IMPORTANT FINDINGS AND RECOMMENDATIONS ON

CHEMICAL USE IN AQUACULTURE IN SOUTHEAST ASIA

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Important Findings and Recommendations on Chemical Use in Aquaculture in Southeast Asia

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Supported by the
Government of Japan - Trust Fund V
Project on Food Safety of Aquaculture Products
in Southeast Asia

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ISBN: 978-971-9931-03-4

Published and printed by
Southeast Asian Fisheries Development Center
Aquaculture Department
Tigbauan, Iloilo, Philippines

FOREWORD

We are all aware that aquaculture is currently the fastest growing food production sector with an average annual growth rate of about 7.8% from 1990-2010. This growth rate is substantially higher compared to those of the other sectors like poultry, pork and beef that grew only from 1.0 - 4.6% during the same period. In addition, half of the fish that we consume or we see in the market today is produced in aquaculture farms. Considering that aquaculture supplies significant volume of fish food to the world population, food safety of aquaculture products becomes highly important. Indeed, food safety of aquaculture products is one of the important considerations for acceptance in the international trade and has been given more importance in the Southeast Asian region with the upcoming ASEAN Economic Integration (AEI). It is foreseen that with AEI, there will be free flow of goods and services between and among countries within the region, thus it becomes imperative that the aquaculture products traded conforms to food safety standards to make sure that these products are safe for utilization and for human consumption. The information contained in this publication is very timely and relevant for all aquaculture farmers in the region. Although not a complete listing, these important findings and recommendations on the use of certain chemicals in aquaculture in the region will be a helpful guide for the farmers during their operations. This supplements the recently published *ASEAN Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals.*

FELIX G. AYSON, D. Sc.
AQD Chief

FOREWORD

As a co-manager of the Government of Japan Trust Fund, it is my pleasure to present to you this monograph *Important Findings and Recommendations on Chemical Use in Aquaculture in Southeast Asia.*

This publication is the result of the study program *Promotion of sustainable aquaculture and resource enhancement in Southeast Asia: Food safety of aquaculture products in Southeast Asia* supported by Government Japan Trust Fund V (2010-2014).

The goal of the program, which is to establish and disseminate the publications on safe aquaculture products and on the use of antibiotics and chemicals, is very relevant to one of the important missions of SEAFDEC/AQD that is to provide safe aquaculture food products for the people of the ASEAN region.

Based on the FAO report in 2012, food safety still remains a major concern facing the seafood industry and it is a critical component in ensuring food and nutrition security worldwide. The issue of food safety is even more important in view of the growth in international fish trade, which has undergone tremendous expansion during the last three decades. For international fish trade, countries have enacted national and regional regulations to control seafood entering or exiting their territories.

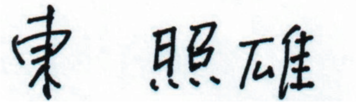
I hope that this publication will be utilized not only for the promotion on food safety awareness for stakeholders through seminars and lectures, but also for some possible action or policy formulations for the trade of aquaculture products by governments of the ASEAN member countries.

TAKURO SHIBUNO, Ph.D.
Deputy Chief and GOJ Trust Fund Co-Manager

FOREWORD

Under the program of Fisheries Consultative Group of the ASEAN (Association of South-East Asian Nations) - SEAFDEC (Southeast Asian Fisheries Development Center) Strategic Partnership Mechanism, a research project entitled “Food Safety of Aquaculture Products in Southeast Asia” was implemented by SEAFDEC/AQD (Aquaculture Department of SEAFDEC) from 2010 to 2014 with the financial support of the Government of Japan Trust Fund V (GOJ-TF5). The objectives of the project were to 1) contribute to establishment of guidelines on the production of safe aquaculture products; 2) determine the presence and levels of commonly used chemicals in aquaculture in aquaculture products; 3) compile and disseminate SEAFDEC guidelines on the use of antibiotics and chemicals in aquaculture; and 4) implement training course/workshop to promote food safety awareness in the ASEAN region. Along with the said objectives, research activities, particularly focusing both on withdrawal periods of antibiotics often used in aquaculture and on surveillance of chemical contaminants in aquaculture products and feeds, were implemented (in 2010-2014). In addition, knowledge and technologies on food safety of aquaculture products were disseminated to local and national government staff, practitioners, stakeholders, etc. through an international seminar (in January 2011) and an international training course (in November 2013). Furthermore, AQD organized *The International Workshop on Food Safety of Aquaculture Products in the Southeast Asia – Challenges in Sustaining the Food Safety of Aquaculture Products* in May 2013 to promote and influence the regional initiatives in securing wholesome and safe aquaculture commodities in the ASEAN region. The publication of this volume consolidates the activities of the project which AQD has pursued during the period of the GOJ-TF5. Although the title of this volume does not include the word “guideline,” this volume surely includes and refers to the ASEAN guidelines recently published with the title of *Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals* courtesy of the ASEAN secretariat based on the understanding of the collaboration between ASEAN-SEAFDEC, as well as the basic and new findings of AQD scientists accomplished through the research activities, which were not covered by the ASEAN guidelines. Although food safety of aquaculture products would be strictly required in the ASEAN region in the future, I believe that this volume will make a great contribution to the requirement.

Finally, I would like to express my sincerest compliment to the three authors of this volume, Drs. Relicardo M. Coloso and Mae R. Catacutan, and Ms. Margarita T. Arnaiz, particularly to Dr. Coloso, the former Head of Research Division, SEAFDEC/AQD, for his persevering editorial work and collaboration as well as his own reflections and studies.



TERUO AZUMA, Ph. D.
Former Deputy Chief, SEAFDEC/AQD and GOJ-Trust Fund Co-Manager
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PREFACE

One of the major problems confronting the aquaculture industry today is the widespread use of chemicals as chemotherapeutic agents for disease prevention and control or those found in feeds ingredients and feeds to allow the industry to meet the increasing demand for aquaculture products. However, the worldwide concern for food safety has led to stricter government regulations on the use of chemicals especially those that are banned or regulated due to their adverse effects on human health, the environment, and the development of pathogen resistance. Thus, fish farmers are facing the challenge of producing safe food from farm to fork.

We have done some work on this area and we wanted to explore it with more depth. It was fortuitous that in 2010 the Government of Japan Trust Fund in its programs under the ASEAN-SEAFDEC FCG Mechanism welcomed the idea and approved the project on Food Safety of Aquaculture Products in Southeast Asia. The accumulation and withdrawal of some antibiotics and chemicals have already been studied in developed countries. However, data on these aspects were generated using their species and under environmental conditions that are vastly different from the conditions prevalent in the Southeast Asian region. Moreover, there are very limited data available on the withdrawal periods of antibiotics and the presence of chemical residues in aquaculture products from the region, a considerable portion of which are exported to developed countries including Japan. Thus, these issues of food safety of aquaculture products is an urgent matter for SEAFDEC and it should take the lead in establishing regional guidelines on the right usage of antibiotics and other chemical inputs that will allow farmers to increase production of safe food. The results of this project would also be useful for the formulation of policy recommendations for a concerted action by governments of the ASEAN member countries.

Concurrent with the Food Safety Project, Malaysia also undertook the responsibility to come up with a set of harmonized guidelines for the use of chemicals in aquaculture and measures to eliminate the use of harmful chemicals for the ASEAN. We also participated in the series of workshops that were conducted to finalize the guidelines an approved copy of which is attached in the Appendix of this monograph. Due to this development, we had to determine the focus of this monograph so as not to duplicate the contents of the ASEAN Guidelines. With more than a decade of on going work at SEAFDEC Aquaculture Department on various aspects of the use of chemicals in aquaculture, we then decided to concentrate on our work on surveillance of antibiotics and pesticide residues in aquaculture products and the withdrawal periods of antibiotics, oxytetracyline and oxolinic acid, in various species of aquatic organisms of importance to aquaculture in the region. Other related research studies we had previously conducted in the Department were also included to present a unified account of the evolution of this area from the scattered works and literature. Hopefully, going through the monograph, the aquaculturist, fish farmer, research students, professionals and other stakeholders will have an overall grasp of the central ideas in this important issue in aquaculture today. If this is achieved then we would have succeeded in our humble efforts. We also hope that the monograph will be useful to experts as a reference for looking up related topics on chemical use in aquaculture.

The actual writing of the monograph took several months and the unwavering encouragement and support from several people. We are particularly indebted to the Government of Japan Trust Fund V for the financial support for the research studies and the international seminars and workshops held under the auspices of the Project. The former GOJ-TF coordinator and SEAFDEC/AQD Deputy Chief, Dr. Teruo Azuma, and later on succeeded by Dr. Takuro Shibuno, provided a lot of encouragement and impetus to write and publish the monograph. The Publications Review

Committee of SEAFDEC/AQD went over the manuscript and provided useful comments and suggestions to improve it. We also deeply acknowledge the help of our colleagues at the Training and Information Division, Development Communication Section, in preparing the layout for this monograph for publication. Without the enthusiasm and help from all of them, this work would not have been completed.

RM Coloso
MR Catacutan
MT Arnaiz

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BACKGROUND AND INTRODUCTION

The use of antibiotics and other chemicals in aquaculture is widely practiced to help meet the increasing demand for aquaculture food. These antibiotics and chemicals detected in aquaculture products appear to derive from material inputs during rearing, mostly from contaminated feed ingredients and therapeutants for prevention or treatment of diseases. Thus, cultured shrimps and fish in various stages from hatcheries to grow-out ponds are exposed to chemical contamination. Consequently, with the ever-growing and worldwide concern for food safety, fish farmers are faced with the challenge of producing safe food from farm to fork. Government regulations are becoming stricter on the uncontrolled use of chemicals due to their adverse effects on human health, the environment and the development of pathogen resistance. Many chemicals have already been banned and the use of some is being regulated. The spectrum of allowable chemicals for aquaculture is becoming narrower, with the trend towards the use of environment-friendly mitigating agents geared to a more responsible approach to aquaculture.

Comprehensive information on the use of chemicals in aquaculture in Asia with emphasis on the various aquaculture systems and species to which chemicals are applied as well as the various country regulations regarding their distribution and use was presented during the *Expert Meeting on the Use of Chemicals in Aquaculture in Asia at the SEAFDEC/AQD* in 1996 (Arthur et al. 2000). Concerns for the safe, effective and minimal use of chemicals to protect human health and the environment are also reflected in the FAO Code of Conduct for Responsible Fisheries (FAO 1995) which called for relevant regulations on the use of chemicals in aquaculture. A wide range of chemicals are being used in aquaculture worldwide in different aquaculture systems and for various reasons. Many chemicals greatly help in ensuring efficient and productive farm and hatchery operations and most have little or no adverse impacts on human health and the environment if applied appropriately. However, sustained efforts are needed to update the general information base on chemical usage in aquaculture in Asia and understand the realities and uncertainties in the regulatory frameworks governing the use of chemicals to ensure food safety and minimal impacts on public health and the environment. Many countries are now imposing strict food safety requirements (maximum residue levels and monitoring banned chemicals) on imported aquaculture products, which will likely pose significant difficulties to countries exporting aquaculture products in the future.

After a series of regional workshops on *Harmonization of Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals* organized by the Department of Fisheries of Malaysia starting in 2009 and then in 2010 and 2012 in Kuala Lumpur and participated in by representatives of ASEAN Members States (AMS), a draft of the ASEAN Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals has been circulated among the AMS prior to its adoption. The Guidelines has been developed to help national regulators and stakeholders in managing the diverse use of chemicals in aquaculture and has been designed so it can be implemented within the specific policy and legal framework of each member state. It outlines the roles and responsibilities of the competent authority or national regulators, the manufacturers and traders of chemicals, and the aquaculturists in each AMS regarding the safe methods to manufacture, procure, use and dispose of chemicals to ensure food safety, and protection of public health and the environment. It also outlines the channels of communication of the competent authorities with the national stakeholders, other ASEAN competent authorities and relevant regional organizations about the use of chemicals and current laws and regulations regarding chemicals in aquaculture as well as the manner to monitor the progress of the competent authorities in the implementation of the Guidelines. The Guidelines also presents a list of commonly used chemicals and drugs in aquaculture among the AMS which have been deliberated and agreed upon during the regional workshops. In the appendices, a list of competent authorities in the AMS (Appendix I), a list of some common chemicals use in aquaculture in the AMS, the indicated use and dosages as well as withdrawal periods if known (Appendix II), and a comprehensive list of chemicals used as well as a list of banned chemicals in aquaculture (Appendix III) in the AMS are presented. Upon adoption of the Guidelines by the ASEAN, SEAFDEC will support and promote its adoption among SEAFDEC Member countries and complement this with guidelines on the use of antibiotics and other chemical inputs based on scientific information gathered from its current project on Food Safety funded by the Government of Japan Trust Fund V.

The mechanisms of accumulation and withdrawal of antibiotics and chemicals have already been well studied in developed countries. However, these data were generated using their species and under environmental conditions that are different from the conditions prevalent in the Southeast Asian region. Moreover, there are very limited data available on the withdrawal period of antibiotics and the presence of chemical residues in aquaculture products from the region, a considerable portion of which are exported to developed countries including Japan. Considering the growing-awareness on issues of food safety of aquaculture products, it is an urgent matter that SEAFDEC should help establish, support and promote regional guidelines on the right usage of antibiotics and other chemical inputs that will allow farmers to increase production of safe aquaculture products using environment-friendly technologies. The Guidelines will also be useful for the formulation of policy recommendations for a concerted action by governments of the AMS.

DEFINITION OF TERMS

Adulteration
Mixing something impure with something genuine, or an inferior article with a superior one of the same kind. Adulteration usually refers to mixing other matter of an inferior and sometimes harmful quality with food or drink intended to be sold. As a result of adulteration, food or drink becomes impure and unfit for human consumption. The U. S. Food and Drug Administration prohibits transportation of adulterated foods, drugs, and cosmetics in interstate commerce, as provided under the Food, Drug and Cosmetic Act (21 U.S.C.A. § 301 et seq. [1938]). State and local agencies, acting under the authority of local laws, do the same to ban the use of such impure goods within their borders.

Antibiotics
Drugs of natural or synthetic origin that have the capacity to kill or inhibit the growth of microorganisms, non-toxic to the host, and are used in order to serve as treatment for disease

Antioxidant (in feeds and feed stuffs)
Substances that prevents rancidity of fats, destruction of vitamins A and E and pigmenters in stored feed

Bioaccumulation
The accumulation of substances, such as pesticides, or other organic chemicals in an organism

Chemotherapy
Use of chemicals to treat diseases

Chromatogram
A plot of analyte concentration signal as a function of elution time

Contaminant
Something that makes a place or a substance (such as water, air, or food) no longer suitable for use; something that contaminates a place or substance

Food safety
Assurance that food will not cause harm to the consumer when it is prepared and/ or eaten according to its intended use

Gas Chromatograph – Electron Capture Detector (GC-ECD)
An instrument for carrying out separations with the use of a gaseous mobile phase and a liquid or a solid stationary phase and an electron capture detector

Hazard
A biological, chemical or physical agent in, or condition of, food with the potential to cause and adverse health effect

High Performance Liquid Chromatograph (HPLC)
An instrument for carrying out separations in which the mobile phase is a liquid, often forced through a stationary phase (or column) by pressure

High-throughput analysis
A process or analysis that is scaled up, usually via increased levels of automation using robots. For instance, high-throughput screening refers to the rapid *in vitro* screening of large numbers of compound libraries (of tens to hundreds of thousands of compounds), using robotic screening assays. High-throughput sequencing involves the application of rapid sequencing technology at the scale of whole genomes

Integrated pest management (IPM)
An ecosystem-based strategy that focuses on long-term prevention of pests or their damage through a combination of techniques such as biological control, habitat manipulation, modification of cultural practices, and use of resistant varieties

Kidney tubules
Small structures in the kidney that filter the blood and produce the urine

Liquid chromatography-tandem mass spectrometry
A method where a sample mixture is first separated by liquid chromatography before being ionised and characterised by mass-to-charge ratio and relative abundance using two mass spectrometers in series

Maximum residue level (MRL)
Maximum concentration of residue to be legally permitted or acceptable in or on a food

Molluscicide
An agent for destroying molluscs, which are usually used in agriculture or gardening, in order to control gastropod pests specifically slugs and snails which damage crops or other valued plants by feeding on them; also known as snail baits or snail pellets

Occupational Safety and Hazard Administration (OSHA)
OSHA is the main federal agency under the U.S. Department of Labor and charged with the enforcement of safety and health legislation in the US region

Organotin compounds
Stannanes are chemical compounds based on tin with hydrocarbon substituents

Oxidation (of fats)
Degradation by oxygen in the air; in feeds, the unsaturated fatty acids will lose hydrogen to form a free radical and will react with oxygen

Permissible Exposure Levels (PEL)
Regulatory limits on the amount or concentration of a substance in the air. They may also contain a skin designation. OSHA PELs are based on an 8-hour time weighted average (TWA) exposure

Pesticide
A substance used for destroying insects or other organisms harmful to cultivated plants or to animals

Prooxidant (in feeds and feedstuffs)
As opposed to antioxidants, they promote oxidation

Rancidity
Unpleasant, stale smell or taste, as a result of the chemical decomposition (hydrolysis/autoxidation) of fats, oils and other lipids

1 Survey of Antibiotic and Pesticide Residues in Aquaculture Products in the Philippines

BY MR CATACUTAN, RM COLOSO AND MT ARNAIZ

A survey in the Philippines in the early and mid-1990s (Lacierda et al.,2008) revealed that more than 100 chemicals and biological products are used in aquaculture production starting from pond preparation until harvest including chemicals for disease prevention and control. The survey comprises groups of chemicals and some are namely, soil and water conditioners, fertilizers, pesticides, probiotics and feed additives. A probable increase in the usage (volume) and number of chemicals throughout the years could be inferred since world aquaculture production and the number of species for culture also increased (Tacon & Metian, 2008). For health reasons of consumers and the safety regulations imposed by importing countries on aquaculture products, there is a pressing need to survey the chemicals used in aquaculture at present. In line with the promotion of food safety awareness in the region with regards to fish, the objective of this survey was to determine levels of commonly used antibiotics and pesticides in aquaculture that maybe present in aquaculture products such as fish and shrimps.

Chemistry of Oxytetracyline (OTC) and Oxolinic Acid (OXA)

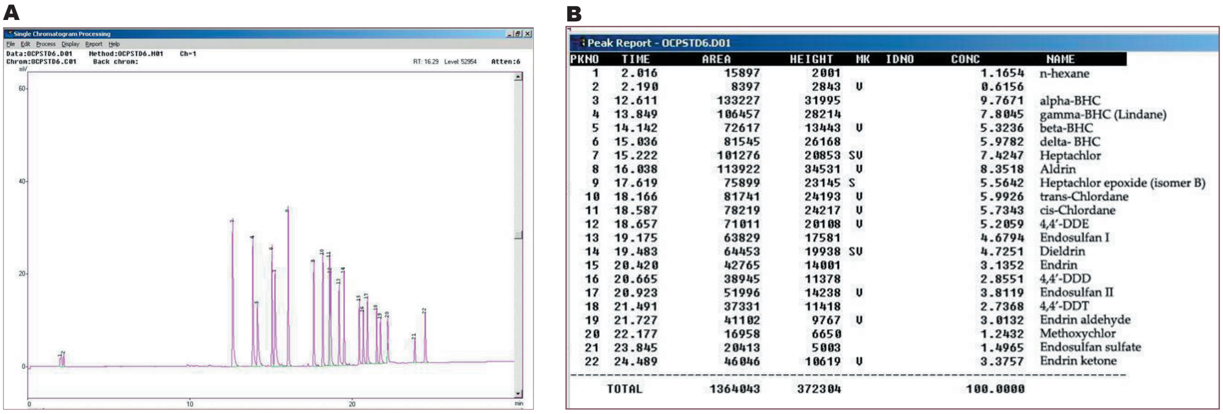
- **Oxytetracycline (OTC).** Oxytetracycline or OTC is crystalline and yellow in color with a chemical formula of $C_{22}H_{25}ClN_2O_9$ and molecular weight of 496.89 (g/mol). This antibiotic can cause reproductive toxicity (Category 2) and a possible risk to the unborn child. OTC is harmful when swallowed, can cause skin and eyes irritation and respiratory tract irritation if inhaled. The LD50 oral in mouse is 6.696 mg/kg (Sigma-Aldrich.com).

- **Oxolinic Acid (OXA).** Oxolinic acid or OXA is solid, clear, and very faintly yellow in color with a chemical formula of $C_{13}H_{11}NO_5$ and molecular weight of 261.23 (g/mol). This antibiotic is harmful if swallowed. In rats, oral ingestion causes acute toxicity at LD50 of 525 mg/kg while for dermal it is 2,000 mg/kg, and also carcinogenic when ingested and can cause damage to rat DNA (Sigma-Aldrich.com).

- **Organochlorine Pesticides (OCPs).** The standard of OCPs used in this study was composed of 20 specific pesticides with chemical formulae ranging from $C_6H_6Cl_6$ to $C_{12}H_8Cl_{60}$ and molecular weights of 290.83 g/mol to 422.92 g/mol. These OCPs are manufactured chemicals and are not naturally occurring in the environment. These are harmful and toxic substances (restek.com). One of these is methoxychlor which can affect the organ systems such as the endocrine and reproductive systems. Endosulfan on the other hand, can alter the gonad structure in juvenile and adult zebrafish and has been detected in significant levels in human milk (Wiley and Krone, 2001; Campoy et al., 2001).

Sample Collection and Analytical Methods

Samples of aquaculture products such as fish and crustaceans from either of the following culture systems: ponds, cages or pens were obtained from the three major islands of the Philippines (Luzon, Mindanao & Visayas). Fresh samples were also obtained from wet markets in some localities visited. Live or fresh samples were immediately placed in styrofoam boxes with ice to avoid spoilage. Carefully labeled samples were packed and transported to SEAFDEC/AQD in Tigbauan and kept in the freezer until processed for the assays of residues of antibiotics and pesticides.



PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	2.016	15897	2001			1.1654	n-hexane
2	2.190	8397	2843	V		0.6156	
3	12.611	133227	31995			9.7671	alpha-BHC
4	13.849	106457	28214			7.8045	gamma-BHC (Lindane)
5	14.142	72617	13443	V		5.3236	beta-BHC
6	15.036	81545	26168			5.9782	delta- BHC
7	15.222	101276	20853	SV		7.4247	Heptachlor
8	16.038	113922	34531	V		8.3518	Aldrin
9	17.619	75899	23145	S		5.5642	Heptachlor epoxide (isomer B)
10	18.166	81741	24193	V		5.9926	trans-Chlordane
11	18.587	78219	24217	V		5.7343	cis-Chlordane
12	18.657	71011	20108	V		5.2059	4,4′-DDE
13	19.175	63829	17581			4.6794	Endosulfan I
14	19.483	64453	19938	SV		4.7251	Dieldrin
15	20.420	42765	14001			3.1352	Endrin
16	20.665	38945	11378			2.8551	4,4′-DDD
17	20.923	51996	14238	V		3.8119	Endosulfan II
18	21.491	37331	11418			2.7368	4,4′-DDT
19	21.727	41102	9767	V		3.0132	Endrin aldehyde
20	22.177	16958	6650			1.2432	Methoxychlor
21	23.845	20413	5003			1.4965	Endosulfan sulfate
22	24.489	46046	10619	V		3.3757	Endrin ketone
TOTAL		1364043	372304			100.0000	

Figure 1.1 The chromotogram (A) of the organochlorine pesticides (OCPs) standard showing the peaks and the retention time in minutes (B).

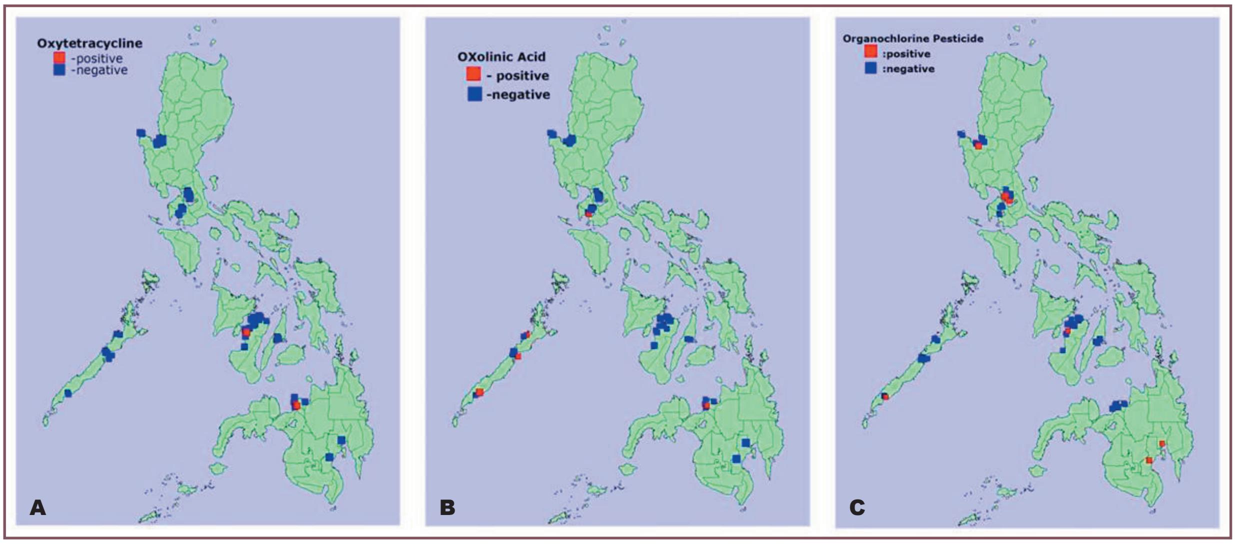


Figure 1.2 Areas sampled in the Philippines showing (A) results of OTC (Oxytetratcycline) analysis, (B) results of OXA (Oxolinic acid) analysis, and (C) showing results of OCP analysis.

The antibiotics, OTC and OXA were determined in samples by HPLC. For OTC assay, the extraction solvent system of metaphosphoric acid and dichloromethane (DCM) was used while for OXA, the extraction solvent system was acetonitrile and Na_2SO_4. The OCP in samples were determined by extraction using acetonitrile and purification with Florisil column chromatography and assayed by Gas Chromatography with electron capture detector (GC-ECD). Confirmatory tests for OTC, OXA and OCPs were done in some samples.

The standard for the organochlorines showed peaks in the chromatograph with a range of retention time in minutes from 6.188 (alpha-BHC) to 12.179 (Endrin ketone) (Figure 1.1A and 1.1B).

Results and Discussion

A total of 69 aquaculture products were obtained and processed to determine/check for presence of residues of antibiotics (OTC and OXA), and pesticides (OCPs). These comprised of the following: milkfish, tilapia, sea bass, snapper, grouper, rabbitfish, carp, catfish, silver perch, tiger shrimp, white shrimp and freshwater prawn. Some samples were positive for the antibiotics and OCPs residues and these comprised samples of low and high value fish species (Tables 1, 2 and 3). The assessment of results were based on the values set for TWA (8-hr time weighted average) and PEL (Permissible Exposure Limits) values based on OSHA (Occupational Safety and Health Administration, US based) and MRLs (Maximum Residue Limits, Japan Food Chemical Research) (Table 4 and

Annex A). The antibiotic OXA in one sample exceeded the PEL and MRL limits (*P. vannamei*) in Mindanao. In addition, the most common OCP in aquaculture products is Methoxychlor (Annex B) detected in samples from the three regions (Luzon, Visayas, and Mindanao). In one sample (*Macrobrachium sp.*) from Luzon, the level of the Endosulfan I (0.01440 ppm) was found harmful based on PEL and MRL (Table 4). The same sample contained a high level of Endrin ketone (0.02582 ppm) but there is no established PEL and MRL yet for this chemical, however, Endrin has PEL limit of 0.00642 and MRL limit of 0.005. The other OCPs detected that were below the MRL were, Heptachlor epoxide isomer B, Endrin, 4,4'-DDT and Trans-Chlordane. The results, positive and negative values are indicated in sampled areas in the three regions of the country (Figure 1.2).

Among the antibiotics, OXA was detected in five samples compared to only 3 samples with OTC. The antibiotic OXA has no established PEL but has an MRL, such that the use of OXA has a basis for regulation based on MRL. For the OCPs, the most commonly found are Methoxychlor and groups of Endosulfan and Endrin.

The antibiotic OXA is an effective antibacterial against infectious *Aeromonas* sp. and *Flavobacterium columnare*, while OTC is indicated for treatment of infectious diseases caused by Gram positive and Gram negative microorganisms such as *Mycoplasma pneumonia*, *E. coli* and *Haemophilus influenza* (Argent Chem Lab.). For fish, OXA is added in the feed at 30 mg/kg of fish per day; while OTC it is immersion in water at 200-700 mg/L of water for 2-6 hours depending on fish species (www.fda.gov). For waste disposal of both antibiotics, these substances can be mixed with a combustible solvent and burned in an incinerator equipped with afterburner and scrubber. For OXA, fish treated with this antibiotic may not be used for human consumption, and for OTC, to be used by qualified personnel for R & D and other authorized use only.

The organochlorine endosulfan is cited as one of the most common insecticide used in Asian aquaculture (Rico et al., 2012). In the Philippines it is used during pond preparation of brackish water ponds for milkfish and tiger shrimp culture at 0.1 ppm or at 8%. (Lacierda et al., 2008). Also, endosulfan is used as pesticides in agricultural products such as beans, cabbage, eggplant and many others to control growth of insects. The presence of this particular OCP in aquaculture products could be traced up from its use in pond preparation and in agricultural products and that endosulfan can stay in the soil for several years and is washed down into rivers during rainy season (Coloso, 2003). In this present report, endosulfan was detected in aquaculture products from Luzon (harmful level) and Visayas. The presence of other OCPs in aquaculture products could probably follow the same path since water from rivers are utilized in aquaculture ponds.

Conclusion and Recommendations

The antibiotics OXA and OTC were detected in aquaculture products while the most common OCP was Methoxychlor detected in samples from the three regions in the Philippines. For the other OCPs, namely Endrin ketone/Endrin and Endosulfan I, only sample which was Macrobrachium from Luzon was detected to contain these chemicals in harmful levels based on PEL and MRL. It is recommended that in future, heavy metals be also determined in aquaculture products and in feeds.

Acknowledgement

The commendable technical assistance of Ms. Jessebel Valera is highly acknowledged.

Table 1.1 Levels of Oxytetracycline (OTC), Oxolinic Acid (OXA), and the Organochlorine Pesticides (OCPs) in fish samples from Luzon

Aquatic Product	No. of Samples Analyzed	No. of (+) Samples	Residual Analysis (ppm)		
			OTC	OXA	OCP
Ayungin or Silver perch	1	1	No spls	No spls	0.00074 (Heptachlor epoxide isomerB)
					0.00093 (Endrin)
Bangus or Milkfish	10	1	-	-	0.00680 (Methoxychlor)
Biya or Goby	1		No spls	No spls	-
Carp (Big Head)	1		-	-	-
Carp (Common)	1		-	-	-
Dalag or Mudfish	1		No spls	No spls	-
Kanduli or Catfish	1	1	-	-	0.00353 (Endrin)
					0.00255 (4,4'-DDT)
					0.03255 (Methoxychlor)
Shrimp (*Macrobachium* sp.)	1	1	No spls	No spls	0.01440 (Endosulfan I)
					0.02582 (Endrin ketone)
Shrimp (*P. monodon*)	1	1	-	-	0.00124 (trans-Chlordane)
Siganid	2		-	-	-
Tilapia (Nile)	9	1	-	0.00496	0.27146 (Methoxychlor)
Tilapia (Red)	2	1	-	-	0.27425 (Methoxychlor)
Total Samples Analyzed	31		27	27	31

Table 1.2 Levels of Oxytetracycline (OTC), Oxolinic Acid (OXA), and the Organochlorine Pesticides (OCPs) in fish samples from Visayas

Aquatic Product	No. of Samples Analyzed	No. of (+) Samples	Residual Analysis (ppm)		
			OTC	OXA	OCP
Bangus or Milkish	12	1	-	0.00830	0.01745 (Heptachlor)
					0.03307 (Methoxychlor)
Grouper	2	1	-	0.02004	-
		1		0.01121	
Seabass	2		-	-	-
Shrimp (*P. monodon*)	5	1	2.51844	-	0.00240 (Endosulfan II)
				-	0.00345 (Endosulfan sulfate)
Siganid	1		-	-	-
Snapper	1		-	-	-
Tilapia	4		-	-	-
Total Samples Analyzed	27		26	26	27

Table 1.3 Levels of Oxytetracycline (OTC), Oxolinic Acid (OXA), and the Organochlorine Pesticides (OCPs) in fish samples from Mindanao

Aquatic Product	No. of Samples Analyzed	No. of (+) Samples	Residual Analysis (ppm)		
			OTC	OXA	OCP
Bangus or Milkfish	5	1	0.04046	0.01006	0.03828 (Methoxychlor)
		1			0.00187(Aldrin)
Seabass	1		-	-	-
Shrimp (P. monodon)	1		-	-	-
Shrimp (P. vannamei)	1	1	0.78121	-	-
Siganid	1		-	-	No spls
Snapper	1		-	-	-
Tilapia	1		-	-	-
Total Samples Analyzed	11		11	11	10

Notes for Tables 1.1, 1.2 and 1.3:
ppm = µg residue/g sample; No spls = Sample was not enough for the analysis; **Red Values = samples exceed there MRLS for Agricultural chemicals (based on Japan); Black Values = samples DO NOT exceed there MRLS for Agricultural chemicals (based on Japan); "-" = symbolizes absence of chemical contaminants in the samples (negative)**

Table 1.4 The list of Minimum Residue Limits (MRLs) for Agricultural Chemicals (Japan Food Chemical Research)*

Chemical Contaminants	OSHA Exposure Limits set by US. (ppm TWA)	MRLs for Agricultural Chemicals-The Japan Food Chemical Research (ppm)							
		Salmoniformes	Anguilliformes	Perciformes	Other fish	Shelled Molluscs	Crustaceans	Other Aquatic animals	Agricultural crops & poultry supply
Antibiotics									
Oxytetracycline (OTC)	0.02460	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01-0.60
Oxolinic Acid (OXA)	No PEL established	0.1	0.1	0.06	0.05	-	0.03	-	0.02-20.0
OC Pesticides									
alpha-BHC	No PEL established	-	-	-	-	-	-	-	0.2
gamma-BHC (Lindane)	0.04204	-	-	-	-	-	-	-	0.2
beta-BHC	No PEL established	-	-	-	-	-	-	-	0.2
delta-BHC	No PEL established	-	-	-	-	-	-	-	0.2
Heptachlor	0.00327	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.006-10.0
Aldrin	0.00335	0.1	0.1	0.1	0.1	0.1	0.1	0.1	ND, 0.006-0.20
Heptachlor epoxide (isomer B)	0.00296	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.006-10.0
trans-Chlordane	0.02983	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.002-0.50
cis-Chlordane	0.02983	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.002-0.50
4,4'-DDE	No PEL established	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.02-5.0
Endosulfan I	0.00601	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004-30.0
Dieldrin	0.01605	0.1	0.1	0.1	0.1	0.1	0.1	0.1	ND, 0.006-0.20
Endrin	0.00642	0.005	0.005	0.005	0.005	0.005	0.005	0.005	ND, 0.005-0.10
4,4'- DDD	No PEL established	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.02-5.0
Endosulfan II	0.00601	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004-30.0
4,4'- DDT	0.06897	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.02-5.0
Endrin aldehyde	No PEL established	No MRLs yet							
Methoxychlor	0.70734	-	-	-	-	-	-	-	0.01-7.0
Endosulfan Sulfate	No PEL established	No MRLs yet							
Endrin ketone	No PEL established	No MRLs yet							

***Notes:** Salmoniformes (such as salmon and trouts); Anguilliformes (such as eel); Perciformes (such as bonito, horse mackerel, mackerel, sea bass, sea bream and tuna); TWA= 8-hour time weighted average; PEL= Permissible Exposure Limits; TLV= Threshold Limit Values OSHA= Occupational Safety and Health Administration.

Methoxychlor

- CAS ID #: 72-43-5
- Affected Organ Systems: Endocrine (Glands and Hormones), Neurological (Nervous System), Reproductive (Producing Children)

Cancer Effects: None

Chemical Classification: Pesticides (chemicals used for killing pests, such as rodents, insects, or plants)

Summary: Methoxychlor is a manufactured chemical that does not occur naturally in the environment. Pure methoxychlor is a pale-yellow powder with a slight fruity or musty odor. Methoxychlor is used as an insecticide against flies, mosquitoes, cockroaches, chiggers, and a wide variety of other insects. It is used on agricultural crops and livestock, and in animal feed, barns, grain storage bins, home gardens, and on pets. Methoxychlor is also known as DMDT, Marlate®, or Metox®.

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2 Withdrawal Periods of Antibiotics, Oxytetracycline, and Oxolinic Acid, in Fish Species Cultured in the Tropics

BY MT ARNAIZ, RM COLOSO, AND MR CATACUTAN

Food safety is one of the major concerns of products derived from aquaculture. Farm inputs, e.g. drugs and agrochemicals, introduced whether intentionally or unintentionally during culture, may contaminate and remain in the product and become a hazard to the consumers. Chemical hazards in aquaculture products, among them drugs used for the chemotherapy of bacterial infection in fish and other cultured aquatic animals present a negative impact in aquaculture. Fish farmers often result to this treatment in order to save their cultured stock when threatened with infection, although a general conception nowadays is the discouragement of its use, being considered only as the "last recourse." Drugs, specifically antibiotics, have a long history of successful use in aquaculture (Alderman, 1980).

One of the most widely used antibacterial agents for therapy of systemic bacterial infections in farmed fish is oxytetracycline (OTC). It is a pale yellow to tan crystalline powder, MW 460.44, molecular formula $C_{22}H_{24}N_2O_9$ and chemical structure as shown in Figure 2.1. As an antibacterial, OTC's mode of action is by inhibiting the ability of bacteria to produce proteins. Without these proteins, bacteria cannot grow and multiply, therefore, it stops the spread of infection and the remaining bacteria are killed by the immune system or eventually die. Oxytetracycline free base is highly insoluble in water but readily forms soluble salt. One commonly used salt in aquaculture medicated feeds is the form oxytetracycline hydrochloride. Oxytetracycline can be given orally thru feeds and the typical dosage is 75 mg/kg body weight fish per day for 4 to 10 days. The recommended maximum residue limit (MRL) is 0.2 mg/kg for fish muscle (FAO FNP 41/14).

Another antibacterial agent also being used is the oxolinic acid (OXA), a white crystalline powder, and has a structure shown in Figure 2.2. OXA is a synthetic antimicrobial agent belonging to the group 4-quinolone. Quinolones are bactericidal which inhibit the bacterial enzyme DNA-gyrase resulting in breaks in the bacterial DNA coiling. OXA is efficacious against gram negative bacterial pathogens of fish. It can be administered orally through feeds with daily dosages of 12-20 mg/kg, or 30 mg/kg BW for 5 to 10 days. In fin fish, the maximum residue limit (MRL) is 100 ug/kg (EMEA/CVMP/41090/2005).

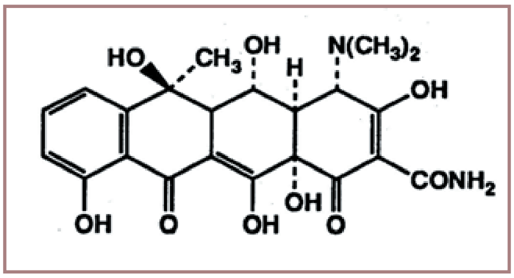

Figure 2.1. Structural formula of oxytetracycline

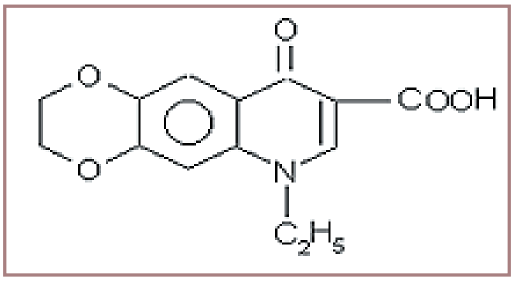

Figure 2.2. Structural formula of oxolinic acid

One of the draw backs in the use of antibiotics is the development of antibiotic resistant bacteria that could compromise the treatment of human infection. Although it cannot be assumed that there is a causal relationship between antibiotics and antibiotic-resistant bacteria, the overwhelming evidence has made this theory widely accepted among scientists (Mortazavi, 2014).

Residues of antibiotics present in fish may pose a risk to the health of the consumers. As mentioned by Mortazavi, humans who were exposed for long to antibiotic residues may disrupt their natural intestinal flora and could become susceptible to entering pathogenic bacteria.

For food safety reasons, cultured aquatic animals must have withdrawn all the residues or even at least, to acceptable levels before they are harvested for consumption, in order to prevent the entry of these contaminants from the "farm to fork" food chain, although cooking may reduce the residual concentration of OTC in fish (Sharafati, Chakeshtori, et al. 2013). Drug excretion is influenced by the temperature, the elimination time generally expressed in degree-days, which is the product of the temperature in ^{o}C and the withdrawal period in days (Inglis, 1996). Most previous studies reported results only in withdrawal periods for specified temperature or temperature ranges.

Residue depletion studies have been conducted on various cold water fishes, such as rainbow trout, *Salmo gairdneri* (Bjorklund and Bylund, 1990). Temperature related absorption and excretion studies done on different temperature ranges on rainbow trout *Onchorynchus mykiss* (Bjorklund, Eriksson and Bylund, 1992), and on *Salmo gairdneri* (Jacobsen, 1989). Hybrid striped bass, *Morone saxatilis*, tilapia, pure strain *Oreochromis niloticus* (Chen, et al. 2005 and Paschaol), were among those few studies on warm water fish.

The Southeast Asian Fisheries Development Center, Aquaculture Department, with the financial support from the Government of Japan, has conducted studies on the withdrawal periods of different fish species cultured in the tropics. These studies aim to estimate the depletion of two commonly used antibiotics, OTC and OXA, from several species cultured in the tropics: milkfish, *Chanos chanos*, hybrid red tilapia (*O. mossambicus*-hornorum hybrid X *O. niloticus*); mangrove red snapper, *Lutjanus argentimaculatus;* and orange-spotted grouper*, Epinephelus coioides.*

In the absence of withdrawal studies on specific species and temperature, fish farmers often follow antibacterial's manufacturer's recommended withdrawal period. The specificity of this study in terms of fish species, culture and temperature range is geared towards a more reliable estimation of the withdrawal period in tropical aquaculture.

Drug administration

The antibiotics were given through feeds which were mixed with SEAFDEC/AQD formulated diet for grow-out culture of species being studied. For every kilogram of diet formulation, 4.54 g of OTC was added. In contrast, OXA-mixed diet was added with 1.52 g active ingredient per kilo of the formulated diet. The antibiotic-mixed diets were given to the fish for ten days with the daily dose of the drugs equivalent to 75 mg/kg body weight for OTC and 30 mg/kg body weight for OXA. The daily feeding ration was about 2% of body weight. The test animals were given non-medicated feeds prior to and post antibiotic-mixed diet feeding.

Fish samples were either kept in 250L capacity fiber glass tanks with flow-through water system or large capacity concrete tanks compartmentalized by hapa nets. Water temperature and salinity were monitored daily.

After the 10-day antibiotic-mixed diet feeding, triplicate samples were retrieved at every collection period done regularly on a 3-day interval. The drug residues were extracted and analyzed using the procedure described below.

The experimental data of concentration was plotted versus time and the decay curve was evaluated by regression analysis. Equations for the time expressed in days, and the concentration in parts per million, were obtained from the regression curve. The withdrawal period can be estimated when the concentration is assumed zero.

Drug residue analysis

a. Oxytetracycline (OTC). The residues of OTC from the muscles of milkfish were analyzed using the high performance liquid chromatograph (HPLC) following the procedure of Carignan, et al. (1993). Briefly, residues are extracted using dichloromethane and 1% metaphosphoric acid solution, homogenized and centrifuged. Solvent removal was done by rotary evaporation and the final solution was filtered through membrane filter and injected in HPLC. The mobile phase of the HPLC system is composed of 0.025M oxalic acid-acetonitrile-terahydrofuran added with octane sulfonic acid sodium salt, and pumped at a rate of 1.0 mL/min, across a reversed phase octadecylsilyl (ODS) column, 5 µm, 4.6 X 150 mm. UV detector was set at 355 nm with 20 µL sample volume injected. Quantitation was based on calibration curves prepared from OTC standard solutions.

b. Oxolinic Acid (OXA). HPLC method was likewise employed for the evaluation of oxolinic acid. The procedure of Ng Poh Chuan et al. (2014) for the detection of oxolinic acid was followed. Accordingly, the residues were extracted with acetonitrile, homogenized and filtered into a separatory funnel containing acetonitrile-hexane solution. The solvent with the extract was drained and the solvent removed by rotary evaporation. Final extract layer from solvent-solvent extraction was pipetted out and filtered in membrane filter prior to HPLC injection. Twenty microliter of the clean sample was injected in an HPLC system with fluorescence detector set at 337 nm excitation and 365 nm emission wavelengths. The mobile phase system consisting of acetonitrile – methanol - 0.1M citric acid system was used and pumped at a rate of 0.5 mL/min. across a reversed phase octadecylsilyl (ODS) column, 5 µm, 4.6 X 150 mm. Quantitation was based on calibration curves generated from prepared standard OXA solutions.

Estimation of withdrawal periods

a. Milkfish. Milkfish is the most commonly cultured species in the Philippines and is now becoming a popular export aquaculture commodity. Its commercial production is one of the major concerns of the aquaculture industry in the country. Most often, monoculture techniques is used however, polyculture system is also being practiced and the milkfish-shrimp polyculture is quite common. Occurrence of milk fish mortalities has been reported and these were attributed to pathogenic bacteria.

Milkfish samples (BW 85±15 g, mean±SD) were kept in six hapa nets measuring 0.5m x 0.5m x 1m, suspended in an 8-ton capacity canvass-lined concrete tank with a flow-through seawater system. Temperature and salinity (mean±SD) during treatment were 29.6±1.0°C and 33±1.0 ppt, respectively and during withdrawal experiment, temperature and salinity were 30.4±0.55°C and 32±1.0 ppt

Regression analysis of the decay curve estimated the time to wash out OTC residues from the muscle of milkfish to be 22 days. In contrast, the value obtained for OXA was 27 days.

b. Hybrid red tilapia. Tilapia is also commonly cultured in the Philippines. It is considered a disease-resistant species, however, intensified tilapia culture may lead to development of bacterial infections.

Tilapia samples (BW 120.32 ±32 g, mean± SD) were kept in 250L capacity fiber glass tanks with flow-through water system. The experiment was conducted using freshwater and tank water temperature was 29.2°C±0.64 (mean ± SD).

Results showed that the estimated withdrawal period of OTC administered to tilapia at a dose of 75mg/kg fish per day was 26 days while that of oxolinic acid (OXA) given at a dose of 30 mg/kg fish per day, was 17 days.

c. **Mangrove red snapper.** Mangrove red snapper is one of the high value fish food species being cultured in the Philippines and in other Southeast Asian countries. Incidence of bacterial infection has been reported and chemotherapy is sometimes employed.

Snapper fish samples (86±15.6 g body weight) were stocked at 13 pcs per tank in 250L capacity fiber glass tanks with a flow-through seawater system. Water temperature during experimentation was 27.7°C ± 0.77 (mean ± SD).

When the concentration versus time data were plotted with regression analysis, the estimated withdrawal periods from mangrove red snapper, were 21 and 18 days for OTC and OXA, respectively.

d. **Orange- spotted grouper.** Groupers are also among fish food species that are of high economic value. They are being cultured in the Philippines and in other countries in Asia. Infectious agents, have caused diseases on cultured groupers, which lead them to be treated with antibiotics.

In 250L capacity fiberglass tanks, grouper juveniles with body weights 164± 25.91 g (mean ± SD), were stocked at 13 pcs/tank. Water temperature during the experiment was 28°C ± 1 (mean ± SD). From the decay curve of the experimental data, the estimated time to eliminate OTC residues from the muscle of orange spotted grouper was 21 days. In contrast, the value obtained for OXA was 17 days.

e. **Black tiger shrimp.** Black tiger shrimp, is a marine crustacean which is also widely cultured in the Philippines and in other Asian countries.

P. monodon samples, average BW 50.88 g, were kept in aerated, seawater flow through 250L capacity fiber glass tanks stocked at 3 pcs/tank. Water temperature during the experiment ranged from 29-31°C and salinity of 28-32 ppt. OTC was added to shrimp feed formulation in a ratio of 5 g/kg feeds while in OXA treatment 4.5 g/kg of feed OXA was added. Shrimps were fed with antibiotic-mixed diet for seven days with a daily ration of the feeds at 3% of BW, spread into three times a day.

The estimated withdrawal period of OTC was found to be 17 days while 19 days for OXA.

Table 2.1 Summary of results

Species	Withdrawal period, days		Temperature, oC
	Oxyteracycline (OTC)	Oxolinic acid (OXA)	
Milkfish	22	27	28.7 - 31.5
Hybrid red tilapia	26	17	28 - 30
Mangrove red snapper	21	18	26 – 29.5
Orange- spotted grouper	21	17	25 – 29.5
Black tiger shrimp	17	19	29-31

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3 Ethoxyquin

BY MT ARNAIZ

Ethoxyquin is a quinoline-based antioxidant used for the preservation of food and feeds. It is also a pesticide (under commercial names such as "Stop-Scald") used to control scald on pears after harvest. Ethoxyquin is also commonly used in spices to prevent color loss due to oxidation of the natural carotenoid pigments.

Ethoxyquin, 6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline, has a molecular formula $C_{14}H_{19}NO$, formula weight 217.31 and chemical structure as shown in Figure 3.1. It is a clear light yellow to dark brown viscous liquid (Figure 3.2), has a Mercaptan-like odor, discolors and stains badly. Ethoxyquin is stable, combustible and incompatible with oxidizing agents and strong acids. On exposure to light and air, it polymerizes and darkens in color. It is soluble in organic solvent (50 mL/L in ethanol) and has very low solubility in water (<0.1 g/100 mL at 20°C) (CAS Database reference).

In feeds and feedstuffs, oxidation occurs resulting in rancidity of fats, destruction of Vitamins A, D, and E, pigmenters (carotenoids) and amino acids which results to lowered biological energy values for the diet. Qualifications for an antioxidant to be useful in animal feeding include (a) must be effective in preserving animal and vegetable fats, vitamins, and other feed qualities subject to oxidative destruction; (b) it must be non-toxic to man and to farm animals (i.e. chicken, swine, fish, etc); (c) should be effective at very low concentration; and (d) low cost to be economically practical. The commonly used feed antioxidants for feeds and feed ingredients which were found to be outstandingly efficient and economical are ethoxyquin (EQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). Among the three, EQ has demonstrated to be the most effective, followed closely by BHT and BHA (Rumsey, G.L).

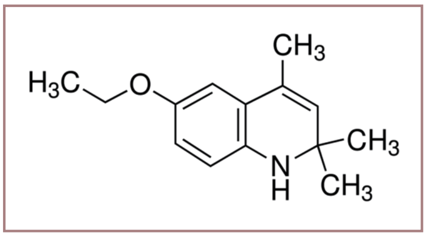

Figure 3.1. Structural formula of ethoxyquin

Figure 3.2. Ethoxyquin

Photos courtesy of http://www.mpbio.com/product.php?pid=02157963 (Figure 3.1) & http://www.asia.ru/en ProductInfo/440832.html (Figure 3.2)

Table 3.1. Tolerances of ethoxyquin

Product	Tolerance level (parts per million)
Uncooked fat of meat from animals except poultry	5
Uncooked liver and fat of poultry	3
Uncooked muscle meat of animals	0.5
Poultry eggs	0.5
Milk	0.0

Ethoxyquin has been known to be one of the best feed antioxidants, however, a review of its use as animal feed (Błaszczyk, 2013) cited that it is responsible for a wide range of health-related problems in dogs, as well as in humans. Ethoxyquin may also show prooxidant properties when too high concentration is used. Dogs are more susceptible to its harmful effects, the symptoms observed were liver, kidney, thyroid and reproductive dysfunction, teratogenic and carcinogenic effects, allergic reactions, and a host of skin and hair abnormalities. The same characteristic symptoms and pathologies, such as weight loss, liver and kidney damage, and alterations of alimentary duct were observed in different species of the majority of animals treated with ethoxyquin at concentrations higher than those permitted in animal feed. Further in Błaszczyk's review article, there were some studies showing cytotoxic effects of ethoxyquin (purity>97%). It induces apoptosis (programmed cell death) in *in vitro* cultured human lymphocytes, the number being dependent on the treatment time. It caused also DNA damage in the comet assay, however, most lesions could be repaired by cellular DNA repair systems. By using chromosome aberration test, the unrepaired DNA damage induced by ethoxyquin could lead to permanent changes in genetic material. These anti-oxidant induced chromosomes aberrations in human lymphocytes and Chinese hamster ovary cells, are known to have serious biological consequences.

Ethoxyquin may be safely used as an antioxidant for preservation of color in the production of chili powder, paprika, and ground chili at levels not in excess of 100 parts per million (US FDA 21CFR172.140). It may be safely used in animal feeds, provided the maximum quantity of the additive permitted to be used and to remain in or on the treated article shall not exceed 150 parts per million (US FDA 21CFR 573.380), and should be intended for use only as:

1) chemical preservative for retarding oxidation of carotene, xantophylls, and vitamins A and E in animal feed and fish food; and
2) as an aid in preventing the development of organic peroxides in canned pet food. Further, established tolerances for residues of ethoxyquin in or on edible products of animals is shown in Table 3.1.

In 2012, Japan lowered the the residual limit of ethoxyquin in shrimp to 0.01 ppm. Consignments containing more than this allowable limit were rejected. This beleaguered the countries which considers Japan as one of their top export market. Shrimp exports from Vietnam experienced 100% inspection in that year. India's shrimp export to Japan also suffered from this move of the Japanese government. In the Philippines, shrimp/prawn growers were also alarmed, especially those from the province of Negros Occidental in Western Visayas region, one of the top shrimp/prawn growers in the country. Among the efforts put forth to alleviate the situation, in addition to their agreements and requirements with feed suppliers, is strengthening their capacity in the analysis of ethoxyquin in feeds and in produce. However, in January 2014, Japan formally announced that they increased the allowable limit from 0.01 ppm to 0.2 ppm (http://vietfish.org/20140219032211848p49c82/japan-raises-maximum-residue-level-for-ethoxyquin.htm), a twenty-fold increase, but still lower than that of the US FDA's limit of 0.5 ppm for uncooked muscle meat of animals. This move however, may have brought some relief to exporting countries like Vietnam, India, and the Philippines. Among the food products listed that have established maximum residue level (MRL) of ethoxyquin in Japan, crustaceans ranked second to chicken and other poultry muscle, which have 0.1 ppm, while in fish, MRL is 1 ppm (Japan Food Chemical Research Foundation). Studies on the withdrawal period of ethoxyquin from the muscles of fish and shrimps are very limited, if there is any.

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4 Organotin Compounds

BY RM COLOSO

Organotin compounds, such as tributyltin and triphenyltin, are tributyl or triphenyl derivatives of tetravalent tin. They are colorless solids with low vapor pressures, lipophilic, and are slightly water soluble. The derivatives can either be in the form of hydride, hydroxide, chloride, acetate or oxide as shown in Figure 4.1.

Tributyltin and triphenyltin have been extensively used as algicides and molluscicides in anti-fouling products since the 1960s. In the Philippines and other Southeast Asian countries, triphenyltin (brand names Aquatin, Brestan or Telostan) has long been used as a molluscicide in brackish water earthen ponds to control the population of pond snails Cerithidea cingulata in the culture of milkfish (Coloso et al., 1998). The use of organotins has been restricted in many countries including the Philippines because of their effects on aquatic organisms and persistence in the environment. These chemicals render the soil sterile, are non-biodegradable, bioaccumulate in fish and snails, and are hazardous to humans. They are harmful to various animal species by affecting the immune and reproductive systems, imposex, development of males sex organs in female gastropods, by increasing androgen levels (an endocrine disruptor), hyperplasia in endocrine organs, apoptosis in thymus cells, calcium release in sarcoplasmic reticulum cells, and the eyes. In humans, toxic effects experienced during spraying of triphenyltin formulations include brief loss of consciousness, headache, nausea, vomiting, photophobia, contact dermatitis, and allergic reactions (Sekizawa, 1999). Despite the ban, the clandestine use of triphenyltin in brackish water ponds continues to threaten the aquaculture industry, environment, and humans. High residues of triphenyltin have been found in pond sediments and soil as well as in milkfish tissues (intestine, liver and flesh) (Coloso and Borlongan, 1999).

To control the population of snails in brackish water ponds, the concept of integrated pest management has been proposed (Bagarinao and Lantin-Olaguer, 2000). Ponds should be completely drained, dried, and cracked under the sun to kill adult snails and eggs. Adult snails may be collected and gathered for shellcraft making. Snails remaining in puddles may be killed by alternative treatments such as metaldehyde and nicotine found in tobacco dust (Coloso et al., 1998; Borlongan et al., 1998, Borlongan et al., 1996). In heavily infested ponds, a dose of 120 kg/ha metaldehyde (10% formulation) under both wet and dry conditions is recommended. In moderately infested ponds, a dose of 80 kg/ha metaldehyde (10% formulation) and 120 kg/ha in dry and wet conditions, respectively, are recommended (Coloso et al., 1998). Furthermore, the 72-h LC99 of nicotine in tobacco dust was shown to be about 24 kg/ha nicotine under laboratory conditions. Tobacco dust contains about 2.8% nicotine and its effective application rate depends on the nicotine content (Borlongan et al. 1998). During pond preparation, the remaining snails can be eradicated by treatment with nitrogen fertilizers and lime. The entry of water into the ponds can be done when veliger counts are low.

Organotins have been included among banned pesticides in the Philippines, Brunei, and Singapore, but continue to be allowed in Indonesia, Thailand, and Vietnam (ASEAN Guidelines, in preparation). The illegal importation of triphenyltin into the Philippines has continued precisely because it is allowed and continues to be applied in ponds in neighboring countries. A uniform implementation of the ban in Southeast Asian countries and the ASEAN will be helpful in limiting the use of this chemical in aquaculture. In the Philippines, the ban on triphenyltin usage in milkfish ponds should be strictly implemented to reduce the threat of this pesticide to the environment, natural resources, aquaculture products, and people.

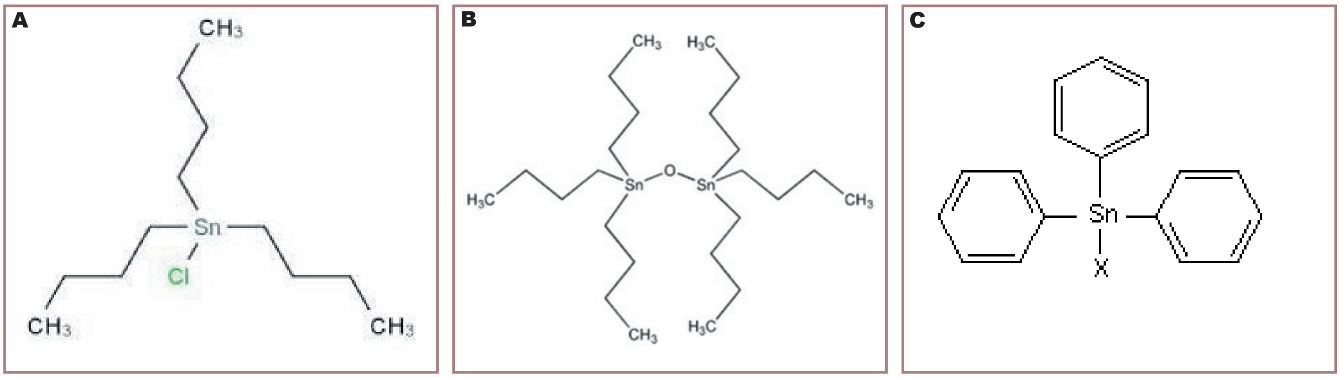

Figure 4.1. (A) Tributyltin chloride, (B) Tributyltin oxide, and C) Triphenyltin

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5 Melamine

BY RM COLOSO

Melamine is an organic compound with a triazine skeleton (Figure 5.1A). It contains 67% nitrogen by mass and is combined with formaldehyde to make melamine resin used in the manufacture of formica, melamine dinnerware, laminate flooring, and dry erase boards. Melamine also combines with cyanuric acid (Figure 5.1B) and related compounds to form melamine cyanurate crystals (Figure 5.1C), which have been found as contaminants or biomarkers in cases of protein adulterations. Melamine is sometimes illegally added to food products and feed ingredients to increase the apparent protein content. In proximate analyses of crude protein using the Kjeldahl method, protein levels of feed ingredients are estimated by measuring the nitrogen content and multiplying this by a factor of 6.25, thus the protein levels of poor quality ingredients can be intentionally increased by adding nitrogen-rich compounds such as melamine. In incidents that caused global concerns about food safety in 2007 and 2008, melamine has been intentionally added to animal feed or food products for humans (such as powdered milk or infant formulas) causing severe kidney damage to children and pets poisoned by melamine-adulterated products.

Method of detection

The U. S. Food and Drug Administration and the Japanese Ministry of Health, Labor and Welfare have issued methods based on liquid chromatography-mass spectrometry (LC/MS) detection of melamine and cyanuric acid after hydrophilic interaction liquid chromatographic (HILIC) separation.

The existing methods for melamine determination using liquid chromatography – mass spectrometry (LC/MS) after solid phase extraction are often complex and time consuming. Improvements using electrospray ionization methods coupled with mass spectrometry allow a rapid and direct analysis of samples with complex matrices where the native liquid samples are directly ionized under ambient conditions in their original solution.

Ultrasound-assisted extractive electrospray ionization mass spectrometry (EESI-MS) has been developed for a rapid detection of melamine in untreated food samples. Ultrasound is used to nebulize the melamine-containing liquids into a fine spray. The spray is then ionized by extractive spray ionization and analysed using tandem mass spectrometry (MS/MS). An analysis requires 30

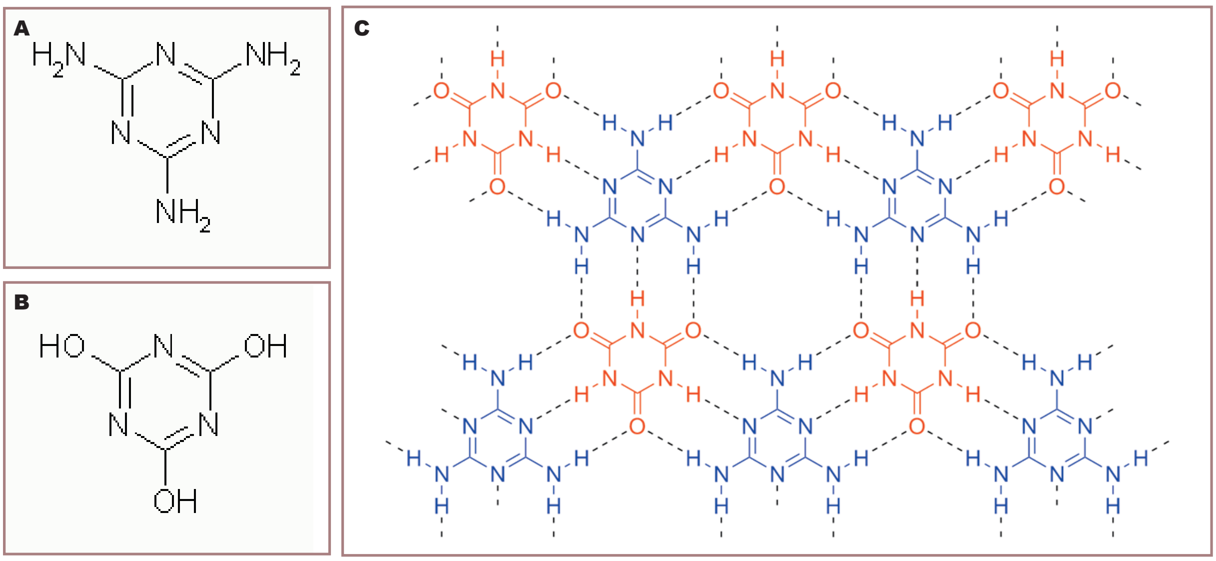

Figure 5.1. Chemical structures of (A) Melanine, (B) Cyanuric Acid and (C) Melamine Cyanurate Crystals

seconds per sample. The limit of detection of melamine in milk for example is a few nanograms of melamine per gram.

A simpler instrumentation and a faster method by using a low-temperature plasma probe to ionize the samples and mass spectrometry technique now allows high-throughput analysis of melamine traces in complex mixtures.

Toxicity

Melamine is an eye, skin, and lung irritant. If swallowed or inhaled it goes to the bloodstream and ultimately finds its way into the kidney tubules. In the kidney, melamine and cyanuric acid are concentrated forming large numbers of crystals which can deposit in and damage the cells lining the kidney tubules causing the kidney to malfunction. Renal failure has been observed in humans, other mammals, and fish.

Tissue (plasma, muscles, kidneys, liver, and gills) deposition of melamine and cyanuric acid following continuous voluntary feeding was observed in rainbow trout (Liu et al. 2014). Upon withdrawal, the melamine and cyanuric acid concentration in the tissues decreased exponentially. It was also noted that melamine is converted into cyanuric acid in trout. Muscles residues and renal crystals also formed in catfish and rainbow trout given melamine and cyanuric acid, persisting for weeks after the single dose (Reimschuessel et al. 2010). Studies have also shown a much slower elimination of melamine and cyanuric acid from rainbow trout body (Xue et al. 2011). Crystal formation in trout kidney was found to be both dose and time dependent (Pacini et al. 2014). Even after six weeks of withdrawal, the crystals persisted in fish that received the high dosage. Furthermore, crystals within multifocal hemocytic granulomas in the antennal gland tubules and peritubular hemal sinuses of penaeid shrimps that were very similar to melamine-cyanuric acid-induced crystals in mammalian kidney with melamine induced renal failure have also been observed in shrimps fed melamine-contaminated feed (Lightner et al., 2009).

Summary and recommendations

Melamine is an adulterant that can be added to feed ingredients for aquafeeds to artificially inflate the apparent protein content. Together with cyanuric acid, it has been found that crystals formed from melamine and cyanuric acid can cause kidney damage in mammals, fish, and shrimp. If in doubt of the source and quality of feed ingredients and aquafeeds, samples should be submitted for melamine and cyanuric acid analysis. Their presence in feed ingredients and aquafeeds are biomarkers for contamination, adulteration or intentional addition to increase crude protein levels. The United Nations' Codex Alimentarius Commission has set the maximum amount of 1 mg/kg melamine in powdered infant formula and 2.5 mg/kg in other foods and animal feed. While not legally binding, the recommended levels can serve as basis for banning the importation of products with excessive levels of melamine.

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GUIDELINES FOR THE USE OF CHEMICALS IN AQUACULTURE AND MEASURES TO ELIMINATE THE USE OF HARMFUL CHEMICALS

one vision
one identity
one community

Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals

The ASEAN Secretariat

Jakarta

The Association of Southeast Asian Nations (ASEAN) was established on 8 August 1967. The Member States of the Association are Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Viet Nam.

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Catalogue-in-Publication Data

Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals.
Jakarta: ASEAN Secretariat, December 2013

639.8959
1. Chemicals – Drugs – Aquaculture
2. Fisheries – Food Safety – ASEAN

ISBN 978-602-7643-64-2

Cover photos by:
SEAFDEC (Southeast Asian Fisheries Development Center).

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1. ACKNOWLEDGEMENTS

On behalf of Malaysia, the drafting committee would like to extend her appreciation to all ASEAN Member States (AMS) who had participated in developing the guidelines. Special thank goes to the Director General of Fisheries Malaysia, YH Dato' Ahamad Sabki Bin Mahmood for his continuous support to the committee's work throughout the process.The committee also expresses gratitude to Mr. Mohamad Shaupi Bin Derahman, currently the Deputy Director General of Fisheries (Operations) for his guidance and interest throughout the three workshops that had been conducted. The guidelines could not have been completed without the brains of the resource persons who had contributed immensely in finalising the guidelines. The dedicated resource persons are the Dr. Hassan Bin Hj. Mohd Daud, Faculty of Veterinary Medicine, University Putra Malaysia, Dr. Relicardo M. Coloso, SEAFDEC Aquaculture Department and Dr. Eduardo Leano, Network of Aquaculture Centres in Asia-Pacific (NACA).

Sincere appreciation also goes to the ASEAN Secretariat for facilitating all the needed assistance by Malaysia as the lead to this initiative. The drafting committee, as appears in **Appendix III**, who basically consists of everyone contributed to the three workshops, is very grateful to all the people who had exerted their efforts in developing the guidelines. The committee is also deeply indebted to the Department of Fisheries Malaysia's staff for their much need valuable technical support which made it possible for the guidelines to be concluded.

2. AIM AND PURPOSE

This set of guidelines has been developed to help national regulators and stakeholders on managing the diverse use of chemicals in aquaculture. It recognising the existing variation in capacity among AMS but has been designed so that it could be adopted and implemented within the specific policy and legal framework of each country.

This document aims to provide guidance for Competent Authorities (CAs) in standards setting / regulating the use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemical among AMS. AMS are encouraged to assess and review gaps at their national level with regard to chemicals used in aquaculture, as listed in this document.

Furthermore, it would also help to develop measures to eliminate the use of harmful chemicals in aquaculture; and it is further envisaged that a harmonised regional set of guidelines for the use of chemicals in aquaculture for ASEAN could be produced.

The purpose of this set of guidelines is to list the major chemicals and other substances commonly used in AMS. This set of guidelines will also list the banned chemicals that should not be used or practiced by farmers or aquaculturist in all AMS. The list was compiled and agreed from previous workshops to assess and review gaps exist among AMS with regards to chemical use in aquaculture.

3. FOREWORD

The 9th ASEAN Summit in Bali, Indonesia in October 2003 adopted the declaration of ASEAN Concord Bali II (Bali Concord II) aimed at making the Southeast Asian region a more dynamic and stronger segment of the global supply chain and the world economy. Under the Declaration, ASEAN has committed to deepen and broaden its internal economic integration linkages, with the participation of the private sector, to realize an ASEAN Economic Community (AEC).

In the following year (2004), ASEAN at its 10th Summit in Vientiane saw the Leaders signed the Framework Agreement for Integration of Priority Sector. An individual protocol including ASEAN Sectoral Integration Protocol for Fisheries was developed and signed. Attached to the protocol is the ASEAN Roadmap for Integration of Fisheries Sector. It lays down both specific measures that are of direct relevance to fisheries sector as well as common issues that cut across all priority integration section to be implemented with timeline until 2010.

In the wake of slow integration of priority sectors, 12th ASEAN Summit in 2006 Philippines adopted a second phase of that Agreement. The previously set timelines in 2004 was extended in view of real situation and constraint. Prior to that, at the 13th meeting of ASEAN Sectoral Working Group on Fisheries (ASWGFi) in Myanmar in 2005, Malaysia along with Singapore and Thailand had been tasked to lead the implementation of several specific issues in view to accelerate the integration of fisheries sector.

During the 13th ASEAN Summit in Singapore in November 2007, the ASEAN Leaders signed the Declaration on the ASEAN Economic Community Blueprint to realise the AEC. The role of the AEC Blueprint is to offer AMS a short to medium term strategic plan towards 2015. Under the AEC Blueprint, a number of actions have been identified for integration of food, agriculture and forestry sectors. Based on the goals and actions outlined in the AEC Blueprint, a Scorecard was developed for monitoring the progress of the implementation of AEC Blueprint over the period from 2008 to 2015.

The Regional Workshop on Implementing the ASEAN Roadmap for Integration of Fisheries Sector was timely held from 16-18 January 2008, in Bangkok prior to the convening of 14th ASEAN Summit. The Roadmap has provided a good guide both for Lead AMS as well as other AMS as to how to carry out their responsibilities.

Subsequently, during the 14th ASEAN Summit, the Leaders signed the Cha-am Hua Hin Declaration on the Roadmap for the ASEAN Community (2009-2015), comprising three pillars: Political Security Community, Economic Community, and Socio Cultural Community. As a result, the Leaders agreed that the Roadmap shall replace the Vientiane Action Programme (VAP).

Based upon the AEC Scorecard, Malaysia further undertook the responsibility to promote harmonization as laid down in its measure 1.8 for ASEAN to *"Harmonise guidelines for the use of chemicals in aquaculture and measures to eliminate the use of harmful chemicals by 2009"*. In the interest of the time and to ease draft guidelines preparation, a questionnaire was carried out in 2009 to all AMS. Specific recommendations by the Regional Workshop on Implementing the ASEAN Roadmap for Integration of Fisheries Sector held in Thailand in 2008 guided Malaysia to form a Working Committee (WC), and this Committee was tasked to maintain networking until the end of the process, including circulating the final draft of the guidelines.

The First Regional Workshop on Harmonisation of Guideline for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemical was held on 2-3 December 2009. The objectives of this first workshop were to assess and review gaps exist among AMSs with regards to chemical use in aquaculture, and to develop a common list of harmful chemicals used in aquaculture. Delegates attending the workshop updated the details of the questionnaire and gave clarifications on the current status of the use of chemicals in aquaculture in their respective countries.

On 25-26 November 2010, Malaysia hosted the Second Regional Workshop on Harmonisation of Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals. This workshop was specifically conducted to list the commonly use chemicals in aquaculture, and to develop a harmonised guideline for ASEAN.The Third Regional Workshop to Finalise Guideline for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemical was again hosted by Malaysia on the 9-12 July 2012. This third and final workshop was carried out to develop and finalise common measures to eliminate the use of harmful chemical in aquaculture; and to finalise the draft harmonised guideline for the use of chemicals in aquaculture for ASEAN from all ASEAN Member States.

4. BACKGROUND AND INTRODUCTION

Aquaculture activity is an important fishery sector for the production of fish for food consumption and ornamental purpose. Aquaculture is an established and growing industry to all ASEAN Member States, and an increasingly important supplier of foods for consumers. Aquaculture is an important contributor to the agriculture economy, and provides as a source of income to the community who depends on it entirely to their social-economic livelihood.

All aquaculture operations will have a demand for drugs and other chemicals. The use of these drugs and chemicals may be used for: i) Disinfectants, ii) Herbicides, piscicide and pesticides used in pond maintenance, iii) Spawning aids, and iv) Vaccines used in disease prevention. There may be other reasons why these chemicals are used in aquaculture operations, but it is critical that aquaculturists should have access to regulated and controlled chemicals that are safe and effective and apply them in a manner that is consistent with their intended use, best management practices, and relevant rules and regulations.

Aquaculture its should be aware of the risks involved when handling chemicals, and their potential impacts on the environment and food safety.The use of chemicals and drugs should have a sufficient regulatory system in place. The uncontrolled and indiscriminate use of antibiotic or drugs in aquaculture may lead to the emergence of antimicrobial resistant (AMR) organisms.

These benefits too are outweighed by concerns with regard to the use of chemicals in aquaculture. These include human health concerns related to the use of feed additives, chemotherapeutants, hormones, disinfectants and vaccines; and also concerns related to product quality such as issues of chemical residues in aquaculture products.

The practice of using drugs and chemicals in aquaculture operations in the ASEAN region currently are not fully regulated and controlled by Competent Authorities. Aquaculturists is currently applying ‘best practiced’ and ‘know-how’ on using this chemicals.

This guideline is intended to serve as a resource to assist aquaculturists to use regulated drugs and chemicals legally and properly. The principles outlined in this guideline are intended to provide directions for the use of drugs and other chemicals in ways that ensure the safety of treated animals, end-users, consumers and to the environment.

5. TERMS AND DEFINITION

Anesthetic

A substance or drug that causes temporary loss or bodily sensation.

Antibiotic

A substance produced by or derived from certain fungus, bacteria or other microorganism, that can destroy or inhibit the growth of other microorganism and is used to prevent and treat infectious diseases.

Antimicrobial

An agent that kills microorganisms or suppresses their multiplication or growth.

Aquaculture

Science, art and business of cultivating aquatic organism under controlled condition.

Aquaculturist

A person who engages in aquaculture.

Chemical

A substance, pure or mixture, with distinct molecular composition that is produced by or used in a chemical process.

Chemotherapeutant

An agent used for treatment or disease on animals or humans using chemicals or drugs that are selectively toxic to the causative agent of the disease such as bacteria, virus or other microorganism.

Competent Authority (CA)

A body or organisation legally qualified or sufficient to perform an act such as regulation, organisation, certification, and etc.

Culture system preparation

Methods or procedures to prepare the tank, pond or floating cages prior to the introduction of culture animals.

Disinfectant

An agent such as a chemical, heat or radiation that destroys, neutralises or inhibits the growth of disease carrying organism. Disinfectants are generally applied to equipment and structures and are not intended to have therapeutic effect on cultured animals. In aquaculture disinfectants can also include compounds used to destroy microorganisms living on the surface of fish eggs.

Drug

Any chemical compound used in the diagnosis, treatment, or prevention of disease or other abnormal condition.

Fish

Any aquatic animal or plant life, sedentary or not, and includes all species of finfish, crustacean, mollusca, aquatic mammals, or their eggs or spawn, fry, fingerling, spat or young, but does not include any species of otters, turtle or their eggs.

Hormone

A substance, usually a peptide or steroid produced by one tissue and carried by the blood stream to another to effect physiological activity such as growth and metabolism.

Maximum Residue Limit (MRL)

The maximum concentration of residue resulting from the use of veterinary drugs and aquaculture chemical (expressed in mg or kg on a fresh weight basis) that is legally permitted or recognised as acceptable in fish and fish products.

Pest

Unwanted species of animals or plants detrimental to aquaculture.

Pesticide

A substance used to control pest.

Piscicide

A piscicide is a chemical substance which is poisonous to fish. The primary use for piscicides is to eliminate parasitic and invasive species of fish in a body of water.

Withdrawal period

The period of time between last administration or exposure of fish to a veterinary drug, and point of harvest to ensure that the concentration of the veterinary drug in their edible flesh complies with the maximum permitted residue limits for human consumption.

6. CHEMICALS IN AQUACULTURE

There are different classifications of "chemicals" and "drugs" being used in aquaculture. In aquaculture, chemicals are classified according to its nature, functions and intended purpose.

In using drugs or chemicals, it is important for all aquaculturists and operators to observe the withdrawal periods while applying drugs and chemicals in fisheries produce intended for human consumption. Concerns regarding the over-use and misuse of certain drugs and chemicals for which proper risk assessments with respect to the target species, human factors and the environment have not been fully conducted and documented.

Product withdrawal times must be observed to ensure that a product used in a target animal does not exceed legal tolerance levels in the animal tissue at the time the edible portion is made available for human consumption. Following proper withdrawal times helps to ensure that products reaching consumers are safe and wholesome. Withdrawal information is found on the product label, package insert, or feed tag of any approved product.

Chemicals have many uses in aquaculture. They are used in:

i. culture system preparation
ii. pest control
iii. soil and, or water quality management
iv. feed and supplements
v. transportation of live fish
vi. breeding
vii. disease treatment and control
viii. health management

Drugs

All drugs used to control mortality associated with bacterial diseases or infestation density of parasites, sedate or anesthetize fish, induce spawning, change gender, or in any other way change the structure or function of aquatic species must be approved by the CAs.

It is illegal and prohibited to use unapproved drugs for any purpose, and approved drugs in a manner other than that specified on the product label.

7. ROLES AND RESPONSIBILITY

7.1. Competent Authority

A Competent Authority (CA), or its national regulator(s) in each AMS as listed in **Appendix I** shall be responsible for the following:

a) technical, diagnostic capacity and capability in the country.
b) coordinate with other relevant agencies within the country.
c) approval and/or registration of aquaculture premises.
d) approval and/or registration of third party service provider (laboratory, quarantine facilities).
e) approval and/or registration of manufacturers and traders of chemicals and drugs for use in aquaculture.
f) establish and regular update a national list of chemicals for aquaculture purposes.
g) to create awareness among aquaculturists through extension and awareness programs.
h) communicate with other AMS (refer to para 8.3).
i) carry out monitoring, inspection and surveillance activities.
j) to regulate the import, manufacture and trade of chemicals and products.
k) to evaluate and verify efficacy and safety of chemicals intended for use in aquaculture systems.
l) to carry out enforcement activities for non-compliance to national practice and/or legislations.

7.2. Manufacturers and Traders

Manufacturers and traders of chemicals in each AMS, shall be responsible for the following:

a) registration and/or approval of the chemicals and products with the Competent Authority, in accordance with national requirements.
b) chemicals to be registered should use its generic name.
c) registration and/or approval of the aquaculture premises with the Competent Authority, in accordance with national requirements.
d) technical information of the chemicals.
e) to provide necessary instructions on the intended use on the label.
f) communicate with national CAs on the requirements of the chemicals.
g) obtain prior approval from national CAs for importation of chemicals or drugs.
h) cooperate with national CAs on trace ability, inspection, audit and other related activities.

7.3. Aquaculturist

Aquaculturist shall be responsible for the following:

a) obtain registration and/or approval of aquaculture premise from national or local CAs.
b) consult with national or local CAs and manufacturers or traders on the proper and safe methods to use and for disposal of the chemicals.
c) comply to national legislation involving the procurement, use and disposal of chemicals.
d) understand the correct application of chemicals in order to increase their effectiveness and minimise adverse impacts.
e) aware of the minimise implications of the use of chemicals to food safety, public health and environment.
f) maintain records of chemicals and its usage for traceability.

8. COMMUNICATION

8.1. The CAs will actively communicate with the following stakeholders about the use of chemicals:

a) Manufacturers and traders.
b) Suppliers and other service providers to aquaculture farms.
c) Aquaculturists.
d) Relevant national CAs.
e) Relevant national government agencies, academic and research institutions.
f) Other ASEAN CAs bilaterally and multilaterally through ASEAN Secretariat or ASWGFi.

8.2. The CA will advise national stakeholders about:

a) new or amended laws and regulations.
b) procedures and guidelines for proper use of chemicals.
c) list of prohibited chemicals.
d) Maximum Residue Limits (MRLs) of chemicals in fish for consumption.
e) the updated *Guidelines for The Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals*

8.3. The CA will notify other ASEAN CAs and other relevant international organizations such as NACA, SEAFDEC and ASWGFi about:

a) Current laws and regulations regarding chemicals in aquaculture.

b) Contact person(s) from each CA.

9. REGISTRATION

The CA will set requirements for registration of aquaculturists, manufacturers and traders as follows:

9.1. Aquaculturists

a) Business registration with relevant government authority.

b) Inspection of registered premises and farms.

9.2. Manufacturers and traders

a) Business registration with relevant government authority.

b) Registration with trade or customs authority, where required.

9.3. Accredited laboratories

a) Accreditation with relevant national CAs.

b) Accreditation with the national and/or international accreditation body.

10. MONITORING PROGRESS OF CAs IN THE IMPLEMENTATION OF THE GUIDELINES FOR THE USE OF CHEMICALS IN AQUACULTURE AND MEASURES TO ELIMINATE THE USE OF HARMFUL CHEMICALS

10.1. The ASWGFi will establish a network among CAs to collate, summarise and analise compliance with the guidelines among the AMS.

10.2. Where non-compliance with guidelines or related problems affect trade in aquaculture products between AMS, the CAs will mutually work to resolve the issues.

11. COMMONLY USED CHEMICALS AND DRUGS IN AQUACULTURE

The list provided below are list of commonly used chemicals in aquaculture in each AMS, which have been agreed and deliberated upon during the Regional Workshops on Harmonisation of Guidelines for the Use of Chemicals in Aquaculture and Measures to Eliminate the Use of Harmful Chemicals, held in Kuala Lumpur in 2010 and 2012. Special care should be considered while using these chemicals and drugs so as not to expose danger to the fish or human. Please seek advice from the Competent Authority or local Veterinarian if in doubt.

Antibiotics/Antimicrobials

Oxolinic acid

Erythromycin

Sulfonamides

Oxytetracyclines

Sulfamerazine

Disinfectants

Benzalkonium Chloride (BKC)

Calcium Hypochlorite/sodium hypochlorite

Lime

Formalin

Sodium chloride

Potassium permanganate

Acetic acid

Acriflavin

Hydrogen peroxide

Iodine

Culture system preparation

Lime

Zeolite

Calcium chloride

Sodium thiosulphate

Anaesthetics

Tricane methanesulphonate

Eugenol, Aqui-S

Hormones

HCG

LHRHa

17” methyltestosterone

Chemotherapeutants

Copper sulfate

Sodium chloride

Formalin
Hydrogen peroxide

Praziquantel

Pottasium permanganate

Methylene blue

Pisicides

Saponin

Rotenone

Organophosphates (dichlorvos, dichlorovos, dipterex, neguvo)

Compound	Indication(s)	Dose
Acetic Acid	Parasiticide for fish	1000-2000 ppm dip for 1-10 minutes
Calcium chloride	Used to aid in egg hardening Used to aid in maintaining osmotic balance during fish holding and transport	10-20 ppm $CaCO_3$ (eggs) ≤150 ppm $CaCO_3$, indefinitely (fish)
Sodium chloride (salt)	Used as an osmoregulatory aid to relieve stress and prevent shock in fish Parasiticide for fish	0.5-1.0% indefinitely 3% dip for 10-30 min
Iodine	Egg surface disinfectant	100 ppm for 10 min during or after waterhardening

Appendix II will list the drugs and chemicals currently being used, or prohibited by each ASEAN Member States. The list has been exhaustedly deliberated and provided by AMS to provide the current practices in their aquaculture activities.

A) FORMALIN

Approved Indications:

Formalin is approved for: (a) for the control of external protozoa (*Chilodonella* spp., Costia spp., *Epistylis* spp., *Ichthyophthirius* spp., *Scyphidia* spp. and *Trichodina* spp.), and the monogenetic trematode parasites (*Cleidodiscus* spp., *Dactylogyrus* spp., and *Gyrodactylus* spp.) on all finfish, (b) for the control of fungi of the family Saprolegniaceae on all finfish eggs and (c) for the control of external protozoan parasites (*Bodo* spp., *Epistylis* spp., and *Zoothamnium* spp.) on penaeid shrimp.

Dosage:

For The Control of Fungi of the Family Saprolegniaceae on Finfish Eggs:

Aquatic Species	Administer in Hatchery Systems (µL/L)
Eggs of all finfish except Acipenseriformes	1000 to 2000 for 15 minutes**
Eggs of Acipenseriformes	up to 1500 for 15 minutes**

**Apply in constant flow water supply of incubating facilities. A preliminary bioassay should be conducted on a small sub-sample of finfish eggs to determine sensitivity before treating an entire group. This is necessary for all species because egg sensitivity can vary with species or strain and the unique conditions at each facility.

Precautions:

Can cause central nervous system (CNS) depression. Slightly irritating to the respiratory system. May cause sensitisation by inhalation. Reports have associated repeated and prolonged occupational over exposure to solvents with permanent brain and nervous system damage. Toxic if inhaled. Harmful if swallowed. Causes bums. May be fatal or cause blindness if swallowed. Harmful in contact with skin, may cause sensitisation cause blindness. Corrosive to eyes.

Withdrawal Period: None.

B) HYDROGEN PEROXIDE

APPROVED INDICATIONS:

For the control of mortality in freshwater-reared finfish eggs due to saprolegniasis.

- 500 to 1,000 mg/L for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all coldwater and coolwater species of freshwater-reared finfish eggs.
- 750 to 1,000 mg/L for 15 minutes in a continuous flow system once per day on consecutive or alternate days until hatch for all warmwater species of freshwater-reared finfish eggs.

For the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium branchiophilum.*

- 100 mg/L for 30 minutes or 50 to 100 mg/L for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath.

For the control of mortality in freshwater-reared coolwater finfish and channel catfish due to external columnaris disease associated with *Flavobacterium*.

- 50 to 75 mg/L for 60 minutes once per day on alternate days for three treatments in a continuous flow water supply or as a static bath (coolwater species of freshwater-reared finfish (except northern pike & paddlefish) and channel catfish).
- 50 mg/L for 60 minutes once per day on alternate days for three treatments in continuous flow water supply or as a static bath (coolwater species of freshwater-reared finfish fry (except northern pike, pallid sturgeon, and paddlefish) and channel catfish fry).

Precautions:

Hydrogen peroxide is a strong oxidiser and personal protective equipment should always be used when handling this chemical (Note: Prolonged exposure may cause skin irritation or burns).

Withdrawal Period: None.

C) TRICAINE METHANESULFONATE (or MS 222)

Approved Indications:

Tricaine methanesulfonate is approved for the temporary immobilisation of fish, amphibians, and other aquatic, cold-blooded animals. It has been recognised as a valuable tool for the proper handling of these animals during manual spawning (other aquatic), weighing, measuring, marking, surgical operations, transport, photography, and research.

Dosage: 10-1,000 mg/L.

Precautions:

May cause skin irritation. May be harmful if absorbed through the skin. May cause eye irritation. Dust may be irritating to the mucous membranes and upper respiratory tract. May be harmful if inhaled. May be harmful if swallowed.

Practical Administration:

Do not use within 21 days of harvesting fish for food.

Withdrawal Period: 21 days

D) COPPER SULFATE

Approved Indications:

Administer as a static bath to control Ichthyophthiriasis (Ich) on catfish and mortality associated with Saprolegniasis in all finfish species.

For the treatment of ichthyophthiriasis (*Ichthyophthirius multifiliis*) on Ictalurid catfish cultured in earthen ponds.

- Administer 0.4 to 1 mg/L per 100 mg/L total alkalinity (as $CaCO_3$) as an indefinite exposure once daily for 5 to 11 consecutive days.

To control mortality associated with Saprolegniasis on channel catfish eggs.

- Administer 10 mg/L for Administer 10 mg/L to the water of a flow-through hatching trough once daily until the embryos (eggs) develop eyes; flow rate should allow for 1 exchange every 30 minutes.

Withdrawal period: 7 days.

E) POTASSIUM PERMANGANATE

Approved Indications:

Administer as a static bath to control external protozoan and metazoan parasites, and bacterial and fungal infections in a variety of warmwater fish species.

Dosage:

Use at a dosage of 1 - 10 mg/L for 1 hour. Although a single treatment event is generally efficacious, repeated treatments may be used.

Withdrawal period: none for fish that are not susceptible

F) LHRHa (Luteinizing Hormone–Releasing Hormone)

Administer by injection to enhance gamete maturation in a variety of variety of fish species.

Dosage:

- LHRHa is available in vials containing 1, 5, or 25 mg LHRHa/vial. LHRHa should be diluted with sterile physiological saline immediately prior to intended use.

- Standard hormone dose rates are 5 to 20 µg LHRHa/kg BW. Although higher dose rates may be used, the total dose may not exceed 100 µg/kg BW.
- LHRHa should be dissolved in sterile physiological saline and administered as either an intraperitoneal (IP) or intramuscular (IM) injection. Intraperitoneal injections are typically administered in females whereas IM injections are typically administered in males.
- The LHRHa dose may be administered as a single injection or multiple injections depending on the species or strain treated. Multiple treatment regimens will generally consist of a single "priming" dose followed by a single "resolving" dose.
- LHRHa treatment has been shown to be most effective when administered during the final stages of gamete maturation. In most cases, LHRHa will be used within 4 weeks of the time fish are normally expected to spawn.

Withdrawal period: 14 days.

G) LIME

Lime is widely used to neutralise acidity, increase total alkalinity, and to increase total hardness in the soil and water of grow-out ponds for fish and shrimp.

Dosage:
The most common preparations/forms are agricultural limestone [calcite-($CaCO_3$) and dolomite ($MgCO_3$)], hydrated or slaked lime [$Ca(OH)_2$] and burnt lime or quick lime (CaO). During pond preparation, lime is applied to the pond bottom at doses of 100-8,000 kg/ha or to the water during the rearing period at 10-500 kg/ha. Liming is also practised (using different procedures) to neutralise acid sulphate resulting from oxidation of pyrites in ponds constructed in mangrove areas. Burnt lime is also used (at 50-100 g/m^2) in conjunction with ammonium phosphate to kill pests and predators.

APPENDIX I

COMPETENT AUTHORITY (IES) IN ASEAN COUNTRIES REGULATING AND MONITORING THE USE OF CHEMICAL IN AQUACULTURE

COMPETENT AUTHORITY			
COUNTRY	**REGULATION ON CHEMICAL USED IN AQUACULTURE**	**VETERINARY DRUGS**	**PESTICIDE**
Brunei Darussalam	**Contact Agency:** Department of Fisheries, Ministry of Industry and Primary Resources Department of Pharmaceutical Services, Ministry of Health		Department of Agrifood and Agriculture, Ministry of Industry and Primary Resources
Cambodia	**Contact Agency:** Fisheries Administration Ministry of Agriculture, Forestry and Fisheries		
Indonesia	**Contact Agency:** Directorate of Fish Health & Environment, Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries		Pesticide Commission, Ministry of Agriculture
Lao PDR	**Contact Agency:** Department of Livestock and Fisheries Ministry of Agriculture and Forestry		
Malaysia	**Contact Agency:** Department of Fisheries, Ministry of Agriculture and Agro-Based Industry Ministry of Health	National Pharmaceutical Control Bureau, Ministry of Health Department of Veterinary Services, Ministry of Agriculture and Agro-Based Industry Food Safety and Quality Division, Ministry of Health	Pesticide Board, Department of Agriculture, Ministry of Agriculture and Agro-Based Industry Food Safety and Quality Division, Ministry of Health

Myanmar	**Contact Agency:** Department of Fisheries, Ministry of Livestock and Fisheries	Department of Fisheries, Ministry of Livestock and Fisheries	Department of Fisheries, Ministry of Livestock and Fisheries
Philippines	**Contact Agency:** Bureau of Fisheries and Aquatic Resources of Department of Agriculture	Food and Drug Administration Department of Health	Fertiliser and Pesticide Authority
Singapore	**Contact Agency:** Agri-Food and Veterinary Authority of Singapore		
Thailand	**Contact Agency:** Department of Fisheries, Ministry of Agriculture and Cooperative	Food and Drug Administration (FDA), Ministry of Public Health	Pesticide Board, Department of Agriculture, Ministry of Agriculture and Cooperative
Viet Nam	**Contact Agency:** Department of Animal Health, Ministry of Agriculture and Rural Development Department of Fisheries, Ministry of Agriculture and Rural Development	Department of Animal Health, Ministry of Agriculture and Rural Development	Plant Protection Department, Ministry of Agriculture and Rural Development

APPENDIX II

List of chemicals used in Aquaculture by ASEAN Member States

Explanatory note –
PROHIBITED – Total banned
NO – Currently not used
NDA – No Data Available (Status not known)
YES – Allowed to be used with recommended MRL and with withdrawal Period

A) ANTIBIOTICS/ANTIMICROBIAL

FISH FOR FOOD CONSUMPTION:

	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
Antibiotics/ Antimicrobials								
Tetracyclines *	PROHIBITED	PROHIBITED	YES	YES	YES	NO	YES	NO
Nitrofurans	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED
Chloramphenicol	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED
Oxolinic acid *	PROHIBITED	NO	YES	PROHIBITED/ YES	YES	YES	YES	NO
Erythromycin *	PROHIBITED	NO	YES	YES	YES	NO	NO	NO
Dimetridazole/Metronidazole	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	NO	PROHIBITED
Elbaju/Ebazine	PROHIBITED	NO	YES	NDA	NDA	NDA	NO	NO
Sulfonamides *	PROHIBITED	NO	YES	YES	YES	NO	YES	YES
Oxytetracyclines *	PROHIBITED	NDA	YES	YES	YES	NO	YES	YES
Chlortetracycline *	PROHIBITED	NO	YES	YES	YES	NO	YES	NO

Sulfamerazine *	PROHIBITED	NO	YES	NDA	YES	NO	YES	YES
Nifurpirinol	PROHIBITED	PROHIBITED	YES	NDA	NO	PROHIBITED	NO	NO
Amoxicilin	PROHIBITED	NO	YES	YES	YES	NO	NO	NO
Doxycyclin	PROHIBITED	NO	NDA	NDA	YES	NO	NO	YES
Enrofloxacin *	PROHIBITED	NDA	NDA	YES	YES	NO	PROHIBITED	PROHIBITED
Florfenicol	PROHIBITED	PROHIBITED	NDA	NDA	YES	NO	NO	YES
Norfloxacin	PROHIBITED	NO	NDA	NDA	YES	NO	NO	NO
Rifamicin / or Rifampicin??	PROHIBITED	NO	NDA	NDA	YES	NO	NO	NO
Ciprofloxacin	PROHIBITED	NO	NDA	NDA	NDA	NO	NO	NO
Sarafloxacin	PROHIBITED	NO	NDA	NDA	NDA	NO	YES	NO
Ormethoprim	PROHIBITED	NO	NDA	NDA	NDA	NO	YES	YES
Sulfadimethoxin + Ormethoprim *	PROHIBITED	NO	NDA	NDA	NDA	NO	YES	YES
Sulfadimethoxin + trimethoprim *	PROHIBITED	NO	NDA	NDA	YES	NO	YES	YES

*Residues with Maximum Residual Limit

ORNAMENTAL FISH:

	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
Antibiotics/ Antimicrobials	NO ANTIBIOTIC							
Tetracyclines	YES	YES	YES	YES	YES	YES	YES	NO
Nitrofurans	PROHIBITED	YES	PROHIBITED	PROHIBITED	YES	PROHIBITED	PROHIBITED	PROHIBITED
Chloramphenicol	PROHIBITED	YES	PROHIBITED	PROHIBITED	NO	PROHIBITED	PROHIBITED	PROHIBITED
Oxolinic acid	YES	YES	YES	YES	YES	YES	YES	NO
Erythromycin	NDA	YES	YES	YES	YES	YES	NO	NO
Dimetridazole/ Metronidazole	PROHIBITED	PROHIBITED	PROHIBITED	PROHIBITED	NO	NO	NO	PROHIBITED
Elbaju/Ebazine	NDA	YES	YES	NDA	NO	NDA	NO	NO
Sulfonamides	NDA	YES	YES	YES	YES	YES	YES	YES
Oxytetracyclines	YES	YES	YES	YES	YES	YES	YES	YES
Chlortetracyclin	YES	YES	YES	YES	YES	NDA	YES	NO
Sulfamerazine	NDA	NO	YES	NDA	YES	NDA	YES	YES
Nifurpirinol	NDA	NDA	YES	NDA	NO	NDA	NO	NO
Amoxicilin	NDA	NDA	NDA	YES	YES	NDA	NO	NO
Doxycyclin	NDA	NO	NDA	NDA	YES	NO	NO	YES
Enrofloxacin	NDA	NO	NDA	YES	YES	NO	PROHIBITED	PROHIBITED
Florfenicol	YES	NO	NDA	NDA	YES	NO	NO	YES
Norfloxacin	NDA	NO	NDA	NDA	YES	NO	NO	NO
Rifamicin	NDA	NO	NDA	NDA	YES	NO	NO	NO
Ciprofloxacin	NDA	NO	NDA	NDA	NDA	NO	NO	NO
Sarafloxacin	NDA	NO	NDA	NDA	NDA	NO	YES	NO
Ormethoprim	NDA	NO	NDA	NDA	NDA	NO	YES	YES
Sulfadimethoxin + Ormethoprim	NDA	NO	NDA	NDA	YES	NO	YES	YES
Sulfadimethoxin + trimethoprim	NDA	NO	NDA	NDA	YES	NO	YES	YES

B) DISINFECTANTS

Disinfectants	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
BKC	**NO**	YES	YES	YES	**YES**	**YES**	YES	YES
Calcium Hypochlorite	YES	YES	YES	YES	YES	YES	YES	YES
Lime	YES	YES	YES	YES	YES	YES	YES	YES
Formalin	YES	YES	YES	YES	YES	YES	YES	YES
Sodium chloride	**NO**	YES	YES	YES	YES	YES	**NO**	**NO**
Potassium permanganate	**NO**	YES	YES	YES	YES	YES	YES	**NO**
Methylene blue	**NO**	YES	**YES**	YES	**YES**	**NDA**	YES	**NO**
Malachite green	**PROHIBITED**	PROHIBITED	**PROHIBITED**	PROHIBITED	**PROHIBITED**	**PROHIBITED**	**PROHIBITED**	**PROHIBITED**
Copper sulphate	**YES**	YES	YES	YES	**NO**	**NDA**	**NO**	**YES**
Acetic acid	**NO**	YES	NO	YES	**NO**	**NDA**	YES	**YES**
Acriflavin	**NO**	NO	NO	NDA	**NO**	NO	YES	**NO**
Hydrogen peroxide	**NO**	YES	YES	YES	YES	YES	YES	**YES**
Sodium hypochlorite	**YES**	NO	YES	YES	YES	YES	YES	**NO**
Iodine	**YES**	YES	YES	YES	YES	YES	YES	**YES**
Cypermethrin	**NO**	NO	**NO**	NDA	YES	**NO**	**NO**	**PROHIBITED**
Potasium monopersulfate	**NO**	YES	**NO**	NDA	YES	**NO**	YES	**NO**
Omnicide	**NO**	YES	**NO**	NO	YES	**NO**	**NO**	**YES**
Trichlorfon	**NO**	NO	**NO**	NDA	YES	**NO**	**NO**	**NO**
Glutaraldehyde	**NO**	YES	**NO**	NDA	**NO**	**NO**	YES	**YES**
Chloramin T	**NO**	NO	**NO**	NDA	**YES**	**NO**	**NO**	**YES**
Sodium Dichloroisoyanurate	**NO**	NO	**NO**	NDA	**NO**	**NO**	**YES**	**YES**

Tricholoroicyanuric acid	**NO**	NO	**NO**	NDA	**NO**	**NO**	**NO**	**YES**
Myristalkonium chloride	**NO**	NO	**NO**	NDA	**NO**	**NO**	**NO**	**YES**
Ethylenediamine tetraacetic acid (EDTA)	**NO**	NO	**NO**	NDA	**NO**	**NO**	**NO**	**YES**
Pottasium peroxymonosulfate	**NO**	YES	**NO**	NDA	**NO**	**NO**	**YES**	**NO**

C) CHEMOTHERAPEUTANTS AGENTS

FISH FOR FOOD CONSUMPTION:

Chemotherapeutants	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
Acriflavin	**NDA**	YES	**YES**	NDA	**NDA**	**NO**	**YES**	**NO**
copper sulfate	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
trichlorfon	**NO**	PROHIBITED	**YES**	NDA	**YES**	**NO**	**YES**	**NO**
trifluralin	**NO**	PROHIBITED	**NO**	YES	**YES**	**NO**	**YES**	**PROHIBITED**
Cypermethrin	**NO**	NO	**NO**	NDA	**YES**	**NO**	**NO**	**YES**
sodium chloride	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
formaldehyde	**YES**	YES	**YES**	YES	**YES**	**NO**	**YES**	**YES**
hydrogen peroxide	**NO**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
praziquantel	**NO**	NO	**YES**	NDA	**YES**	**NO**	**YES**	**YES**
potasium permanganate	**YES**	YES	**YES**	YES	**YES**	**NO**	**YES**	**NO**
methylene blue	**NO**	YES	**YES**	YES	**YES**	**NO**	**YES**	**NO**
Bronopol	**NO**	NO	**NO**	NDA	**NO**	**NO**	**NO**	**YES**
Levamisol	**NO**	NO	**NO**	NDA	**NO**	**NO**	**NO**	**YES**

ORNAMENTAL FISH:

Chemotherapeutants	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
Acriflavin	**YES**	YES	**YES**	NDA	**NDA**	**YES**	**YES**	**NO**
copper sulfate	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
trichlorfon	**NDA**	PROHIBITED	**YES**	NDA	**YES**	**NO**	**YES**	**NO**
trifluralin	**NDA**	PROHIBITED	**NO**	YES	**YES**	**NO**	**YES**	**PROHIBITED**
cypermethrin	**NDA**	NO	**NO**	NDA	**NDA**	**NO**	**NO**	**YES**
sodium chloride	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
formaldehyde	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
hydrogen peroxide	**NO**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
praziquantel	**NDA**	NO	**YES**	NDA	**YES**	**YES**	**YES**	**YES**
potassium permanganate	**NO**	YES	**YES**	YES	**YES**	**YES**	**YES**	**NO**
methylene blue	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**NO**
Bronopol	**NDA**	NO	**NO**	NDA	**NO**	**NO**	**YES**	**YES**
Levamisol	**NDA**	NO	**NO**	NDA	**NO**	**NO**	**NO**	**YES**

D) PISCICIDE (FOR USE IN POND PREPARATION OR EARLY CULTURE ONLY)

Piscicide	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
saponin	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**YES**
Rotenone	**YES**	YES	**YES**	YES	**YES**	**NDA**	**YES**	**YES**
Organophosphates (OPs) - The two most commonly used OPs are dichlorvos (dichlorovos) and trichlorfon (dipterex, and neguvon).	**YES**	YES	**YES**	YES	**YES**	**NDA**	**YES**	**NO**

cyanide	**PROHIBITED**	NO	**NO**	NO	**NO**	**NO**	**PROHIBITED**	**NO**
fentin acetate	**NO**	YES	**NDA**	NDA	**NO**	**NO**	**YES**	**NO**
Deltamethrine	**NDA**	NO	**NDA**	NDA	**NDA**	**NO**	**PROHIBITED**	**PROHIBITED**

E) HORMONES

FISH FOR FOOD CONSUMPTION:

Hormones	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
HCG	**YES**	NO	**YES**	YES	**YES**	**NO**	**YES**	**YES**
LHRHa	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**NDA**
GnRHa	**NO**	YES	**YES**	YES	**NO**	**NO**	**YES**	**NDA**
Ovaprim	**NO**	YES	**YES**	YES	**NO**	**NO**	**NO**	**NO**
Pituitary extract	**YES**	NDA	**NO**	YES	**YES**	**NO**	**YES**	**NO**
Puberogen	**NO**	NO	**YES**	NDA	**NO**	**NO**	**YES**	**NO**
17" methyltestosterone	**YES**	PROHIBITED	**YES**	YES	**YES**	**NO**	**YES**	**NO**
androgen	**NO**	NO	**NO**	NDA	**NO**	**NO**	**YES**	**NO**
17 - Beta estradiol	**NO**	NO	**NO**	YES	**NO**	**NO**	**YES**	**NO**
Ovatide	**NO**	NO	**YES**	NDA	**NO**	**NO**	**NO**	**NO**

F) HORMONES

ORNAMENTAL FISH

Hormones	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
HCG	**YES**	NO	**YES**	YES	**YES**	**YES**	**YES**	**YES**
LHRHa	**YES**	YES	**YES**	YES	**YES**	**YES**	**YES**	**NDA**

Ovaprim	**NO**	YES	**YES**	YES	**NO**	**NO**	**NO**	**NO**
Puberogen	**NO**	NO	**YES**	NDA	**NO**	**NO**	**YES**	**NO**
androgen	**NO**	NO	**NO**	NDA	**NO**	**NO**	**YES**	**NO**
Ovatide	**NO**	NO	**YES**	NDA	**NO**	**NO**	**NO**	**NO**

G) ANAESTHETICS (FOR USE BOTH ON FISH FOR FOOD CONSUMPTION AND ORNAMENTAL FISH)

Anaesthetics	**Brunei Darussalam**	**Indonesia**	**Malaysia**	**Myanmar**	**Philippines**	**Singapore**	**Thailand**	**Viet Nam**
Tricane methanesulphonate (TMS222)	**YES**	**YES**	**YES**	YES	**YES**	**YES**	**YES**	**NDA**
Eugenol, Aqui-S	**NDA**	**YES**	**YES**	YES	**YES**	**NO**	**YES**	**YES**
Quinaldine	**NDA**	**NO**	**NO**	NDA	**NO**	**NO**	**YES**	**NO**
Tranquil (Aquacalm)	**NDA**	**NO**	**YES**	NDA	**NO**	**NO**	**NO**	**NO**
Benzocaine	**NDA**	**NO**	**YES**	NDA	**NO**	**NO**	**YES**	**NDA**
phenoxy ethanol	**NDA**	**YES**	**NO**	NDA	**YES**	**NO**	**YES**	**NO**

H) CULTURE SYSTEM PREPARATION

Culture System Preparation	**Brunei Darussalam**	**Indonesia**	**Malaysia**	**Myanmar**	**Philippines**	**Singapore**	**Thailand**	**Viet Nam**
Calcium Hypochlorite	**YES**	**YES**	**YES**	YES	**YES**	**YES**	**NO**	**YES**
lime	**YES**	**YES**	**YES**	YES	**YES**	**YES**	**YES**	**YES**
urea	**YES**	**YES**	**YES**	YES	**YES**	**NO**	**NO**	**NO**
zeolite	**YES**	**YES**	**YES**	YES	**YES**	**NDA**	**YES**	**YES**
calcium chloride	**NO**	**YES**	**YES**	YES	**YES**	**NDA**	**NO**	**YES**
EDTA	**YES**	**NO**	**NO**	YES	**NDA**	**NO**	**YES**	**YES**
Sodium thiosulphate	**YES**	**YES**	**YES**	YES	**YES**	**NO**	**YES**	**YES**

I) BANNED CHEMICALS

FISH FOR FOOD CONSUMPTION:

Banned chemicals	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore	Thailand	Viet Nam
Enrofloxacin		NDA			YES	PROHIBITED	**YES**	**YES**
Cypermethrin		YES			YES			**YES**
Deltamethrine		YES			NDA			**YES**
Malachite green	**YES**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Nitrofurans	**YES**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Chloramphenicol	**YES**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Beta-agonist	**YES**	**YES**	**YES**		PROHIBITED	PROHIBITED	**YES**	**YES**
Nitroimidazoles	**YES**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
organotin	**YES**	**NO**	**NDA**	NDA	PROHIBITED	PROHIBITED	**NO**	**NO**
organochlorin	**YES**	**YES**	**NO ***	NDA	PROHIBITED	PROHIBITED	**YES**	**NO**
Crystal violet	**YES**	**YES**	**YES**	NDA	PROHIBITED	PROHIBITED	**NO**	**YES**
*selected organophosphates	**YES**	**NO**	**YES**	YES	PROHIBITED	PROHIBITED	**NO**	**NO**
Trichlorfon (Dipterex)	**NDA**	**YES**	**YES**	YES	YES	PROHIBITED	**NO**	**YES**
Chloroform	**NDA**	**YES**	**NOT KNOWN**	PROHIBITED	NDA	PROHIBITED	**YES**	**YES**
Chlorpromazine	**NDA**	**YES**	**YES**	PROHIBITED	NDA	PROHIBITED	**YES**	**YES**
Colchicine	**NDA**	**YES**	**YES**	PROHIBITED	NDA	PROHIBITED	**YES**	**YES**
Dapsone	**NDA**	**YES**	**YES**	PROHIBITED	NDA	PROHIBITED	**YES**	**YES**
Ronidazole (nitroimidazole)	**NDA**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Ipronidazole	**NDA**	**NOT KNOWN**	**YES**		PROHIBITED	PROHIBITED	**YES**	**YES**
Clenbuterol	**NDA**	**NOT KNOWN**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**

Glycopeptides	**NDA**	**NOT KNOWN**	**NOT KNOWN**		NDA	PROHIBITED	**YES**	**YES**
Diethylstilbestrol (stilbene)	**NDA**	**YES**	**YES**		PROHIBITED	PROHIBITED	**YES**	**YES**
Trifluralin	**NDA**	**YES**	**NDA**		YES	PROHIBITED	**NO**	**YES**
Dimetridazole (nitroimidazole)	**NDA**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Metronidazole (nitroimidazole)	**NDA**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**

***Residues with Maximum Residual Limit (MRL)**

ORNAMENTAL FISH:

Banned chemicals	Brunei Darussalam	Indonesia	Malaysia	Myanmar	Philippines	Singapore[b]	Thailand	Viet Nam
Enrofloxacin		NO			YES	PROHIBITED	**YES**	**YES**
Cypermethrin		NO			NDA			**YES**
Deltamethrine		NO			NDA			**YES**
Malachite green	**YES**	**YES**	**YES**	PROHIBITED	PROHIBITED	**NO**	**NO**	**YES**
Nitrofurans	**YES**	**YES**	**YES**	PROHIBITED	YES	PROHIBITED	**YES**	**YES**
Chloramphenicol	**YES**	**YES**	**YES**	PROHIBITED	NO	PROHIBITED	**YES**	**YES**
Beta-agonist	**YES**	**YES**	**YES**		NO	**NO**	**YES**	**YES**
Nitroimidazoles	**YES**	**YES**	**YES**	PROHIBITED	NDA	PROHIBITED	**YES**	**YES**
organotin	**YES**	**NO**	**NOT KNOWN**		PROHIBITED	**NO**	**NO**	**NO**
organochlorin	**YES**	**YES**	**NO**		PROHIBITED	**NO**	**YES**	**NO**
Crystal violet	**YES**	**YES**	**NO**	NDA	PROHIBITED	**NO**	**NO**	**YES**

*selected organophosphates	**YES**	**NO**	**YES**	YES	PROHIBITED	**NO**	**NO**	**NO**
Trichlorfon (Dipterex)	**NOT KNOWN**	**YES**	**NO**	YES	YES	**NO**	**NO**	**YES**
Chloroform	**NOT KNOWN**	**YES**	**NOT KNOWN**	PROHIBITED	NDA	**NO**	**YES**	**YES**
Chlorpromazine	**NOT KNOWN**	**YES**	**YES**	PROHIBITED	NDA	**NO**	**YES**	**YES**
Colchicine	**NOT KNOWN**	**YES**	**YES**	PROHIBITED	NDA	**NO**	**YES**	**YES**
Dapsone	**NOT KNOWN**	**YES**	**YES**	PROHIBITED	NDA	PROHIBITED	**YES**	**YES**
Ronidazole (nitroimidazole)	**NOT KNOWN**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Ipronidazole	**NOT KNOWN**	**NOT KNOWN**	**YES**		PROHIBITED	**NO**	**YES**	**YES**
Clenbuterol	**NOT KNOWN**	**NOT KNOWN**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Glycopeptides	**NOT KNOWN**	**NOT KNOWN**	**NOT KNOWN**		NDA	PROHIBITED	**YES**	**YES**
Diethylstilbestrol (stilbene)	**NOT KNOWN**	**YES**	**YES**		PROHIBITED	**NO**	**YES**	**YES**
Trifluralin	**NOT KNOWN**	**YES**	**NOT KNOWN**		YES	**NO**	**NO**	**YES**
Dimetridazole (nitroimidazole)	**NOT KNOWN**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**
Metronidazole (nitroimidazole)	**NOT KNOWN**	**YES**	**YES**	PROHIBITED	PROHIBITED	PROHIBITED	**YES**	**YES**

[b]For Singapore, no specific regulation for ornamental fish

APPENDIX III

DRAFTING COMMITTEE

Resource Persons

1. Dr. Hassan Bin Hj. Mohd Daud
   Faculty of Veterinary Medicine
   University Putra Malaysia, Serdang, Malaysia

2. Dr. Relicardo M. Coloso
   SEAFDEC Aquaculture Department,
   Tigbauan, Iloilo, Philippines

3. Dr. Eduardo M. Leano
   Network of Aquaculture Centres in Asia-Pacific (NACA)
   Bangkok, Thailand

4. Mr. Suriyan Vichitlerkarn
   ASEAN Secretariat

Brunei Darussalam

Mdm Laila Bt. Hj. A. Hamid
Mdm Noorizan Bt. Hj. Abd. Karim
Mdm Wanidawati Tamat

Cambodia

Mr. Chhoun Chamnan
Mr. Chin Da

Indonesia

Mdm Maysaroh Mawardi
Mdm Refnita Eliza
Drh. Siti Fatimah
Mdm Mufidah Fitriati

Malaysia

Mr. Mohamad Shaupi Bin Derahman
Mr. Abdul Rahman Bin Abdul Wahab
Mr. Zulkafli Abd Rashid
Dr. Kua Beng Choo
Mr. Adrian F. Vijiarungam
Mr. Hj. Raihan B. Sh. Hj. Ahmad
Ms. Hemalatha a/p Raja Sekaran
Mr. Jamaludin Ibrahim
Dr. Siti Zahrah Bt. Abdullah
Mdm Maria Afiza Bt. Omar
Mdm Zaleenah Bt. Zainuddin
Mdm Azlinda Bt. Abdul Samad
Mdm Maznah Bt. Othman

Philippines

Dr. Simeona E. Regidor
Dr. Sonia S. Somga

Singapore

Dr. Kelvin Lim Zhi Jian
Ms. Phua Hoon Hong

Mr. Tan Yit Wee
Dr. Chong Shin Min

Mr. Johari Daud
Mdm Nik Haiha Nik Yusof
Mr. Saberi Mawi
Mr. Kaharudin Md Salleh
Dr. Hamdan Bin Ahmad
Mr. Arthur Besther Sujang
Mr. Yusri bin Ismail

Thailand

Dr. Kom Silapajarn
Mr. Youngyut Predalumpaburt
Mdm Mukda Uttarapong

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Ms. Nguyen Thi Thu Trang
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