

Thailand

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1. Introduction

In 2002, some Southeast Asian countries' aquacultured shrimp and fish were found to contain nitrofurans and chloramphenicol residues. In the past 2 years, some countries also found Malachite Green and Leuco-Malachite Green contamination in their aquacultured shrimp and fish. The Southeast Asian countries thus addressed these contamination problems immediately. This Japanese Trust Fund II project is very helpful for the monitoring and controlling of the occurrences of these chemical residues in Southeast Asian countries and to ensure traceability of the fish and fish products in importing and exporting countries.

2. Objectives And Goals

- To survey the level of drug residues used in ASEAN countries, and
- To ensure the food safety for consumer.

3. Survey Methodologies

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

The samples were collected from the Central, Western and Southern part of Thailand. The raw materials of black tiger prawn (*Penaeus monodon*), freshwater shrimp and crab were collected from at least 5 samples/farm and 5-7 samples/lot/month. For samples collected from the factories, a sampling plan following the CODEX sampling criteria was used. Samples were kept in plastic bag and properly packed in a cool condition (temperature below 10°C) during transportation. Upon arriving in the laboratory, the sample preparation was conducted immediately and the blended samples were kept in the freezer below -18°C.

b. Method of Analysis

Nitrofurans (NF) (Metabolite) by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS):

1 g of sample was weighed into a centrifuge tube, and extracted with 0.5 ml of 1M hydrochloric acid (HCl) and 150 µl of 2-nitrobenzaldehyde (2-NBA) solution. The tube was placed in the water bath at 37°C for 16± 2 hrs. The metabolites were simultaneously released from the sample tissue and the drug residues were derivatised in the acid medium containing 2-NBA. The nitrophenyl derivatives produced were AOZ, AHD, AMOZ and SEM. They are detected and quantified using HPLC tandem mass spectrometry with ESI (Electro spray) and positive mode.

Note:

All chemicals used were analytical and HPLC grade. The LC/MS/MS used to detect Nitrofurans (metabolites) was Micromass system.

Chloramphenicol (CAP) by ELISA :

3 g of sample was weighed into a centrifuge tube. The samples were homogenized in ethyl acetate and then evaporated to dryness. The residue was reconstituted in iso-octane/chloroform and a buffer. The ELISA is a competitive enzyme immunoassay for the screening in various matrices on the presence of this broad spectrum antibiotic.

Malachite Green (MG) and Leuco-Malachite Green (LMG) by LC/MS/MS:

1 g of sample was weighed into a centrifuge tube. The sample was homogenized in McIlvaine buffer:Methanol (50:50 by volume) and N, N, N', N'-tetramethyl-1,4-phenylenediamine dihydrochloride was used for the extraction process. The sample was then centrifuged and passed through the Solid-Phase Extraction (SPE) cartridge (Waters OASIS, MCX) for the clean-up step. The samples

after the SPE step was then dried under nitrogen gas and re-dissolved with 50 % acetonitrile.

Note:

All chemicals used were analytical and HPLC grade. The LC/MS/MS used to detect Malachite Green and Leuco-Malachite Green is API (Applied Biosystem) Model 4000.

c. Limit of Detection and Limit of Quantification

The Minimum Required Performance Limit (MRPL) for Nitrofurans (metabolites) is 1.00 ppb. The LOD and LOQ for all metabolites are 0.02 and 0.10 ppb, respectively.

The MRPL for Chloramphenicol is 0.30 ppb. The LOD and LOQ are 0.01 and 0.03 ppb, respectively.

The MRPL for Malachite Green and Leuco-Malachite Green are 2.00 ppb. The LOD are 0.02 and 0.01 ppb respectively. The LOQ for both residues are 0.10 ppb.

d. National Regulatory Limits

For **Thailand regulation**, all residues that mentioned above are prohibited for use for fish/shrimp aquaculture.

The national regulatory limit for Nitrofurans (metabolites) is in-line with this MRPL limit; 0.30 ppb for AOZ and AMOZ and 1.00 ppb for AHD and SEM.

The national regulatory limit for Chloramphenicol is 0.30 ppb.

The national regulatory limit for Malachite Green and Leuco-Malachite Green is in-line with the MRPL limit at 2.00 ppb.

EU regulation

Nitrofurans (metabolites): 1.00 ppb
Chloramphenicol: 0.30 ppb

Total Malachite green and Leuco-Malachite Green: 2.00 ppb

Canada Regulation

Nitrofurans (metabolites): Not detected
Chloramphenicol: Not detected
Total Malachite Green and Leuco-Malachite Green: 1.00 ppb

USA Regulation

Nitrofurans (metabolites): 1.00 ppb
Chloramphenicol: 0.30 ppb
Total Malachite Green and Leuco-Malachite Green: 1.00 ppb

4. Results And Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

Year of participation	Pro-gram Name	Analyte Tested	Re-ported results	True value	z-score
2008	AFSSA, EU CRL	MG and LMG	0.775	0.875	-0.479
2008	FAPAS	CAP	0.35	0.61	-2.0
2007	FAPAS	NF	0.24	0.40	-1.8

b. Survey Results and Discussion

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppb) – wet weight basis	Max. value of result (ppb) – wet weight basis	Average value of result (ppb) – wet weight basis	Average Recovery (%)	Remarks
		Common name	Scientific name						
2005	Nitrofurans (Metabolites)	Black tiger shrimp	<i>Penaeus monodon</i>	40	ND	0.25 (SEM)	0.14	85 %	Fresh from farm
	Chloramphenicol	Black tiger shrimp	<i>Penaeus monodon</i>	40	ND	0.02 (< LOQ)	0.02 (< LOQ)	80%	Fresh from farm
	Nitrofurans (Metabolites)	Black tiger shrimp	<i>Penaeus monodon</i>	40	ND	ND	ND	85 %	Fresh/frozen from factory
	Chloramphenicol	Black tiger shrimp	<i>Penaeus monodon</i>	40	ND	ND	ND	80%	Fresh/frozen from factory
2006	Nitrofurans (Metabolites)	Black tiger shrimp	<i>Penaeus monodon</i>	45	ND	ND	ND	80 %	Fresh from farm
	Chloramphenicol	Black tiger shrimp	<i>Penaeus monodon</i>	45	ND	0.01 (< LOQ)	ND	82%	Fresh from farm
	Nitrofurans (Metabolites)	Black tiger shrimp	<i>Penaeus monodon</i>	45	ND	ND	ND	80 %	Fresh/frozen from factory
	Chloramphenicol	Black tiger shrimp	<i>Penaeus monodon</i>	45	ND	ND	ND	82%	Fresh/frozen from factory
2007	Nitrofurans (Metabolites)	Freshwater shrimp	<i>Penaeus monodon</i>	20	ND	ND	ND	88%	Fresh from farm
	Chloramphenicol	Freshwater shrimp	<i>Penaeus monodon</i>	20	ND	ND	ND	82%	Fresh from farm
	Malachite green and Leuco-malachite green	Freshwater shrimp	<i>Macrobrachium rosenbergii</i>	20	ND	ND	ND	90%	Fresh from farm
	Nitrofurans (Metabolites)	Crab	<i>Scylla serrata</i>	10	ND	ND	ND	88%	Fresh/frozen from factory
Chloramphenicol	Crab	<i>Scylla serrata</i>	10	ND	ND	ND	ND	82%	Fresh/frozen from factory

	Malachite green and Leuco-malachite green	Crab	<i>Scylla Serrata</i>	10	ND	ND	ND	90%	Fresh/frozen from factory
2008	Nitrofurans (Metabolites)	Freshwater shrimp	<i>Penaeus monodon</i>	9	ND	ND	ND	80%	Fresh from farm
	Chloramphenicol	Freshwater shrimp	<i>Penaeus monodon</i>	9	ND	ND	ND	80%	Fresh from farm
	Malachite green and Leuco-malachite green	Freshwater shrimp	<i>Macrobrachium rosenbergii</i>	9	ND	ND	ND	86%	Fresh from farm
	Nitrofurans (Metabolites)	Crab	<i>Scylla serrata</i>	6	ND	ND	ND	80%	Fresh/frozen from factory
	Chloramphenicol	Crab	<i>Scylla serrata</i>	6	ND	ND	ND	80%	Fresh/frozen from factory
	Malachite green and Leuco-malachite green	Crab	<i>Scylla serrata</i>	6	ND	ND	ND	86%	Fresh/frozen from factory

ND: Not Detected

c. Corrective Actions (if applicable)

For any detection of the prohibited residues, the farmer or processor will be informed of the violation. If the violation is found in finished products, the processor must conduct a self-monitoring program to control the raw material reception. At farm/hatchery level, responsible DOF offices will conduct a follow-up inspection to determine the possible sources of contamination and to ensure the implementation of proper corrective actions and preventive measures.

5. Problems and Challenges Encountered

Not applicable.

6. Recommendations and Suggestions for Future Follow up Action

Not applicable.