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).

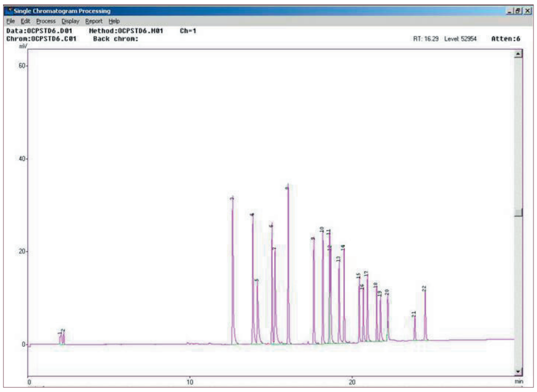
- **Oxolinic Acid (OXA).** Oxolinic acid or OXA is solid, clear, and very faintly yellow in color with a chemical formula of $C_{13}H_{11}NO_5$ and molecular weight of 261.23 (g/mol). This antibiotic is harmful if swallowed. In rats, oral ingestion causes acute toxicity at LD50 of 525 mg/kg while for 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).

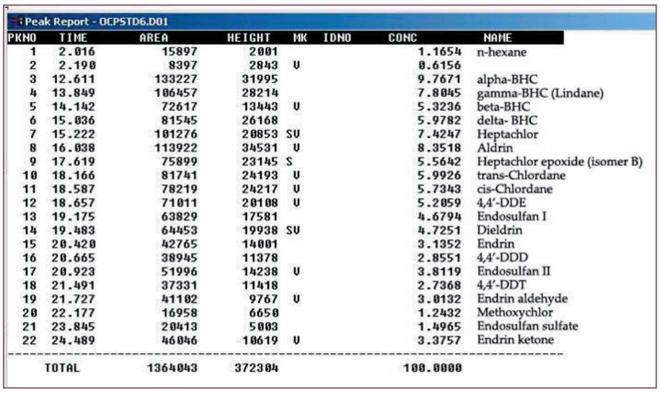
Sample Collection and Analytical Methods

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.

A



B



Peak Report - OCPSTD6.D01

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).

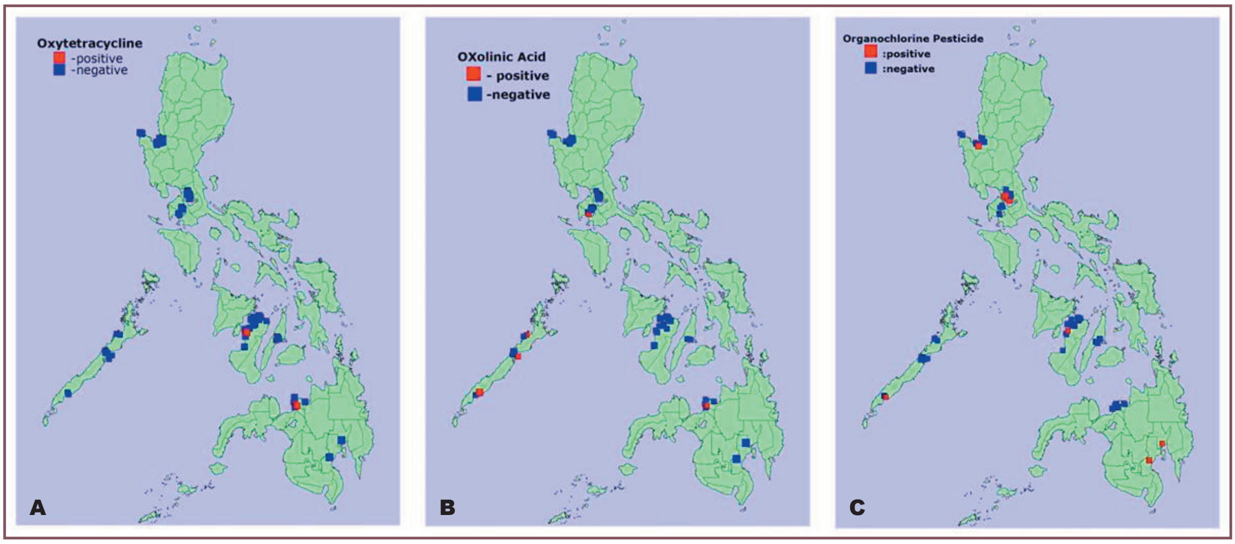


Figure 1.2 Areas sampled in the Philippines showing (A) results of OTC (Oxytetratcycline) analysis, (B) results of OXA (Oxolinic acid) analysis, and (C) showing results of OCP analysis.

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®.

References

Arthur, J. R., Lavilla-Pitogo, C. R. and Subasinghe, R. P. 2000.Use of Chemicals in Aquaculture in Asia. Proceedings of the meeting on the Use of Chemicals in Aquaculture in Asia, 20-22 May 1996, Tigbauan, Iloilo, Philippines, aquaculture Department, SEAFDEC/AQD. 235 pp.

FAO. 1995. Code of Conduct on Responsible Fisheries, FAO, Rome. 41 pp.

Campoy, C., Jimenez, M., Olea-Serrano, M.F., Moreno-Frias, M. Canabate, F., Olea, N. Bayes, R. and Molina-Font, J.A. 2001. Analysis of organochlorine pesticides in human milk: preliminary results. Early Hum. Dev. 65:S 183-190.

Carignan, G., Carrier, K and Sved, S. 1993. Assay of Oxytetracycline residues in salmon muscle by Liquid Chromatography with Ultaviolet detection. J. of AOAC International. 76 (2) 325-328.

Coloso, R.M. 2003.Endosulfan: a hidden menace. SEAFDEC Asian Aquaculture.V-25.April-June issue, 1-6pp.

Coloso. R. and Borlongan, I. 1999. Significant organotin contamination of sediment and tissues of milkfish in brackish water ponds. Bull. Environ. Contam.Toxicol. 63:297-304.

Londershausen, M. 2009. Presentation Title "Aquaculture in the light of regulatory requirements in import countries" presented during a conference on "Innovations to Sustain Sea-food Supply" on March 12, 2009, BITEC, Bangkok.

Lacierda, E., de La Pena L. & Lumanlan-Mayo, S. 1996. Use of Chemicals in Aquaculture in the Philippines. In: Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia (JR Arthur, CP Lavilla-Pitogo, CR Subasinghe, Eds.), 20-22 May 1996, Tigbauan, Iloilo, Philippines, Aquaculture Department, Southeast Asian Fisheries Development Center, Philippines. 155-184 p.

Lacierda, E., Corrre V., Yamamoto, A., Koyama, J. and Matsuoka, T. 2008. Current status on the Use of Chemcials and Biological Products and Health Management Practices in Aquaculture Farms in the Philippines.Mem. Fac. Fish. Kagoshima Univ. 57: 37-45.

MFRD Report. 2008. Japanese Trust Fund II. Project on Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products (2004-2008), Technical Compilation of Heavy Metals, Pesticide Residues, Histamine and Drug Residues in Fish and Fish Products in Southeast Asia. p 212.

Rico, A., Satapornvanit, K., Haque, M., Min, J, Nguyen P., Telfer, T. and van den Brink, P. 2012. Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. Reviews in Aquaculture 4: 75-93.

Tacon, A. and Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded feeds: Trends and future prospects. Aquaculture 285:146-158.

Training Manual on Pesticide Residue Analysis, 6-15 June 2005, Singapore. MFRD. Singapore (Modified).

Willey, J.B. and Krone, P.H. 2001. Effects of endosulfan and nonylphenol on the primordial germ cell population in pre-larval zebrafish embryos Aquat. Toxicol. 54: 113-123.