimp products (*Penaeus vannamei* and *Penaeus monodon*). The results showed that all of samples analysed for CAP and NF (AOZ and AMOZ) were negative (not detected).

Table 1. Data Monitoring of Drug Residues in Shrimp Conducted in Lampung, North Sumatera, East Java, West Java and South Sulawesi in 2005.

No.	Location	Time of Sampling	Species/ Products	Number of sample	CAP (ppb)
1.	Lampung	July	*P. monodon*	3	ND
		October	*P. monodon*	4	ND
		December	*P. monodon*	3	ND
2.	North Sumatera	July	*P. monodon*	4	ND
		October	*P. vannamei*	4	ND
		December	*P. monodon*	3	ND
3.	East Java	July	*P. vannamei*	6	ND
		October	*P. vannamei*	6	ND
		December	*P. vannamei*	2	ND
4.	West Java	July	*P. vannamei*	3	ND
		October	*P. vannamei*	2	ND
		December	*P. vannamei*	2	ND
5.	South Sulawesi	July	*P. vannamei*	1	ND
		October	*P. vannamei*	2	ND
		December	*P. vannamei*	3	ND

Table 2. Data Monitoring of Drug Residues in Shrimp Conducted in Lampung, South Sumatera, East Java, South Sulawesi and East Kalimantan in 2006.

No.	Location	Time of Sampling	Species / Products	Number of sample	CAP (ppb)	Nitrofuran (ppb)
1.	Lampung	May	*P. monodon*	4	ND	ND
		July	*P. monodon*	3	ND	ND
		Sep.	*P. monodon*	4	ND	ND
		Nov.	*P. monodon*	3	ND	ND
2.	South Sumatera	May	*P. monodon*	4	ND	ND
		July	*P. monodon*	8	ND	ND
		Sep.	*P. vannamei*	4	ND	ND
		Nov.	*P. monodon*	2	ND	ND
3.	Jakarta	May	*P. monodon**	3	ND	ND
		July	*P. vannamei**	8	1 sample (0.46 ppb)	ND
		Sep.	*P. vannamei**	3	1 sample (2.5 ppb)	ND
		Nov.	*P. vannamei**	3	1 sample (0.17 ppb)	ND
4.	East Java	May	*P. vannamei**	8	ND	ND
		July	*P. vannamei*	6	ND	ND
		Sep.	*P. vannamei*	7	ND	ND
		Nov.	*P. vannamei*	6	ND	ND

5.	South Sulawesi	May	*P. vannamei*	2	ND	ND
		July	*P. vannamei*	2	ND	ND
		Sep.	*P. vannamei*	4	ND	ND
		Nov.	*P. vannamei*	6	1 sample (0.99 ppb)	ND
6.	East Kaliman-tan	May	*P. monodon*	6	ND	ND
		July	*P. vannamei*	9	1 sample (0.18 ppb)	ND
		Sep.	*P. vannamei*	9	1 sample (0.54 ppb)	ND
		Nov.	*P. vannamei*	3	1 sample (1.42 ppb)	ND

Table 3. Data Monitoring of Drug Residues in Shrimp Conducted in North Sumatera, *Lampung, Jakarta, West Java, East Java and South Sulawesi in 2007.*

No.	Location	Time of Sampling	Species/ Products	Number of sample	CAP (ppb)	Nitrofuran (ppb)
1.	North Sumatera	April	*P. vannamei*	3	ND	-
		June	*P. vannamei*	2	ND	-
		August	*P. vannamei*	3	ND	-
2.	Lampung	April	*P. monodon*	3	ND	-
		June	*P. monodon*	4	ND (1 sample 0.31 ppb)	-
		August	*P. monodon*	3	ND	-
3.	Jakarta	April	*P. vannamei**	2	ND	-
			*P. monodon**	2	ND	-
		June	*P. vannamei**	3	ND	-
			*P. monodon**	2	ND	-
		August	*P. vannamei*	3	ND	-
4.	West Java	April	*P. vannamei*	4	ND	-
		June	*P. vannamei*	4	ND	-
		August	*P. vannamei*	3	ND	-
5.	East Java	March-April	*P. vannamei*	2	ND	ND
			*P. monodon**	2	ND	ND
		June	*P. vannamei*	2	ND	ND
			*P. monodon**	3	ND	ND (1 sample: 1.15 ppb)
		July-August	*P. vannamei*	2	ND	ND
			P. monodon	2	ND	ND
6.	South Sulawesi	April	*P. vannamei*	2	ND	-
		June	*P. vannamei*	2	ND	-
			P. monodon	2	ND	-
		August	*P. vannamei*	3	ND	-

Table 4. Data Monitoring of Drug Residues in Shrimp Conducted in North Sumatera, Lampung, Jakarta, West Java, East Java and South Sulawesi in 2008.

No.	Location	Time of Sampling	Species / Products	Number of sample	CAP *(ppb)*	Nitrofuran *(ppb)*
1.	North Sumatera	February	*P. vannamei**	2	ND	-
		April	*P. vannamei**	4	ND	-
2.	Lampung	February	*P. vannamei*	3	ND	-
		April	*P. vannamei*	4	ND	-
3.	Jakarta	February	*P. vannamei**	4	ND	-
		April	*P. vannamei**	4	ND	-
4.	West Java	February	*P. monodon*	2	ND	-
		April	*P. vannamei*	1	ND	-
5.	East Java	February	*P. vannamei**	4	ND	ND *(AOZ & AMOZ)*
		April	*P. vannamei**	4	ND	ND *(AOZ & AMOZ)*
6.	South Sulawesi	February	*P. monodon & vannamei*	6	ND	-
		April	*P. monodon & vannamei*	6	ND	-

Note:
Methods of Analysis: HPLC and LC-MS/MS for Nitrofurans (AOZ & AMOZ)
ND = Not Detected
* Sample taken from fish processing plant

c. Corrective Action

The corrective action will be taken when the results of monitoring does not comply with the standard or higher than the standard. The corrective action process for non-compliant results is as follows:

1. Carry out the investigation such as traceability to the fishing ground or aquaculture areas.
2. Verification of sampling and analysis methods.
3. Perform repeat sampling.
4. Exclusion of products with higher than the maximum residue limit allowed from establishment or continue processing as products not for human consumption.
5. Intensively control the drug residues in fish during aquaculture or farming.

Moreover, in order to ensure the food safety of aquaculture products, Indonesia Government undertakes continuous actions such as: (1) Surveillance of stakeholders to ensure their compliance with rules and regulations in the standards/procedure and (2) Obtain data and information periodically on the level of veterinary drug residues and contaminants in aquaculture products.

5. Problems and Challenges Encountered

Problems faced during implementation of the drug residues monitoring and surveys are:

1) Lack of suitable equipment such as LC-MS/MS especially for analysing nitrofurans and it's derivatives (between 2004 to 2006).
2) Laboratory personnel are not trained/qualified to perform drug residues analysis using HPLC and LC-MS/MS.
3) Lack of networking system to harmonize the standard and analysis methods for drug residues among ASEAN countries.
4) Lack of law enforcement in Indonesia to control the distribution of chemicals and veterinary drugs.
5) Implementation of the National Residues Control Plan (NRCP) due to the stringent requirements set by importing countries such as European Union (EU).

6. Recommendations and Suggestions for Future Follow up Action

1) Need training program for methods validation and verification especially in the determination of the limit of detection and minimum residue performance limit for drug residues.
2) Need training on LC-MS/MS for analysing nitrofurans and it's derivatives such as FTD, NFZ, NFT and FZD.
3) Need to establish networking system to develop the analysis method for drug residues among ASEAN countries.
4) Need to establish a harmonised standard among ASEAN countries for the maximum level of drug residues when conducting the NRCP. This will fulfill the requirements set by the importing countries such as EU.
5) Need to improve the accuracy of testing results by participating in proficiency testing program especially for drug residues among ASEAN countries

MALAYSIA

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1. Introduction

Malaysia, like most countries in the world, does not permit the use of chloramphenicol in fish and fish products as a safety health measure for consumers. Despite the ban, chloramphenicol residue had been detected in fish and fish products. On 3rd and 5th May of 2004, Belgium had notified EU member states that Chloramphenicol had been detected in peeled undeveined (PUD) frozen shrimps and PUD red shrimps from Malaysia. There could be two possibilities for such occurrence of chloramphenicol residues in fish and fish products:

(i) Very low concentrations of the drug may be a result of naturally produced chloramphenicol by micro-organisms, residues from previous uses of the drug or by intentional uses of the drug followed by long withholding times before harvesting.
(ii) Higher concentrations of the drug were more likely a result of recent intentional uses and failure to observe sufficient withdrawal times before harvesting.

Chloramphenicol is a broad-spectrum antibiotic with historical veterinary uses in all major food-producing animals and with current uses in humans and companion animals. Chloramphenicol was evaluated previously by the European Committee at its twelfth, thirty-second and forty-second meetings. A number of other agencies had also reviewed chloramphenicol. Some of the agencies were International Agency for Research on Cancer (IARC), 1990; European Committee for Veterinary Medicinal Products, 1994; United States Food and Drug Administration, 1985. Deficiencies identified were in the lack of data on the toxicity of chloramphenicol, including information necessary for the assessment of carcinogenicity and effects on reproduction. An acceptable daily intake (ADI) has never been allocated and consequently a maximum residue limit (MRL) has not been assigned. This has resulted in the restriction of the use of chloramphenicol in veterinary medicine to use in non-food products. The legal basis of the system is Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (O.J.EU No L 31 of 1 February 2002).

2. Objectives And Goals

- To carry out monitoring program to check on the illegal usage of chloramphenicol for official control and generate useful database for management control.
- Department of Fisheries Malaysia (DoFM) had implemented the SPS Monitoring Program in accordance with the WTO requirements to ensure that fish produced in the country is safe to eat and is of good quality.
- With the JTF program being proposed for 2007/2008, it is expected that the on-going SPS program will be enhanced through training and the increase in monitoring activities.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

In 2007, 2 samplings were carried out for this

activity. The first sampling was carried out on 6 Jun 2007. Eight samples were collected from Lakudang Sdn. Bhd at Kuala Kurau, Perak. For the second sampling on 28 August 2007, 16 samples were taken from Eastern Global located at Parit Buntar, Perak. The type of samples collected was frozen Black Tiger Prawn (*P. monodon*). Weight for each package sample ranged from 0.5 kg to 1.8 kg.

b. Method of Analysis

Sample Preparation

The adopted EU method for chloramphenicol (CAP) analysis is as outlined below:

a. Edible fish tissue was blended using a Waring blender.
b. 5 g of homogenized tissue was weighted into a coded test tube.
c. MMS (Matrix Matched Standards), MMRS (Matrix Matched Recovery Standards) and test samples were prepared according to table 1.
d. 10 ml extraction solution, which was HPLC grade water, was added.
e. The mixture was then centrifuged for 15 min at 2700 rpm.
f. 3 ml of supernatant was transferred into the Extrelut® NT3 column and left to stand for 20 minutes.
g. The sample was then eluted with 15 ml dichloromethane.
h. The eluate was collected and evaporated to dryness.
i. The dried residue was re-dissolved in 0.5 ml water.
j. 2 ml toluene was added and the mixture was vortexed for 10 s.
k. The aqueous phase was transferred into a LC-MS vial. This was carried out for all samples, except for MMRS.
l. MMRS was prepared according to table 2.
m. Analysis was carried out using the LC-MS/MS.

Table 1. preparation of quality control samples and samples.

Code MMS sample	Spike level in sample (µg/kg)	Standard solution (25 µg/l) (µl)	Internal Standard (50 µg/l) (µL)
MMS A	0.05	10	50
MMS B	0.1	20	50
MMS C	0.2	40	50
MMS D	0.3	60	50
MMS E	0.5	100	50
MMS F	1.0	200	50
MMRS A	0.2	-	50
MMRS B	0.5	-	50
QC A	0.2	40	50
QC B	0.5	100	50
TS 1			50
TS 2			50
TS 3			50
TS 4			50

Table 2. Preparation of the MMRS after evaporation of dichloromethane.

Code MMS sample	Spike level in sample (µg/kg)	Standard solution (10 µg/l) (µl)	Aqueous phase (µl)
MMRS A	0.2	11	239
MMRS B	0.5	28	222

LC Conditions

Column : C18
Particle size : 5µm, 3µm or 1.9µm
Diameter : 50 mm x 2.1 mm

Gradient program
Mobile Phase A : HPLC Grade Acetonitrile
Mobile Phase B : HPLC Grade Water

No.	Time (min)	A (%)	B (%)	C (%)	D (%)	Flow Rate (ul/min)
1.	0:00	10	90	0	0	200
2.	0.99	10	90	0	0	200
3.	1.00	90	10	0	0	200
4.	4:99	90	10	0	0	200
5.	5:00	10	90	0	0	200
6.	8:00	10	90	0	0	200

Volume of sample injection : 10 -25 ul

MS Conditions

Ion Source (polarity) : ESI Negative mode

System check

Fore Pump Pressure (Torr) : 1 – 2
Ion Gauge Pressure (Torr) : Standby mode = 3 – 4 e^{-6}
Scanning = 1 – 2 e^{-5}

c. Limit of Detection and Limit of Quantification

Limit of detection and limit of quantification in the analysis are 0.01 pbb and 0.05 ppb respectively.

d. National Regulatory Limits

In Malaysia, the use of chloramphenicol in food is absolutely banned. There is, however, no Minimum Required Performance Limit (MRPL) set. For the European Union, the MRPL for chloramphenicol is 0.3 ppb.

4. Results and Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Malaysia did not participate in any inter-laboratory proficiency testing for drug residues.

b. Survey Results and Discussion

Year of analysis & Sampling location	Analyte	Fish Sample Analysed		No. of samples analysed	Min. value of results (ppb) – wet weight basis	Max. value of results (ppb) – wet weight basis	Average value of results (ppb) – wet weight basis	Average Recovery (%)
		Common Name	Scientific Name					
Kuala Kurau, Perak (6/6/2007)	CAP	Frozen Black Tiger	*Penaeus Monodon*	8	Not Detected	Not Detected	-	84.47
Parit Buntar, Perak (28/8/2007)	CAP	Frozen Black Tiger	*Penaeus Monodon*	16	Not Detected	Not Detected	-	75.53

Figures. 1 and 2 show the full scan spectrum of CAP and product scan of the ion *m/z* 321. All fragments were detected. The ion *m/z* 152, giving the strongest signal, was used as quantifier.

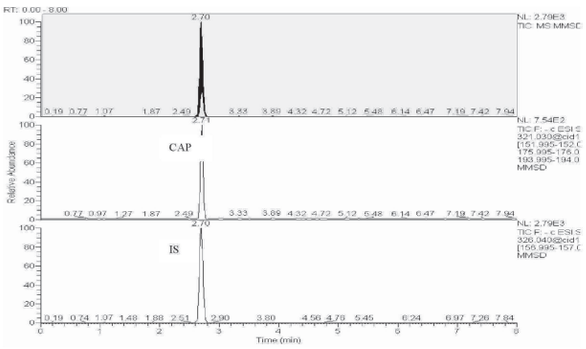

Figure 1: Mass chromatogram of blank black tiger shrimp extract spiked with CAP 0.5ppb and internal standard (IS).

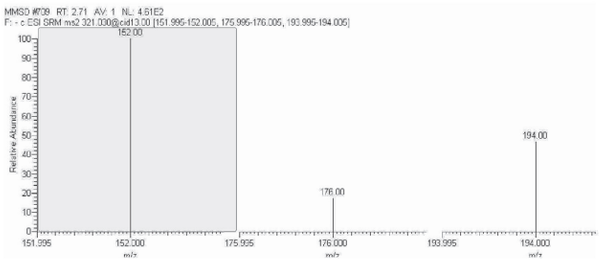

Figure 2: Mass spectrum of CAP product ion *m/z* 152, *m/z* 176 and *m/z* 194.

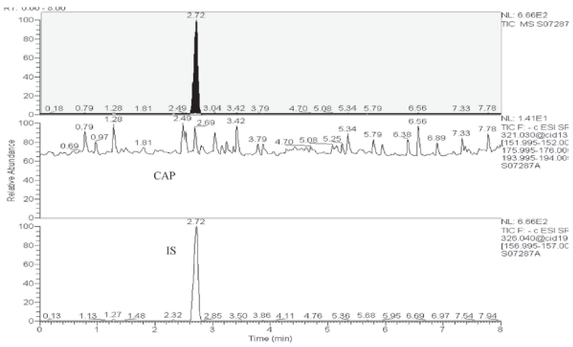

Figure 3: Mass chromatogram of test sample.

Figure 3 shows the mass chromatogram of the test sample. m/z 321 was not detected. Chloramphenicol was not detected in all the samples analyzed, meaning that the concentration of chloramphenicol is lower than EU's MRPL of 0.3 ppb.

c. Corrective Action

Not applicable.

5. Problems and Challenges Encountered

- There was a lack of technical support staff.
- The increase in occurrence of disease infections in cultured fish has resulted in the tendency to increase the drug application in aquaculture practices.
- Good and responsible aquaculture practices should be implemented to achieve sustainability.
- There is a lack of regulatory enforcement.
- Implementation of farm certification.

6. Recommendations and Suggestions for Future Follow Up Action

More allocations should be provided for more samples to be included in the testing. Sampling points should also be increased to cover a wider area so and have a more representative data of the whole country.

Myanmar

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1. Introduction

Myanmar has a total area of 228,781 km^2 and an Exclusive Economic Zone (EEC) of 486,000 km^2 (CS0, 2004). It has a coastline of 2,800 km with swamplands along the coast totaling to about 0.5 million hectares. The country's fisheries operation is classified into marine and inland fisheries. Marine fisheries include both coastal and offshore fisheries while inland fisheries cover freshwater capture fishery and aquaculture. Aquaculture is categorized under inland fishery, which covers an area of about 70279.43 hectares. Coastal aquaculture, to date, is mostly devoted to Shrimp farming with relatively smaller production of Mud Crab and Grouper. The exported amount of fish and fish products was 0.34 million metric tons valued at US$ 466 million in 2006 to 2007. Myanmar has been exporting fish and fish products, including frozen, chilled, live and dried forms, to 33 different countries.

2. Objectives And Goals

- To ensure that aquaculture products of Myanmar are free from drug residues, such as chloramphenicol and nitrofuran.
- To set up the Monitoring Program on Drug Residues in Aquaculture Products.
- To supply aquaculture products that comply with the international market standard and food safety requirements for drug residues.
- To collect and deposit data in the database of the Fish and Fish Products Saftey Information Network.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling Method

random sampling

n=9

Sampling Locations

Yangon Division (Kyauktan, Khayaung &Twente Aquaculture Zone)

Ayeyarwaddy Division (Pantanaw Aquaculture Zone, Ghani Win & Myanmar Seafood processing Plant)

Tanintharyi Division (United Myeik & Pyi Phyo Tun Processing Plant)

Rakhine State (Thantwe Marine Processing Plant)

Species & Sampling Size in 1st Quarter (Oct 2006)

Giant Freshwater Prawn, aquacultured (26/30 size x1 kg)

Black Tiger Shrimp, aquacultured (41/45 size x 1 kg)

Giant Freshwater Prawn, captured (21/25size x 3 kg)

Black Tiger Shrimp, captured (36/40 size x 1 kg)

Species & Sampling Size in 2nd Quarter (Dec 2007-Apr 2008)

Tilapia (0.25-0.5 kg x 7)

River catfish (3-4 kg x 7)

Black Tiger Shrimp (40/45 size x 1 kg)

Rohu (2-3 kg x 7)

Giant Freshwater Prawn (16/20 size x 1 kg)

Pink (16/20 size 1 kg)

White (21 /25 size x 1 kg)

Number of Samples
1st Quarter = 40 (CAP Test only)

2nd Quarter = 133
Total number of samples = 173

Sample Preparation
Only edible portions and fish muscle tissue were used for testing.

b. Method of Analysis

Method of Analysis
(i) ELISA method for Choramphenicol (CAP) Test

(ii) LC/MS/Ms Method for Nitrofuran Test

Method References
(i) 'EURO –DIAGNOST ICA' Method manual, Netherlands for CAP Test

(ii) Journal of Chromatography B, 691(1997) for Nitrofuran Test

Brand of Instrument
(i) ELISA method for CAP
QUALIGENS Strip Reader (ELISA), Italy
Chemical kit: EURO –DIAGNOSTICA Netherlands

(ii) LC/MS/MS method for Nitrofuran
API 4000 LC/MS /MS (Applied Biosystems), USA

c. Limit of Detection and Limit of Quantification

Limit of Detection (LOD):
CAP = 0.025 ppb
Nitrofuran = 0.01 ppb

Limit of Quantification (LOQ):
CAP = 0.3 ppb
Nitrofuran = 1.0 ppb

d. National Regulatory Limits

There were no national regulatory limits in Myanmar. DOF of Myanmar complies and adopts the EU Standards and that of the importing countries.

Maximum Permitted Level in Fish & Fish Products enforced by EU and Japan.

SN	Type of Chemical Hazardous	Product Types	EU	Japan
1.	CAP	All fish products	0.3 ppb	0.5 ppb

SN	Type of Chemical Hazardous	Product Types	EU	Japan
	Nitrofurans			
1.	AOZ	All fish & shell fish products	1.0 ppb	1.0 ppb
2.	AMOZ		1.0 ppb	
3.	AHD		1.0 ppb	
4.	SEM		1.0 ppb	

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

There was no participation in any inter-laboratory proficiency testing.

b. Survey Results and Discussion

Table 1. Results of analysis for Chloramphenicol (CAP) in fish and fish products conducted in 1st quarter (Oct 2006).

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) -wet weight basis	Max. value of results (ppb) -wet weight basis	Average value of results (ppb) -wet weight basis	Remarks
		Common name	Scientific name					
Oct 2006, Pantanaw Aquaculture Zone, Ayeyarwaddy Division	CAP	Giant Fresh water Prawn	*Macrobrachium rosenbergii*	10	0.0148	0.0226	0.01342	Aquacultured (Fresh/ Chilled)
Oct 2006, Kyauk Tan Aquaculture Zone, Yangon Division		Black Tiger Shrimp	*Penaeus monodon*	10	Not Detected	Not Detected	Not Detected	Aquacultured (Fresh/ Chilled)
Nov 2006, United Myeik Processing Plant in Myeik, Tanintharyi Division		Giant Fresh water Prawn	*Macrobrachium rosenbergii*	10	Not Detected	0.0663	0.0172	Captured (Frozen)
Oct 2006, Thantwe Marine Processing Plant in Thantwe, Rakhine State		Black Tiger Shrimp	*Penaeus monodon*	10	Not Detected	0.1682	0.0194	Captured (Frozen)

Table 2. Results of analysis for Chloramphenicol (CAP) in fish and fish products conducted in 2nd quarter (Dec 2007 – Apr 2008).

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) -wet weight basis	Max. value of results (ppb) -wet weight basis	Average value of results (ppb) -wet weight basis	Remarks
		Common name	Scientific name					
Dec 2007, Khayoung Aquaculture Zone, Yangon Division	CAP	Tilapia	*Oreochromis niloticus*	10	Not Detected	0.062	0.0216	Aquacultured (Fresh/ Chilled)
Jan 2008, Ayeyarwaddy Division, Ghani Win Processing Plant		River cat Fish	*Sperata seenghala*	10	Not Detected	Not Detected	Not Detected	Captured (Frozen)
Feb 2008, Rakhine State, Thantwe Marine Processing Plant		Black Tiger Shrimp	*Penaeus monodon*	10	Not Detected	0.061	0.0193	Aquacultured (Frozen)
Mar 2008, Twente Aquaculture Zone, Yangon Division, Annawar Hlwam Processing Plant		Rohu	*Labeo rohita*	10	Not Detected	0.028	0.0045	Aquacultured (Frozen)
Apr 2008, Ayeyarwaddy Division, Myanmar Sea Food Processing Plant		Giant Fresh water Prawn	*Macrobrachium rosenbergii*	10	Not Detected	0.043	0.0064	Captured (Frozen)
Apr 2008 Tanintharyi Division, Phyi Phyo Tun Processing Plant		Pink Shrimp	*Metapenaeus affinis*	10	Not Detected	0.034	0.0074	Captured (Frozen)
Apr 2008, Tanintharyi Division, Phyi Phyo Tun Processing Plant		Indian White Prawn	*Penaeus indicus*	10	Not Detected	0.021	0.0039	Captured (Frozen)

Table 3. Results of analysis for Nitrofurans (NF) in fish and fish products conducted in 2nd quarter (Dec 2007 – Apr 2008).

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) - wet weight basis	Max. value of results (ppb) - wet weight basis	Average value of results (ppb) - wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific Name						
Dec 2007, Khayoung Aquaculture Zone, Yangon Division	NF	Tilapia	*Oreochromis Niloticus*	9	Not Detected	Not Detected	Not Detected	89.3	Aquacultured (Fresh/ Chilled)
Jan 2008, Ayeyarwaddy Division, Ghani Win Processing Plant		River catfish	*Sperata seenghala*	9	Not Detected	Not Detected	Not Detected	90.2	Captured (Frozen)
Feb 2008, Rakhine State, Thantwe Marine Processing Plant		Black Tiger Shrimp	*Penaeus monodon*	9	Not Detected	Not Detected	Not Detected	91.0	Aquacultured (Frozen)
Mar 2008, Twente Aquaculture Zone, Yangon Division, Annawar Hlwam Processing Plant		Rohu	*Labeo rohita*	9	Not Detected	Not Detected	Not Detected	90.3	Aquacultured (Frozen)
Apr 2008, Ayeyarwaddy Division, Myanmar Sea Food Processing Plant		Giant Fresh water Prawn	*Macrobrachium rosenbergii*	9	Not Detected	Not Detected	Not Detected	92.2	Captured (Frozen)
Apr 2008, Tanintharyi Division, Phyi Phyo Tun Processing Plant		Pink Shrimp	*Metapenaeus affinis*	9	Not Detected	Not Detected	Not Detected	87.7	Captured (Frozen)
Apr 2008, Tanintharyi Division, Phyi Phyo Tun Processing Plant		Indian White Prawn	*Penaeus indicus*	9	Not Detected	Not Detected	Not Detected	87.9	Captured (Frozen)

c. Corrective Actions

- Carry out investigation studies such as traceability, from farmers to suppliers and establishments.
- Verification of Sampling and Analytical Methods.

5. Problems and Challenges Encountered

- The budget is limited.
- There was a lack of skilled laboratory personnel.

6. Recommendations and Suggestions for Future Follow up Action

- There is a need for on-site training courses.
- Skills of laboratory personnel on sampling and analysis can be strengthened.
- There is a need to train farmers and aquaculture inspectors on Good Aquaculture Practices (GAP).
- Proficiency testing should be conducted.
- DOF of Myanmar have drawn the Drug Residues Monitoring Plan and results from the project activities are deposited in the database of this plan.

Philippines

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1. Introduction

Successful national food control systems are necessary to protect the health and safety of domestic consumers. They are also essential for ensuring the quality and safety of aquacultured products entering the international trade and making sure that the product conforms to the international requirements.

Trading partners are taking much interest in the way food is produced, processed and marketed and these partners are relying heavily on the governments to take greater responsibility in food quality and safety assurance. They require supplementary efforts and proactive behavior from the authorities and private sector in an increasingly competitive environment in establishing food safety control structures or its equivalent.

The Philippines has to keep up with the dynamic trade requirements, notably with the technical requirements and SPS measures to gain a better access to foreign markets. Trade partners have become more demanding and the corresponding technical requirements tend to become more complex and stringent.

Participation in the Philippines on this Japanese Trust Fund (JTF) II Project, specifically on Regional survey of pesticide residues in fish and fish products and Regional survey of chloramphenicol and nitrofuran in fish and fish products and in Japanese Trust Fund II Project "Food Safety Program: Research and Analysis of Chemical Residues and Contamination :Chemical and Drug Residues in Marine and migratory species and straddling stocks is one of the positive responses of the country to the aforementioned stringent requirements on fish trade.

Food Safety Management

At the Central Office, the food safety management for Fish Inspection and Quality Assurance Services is directly under the Office of the Director and is composed of five units: the Fish Product Testing Laboratory (FPTL), the Marine Biotoxin Laboratory (MBL), the Administrative Support and Product Certification Unit (ASPCU), the HACCP-based Fish Inspection Unit (HFIU) and the Fish Health Management and Quality Assurance Section (FHMQAS).

The FPTL and MBL are in charge of providing services to other units in their respective fields. The HFIU is responsible for the inspection of accredited fish processing plants based on 94/356/EEC, 91/493/EEC and to ensure consistent compliance with EU requirements as per 91/493/EEC, the new European Food Law, and other relevant national rules and regulations on the export of fishery and aquaculture products. The APSCU is responsible for the issuance of product health certificates for fishery and aquaculture products for export to the EU and other markets. To ensure the safety and quality of fishery and aquaculture products for export, the FIQAS implements a HACCP-based inspection program. It coordinates with the FHMQAS, FPTL and the BFAR Regional Offices on the effective implementation of the Quality Assurance Program. The FIQAS supervises the overall inspection of fish processing plants and certification of fishery and aquaculture products for export in coordination with the BFAR Regional Offices. The FHMQAS spearheads the registration of aquaculture farms, residues and health monitoring, as well as provision of diagnostic services, technical and advisory assistance to the aquaculture industry. Its monitoring program includes residues and disease surveillance and the reporting system, aquatic animal health certification, implementation of quarantine procedures, assessment of the health status of stocks of selected fish and the management of other aquatic resource farms in the Philippines.

At the Regional Offices, food safety management is under the responsibility of BFAR regional directors located in 15 regional offices. Each has a Fish Health Unit, linked to the central FHMQAS, and a Fishery Inspection unit, linked to the HFIU and central laboratories.

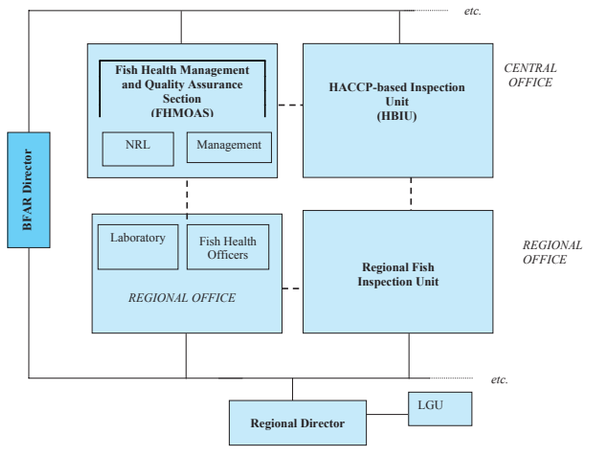
Central Fish Health Management and Quality Assurance Laboratory

The residues monitoring is implemented by the FHMQAS in coordination with the 15 BFAR Regional Offices. The regional directors have full responsibility over their area of jurisdiction. However, matters of policy-determining nature still lie under the jurisdiction of the Bureau Director. The organizational structure and coordination between regional and central offices are presented in Figure 1.

Role of National and Regional Fish Health Officers are as follows:

- Register aquaculture farms.
- Monitor the hygiene of production.
- Disseminate information and educate the aquaculture chain operators on the need of aquatic animal feeds, veterinary drugs and products registration prior to marketing and usage.
- Conduct surveillance and monitoring of aquatic animal feeds, veterinary drugs and products in their areas of responsibility.
- Recommend regulatory actions for any violations on policies and guidelines on registration, manufacturing, distribution and usage of aquatic animal feeds.
- Assist in planning, directing and supervising national programs on aquatic feeds, veterinary drugs and products control.
- Assist in the registration, evaluation and inspection of establishments engaged in manufacturing, distribution and sale of aquatic animal feeds, drugs, products premixes and water solubles, whenever necessary

Figure 1. Organizational structure for residues monitoring and coordination between regional and central departments.



Coordination of the Activities of Central and Regional Departments

The FHMQAS develops and updates standardized routine procedures and guidelines for the operation of the regional fish health laboratories. It also supervises the activities and sets directions for the operation of such laboratories. The FHMQAS also provides technical guidance and instructions to the 44 BFAR-designated Regional Fish Health Officers on the execution of diagnostic activities and provide technical assistance on fish health-related and residues monitoring problems. It also provides specialized training on sanitary and phyto-sanitary programs of the government to fishery biologists, extension workers and fish farmers. The section sets national programs for the surveillance and monitoring of aquatic animal feeds, veterinary drugs, products and their control as well as formulates standardized monitoring procedures on field inspections and reporting, to ensure high feed quality and compliance to the regulations of the government. The FHMQAS also supervises the implementation and monitoring activities of the Fish Health Officers deputized as Aquatic Animal Feeds, Veterinary Drugs and Products Control Officers (AAFVDAPCO) nationwide. To harmonize activities, the FHMQAS coordinates with the other sector on the results of monitoring aquaculture farms supplying raw materials to the fish processing plants. Regional Fish Health Officers regularly coordinate with the officers of the Central FHMQAS in the implementation of the official program, including residues monitoring. Reports are also submitted to the Central FHMQAS for consolidation and analysis.

2. Objectives And Goals

Objectives:

- To increase export of aquacultured products like shrimp and milkfish, and marine products like lobster, crab and grouper.
- To develop monitoring and surveillance mechanism for chemical contaminants, for example antibiotic residues, for food safety and quality assurance of aquacultured and marine fish products.
- To develop sampling method and establish baseline information on the level pesticides in fish and fish products.

Goals:

- To ensure food safety and quality of fish and fish product for export.
- To obtain baseline information of the usage and extent of antibiotic contamination for Chloramphenicol and Nitrofurans.
- To develop capability on the analysis of harmful substances, for example in the use of LC/MS/MS, through attending trainings held by SEAFDEC-MFRD, in collaboration with a country in the region with the capability.
- To be able to establish a good network and increase communication with the laboratories in the region.
- To adopt methods used by recognized laboratories, for example, SEAFDEC-MFRD.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Sampling

The sampling for official samples took place at random timings, unknown to the farms, markets and processing plants. Sampling was carried out at variable intervals over the year at farms, markets and receiving areas of processing plants. The processing plants, farms and markets sampling were defined using as much targeting criteria as possible, making sampling homogeneous.

All accredited establishments and registered farms that export products to the EU and other countries were under the scope of the monitoring plan. The number of sampling was defined using the regional production statistics as a minimal level, as well as taking into account the risks and results of past monitoring plans.

All available information of targeted samples were taken into consideration, for example, the use of presently unknown substances, sudden appearance of diseases in particular regions and indications of fraudulent activities.

Collection of samples from farms, auction areas/ markets and processing establishments

Samples were taken from farms, processing plants or purchased from the markets in a random manner. Identification and traceability of the samples back to the source was ensured. Important information like the source was taken. Information of the source was only available up to the provincial level for samples taken from the markets. Measures to prevent contamination of samples were taken during transportation of the samples to the laboratory. Suitable sampling tools, plastic bags and transportation boxes were provided for the collection of samples.

Each sample was equivalent to 1 kg of raw material. Every sample was packed separately into a disposable plastic sample container to prevent contamination and labelled accordingly with a unique number on the container. Each sample container was closed, sealed properly and chilled with ice during transportation to the laboratory. The samples could be kept in a refrigerator for several days. Plastic containers were placed in plastic bags to avoid contact with other samples. Every sample was separately packed in a plastic bag and frozen.

A report was produced after each sampling with at least the following information in it:

- Address of the authority that took the sample.
- Name and surname for identification.
- Sampling date, name and address of the exporter or the person in charge of the animals or the animal products.
- Remarks.

Sending of samples

Preliminary sample preparation was conducted in the regional laboratories. Duplicates of homogenised samples were prepared and frozen for immediate submission. One sample may be subjected for analysis of several analytes. Samples sent to FHMQAL were packed separately in clean sample containers to prevent contamination, coded for easy identification, and accompanied with sampling information for traceability purposes. Samples were chilled with ice bags and stored in

clean durable containers to prevent any damage and contamination during transportation.

Sample reception at the laboratory

At the receiving area, personnel assigned to receive samples checked the following information:

- Temperature of the sample and its visual aspects.
- Packaging and container.
- Labeling, code and accompanied sampling report.
- Quantity and weight of the sample as required by the analysis.

b. Method of Analysis

The Central FHMQAL is responsible for the analysis of residues in foodstuffs of aquatic animal origin. It is located at the BFAR Central Office in Quezon City. The FHMQAL is under the supervision of the chief of the FHMQAS and has five personnel, one in-charge of field operations (veterinarian), one in-charge of the laboratory (veterinarian), a permanent analyst (chemist), two temporary analysts (chemist), and one administrative support for receiving samples and releasing results. Chemists are involved in the analysis of residues.

FHMQAS issued formal instructions to the Fish Health Officers on how to carry out sampling and to ensure the traceability of the samples. It had also implemented quality checks (temperature and visual inspection) on samples received in order to confirm their suitability for analysis for this study. The FMHQAL either performed the analysis in its own facilities or in collaboration with a third-party laboratory i.e. SGS, Phils.

Chloramphenicol and nitrofurans (AOZ and AMOZ) residues in aquaculture and fishery products were analyzed by Enzyme-linked Immunosorbent Assay (ELISA) using the RidaScreen Kit from Germany. The laboratory followed the required % recovery rate required in the kits.

c. Limit of Detection and Limit of Quantification

Chloramphenicol
Limit of detection (LOD): 0.05 ppb
Limit of quantification (LOQ): 0.15 ppb

Nitrofurans (AOZ and AMOZ)
Limit of detection (LOD): 0.10 ppb
Limit of quantification (LOQ): 0.30 ppb

d. National Regulatory Limits

No national standard has been set yet on the maximum residue limits (MRL) for drugs and other substances in aquaculture. The standards used are the lowest ones set by the *Codex Alimentarius*, the United States Food Development Authority (US-FDA) and the European Union (EU). Food-business operators placing products on export markets must comply with these values and they are monitored by the Competent Authority (CA).

Several drugs, including A6 nitrofurans, olaquindox, carbadox and A6 chlorampenicol, are banned through joint administrative orders from the DOH:

a) Department of Health (DOH) and Department of Agriculture (DA) Joint Administrative Order No. 2, Series of 2000- Declaring ban/ phase out of the use of nitrofurans in food-producing animals.

b) DOH Administrative Order No. 91 and DA Administrative Order No. 60, Series of 1990- Declaring ban on the use of chlorampenicol in food-producing animals.

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Table 1. Results of inter-laboratory comparison on Chloramphenicol and Nitrofuran (AOZ).

Inclusive Dates	13 to 26 July 2004		
No. of Participating Laboratories	4		
Type of Analysis	No. of samples	No. of replicates per sample	Average Precision (RSD, %)
Nitrofuran (AOZ)	4	12	61.5
Chloramphenicol	4	12	43.5

Inclusive Dates	31 May to 02 June 2005		
No. of Participating Laboratories	4		
Type of Analysis	No. of samples	No. of replicates per sample	Average Precision (RSD, %)
Nitrofuran (AOZ)	4	12	112.6
Chloramphenicol	4	12	27.4

Inclusive Dates	20 to 28 Nov 2007				
No. of Participating Laboratories	3				
Type of Analysis	Blank Samples		Spiked Samples		
	No. of aliquots	No. of replicates per aliquot	No. of aliquots	No. of replicates per aliquot	Average Precision (RSD, %)
Chloramphenicol	3	9	3	7	25.2

b. Survey Results and Discussion

Table 2. Summary of results of survey on Chloramphenicol and Nitrofurans (AOZ and AMOZ) in aquaculture and marine fish products from 2005-2007.

Year	Sampling location	Sample	No. of samples	Analyte	Results (ppb)	Recovery Rate (%)	Remarks
2005	Processing Plants	Fresh frozen Shrimp *Penaeus monodon*	39	CAP AOZ	ND ND	80-120	As required by the kit
2006	Processing Plants	Shrimp *Penaeus monodon*	72 55	CAP AOZ	ND ND	80-120	As required by the kits
	Farms	Shrimp *Penaeus monodon*	68 64 63	CAP AOZ AMOZ	ND ND ND	80-120	As required by the kits
2007	Public markets	Crabs *Scylla serrata*	40 3	AOZ AMOZ	ND ND	112.82 102.5	-
	Public markets	Lobsters *Panulirus sp.*	32 3	AOZ AMOZ	ND ND	111.62 104	-
	Public markets	Groupers *Epinephelus* spp.	20	AOZ	ND	115.78	-

Laboratory: Central Fish Health Management and Quality Assurance Laboratory
Method of Analysis: Enzyme-linked Immunosorbent Assay (ELISA) RidaScreen Kit
Residues Analysed: Chloramphenicol (CAP), Nitrofuran (AOZ and AMOZ)
Dilution Factor : CAP: 1; AOZ: 2
LOD : CAP : 0.05 ppb; AOZ and AMOZ : 0.10 ppb
LOQ : CAP : 0.15 ppb; AOZ and AMOZ : 0.30 ppb

In 2005, a total of 39 fresh frozen Shrimp (*Penaeus monodon)* samples were collected from the processing plants from August to December. The samples were analyzed for the residues of Chloramphenicol (CAP) and metabolites of Nitrofuran (AOZ) at the Central Fish Health Management and Quality Assurance Laboratory using Enzyme-linked Immunosorbent Assay (ELISA) Rida Screen Kit. The level of detection for chloramphenicol is 0.05 ppb and the level of quantification is 0.15 ppb. For the metabolites of nitrofurans (AOZ), the level of detection is 0.10 ppb and the level of quantification is 0.30 ppb. The samples were tested negative for both CAP and AOZ.

In 2006, a total of 140 fresh frozen Shrimp samples were taken, 72 from the processing plant and 68 from aquaculture farms. All 72 samples from processing plants were analyzed for CAP and 55 of which were also subjected to AOZ analysis. No CAP and AOZ residues were detected in all samples. For the samples taken from the farms, 68 were analyzed for CAP, 64 and 63 of which were also analyzed for AOZ and AMOZ respectively. No CAP and AOZ residues were detected in all samples.

In 2007, marine fish were taken from public markets from March to November for the analysis of metabolites of nitrofuran (AOZ and AMOZ). A total of 40 fresh Mudcrab, 32 Lobster and 20 Grouper *s*amples were collected. For Mudcrab samples, 40 were analyzed for AOZ and 3 of which were also analyzed for AMOZ, for Lobster samples 32 were analyzed for AOZ and 3 for AMOZ. For Grouper samples, 20 samples were analyzed for AOZ. Results showed that no AOZ and AMOZ were detected in all samples. The summary of analysis is presented in Table 2.

Antibiotics have a wide range of use to prevent bacterial growth and there is a use of banned drugs in fish and fish products. Wild caught crabs and lobsters are sometimes treated with the drugs to prevent diseases and prolong the shelf life of the products, however, the number of samples tested positive for chloramphenicol and nitrofuran are very few.

c. Corrective Actions

Presence of residues of banned antibiotics in harvested animals are subjected to the following corrective actions:

The farm owner is immediately informed of the presence of residues of banned antibiotics in shrimp/fish samples obtained from his farm, so that he can take appropriate measures to withdraw his products from export. For the next 12 months, the farm is subjected to more stringent checks for the residues in question. The results are also immediately provided to the concerned auction market owners and exporters who have to recall the products if the products are no longer under their control. No health certificates are issued in cases where banned antibiotics are tested positive.

The presence of residues of banned chemicals in shrimp/fish meat is also reported immediately to the Local Government Units concerned, with the recommendation of the proper action and application of National Laws and Local Ordinances pertaining to pollution and consumer protection. An investigation is carried out to determine the reasons for the presence of residues and the extent of the problem.

In cases of positive findings, the registration of the farm concerned will be suspended and the information will be given to the auction markets and processing plants the farm supplies to. The suspension will be lifted after three successive negative findings.

5. Problems and Challenges Encountered

- Delayed release of funds, which caused problems in the implementation of the project as scheduled.
- There is a need to strengthen capability in analyzing residues with LC/MS/MS as methods used are for screening only.
- There is a need to have certified reference materials for method validation.

6. Recommendations and Suggestions for Future Follow up Action

- SEAFDEC-MFRD must continue its significant role in coordination work and networking among the participating countries.
- To continue activities on the harmonization of protocol procedures on the confirmatory analysis using sensitive equipment like the LC/MS/MS.
- Preparations of risk communications materials for information and education campaign.
- To cover more antibiotics and develop other methods of analysis like antimicrobial sensitivity assay.
- To provide financial assistance for the purchase of equipment and technical support in the implementation food safety program.

Thailand

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1. Introduction

In 2002, some Southeast Asian countries' aquacultured shrimp and fish were found to contain nitrofuran and chloramphenicol residues. In the past 2 years, some countries also found Malachite Green and Leuco-Malachite Green contamination in their aquacultured shrimp and fish. The Southeast Asian countries thus addressed these contamination problem immediately. This Japanese Trust Fund II project is very helpful for the monitoring and controlling of the occurrences of these chemical residues in Southeast Asian countries and to ensure traceability of the fish and fish products in importing and exporting countries.

2. Objectives And Goals

- To survey the level of drug residues used in ASEAN countries, and
- To ensure the food safety for consumer.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

The samples were collected from the Central, Western and Southern part of Thailand. The raw materials of black tiger prawn (*Penaeus monodon*), freshwater shrimp and crab were collected from at least 5 samples/ farm and 5-7 samples/lot/month. For samples collected from the factories, a sampling plan following the CODEX sampling criteria was used. Samples were kept in plastic bag and properly packed in a cool condition (temperature below 10°C) during transportation. Upon arriving in the laboratory, the sample preparation was conducted immediately and the blended samples were kept in the freezer below –18°C.

b. Method of Analysis

Nitrofuran (NF) (Metabolite) by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS):
1 g of sample was weighed into a centrifuge tube, and extracted with 0.5 ml of 1M hydrochloric acid (HCl) and 150 µl of 2-nitrobenzaldehyde (2-NBA) solution. The tube was place in the water bath at 37°C for 16± 2 hrs. The metabolites were simultaneously released from the sample tissue and the drug residues were derivatised in the acid medium containing 2-NBA. The nitrophenyl derivatives produced were AOZ, AHD, AMOZ and SEM. They are detected and quantified using HPLC tandem mass spectrometry with ESI (Electro spray) and positive mode.

Note:
All chemicals used were analytical and HPLC grade. The LC/MS/MS used to detect Nitrofuran (metabolites) was Micromass system.

Chloramphenicol (CAP) by ELISA :
3 g of sample was weighed into a centrifuge tube. The samples was homogenized in ethyl acetate and then evaporated to dryness. The residue was reconstituted in iso-octane/chloroform and a buffer. The ELISA is a competitive enzyme immunoassay for the screening in various matrices on the presence of this broad spectrum antibiotic.

Malachite Green (MG) and Leuco-Malachite Green (LMG) by LC/MS/MS:
1 g of sample was weighed into a centrifuge tube. The sample was homogenized in McIlvain buffer:Methanol (50:50 by volume) and N, N, N', N'- tetra methyl - 1, 4 - Phenylenedia mine dihydro chloride was used for the extraction process. The sample was then centrifuged and passed through the Solid-Phase Extraction (SPE) cartridge (Waters OASIS, MCX) for the clean-up step. The samples

after the SPE step was then dried under nitrogen gas and re-dissolved with 50 % acetonitrile.

Note:
All chemicals used were analytical and HPLC grade. The LC/MS/MS used to detect Malachite Green and Leuco-Malachite Green is API (Applied Biosystem) Model 4000.

c. Limit of Detection and Limit of Quantification

The Minimum Required Performance Limit (MRPL) for Nitrofuran (metabolites) is 1.00 ppb. The LOD and LOQ for all metabolites are 0.02 and 0.10 ppb, respectively.

The MRPL for Chloramphenicol is 0.30 ppb. The LOD and LOQ are 0.01 and 0.03 ppb, respectively.

The MRPL for Malachite Green and Leuco-Malachite Green are 2.00 ppb. The LOD are 0.02 and 0.01 ppb respectively. The LOQ for both residues are 0.10 ppb.

d. National Regulatory Limits

For **Thailand regulation**, all residues that mentioned above are prohibited for use for fish/ shrimp aquaculture.

The national regulatory limit for Nitrofuran (metabolites) is in-line with this MRPL limit; 0.30 ppb for AOZ and AMOZ and 1.00 ppb for AHD and SEM.

The national regulatory limit for Chloramphenicol is 0.30 ppb.

The national regulatory limit for Malachite Green and Leuco-Malachite Green is in-line with the MRPL limit at 2.00 ppb.

EU regulation
Nitrofuran (metabolites): 1.00 ppb
Chloramphenicol: 0.30 ppb
Total Malachite green and Leuco-Malachite Green: 2.00 ppb

Canada Regulation
Nitrofuran (metabolites): Not detected
Chloramphenicol: Not detected
Total Malachite Green and Leuco-Malachite Green: 1.00 ppb

USA Regulation
Nitrofuran (metabolites): 1.00 ppb
Chloramphenicol: 0.30 ppb
Total Malachite Green and Leuco-Malachite Green: 1.00 ppb

4. Results And Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results	True value	z-score
2008	AFSSA, EU CRL	MG and LMG	0.775	0.875	-0.479
2008	FAPAS	CAP	0.35	0.61	-2.0
2007	FAPAS	NF	0.24	0.40	-1.8

b. Survey Results and Discussion

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) – wet weight basis	Max. value of result (ppb) – wet weight basis	Average value of result (ppb) – wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific name						
2005	Nitrofuran (Metabolites)	Black tiger shrimp	*Penaeus monodon*	40	ND	0.25 (SEM)	0.14	85 %	Fresh from farm
	Chloramphenicol	Black tiger shrimp	*Penaeus monodon*	40	ND	0.02 (< LOQ)	0.02 (< LOQ)	80%	Fresh from farm
	Nitrofuran (Metabolites)	Black tiger shrimp	*Penaeus monodon*	40	ND	ND	ND	85 %	Fresh/frozen from factory
	Chloramphenicol	Black tiger shrimp	*Penaeus monodon*	40	ND	ND	ND	80%	Fresh/frozen from factory
2006	Nitrofuran (Metabolites)	Black tiger shrimp	*Penaeus monodon*	45	ND	ND	ND	80 %	Fresh from farm
	Chloramphenicol	Black tiger shrimp	*Penaeus monodon*	45	ND	0.01 (< LOQ)	ND	82%	Fresh from farm
	Nitrofuran (Metabolites)	Black tiger shrimp	*Penaeus monodon*	45	ND	ND	ND	80 %	Fresh/frozen from factory
	Chloramphenicol	Black tiger shrimp	*Penaeus monodon*	45	ND	ND	ND	82%	Fresh/frozen from factory
2007	Nitrofuran (Metabolites)	Freshwater shrimp	*Penaeus monodon*	20	ND	ND	ND	88%	Fresh from farm
	Chloramphenicol	Freshwater shrimp	*Penaeus monodon*	20	ND	ND	ND	82%	Fresh from farm
	Malachite green and Leuco-malachite green	Freshwater shrimp	*Macrobrachium rosenbergii*	20	ND	ND	ND	90%	Fresh from farm
	Nitrofuran (Metabolites)	Crab	*Scylla serrata*	10	ND	ND	ND	88%	Fresh/frozen from factory
	Chloramphenicol	Crab	*Scylla serrata*	10	ND	ND	ND	82%	Fresh/frozen from factory

	Malachite green and Leuco-malachite green	Crab	*Scylla Serrata*	10	ND	ND	ND	90%	Fresh/frozen from factory
2008	Nitrofuran (Metabolites)	Freshwater shrimp	*Penaeus monodon*	9	ND	ND	ND	80%	Fresh from farm
	Chloramphenicol	Freshwater shrimp	*Penaeus monodon*	9	ND	ND	ND	80%	Fresh from farm
	Malachite green and Leuco-malachite green	Freshwater shrimp	*Macrobrachium rosenbergii*	9	ND	ND	ND	86%	Fresh from farm
	Nitrofuran (Metabolites)	Crab	*Scylla serrata*	6	ND	ND	ND	80%	Fresh/frozen from factory
	Chloramphenicol	Crab	*Scylla serrata*	6	ND	ND	ND	80%	Fresh/frozen from factory
	Malachite green and Leuco-malachite green	Crab	*Scylla serrata*	6	ND	ND	ND	86%	Fresh/frozen from factory

ND: Not Detected

c. Corrective Actions (if applicable)

For any detection of the prohibited residues, the farmer or processor will be informed of the violation. If the violation is found in finished products, the processor must conduct a self-monitoring program to control the raw material reception. At farm/hatchery level, responsible DOF offices will conduct a follow-up inspection to determine the possible sources of contamination and to ensure the implementation of proper corrective actions and preventive measures.

5. Problems and Challenges Encountered

Not applicable.

6. Recommendations and Suggestions for Future Follow up Action

Not applicable.

VIETNAM

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1. Introduction

The increase in export of fish and fish products to high-income countries requires strict standards on hygiene and safety. Specific requirements on chemical residues in fish and fish products are consequently set up.

For this reason, surveys and studies activities organized by SEAFDEC and funded by JTF IV were significant, as they contribute to providing an overview on residue levels of chemicals and contaminants in aquaculture fish in South-East Asia. , Member countries would then determine appropriate policies to ensure quality and safety of fish and fish products, meet importing countries' requirements and protect the health of domestic consumers.

In Vietnam, as the competent national authority responsible for ensuring quality, hygiene and safety of fish and fish products since 1997, the National Fisheries Quality Assurance and Veterinary Directorate – NAFIQAVED (now renamed as the National Agriculture, Forestry and Fisheries Quality Assurance Department-NAFIQAD) has been carrying out the Monitoring Program for certain harmful substances and residues in aquaculture animals. NAFIQAD had also actively participated in the survey titled "Research and Analysis of Chemical Residues and Contaminants in fish and fish products" as well as other activities organized by SEAFDEC.

2. Objectives And Goals

Surveys of antibiotic residues are carried out to provide information on the level of histamine present in fish and fish products. The data obtained was deposited into the database of the Fish and Fish Products Safety Information Network. The network's website: www.fishsafetyinfo.com contains general information on fishery hygiene and safety in SEAFDEC's member countries.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

- NAFIQAD led to implement all activities in 2006 to 2008.
- NAFIQAD branches took samples and analyzed samples as required by the activity.
- Activities carried out by NAFIQAD including the following:
  (i) Activities using SEAFDEC's budget;
  (ii) Activities using budget from the Monitoring Program for certain harmful substances and residues in aquaculture animals and certification activities for exported fishery consignments.
- NAFIQAD has signed contracts with its Branches for the implementation of activities, and then is responsible for data analysis and treatment and making report.
- NAFIQAD branches carry out the procedures of sampling, sample preservation, dispatch of samples to laboratories and analysis, in compliance with the Residues Monitoring Program Manual and Quality Manual of NAFIQAD.
- Raw materials of the following species were collected:
  Frozen Black Tiger Shrimps (*Penaeus monodon*)
  Crabs (*Scylla serrata*)
  Bigeye Tuna (*Thunnus albacares*)
  Octopus (*Octopus* spp.)
  Squid (*Loligo edulis*)
  Cuttlefish (*Sepia* spp.)

- The samples were collected from Northern to Southern of Vietnam. Fresh chilled samples were collected from aquaculture farms and from the sea. Frozen samples were collected from processing establishments.

b. Method of Analysis

Screening method for Chloramphenicol (CAP) and Nitrofurans (NF)
Method: Guidance of R-Biopharm using ELISA
Testing method was accredited with ISO 17025 by BoA – VILAS.

Confirmatory method for CAP and NFs for positive samples in NAFIQAVED Branch 4
Method: in-house method 5.2-CL4/ST
Equipment: Tandem LC/MS/MS
Testing method was accredited with ISO 17025 by BoA – VILAS.

Malachite Green (MG) and Leuco-Malachite Green (LMG) analysis
Method: in-house method
Equipment: LC/MS/MS
Testing method was accredited with ISO 17025 by BoA – VILAS.

c. Limit of Detection and Limit of Quantification

- Screening method for CAP, NFs:

Limit of detection (LOD) for CAP: 0.2 ppb

LOD for AOZ and AMOZ: 0.5 ppb

- Confirmatory method for CAP, NFs in case of positive samples in NAFIQAVED Branch 4:

LOD for CAP: 0.1 ppb

LOD for AOZ and AMOZ: 0.5 ppb

- MG/LMG analysis

LOD for MG and LMG: 0.5 ppb

d. National Regulatory Limits

Are not allowed by Vietnam, EU, USA, Japan and Korea

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Remarks
2006	FAPAS/ 2006/ Prawn	AOZ, AMOZ	Passed
2006	FAPAS/ 2006/ Fish	MG	Passed
2007	FAPAS/ 2007/ Fish	MG	Passed

b. Survey Results and Discussion

SEAFDEC's budget

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) – wet weight basis	Max. value of result (ppb) – wet weight basis	Average value of result (ppb) – wet weight basis	Average Recovery (%)
		Common name	Scientific name					
2006	CAP	Crabs	*Portunus pelagicus*	8	ND	ND	ND	70%
		Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	70%
		Frozen Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	70%
		Frozen Boiled Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	70%
	Nitrofuran (AOZ)	Crabs	*Portunus pelagicus*	8	ND	ND	ND	80%
		Marine fish	*Penaeus monodon*	8	ND	ND	ND	80%
		Squid/Octopus	*Sepia* spp./ *Octopus* spp.	8	ND	ND	ND	80%
		Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	80%
	Nitrofuran (AMOZ)	Crabs	*Portunus pelagicus*	8	ND	ND	ND	75%
		Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	75%
		Frozen Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	75%
		Frozen Boiled Black Tiger Shrimps	*Penaeus monodon*	8	ND	ND	ND	75%
2007	CAP	Crabs	*Portunus pelagicus*	10	ND	ND	ND	70%
		Marine fish	/	10	ND	ND	ND	70%
		Squid/Octopus	*Sepia* spp./ *Octopus* spp.	10	ND	ND	ND	70%
		Black Tiger Shrimps	*Penaeus monodon*	10	ND	ND	ND	70%
	Nitrofuran (AOZ, AMOZ)	Crabs	*Portunus pelagicus*	10	ND	ND	ND	80%
		Marine fish	/	10	ND	ND	ND	80%
		Squid/Octopus	*Sepia* spp./ *Octopus* spp.	10	ND	ND	ND	80%
		Black Tiger Shrimps	*Penaeus monodon*	10	ND	ND	ND	80%

2008	CAP	Crabs	*Portunus pelagicus*	5	ND	ND	ND	70%
		Marine fish	/	5	ND	ND	ND	70%
		Squid/Octopus	*Sepia* spp./ *Octopus* spp.	5	ND	ND	ND	70%
		Black Tiger Shrimps	*Penaeus monodon*	5	ND	ND	ND	70%
	Nitrofuran (AOZ, AMOZ)	Black Tiger Shrimps	*Penaeus monodon*	5	ND	ND	ND	80%

Vietnam's budget

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) – wet weight basis	Max. value of result (ppb) – wet weight basis	Average value of result (ppb) – wet weight basis	Average Recovery (%)	Remarks (No. of samples exceeding MRL)
		Common name	Scientific name						
2006	CAP	Crabs	*Portunus pelagicus*	175	0.1	1.3	0.7	70%	49
		Black Tiger Shrimps	*Penaeus monodon*	869	0.1	0.6	0.4	70%	2
		Frozen Black Tiger Shrimps	*Penaeus monodon*	6192	ND	ND	ND	70%	3
		Frozen Boiled Black Tiger Shrimps	*Penaeus monodon*	1992	0.1	0.8	0.5	70%	3
		Others	/	8303	0.1	4.5	1.4	70%	56
	Nitrofuran (AOZ)	Crabs	*Portunus pelagicus*	40	ND	ND	ND	80%	0
		Marine fish	*Penaeus monodon*	660	ND	ND	ND	80%	0
		Squid/ Octopus	*Sepia* spp./ *Octopus* spp.	5774	0.5	1.4	0.8	80%	7
		Black Tiger Shrimps	*Penaeus monodon*	1459	0.5	1.2	0.7	80%	6
		Others	/	5878	0.5	3.2	1.3	80%	16
	Nitrofuran (AMOZ)	Crabs	*Portunus pelagicus*	45	ND	ND	ND	75%	0
		Black Tiger Shrimps	*Penaeus monodon*	659	0.7	0.7	0.7	75%	1
		Frozen Black Tiger Shrimps	*Penaeus monodon*	3834	ND	ND	ND	75%	0
		Frozen Boiled Black Tiger Shrimps	*Penaeus monodon*	1458	ND	ND	ND	75%	0
		Others	/	5671	ND	ND	ND	75%	0

2007	CAP	Crabs	*Portunus pelagicus*	515	0.1	124.1	10.3	70%	15
		Marine fish	/	3,273	0.3	3.3	1.7	70%	9
		Squid/ Octopus	*Sepia* spp./ *Octopus* spp.	3,062	0.1	11.1	3.5	70%	18
		Black Tiger Shrimps	*Penaeus monodon*	24,718	0.1	23.9	3.6	70%	50
		Others	/	2,861	0.1	5.43	1.2	70%	34
	Nitrofuran (AOZ, AMOZ)	Crabs	*Portunus pelagicus*	856	0.5	5.4	1.8	80%	14
		Marine fish	/	1,982	0.5	6.6	2.3	80%	18
		Squid/ Octopus	*Sepia* spp./ *Octopus* spp.	1,230	0.5	8.5	3.1	80%	8
		Black Tiger Shrimps	*Penaeus monodon*	22,403	0.5	51.6	5.7	80%	64
		Others	/	1,300	0.5	2.7	1.5	80%	6
	MG/ LMG	Tra/Basa catfish	*Portunus pelagicus*	22,191	3.3	25	12.2	85%	24
		Black Tiger Shrimps	*Penaeus monodon*	2,913	ND	ND	ND	85%	0
		Others	/	827	4	64	10.7	85%	15

c. Corrective Actions

Not applicable.

5. Problems and Challenges Encountered

Analysis results showed that there were still samples detected with residues of banned substances. This signifies that the use of banned substances in aquaculture does exist. The number of overall violated samples in 2007 was increased to 0.38% from 2006's 0.33%. but the rate of defected samples and total sample in CAP was reduced (2006:0.64%; 2007:0.37%). The number of Vietnamese fishery consignments notified by importing countries due to detection of banned substances residues, such as CAP, NFs, MG/LMG in 2007 is much fewer than previous years. It means that the awareness of the need to stop using banned substances has raised among Vietnamese farmers and the applied control measures for the residues of banned substances in Vietnam proved to be effective.

6. Recommendations and Suggestions for Future Follow up Action

- Due to limited budget for each activity, collected data are not representative enough to reflect the real impact of chemical contaminants and antibiotics residues on fish.
- It would be highly appreciated if the Project could focus on training in new analytical methods, specifically in multi-residues analysis methods in order to improve testing capabilities in fish quality and safety control in the South-East Asia region.

Summary

A total of 8 countries, namely Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam, participated in this JTF II project on research and analysis of chemical residues and contamination in fish and fish products.

From the results of the surveys conducted on heavy metals, pesticide residues, histamine and drug residues, only a few samples have exceeded national or international regulatory limits. It can therefore be concluded that the fish and fish products from these countries are safe for both domestic consumption and export.

From this compilation, it was noted that only a few laboratories participate regularly in recognised inter-laboratory proficiency testing. Participation in inter-laboratory proficiency testing is strongly encouraged as it is a measure of the laboratory's proficiency and staff competency when conducting the test with the analysis method and it gives confidence to the results produced by the laboratory.

Some of the common problems faced by participating laboratories include the lack of budget and the lack of competent laboratory staff. Many laboratories mentioned that the sampling size of the survey was small and the sampling locations were limited. The results of the survey may therefore not be representative enough to reflect the actual situation of the chemical contamination level in the country. In this JTF II project, 6 on-site and 3 regional trainings were conducted for a total of 106 laboratory personnels to transfer technology in the analysis of heavy metals, pesticide residues, histamine and drug residues. However, there is a need to continuously train and upgrade the skills of laboratory personnel to ensure the competency and efficiency.

In conclusion, this project benefitted participating countries by providing baseline information of the level of contamination for heavy metals, pesticide residues, and histamine and drug residues. This information may be useful for the planning of monitoring programs within the country. These results had also given assurance and confidence in the safety of the fish and fish products in the participating countries.

Annex 1

List of Major Importing Countries and their Maximum Residue Limits

Country	Heavy Metals	Pesticide Residues	Histamine
USA	Cadmium: • 3 µg/g for crustaceans • 4 µg/g for clams, oysters and mussels Lead: • 1.5 µg/g for crustaceans • 1.7 µg/g for clams, oysters and mussels Arsenic: • 76 µg/g for crustaceans • 86 µg/g for clams, oysters and mussels Mercury: • 1.0 µg/g methylmercury for all fish Source: U.S. Food and Drug Administration, Centre for Food Safety and Applied Nutrition, Fish and Fishery Products: Hazards and Controls Guide, 3rd Edition, 2001.	• Aldrin / Dieldrin: 0.3 ppm in edible portion of fin fish and shell fish • Benzene hexachloride, BHC (HCH): 0.3 ppm in edible portion of frog legs <u>In edible portion of all fish</u> • Chlordane: 0.3 ppm • DDT, TDE, DDE: 5.0 ppm • Heptachlor and Heptachlor Epoxide: 0.3 ppm Source: FDA Guidelines extracted from Appendix 5, FDA & EPA Safety Levels in Regulations and Guidance, Third edition, June 2001	For tuna, mahi-mahi and related fish: 500 ppm (50 mg/100 g) as the toxicity level, and 50 ppm (5 mg/100 g) as the defect action level (or hazard action level) Source: U.S. Food and Drug Administration, Centre for Food Safety and Applied Nutrition, Fish and Fishery Products: Hazards and Controls Guide, 3rd Edition, 2001.

Country	Heavy Metals	Pesticide Residues	Histamine

EU	Cadmium:
	• 0.05 µg/g in muscle excluding species listed below
	• 0.10 µg/g in muscle of the following fish:
	  • anchovy (*Engraulis species*)
	  • bonito (*Sarda sarda*)
	  • common two-banded seabream (*Diplodus vulgaris*)
	  • eel (*Anguilla anguilla*)
	  • grey mullet (*Mugil labrosus labrosus*)
	  • horse mackerel or scad (*Trachurus species*)
	  • louver or luvar (*Luvarus imperialis*)
	  • sardine (*Sardina pilchardus*)
	  • sardinops (*Sardinops species*)
	  • tuna (*Thunnus species, Euthynnus species, Katsuwonus pelamis*)
	  • wedge sole (*Dicologoglossa cuneata*)
	• 0.30 µg/g in muscle of swordfish (*Xiphias gladius*)
	• 0.50 µg/g in crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae and Palinuridae*)
	• 1.0 µg/g for bivalve molluscs and cephalopods (without viscera)
		<u>In meat and edible offal</u>
		• Chlordane: 0.05 ppm
		• DDT: 1 ppm
		• Dieldrin/aldrin: 0.2 ppm
		• Endrin: 0.05 ppm
		• HCH: 0.3 ppm
		• Heptachlor: 0.2 ppm
		• Hexaclorobenzene: 0.2 ppm
		• Lindane: 0.02 ppm
		
		Source: EU Guidelines extracted from informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC, 5058/VI/98, 3 November 2004.
			<u>For fish species in the Scombridae and Clupeidae family</u>
			• Nine samples must be taken from each batch. These must fulfil the following requirements:
			• The mean value must not exceed 100 ppm
			• Two samples may have a value of more than 100 ppm but less than 200 ppm
			• No sample may have a value exceeding 200 ppm
			
			Source: Directive 91/493 Annex Chapter V: Health control and monitoring of production conditions

	Lead: • 0.30 µg/g in muscle of fish • 0.50 µg/g in crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae and Palinuridae*) • 1.5 µg/g for bivalve molluscs • 1.0 µg/g for cephalopods (without viscera)		

Country	Heavy Metals	Pesticide Residues	Histamine

	Mercury:
	• 0.50 µg/g in fishery products and muscle of fish excluding species listed below
	• 1.0 µg/g in muscle meat of:
	  • anglerfish (*Lophius species*)
	  • atlantic catfish (*Anarhichas lupus*)
	  • bonito (*Sarda sarda*)
	  • eel (*Anguilla anguilla*)
	  • emperor, orange roughy, rosy soldierfish (*Hoplostehus species*)
	  • grenadier (Coryphaenoides rupestris)
	  • halibut (*Hippoglossus hippoglossus*)
	  • marlin (*Makaira species*)
	  • megrim (*Lepidorhombus species*)
	  • mullet (*Mullus species*)
	  • pike (*Esox lucius*)
	  • plain bonito (*Orcynopis unicolor*)
	  • poor cod (*Tricopterus minutes*)
	  • portuguese dogfish (*Centroscymnus coelolepis*)
	  • rays (*Raja species*)
	  • redfish (*Sebastes marinus, S. mentella, S. viviparus*)
	  • sail fish (*Istiophorus platypterus*)
	  • scabbard fish (*Lepidopus caudatus, Aphanopus carbo*)
	  • seabream, Pandora (*Pagellus species*)
	  • shark (all species)
	  • snake mackerel or butterfish (*Lepidocybium flavobrunneum, Ruvettus pretiosus, Gempylus serpens*)
	  • sturgeon (*Acipenser species*)
	  • swordfish (*Xiphias gladius*)
	  • tuna (*Thunnus species, Euthynnus species, Katsuwonus pelamis*)
	• 0.5 µg/g for crustaceans, excluding the brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae and Palinuridae*)
	
	Source: Commission Regulation (EC) No 1881/2006		

Country	Heavy Metals	Pesticide Residues	Histamine
Canada	• Lead: Not more than 0.5 µg/g in fish protein concentrate • Arsenic: Not more than 3.5 µg/g in fish protein concentrate • Mercury: Not more than 0.5 µg/g of mercury in fish and fish products (except swordfish, shark, fresh and frozen tuna) Source: The Fish Inspection Act (R.S.C. 1985 c.F.-12). The Fish Inspection Regulations (C.R.C 1978c 802)	<u>In all fish products</u> DDT and Metabolites (DDD & DDE): 5.0 ppm Other agricultural chemicals or their derivatives: 0.1 ppm Source: Canadian Guidelines extracted Appendix 3, Canadian Guidelines For Chemical Contaminants and Toxins in Fish and Fish Products	For enzyme ripened products, anchovies, anchovy paste and fish sauce: 200 ppm For all other scombroid fish products: 100 ppm Source: The Fish Inspection Act (R.S.C. 1985 c.F.-12). The Fish Inspection Regulations (C.R.C 1978c 802)
Japan	Mercury: • 0.4 µg/g for total mercury • 0.3 µg/g for methylmercury in fish and shellfishes, except tuna fishes, fishes from inland waters and deep-sea Source: Standards Information Service, Japan External Trade Organisation (JETRO), Specifications and Standrds for Foods, Food Additives, etc. Under Food Sanitation Law, March 2001.	Not available	Not available

Annex 2

List of Participating Countries, Regional Surveys Participated and Types of Fish and Fish Products Surveyed

Country	Types of Fish and Fish Products Surveyed for Heavy Metals (Activity 1.1)	Types of Fish and Fish Products Surveyed for Pesticide Residues (Activity 2.2)	Types of Fish and Fish Products Surveyed for Histamine (Activity 3.1)	Types of Fish and Fish Products Surveyed for Chloramphenicol, Nitrofuran, Malachite Green & Leuco-malachite Green (Activity 4.1)
Cambodia	• Frozen Iridescent Shark Catfish (*Pangasianodon hypophthamus*)	• Trey Kes (*Micronema micronema*) • Marbled Sleeper (*Oxyeleotris marmorata*)	• Basa Catfish (*Pangasius bocourti*) • Indo-pacific Mackerel (*Scomberomorus guttatus*) • Grouper (*Epinephelus coioides*)	-
Indonesia	• Fresh/Frozen/Canned/ Loin/Baby Tuna (*Thunnus albacares*) • Fresh Skipjack Tuna (*Katsuwonus pelamis*) • Yellowfin Tuna (*Thunnus albacares*) • Bluefin Tuna (*Thunnus thynnus*) • Red Snapper (*Lutjanus* spp.) • Grouper (*Epinephelus* spp.) • Frigate Mackerel (*Auxis thazard*) • Barred Spanish Mackerel (*Scomberomorus commerson*) • Swordfish (*Xiphias gladius*) • Striped Marlin (*Tetrapturus audax*) • Black Marlin (*Makaira indica*) • Baramundi (*Lates calcarifer*) • Butterfish (*Lepidocybium flavobrunneum*) • Leather jacket (*Aluterus monoceros*) • Kingfish (*Seriolina nigrofasciata*) • Black Tiger Prawns (*Penaeus monodon*) • Shrimp (*Penaeus vannamei*)	-	• Fresh/Frozen/ Canned/Loin/ Baby Tuna (*Thunnus albacares*) • Bluefin Tuna (*Thunnus thynnus*) • Yellowfin Tuna (*Thunnus albacares*) • Skipjack Tuna (*Katsuwonus pelamis*) • Striped Marlin (*Tetrapturus audax*) • Black Marlin (*Makaira indica*) • Swordfish (*Xiphias gladius*) • Red Snapper (*Lutjanus* spp.) • Butterfish (*Lepidocybium flavobrunneum*) • Grouper (*Epinephelus* spp.) • Leather Jacket (*Aluterus monoceros*) • Baramundi (*Lates calcarifer*)	• Shrimp (*Penaeus monodon*) • Shrimp (*Penaeus vannamei)*

Country	Types of Fish and Fish Products Surveyed for Heavy Metals (Activity 1.1)	Types of Fish and Fish Products Surveyed for Pesticide Residues (Activity 2.2)	Types of Fish and Fish Products Surveyed for Histamine (Activity 3.1)	Types of Fish and Fish Products Surveyed for Chloramphenicol, Nitrofuran, Malachite Green & Leuco-malachite Green (Activity 4.1)
Malaysia	• Spanish Mackerel (*Scomberomorus sp.*) • Red Snapper (*Lutjanus sp.*) • Shrimps (*Metapeneaus sp.*) • White Shrimp (*Penaeus vannamei*) • Squid (*Loligo sp.*) • Baramundi (*Lates calcarifer*) • Catfish (*Clarius batrachus*) • Tilapia (*Oreochromis niloticus*) • Green mussel (*Perna viridis*) • Black Tiger shrimp (*Penaeus monodon*) • Grouper (*Epinephelus sp.)* • Cockle (*Anadara granosa*) • Fishcake • Fishball • Surimi Kintokidai A, Big Eye Snapper • Surimi (Threadfin Bream) • Frozen octopus (*Octopus dofleini*) • Frozen cuttlefish (*Sepia inermis*)	• Surimi (threadfin bream) • Surimi Kintokidai A, Big Eye Snapper • Fish ball • Black Tiger Prawn (*Penaeus monodon*) • Frozen Shrimp (*Penaeus vannamei*) • Frozen Squid (*Loligo* spp.)	• Japanese Threadfin Beam, Kerisi (*Nemipterus sp.*) • Mackerel, Kembung (*Rastrelliger sp.*) • Dorab Wolf-Herring, Parang (*Chirocentrus dorab*) • Narrow-barred Spanish Mackerel, Tenggiri (*Scomberomorus commerson*) • Swordfish, Todak (*Xiphias gladius*) • Shortfin Scad, Selayang (*Decapterus macrosoma*) • Hardtail Scad, Cencaru (*Megalaspis cordyla*) • Big Eye Scad, Lolong (*Selar crumenopthlamus*) • Rainbow Sardine, Round Herring, Tamban (*Sardinella fimbriata*) • Yellow-striped trevally, Selar Kuning (*Selaroides leptolepis*) • Anchovies, Ikan Bilis (*Stolephorus indicus*)	• Shrimp (*Penaeus monodon*)

Country	Types of Fish and Fish Products Surveyed for Heavy Metals (Activity 1.1)	Types of Fish and Fish Products Surveyed for Pesticide Residues (Activity 2.2)	Types of Fish and Fish Products Surveyed for Histamine (Activity 3.1)	Types of Fish and Fish Products Surveyed for Chloramphenicol, Nitrofuran, Malachite Green & Leuco-malachite Green (Activity 4.1)
Myanmar	• Tiger Prawn (*Penaeus monodon*) • Snake Head (*Channa striata*) • Yellowtail Catfish (*Pangasius pangasius*) • Frozen Squid (*Loligo duvauceli*) • Frozen Cuttlefish (*Sepia pharaonis*) • Rohu (*Labeo rohita*) • Grouper (*Epinephelus tauvina*) • Seabass (*Lates calcarifer*) • Giant Fresh-water prawn (*Macrobrachium rosenbergii*)	• Rohu (*Labeo rohita*) • Giant Fresh-water prawn (*Macrobrachium rosenbergii*) • Tilapia (*Oreochromis niloticus*) • Mrigal (*Cirrhinus mrigala*) • Snake Head (*Channa striata*) • Common Carp (*Cyprinus carpio*) • Tarpon (*Puntius chola*) • Boal (*Wallago atta*) • Indian Cat Fish (*Clarias batrachus*) • Climbing Perch (*Anabas testudineus*)	• Spanish Mackerel (*Scomberomorus commerson*) • Fresh/Canned Sardine (*Sardinella gibbosa*) • Dried Anchovy (*Stolephorus indicus*) • Fresh/Canned Hilsa (*Tenualosa ilisha*) • Dried Lotia (*Harpodon nehereus*) • Fish paste • Shrimp paste	• Fresh/Frozen Tiger Prawn (*Penaeus monodon*) • Fresh/ Frozen Prawn (*Macrobrachium rosenbergii*) • Tilapia (*Oreochromis niloticus*) • Rohu (*Labeo rohita*) • River Catfish (*Sperata seenghala*) • Pink shrimp (*Metapenaeus affinis*) • White Indian prawn (*Penaeus indicus*)
Philippines	-	• Shrimp (*Penaeus monodon*) • Milkfish (*Chanos chanos*) • Mudcrab (*Scylla serrata*) • Lobster (*Panulirus sp.*)	• Yellowfin Tuna (*Thunnus albacares*) • Blue Marlin (*Makaira nigricans*) • Skipjack Tuna (*Katsuwonus pelamis*) • Milkfish (*Chanos chanos*) • Mackerel (*Scomberomorus* spp.) • Sardine (*Sardinella longiceps*) • Dried Anchovies (*Stolephorus ronquilloi*)	• Fresh frozen shrimp (*Penaeus monodon*) • Crab (*Scylla serrata*) • Lobster (*Panulirus sp.*) • Grouper (*Epinephelus* spp.)

Country	Types of Fish and Fish Products Surveyed for Heavy Metals (Activity 1.1)	Types of Fish and Fish Products Surveyed for Pesticide Residues (Activity 2.2)	Types of Fish and Fish Products Surveyed for Histamine (Activity 3.1)	Types of Fish and Fish Products Surveyed for Chloramphenicol, Nitrofuran, Malachite Green & Leuco-malachite Green (Activity 4.1)
Singapore	• Sea cucumber • Blood cockle (*Anadara granosa*) • Mitre squid (*Loligo chinensis*) • Mud crab (*Scylla serrata*) • Longtail tuna (*Thunnus tonggol*) • Barred Spanish mackerel (*Scomberomorus commerson*)	• Spanish Mackerel (*Scomberomorus commerson*) • Snakeskin Gouramy (*Trichogaster pectoralis*) • Mei Ren Yu • Sand Whiting (*Sillago sihama*) • Yellow-banded Scad (*Selaroides leptolepis* Valencinnes) • Threadfin (*Polynemus indus* Shaw) • Indian Mackerel (*Rastrelliger kanagurta*)	-	-
Thailand	• Frozen Cuttlefish (*Sepia* spp.) • Frozen Baby Octopus (*Octopus* spp.) • Frozen squid (Loligo spp.) • Canned Skipjack Tuna (*katsuwonus pelamis*) • Canned Albacore Tuna (*Thunnus alalunga*) • Canned Yellowfin Tuna (*Thunnus albacares*) • Frozen Vannamei Shrimp (*Penaeus vannamei*) • Frozen Salmon (*Oncorhynchus keta*) • Ribbon Fish（*Trichiurus sp.)* • Imitation Crab Meat (*Nemipterus* spp.) • Frozen Nile Tilapia (*Tilapia nilotica*) • Canned Sardinella (*Sardinella gibbosa*) • Frozen Baby clam (*Paphia undulate*) • Frozen green mussel (*Perna viridis*)	• Frozen Shrimp (*Penaeus vannamei*) • Frozen Nile Tilapia (*Tilapia nilotica*) • Dried Yellowstripe Trevally (*Selaroides leptolepis*)	• Frozen and Canned Skipjack Tuna (*Katuwonus pelamis*) • Dried Anchovy (*Stolephorus* spp.) • Mackerel (*Decapterus sp.*) • Sardine (*Sardinella gibbosa*) • Long Tail (Thunnus tonggol) • Tuna sauce	• Fresh/Frozen Tiger prawn (*Penaeus monodon*) • Freshwater Shrimp (*Macrobrachium rosenbergii*) • Crab (*Scylla serrata*)

Country	Types of Fish and Fish Products Surveyed for Heavy Metals (Activity 1.1)	Types of Fish and Fish Products Surveyed for Pesticide Residues (Activity 2.2)	Types of Fish and Fish Products Surveyed for Histamine (Activity 3.1)	Types of Fish and Fish Products Surveyed for Chloramphenicol, Nitrofuran, Malachite Green & Leuco-malachite Green (Activity 4.1)
Vietnam	• Black Tiger Shrimps (*Penaeus monodon*) • Bigeye Tuna (*Thunnus albacares*) • Tra Catfish (*Pangasius hypophthalmus*) • Basa Catfish (*Pangasius bocourti*) • Clam *(Meretrix lyrata*) • Squids (*Loligo edulis*) • Cuttlefish (*Sepia* spp.) • Octopus (*Octopus* spp.) • Marlin Fish (*Makaira indica*) • Swordfish (*Xiphias gladius*) • Ribbon Fish (*Xiphias gladius*) • Mackerel (*Acanthocybium solandri*)	• Black Tiger Shrimps *(Penaeus monodon*) • Clam (*Meretrix lyrata*) • Basa catfish (*Pangasius bocourti*) • Tra catfish (*Pangasius hypophthalmus*) • Tilapia (*Oreochromis* spp.)	• Dried anchovy (*Stolephorus* spp.) • Frozen/Canned Skipjack Tuna • Frozen/Canned Bigeye Tuna (*Thunnus albacares*) • Canned Sardines (*Sardinella gibbosa*)	• Crabs (*Portunus pelagicus*) • Frozen/Frozen Boiled/ Fresh Black Tiger Shrimps (*Penaeus monodon*) • Bigeye Tuna (*Thunnus albacares*) • Octopus (*Octopus* spp.) • Squid (*Loligo edulis*) • Cuttlefish (*Sepia* spp.)

Common Name	Scientific Name
Anchovy	
Indian Anchovy	*Stolephorus indicus*
Ronquillo'sAnchovy	*Stolephorus ronquilloi*
Cat fish	
Basa catfish	*Pangasius bocourti*
Indian catfish	*Clarius batrachus*
Iridescent Shark catfish	*Pangasianodon hypophthamus*
River catfish	*Sperata seenghala*
Tra Catfish	*Pangasius hypophthalmus*
Yellowtail catfish	*Pangasius pangasius*
Clam	
Clam	*Meretrix lyrata*
Baby Clam	*Paphia undulate*
Crab	
Crab	*Portunus pelagicus*
Mudcrab	*Scylla serrata*
Cuttlefish	
Pharaoh Cuttlefish	*Sepia inermis*
Spineless Cuttlefish	*Sepia pharaonis*
Grouper	
Orange-spotted Grouper	*Epinephelus coioides*
Greasy Grouper	*Epinephelus tauvina*
Mackerel	
Barred Spanish Mackerel	*Scomberomorus commerson*
Frigate Mackerel	*Auxis thazard*
Indian Mackerel	*Rastrelliger kanagurta*
Indo-pacific Mackerel	*Scomberomorus guttatus*
Mackerel	*Rastrelliger sp.*
Mackerel	*Decapterus sp.*
Mackerel, Wahoo	*Acanthocybium solandri*
Marlin	
Black Marlin	*Makaira indica*
Blue Marlin	*Makaira nigricans*
Striped Marlin	*Tetrapturus audax*
Prawn and shrimp	
Black Tiger Prawn	*Penaeus monodon*
Giant Freshwater prawn	*Macrobrachium rosenbergii*
Pink shrimp	*Metapenaeus affinis*
Shrimp	*Metapeneaus sp.*
White Indian prawn	*Penaeus indicus*
White shrimp	*Penaeus vannamei*
Ribbon Fish	
Ribbon Fish	*Trichiurus sp.*
Sardine	
Sardine, Goldstripe *sardinella*	*Sardinella gibbosa*
Indian Oil Sardine	*Sardinella longiceps*
Scad	
Big Eye Scad	*Selar crumenopthlamus*
Hardtail Scad	*Megalaspis cordyla*
Shortfin Scad	*Decapterus macrosoma*
Yellow-banded Scad	*Selaroides leptolepis* Valencinnes

Snapper	
Red snapper	*Lutjanus* spp.
Swordfish	
Swordfish, Ribbon Fish, Ikan Todak	*Xiphias gladius*
Squid	
Mitre squid	*Loligo chinensis*
Squid	*Loligo edulis*
Squid	*Loligo duvauceli*
Threadfin	
Surimi Threadfin Bream	*Nemipterus sp.*
Threadfin	*Polynemus indus* Shaw
Tilapia	
Nile Tilapia	*Tilapia nilotica*
Tilapia	*Oreochromis niloticus*
Tuna	
Albacore Tuna	*Thunnus alalunga*
Bigeye Tuna	*Thunnus albacares*
Bluefin Tuna	*Thunnus thynnus*
Longtail Tuna	*Thunnus tonggol*
Skipjack Tuna	*Katsuwonus pelamis*
Yellowfin Tuna	*Thunnus albacares*
Others	
Boal	*Wallago atta*
Butterfish	*Lepidocybium flavobrunneum*
Climbing perch	*Anabas testudineus*
Cockle / Blood cockle	*Anadara granosa*
Common carp	*Cyprinus carpio*
Dorab Wolf-Herring	*Chirocentrus dorab*
Frozen Salmon	*Oncorhynchus keta*
Green mussel	*Perna viridis*
Hilsa	*Tenualosa ilisha*
Kingfish	*Seriolina nigrofasciata*
Imitation Crab Meat	*Nemipterus* spp.
Leather jacket	*Aluterus monoceros*
Lobster	*Panulirus sp.*
Long Tail	*Thunnus tonggol*
Lotia	*Harpodon nehereus*
Marbled Sleeper	*Oxyeleotris marmorata*
Milkfish	*Chanos chanos*
Mrigal	*Cirrhinus mrigala*
Octopus	*Octopus dofleini*
Rohu	*Labeo rohita*
Round Herring	*Sardinella fimbriata*
Sand Whiting	*Sillago sihama*
Seabass, Baramundi	*Lates calcarifer*
Snakehead	*Channa striata*
Snakeskin Gouramy	*Trichogaster pectoralis*
Tarpon	*Puntius chola*
Trey Kes	*Micronema micronema*
Yellow-striped trevally	*Selaroides leptolepis*

SEAFDEC's Member Countries' Key Regional Laboratories and Key Project Leaders

Information on Key Laboratory in SEAFDEC's Member Country for JTF II Project.

Country	Name of Key Laboratory	Department, Ministry	Address of Laboratory	Telephone number of Laboratory	Fax number of Laboratory	ISO/IEC 17025 accredited?	Types of test available for Fish & Fish products testing in Laboratory ("A" for tests accredited)
Cambodia	-	Fisheries Administration Ministry of Agriculture Forestry and Fisheries	186 Norodom Blvd, P.O. Box 582, Phnom Penh, Cambodia	-	(855) 23 221 485	No	No
Indonesia	National Centre for Fish Quality Control (NCQC) Jakarta	Directorate General of Fish Processing and Marketing. Ministry of Marine Affairs and Fisheries	Jalan Muara Baru Ujung, Penjaringan. Jakarta Utara Indonesia	(62-21) - 669 5586	(62-21) - 669 5593	Accredited for organoleptic, chemical and microbiology by the National Accreditation Committee under the National Standardisation Agency of Indonesia	Chloramphenicol by LC-MS, Chloramphenicol by HPLC-UV/Vis, Nitrofuran and metabolites by LC-MS, Malachite green by HPLC-UV/Vis, Leucomalachite green by HPLC-fluorescence, Pb and Cd by AAS-graphite (A), Hg by CV-AAS (A), Organochlorine and organophosphoric pesticides by GC-MS, Histamine by HPLC-fluorescence and spectrofluorometer (A), TVBN by distillation (A).
Malaysia	Fish Quality Control Centre, Subang	Department of Fisheries	47200 Subang, Selangor Darul Ehsan, Malaysia	60-3-78465143	60-3-78465149	No	Heavy metals: Mercury, lead, Cadmium, Arsenic and Copper Drugs: Chloramphenicol and Nitrofuran Pesticide residues: α-BHC, β-BHC, γ-BHC, δ-BHC, Heptachlor, Aldrin, Heptachlor Epoxide, γ-chlordane, α-chlordane, dieldrin, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT

Myanmar	Supervision of Quality Control Laboratories Fish Inspection & Quality Control Division	Department of Fisheries, Ministry of Livestock and Fisheries	Shu Khin Tha Road, Thaketa P.O Yangon, Myanmar	095-01 708520	095-01-228258 (Head Office)	No	-
Philippines	Fisheries Product Testing Laboratory	Bureau of Fisheries & Aquatic Resources. Department of Agriculture	860 Arcadia Bldg. Quezon Avenue Quezon City	372-50-59 372-50-50	372-50-59	No	Chemical: Ash, Moisture, Crude Protein, Crude Fat, NaCl, Boric Acid, Free Fatty Acid, Peroxide Value, Thiobarbituric Acid No., Histamine, Trimethylamine, Formalin, Cyanide, Total Mecury, Lead, Cadmium, Copper, Water Activity Microbial: Aerobic Plate Count, Anaerobic Plate Count, *Salmonella* & *Shigella*, *Staphylococcus*, Fungi (Yeast & Molds), Total *Coliform* & *E.coli*, *Clostridium perfringens* in water, *Enterococci* in water
	Fish Health Management and Quality Assurance Laboratory	Bureau of Fisheries and Aquatic Resources, Department of Agriculture	860 Quezon Avenue, Quezon City	632-4074421	632-3725055	Not yet Application submitted to Philippine Accreditation Office, awaiting schedule of audit	***1. Disease diagnosis*** Bacteriology Polymerase Chain Reaction to dectect WSSV.KHV, etc. ***2. Residues*** ELISA CAP, Nitrofurans, DES 3. Aqua-Feeds Chloramphenicol Aflatoxin

Country	Name of Key Laboratory	Department, Ministry	Address of Laboratory	Telephone number of Laboratory	Fax number of Laboratory	ISO/IEC 17025 accredited?	Types of test available for Fish & Fish products testing in Laboratory ("A" for tests accredited)
Singapore	Chemistry Laboratory	Marine Fisheries Research Department	2 Perahu Road, Off Lim Chu Kang Road, Singapore 718915	+65-6790 7973	+65-6861 3196	Yes. SAC-SINGLAS.	Moisture, Arsenic, Cadmium, Lead and Mercury methods Accredited
Thailand	Fish Inspection and Quality Control Division	Department of Fisheries, Ministry of Agriculture and Cooperatives	50 Preeda Karnasutha Building, Kaset-klang, Chatuchak, Bangkok 10900	66 2 558 0150-5 ext 13300 and 13303	66 2 558 0139	Yes. Bureau of Laboratory Quality Standards (BLQS)	Nitrofuran (Metabolites), Chloramphenicol, Malachite green, Cd, Oxolinic acid, Oxytetracycline, Histamine, Benzoic and Sorbic acid.
Thailand	Songkhla Fish Inspection and Research Center	Department of Fisheries, Ministry of Agriculture and Cooperatives	79/2 Wichainchom Road, Boyang, Muang Songkhla 90000 Thailand	66 74 440054	66 74 314797	Yes. Bureau of Laboratory Quality Standards, Ministry of Public Health	Histamine , Cd ,Pb, Hg Oxytetracycline, Oxolinic acid , Chloramphenicol Bensoic & Sorbic acid,

Vietnam	Chemistry Laboratory Branch 3, 4 and 6	Ministry of Agriculture and Rural Development – National Agriculture, Forestry and Fisheries Quality Assurance Department	10 Nguyen Cong Hoan Street, Ba Dinh District, Ha Noi City	84-4-8310983; 7714192	88-4-8317221	Yes, ISO 17025 by BoA-VILAS	Heavy metals (Pb, Cd, Hg) using AAS (A), Pesticide residues using GC-ECD (A), Histamine (A), ELISA for CAP, NFs (A), CAP, NFs by Tandem LC/MS/MS (A), MG and LMG by LC/MS/MS (A) Other methods available: Lipophilic toxin (A), PSP toxin (A), ASP toxin (A), Aflatoxin B_1, B_2, G_1, G_2 (A), Methyl testosterone (A), diethyl stilbestrol (DES) (A), Melamine (A), Crystal violet + Leuco Crystal Violet (A), Tetracylines group (A), Quinolones/ Fluoroquinolnes group (A), Sulfonamides group (A), Macrolides Group (A), Flophenicol (A), Trimethoprime (A), Formaldehyde, TVB-N (A), NaCl, Sulphite (A), Nitrogen-ammonia, Boric acid
			Branch 3 – 779 Le Hong Phong, Binh Tan Ward, Nha Trang City, Vietnam	84-58-888248	84-58-884811		
			Branch 4 –30 Ham Nghi, Ben Nghe Ward, Ho Chi Minh City, Vietnam	84-891-43048	84-8-8212613		
			Branch 6 – 386C Cach Mang Thang Tam, An Thoi Ward, Can Tho City, Vietnam	84-71-03880260	84-71-03884697		

Information on Key Project Leader for JTF II Project

Country	Name of Activity Involved	Name of Key Project Leader	Designation	Department, Ministry	Address of Office	Telephone number	Fax number	Email address
Cambodia	1.1: Regional survey of heavy metals in fish and fish products	Dr. Chea Tharith	Deputy Director, Inland Fisheries Research and Development Institute (IFReDI),c/o Fisheries Administration (FiA)	Fisheries Administration Ministry of Agriculture Forestry and Fisheries	186 Norodom Blvd, P.O. Box 582, Phnom Penh, Cambodia	(855) 12467648	(855) 23221485	cheatharit@yahoo.com
Cambodia	2.2: Regional survey of pesticide residues in fish and fish products	Mr Thach Phanara	Vice Chief, Laboratory of Inland Fishery Research and Development Institute	Fisheries Administration Ministry of Agriculture Forestry and Fisheries	186 Norodom Blvd, P.O. Box 582, Phnom Penh, Cambodia	(855) 023221486 (855) 023221485	(855) 23427048	thachphanara@yahoo.com
Cambodia	3.1: Regional survey of histamine in fish and fish products	Mr Em Samy	Vice Chief, Laboratory of Inland Fishery Research and Development Institute	Fisheries Administration Ministry of Agriculture Forestry and Fisheries	186 Norodom Blvd, P.O. Box 582, Phnom Penh, Cambodia	(855) 023221486 (855) 023221485	(855) 023221486 (855) 023221485	emsamy@yahoo.com

Indonesia	1.1: Regional survey of heavy metals in fish and fish products 2.2: Regional survey of pesticide residues in fish and fish products 4.1 Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.	Dr. Simson Masengi	Chief of Sub-Directorate for Processing of Small-Medium Enterprise	Directorate of Fisheries Product Processing, Directorate General of Fish Products Processing and Marketing, Ministry of Marine Affairs & Fisheries	Jl. Merdeka Timur 16, Jakarta Pusat 10110, Indonesia	(62-21) 3500187 (62-21) 3519070	(62-21) 3500187 (62-21) 3519070	simsonmasengi@hotmail.com
Malaysia	1.1: Regional survey of heavy metals in fish and fish products	Mr Azlan Bin Md. Nor	Fisheries Officer	Fish Quality Control Centre, Subang, Department of Fisheries	47200, Subang, Selangor Darul Ehsan, Malaysia	(03) 7846-5143	(03) 7846-5149	azulan77@yahoo.com.sg
Malaysia	2.2: Regional survey of pesticide residues in fish and fish products	Mr Azlan Bin Md. Nor/ Mrs Rohana Shapiin	Fisheries Officer and Assistant Research Officer	Fish Quality Control Centre, Subang, Department of Fisheries	47200, Subang, Selangor Darul Ehsan, Malaysia	(03) 7846-5143	(03) 7846-5149	*rohana_shapiin@yahoo.com*
Malaysia	4.1 Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.	Mr Othman Muhamad / Ms Zarina Zainuddin	Senior Research Officer/ Fisheries Officer	Fish Quality Control Centre, Subang, Department of Fisheries	47200, Subang, Selangor Darul Ehsan, Malaysia	(03) 7846-5143	(03) 7846-5149	zarinazainuddin80@yahoo.com

Country	Name of Activity Involved	Name of Key Project Leader	Designation	Department, Ministry	Address of Office	Telephone number	Fax number	Email address
Myanmar	1.1: Regional survey of heavy metals in fish and fish products 2.2: Regional survey of pesticide residues in fish and fish products 3.1: Regional survey of histamine in fish and fish products 4.1 Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.	Mr Than Winn	Assistant Director	Department of Fisheries, Ministry of Livestock and Fisheries	Supervision of Quality Control Laboratories, Shu Khin Tha Road, Thaketa P.O Yangon, Myanmar	(095) 01-708520	(095) 01-228258 (Head Office)	DOF@ mptmail.net.mm (Head Office)

Philippines	3.1 Regional Survey of Histamine in Fish and Fish Products in Southeast Asia	Ms Belinda S. Raymundo Ms Flordeliza D. Cambia	Chief, Fisheries Product Testing Laboratory Aquaculturist II	Bureau of Fisheries & Aquatic Resources. Department of Agriculture	860 Arcadia Bldg. Quezon Avenue Quezon City	(632) 372-50-59	(632)372-50-59	bfarphtd@yahoo.com
	2.2: Regional survey of pesticides residues in fish and fish products 4.1 Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.	Simeona E. Regidor	Chief- Fish Health Management and Quality Assurance Section	Bureau of Fisheries and Aquatic Resources, Department of Agriculture	860 Quezon Avenue, Quezon City	(632) 407-4421	(632) 372-5055	simeona03@yahoo.com
Singapore	1.1: Regional survey of heavy metals in fish and fish	Ms Saw Huiyi	Senior Research Officer	Marine Fisheries Research Department	2 Perahu Road, Off Lim Chu Kang Road, Singapore 718915	+65-6790 7973	+65- 6861 3196	Saw_Huiyi@ava.gov.sg
Singapore	2.2: Regional survey of pesticides residues in fish and fish products	Ms Tan Lu Hsia	Senior Research Officer	Marine Fisheries Research Department	2 Perahu Road, Off Lim Chu Kang Road, Singapore 718915	+65-6790 7973	+65- 6861 3196	Tan_Lu_Hsia@ava.gov.sg

Country	Name of Activity Involved	Name of Key Project Leader	Designation	Department, Ministry	Address of Office	Telephone number	Fax number	Email address
Thailand	1.1: Regional survey of heavy metals in fish and fish products 2.2: Regional survey of pesticides residues in fish and fish products	Ms Jariya Pucharoen	Food technologist	Samutsakorn Fish Inspection and Research Centre, Department of Fisheries, Ministry of Agriculture and Cooperative	P.O.39, A.Muang, Samutsakorn, 74000 Thailand	(66) 34-413189, (66) 34-413190	(66) 34-857192	jpucharoen1@yahoo.com
Thailand	3.1: Regional survey of histamine in fish and fish products	Ms Thanyaporn Kongchan	Senior Food Technologist	Songkhla Fish Inspection and Quality Control Division, Department of Fisheries, Ministry of Agriculture and Cooperatives	79/2 Wichainchom Road, Boyang, Muang Songkhla 90000 Thailand	(66) 74 440054	(66) 74 314797	thanyaporn_k@hotmail.com
Thailand	4.1 Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.	Mrs Supanoi Subsinserm	Head of Chemical Laboratory	Fish Inspection and Quality Control Division, Department of Fisheries, Ministry of Agriculture and Cooperatives	50 Preeda Karnasutha Building, Kaset-klang, Chatuchak, Bangkok 10900	66 2 558 0150-5 ext 13300 and 13303	66 2 558 0139	supanois@yahoo.com, supanois@fisheries.go.th

Vietnam	1.1: Regional survey of heavy metals in fish and fish products	Mrs Huynh Thi Ngoc Lien	Head of Chemical Laboratory	NAVIQAVED Branch 6, National Fisheries Quality Assurance and Veterinary Directorate	386C Cach Mang Thang Tam, An Thoi Ward, Can tho city, Vietnam	(84) 71-884818	(84) 71- 883257	ngoclienct@yahoo.com
Vietnam	2.2: Regional survey of pesticides residues in fish and fish products	Ngo Hong Phong	Controlling Officer of Laboratory Management Division	National Fisheries Quality Assurance and Veterinary Directorate	10 Nguyen Cong Hoan street, Ba Dinh district, Ha Noi city	(84) 4-8310983 (84) 77-12529	(84) 4-8317221	hongphong.nafi@mofi.gov.vn
Vietnam	3.1: Regional survey of histamine in fish and fish products	Mr Nguyen Anh Dung	Head of Lab. Division	NAVIQAVED Branch 4, National Fisheries Quality Assurance and Veterinary Directorate	30 Ham Nghi, Ben Nghe Ward, Ho Chi Minh city, Vietnam	(84) 8-91-43048	(84) 8-8212613	knhoanafi4@vnn.vn
Vietnam	4.1 Regional survey of chloramphenicol, nitrofuran, malachite green and leuco-malachite green in fish and fish products.	Mr Truong Anh Tuan	Head of Chemical Laboratory	NAVIQAVED Branch 3, National Fisheries Quality Assurance and Veterinary Directorate	779 Le Hong Phong, Binh Tan Ward, Nha Trang city, Vietnam	(84) 58-888248	(84) 58-884811	tuannaf3@yahoo.com