MALAYSIA

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1. Introduction

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

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

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

2. Objectives And Goals

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

3. Survey Methodologies

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

In 2007, 2 samplings were carried out for this

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

b. Method of Analysis

Sample Preparation

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

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

Table 1. preparation of quality control samples and samples.

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

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

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

LC Conditions

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

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

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

Volume of sample injection : 10 -25 ul

MS Conditions

Ion Source (polarity) : ESI Negative mode

System check

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

c. Limit of Detection and Limit of Quantification

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

d. National Regulatory Limits

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

4. Results and Discussion

a. Participation of Inter-laboratory Proficiency Testing and Results

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

b. Survey Results and Discussion

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

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

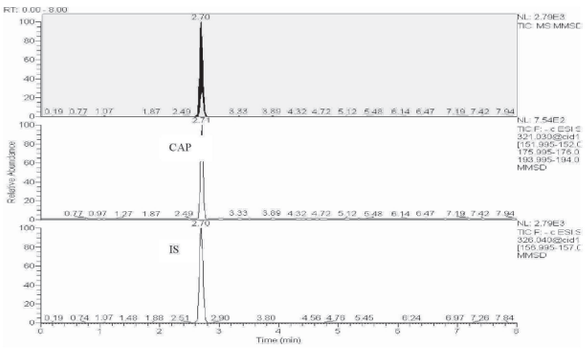

Figure 1: Mass chromatogram of blank black tiger shrimp extract spiked with CAP 0.5ppb and internal standard (IS).

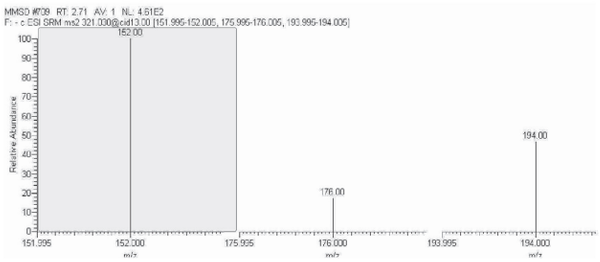

Figure 2: Mass spectrum of CAP product ion *m/z* 152, *m/z* 176 and *m/z* 194.

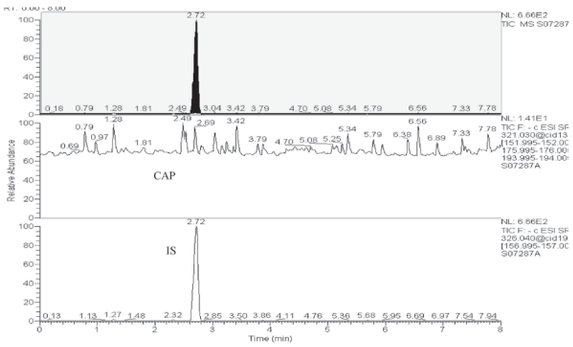

Figure 3: Mass chromatogram of test sample.

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

c. Corrective Action

Not applicable.

5. Problems and Challenges Encountered

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

6. Recommendations and Suggestions for Future Follow Up Action

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