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MALAYSIA

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1. Introduction

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

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

2. Objectives And Goals

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

3. Survey Methodologies

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

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

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

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

b. Method of Analysis

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

Solvents, Reagents and Standards:

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

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Glassware and Apparatus:

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

Preparation of Samples:

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

Sample Extraction:

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

Concentration of Samples:

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

Analyze sample using GC-MS:

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

GC-MS operating condition for pesticide analysis:

Injector :

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

Oven Temperature Program:

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

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

Detector (MS-QP2010 plus):

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

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Auto sampler (AOC-20i+s):

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

Calibration:

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

a. Limit of Detection and Limit of Quantification

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

b. National Regulatory Limits

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

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

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

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

b. Survey Results and Discussion

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

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

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

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

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

5. Problem and Challenges Encountered

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

6. Recommendations and Suggestions for Future Follow up Action

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

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