Singapore

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Preamble

This survey was conducted by the Marine Fisheries Research Department (MFRD) Chemistry Laboratory of the Fish Quality Management and Technology Branch as part of the Japanese Trust Fund II Program on the Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products.

1. Introduction

The Marine Fisheries Research Department (MFRD) Chemistry Laboratory of the Fish Quality Management and Technology Branch is the responsible laboratory for conducting the analysis of heavy metals in fish and fish products in this Japanese Trust Fund II Program on the Research and Analysis of Chemical Residues and Contamination in Fish and Fish Products.

In July 2002, MFRD received a report from Food and Agriculture Organization of the United Nations (FAO) on the traditional fish drying in coastal villages of Bangladesh: Practices, shortcomings and scope for improvements. It had been reported that organochlorine insecticides were widely used in order to control blowfly and beetle infestation during the sun-drying of fish. Typically, the insecticides were applied three times during the process, and reapplied during storage if further infestation was found. Thus MFRD decided to investigate the level of organochlorine pesticide residues in the different types of dried salted fish commonly found in Singapore under this JTF II program.

2. Objectives And Goals

The objective of this activity is to investigate on the level of 15 types of organo-chlorine pesticide residues contamination in dried fish commonly available in Singapore.

Table1. Pesticide analysed for this activity.

S/N	Compounds	Chemical Name
1	α-HCH	alpha-Hexachlorocyclohexane
2	β-HCH	beta-Hexachlorocyclohexane
3	γ-HCH	gamma-Hexachlorocyclohexane
4	δ-HCH	delta-Hexachlorocyclohexane
5	HCB	Hexachlorobenzene
6	Heptachlor	Heptachlor
7	o,p'-DDE	ortho-para- Dichlorodiphenyldichloroethylene
8	p,p'-DDE	para-para- Dichlorodiphenyldichloroethylene
9	o,p'-DDD	ortho-para-Dichlorodiphenyldichloroethane
10	p,p'-DDD	para-para-Dichlorodiphenyldichloroethane
11	o,p'-DDT	ortho-para-Dichlorodiphenyltrichloroethane
12	p,p'-DDT	para-para-Dichlorodiphenyltrichloroethane
13	Aldrin	Aldrin
14	Dieldrin	Dieldrin
15	Endrin	Endrin

3. Survey Methodologies

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

Seven species of dried and dried salted fish were used in this survey. The species selected for analysis were based on their popularity among local consumers. All dried samples were obtained from eight different shops in Pasir Panjang Wholesale Market. The dried fishes were randomly selected and purchased. At least six to ten pieces of fish for each species were collected and were of consumable or "market" size.

The dried fishes were transported at atmospheric temperature. Upon arrival at the laboratory, the samples were identified. The common and scientific names of the fish studied are shown in Table 2. The samples were stored at –10°C until sampled.

Table 2. The common, scientific and indigenous names of the seven fish species surveyed.

Common Names	Scientific Name	Indigenous Name	Denoted by
Spanish Mackerel	*Scomberomorus commerson* (Lacepede)	Tenggiri batang	SM
Snakeskin Gouramy	*Trichogaster pectoralis*	Ikan Sepat Sepat, Ikan Sepat Siam	IS
Mei Ren Yu	-	-	MR
Sand Whiting	*Sillago sihama*	-	SW
Yellow-banded Scad	*Selaroides leptolepis* (Valenciennes)	Selar Kuning	KG
Threadfin	*Polynemus indus* Shaw	Ikan Kurau	KR
Indian Mackerel	*Rastrelliger kanagurta* (Cuvier)	Kembong	IM

Note: A two-letter short form (last column) is assigned to each species.

b. Method of Analysis

The fish samples were sampled and analysed by the Fish Quality Management & Technology Branch. The total length, standard length and body weight of the samples were measured and recorded. Seven samples of each type of species (n = 7) were used for analysis. Only the edible portions were sampled. The sampling procedures were as follows;

i. Clean and scale fish.
ii. Fillet fish to obtain all flesh and skin.
iii. Mince sample rapidly and thoroughly with chopper.
iv. Remove unground material from blade of chopper and mix thoroughly with ground material and mince thoroughly again.
v. Make mince into a burger and divide into equal quarters.
vi. Remove the first quadrant of minced meat for testing.

If necessary, repeat i to vi until sufficient sample weight is obtained for the test.

The in-house method used is a multi-residues method (MRM) that allows the simultaneous detection and quantification of the 15 organochlorine pesticides. The sample extraction procedure and analytical instrumentation are as follows;

Sample Extraction

10 g of sample, to which 100 ml of acetonitrile and 2 spatulas of Celite 545 were added, was homogenized. It was then filtered using the Kiriyama filtration apparatus under vacuum. The filtrate was collected in a round-bottomed flask (RBF) and concentrated to about 10 ml on the rotary evaporator. The spiked sample was treated last.

The extract was then diluted with 150 ml of distilled water and transferred to a 300 ml separatory flask

(F1), which contained approximately 5 g of sodium chloride. 50 ml of n-hexane was used to rinse the RBF and was transferred to the separatory flask. The mixture was manually shaken for 1 minute until all the sodium chloride had dissolved. It was then left to stand for phase separation.

An additional 50 ml of n-hexane was used to rinse the round-bottomed flask, and transferred to a 200 ml separatory flask (F2). The bottom layer of F1 was eluted to F2, which was then shaken manually for 1 minute and left to stand for phase separation. The bottom aqueous layer of F2 was discarded, and the organic top layer was transferred and combined with that in F1. F2 was rinsed twice with 5 ml of n-hexane. The organic phase in F1 was then filtered through a filter funnel plugged with cotton wool at its base and 70% filled with anhydrous sodium sulphate. The filtrate was collected in a new 200 ml RBF. F1, the filter funnel and tip were rinsed with n-hexane.

After liquid-liquid extraction, the extract was evaporated to about 1 to 2 ml and dried with nitrogen gas. As the fatty tissue could not be dried with nitrogen gas, it would remain on the RBF walls as small oil droplets. The following would only be done if there were visible oil droplets (*). If not, 5 ml of n-hexane was added into the RBF.

(*) 20 ml of n-hexane was added to the extract, and transferred into a 125 ml separatory flask. The RBF was rinsed twice with 20 ml of acetonitrile saturated with n-hexane (20 ml twice), and its contents transferred into the separatory flask. The mixture was shaken for 1 minute and the phases were allowed to separate. The bottom acetonitrile layer was eluted into the same RBF. The partitioning and collection was repeated for another two times using 20 ml of acetonitrile saturated with n-hexane. The sample was then concentrated to about 1 to 2 ml and dried with nitrogen gas before it was dissolved in 5 ml of n-hexane and transferred into the chromatographic tube.

To prepare the chromatographic tube, the base of a 20 mm ID tube was plugged with cotton wool and wetted with n-hexane. A suspension of 12 g of florisil and n-hexane was then transferred into the tube. Anhydrous sodium sulphate was then added until a 1cm thick layer above the florisil bed was formed. n-Hexane was used to wash the tube, and the solvent was eluted out until it was 1cm above the ring of anhydrous sodium sulphate.

The extract was then transferred into the tube, and the RBF was rinsed twice with 2 ml of n-hexane. The tube wall was also rinsed with n-hexane. The sample was eluted out of the tube at a rate of 1 drop/second, and collected in a new 200 ml RBF. When 1cm of solution remained above the florisil bed, a separatory funnel containing 120 ml of diethyl ether: n-hexane mixture was attached to the tube. The elution was allowed to continue till completion.

The sample was then concentrated to about 1 to 2 ml on the evaporator and dried completely with nitrogen gas. 5 ml of n-hexane was pipetted into the RBF. The solution was transferred to a small bijou bottle, and nitrogen gas was blown into the airspace above the solution before it was capped, sealed with parafilm, and stored in a 5ºC refrigerator.

Analytical Instrumentation

A fused silica capillary SPB-5 (Supelco, 0.25 mmID, 30 m long, and 0.25 μm film thickness) was used. The Shimadzu Gas Chromatograph Mass Spectrometer (GC/MS) GC17A/QP5050A was operated under the following conditions: helium constant flow 1.0 ml/min, injection temperature 250ºC, detector temperature 230ºC, ionization voltage 70eV, EI ionization method, and injection volume 1μl (splitless). The oven temperature was programmed in the following manner: 80ºC (2min), then 10ºC/min ramp to 320ºC (held for 8min). The MS was operated in the selective ion monitoring (SIM) mode. The retention times and monitor ions were as shown in Table 3.

Table 3. Monitor and determination ions of the 15 organochlorine compounds.

Compounds	Molecular weight/g	m/z ratio of Determination ion	m/z ratio of Identification ion
α-HCH	290.8	219	217
β-HCH	290.8	219	217
γ-HCH	290.8	219	217
δ-HCH	290.8	219	217
HCB	284.8	284	286
Heptachlor	372.5	272	274
o,p'-DDE	318.0	318	316
p,p'-DDE	318.0	318	316
o,p'-DDD	320.0	235	237
p,p'-DDD	320.0	235	237
o,p'-DDT	354.5	235	237
p,p'-DDT	354.5	235	237
Aldrin	364.9	263	265
Dieldrin	380.9	263	265
Endrin	380.9	263	265

c. Limit of Detection and Limit of Quantification

The limit of quantification of all the 15 organochlorine pesticides are 0.005ppm.

d. National Regulatory Limits

Singapore Guidelines extracted from The Sale of Food Act, Chapter 283, Section 56, Food Regulations, 2005 Revised Edition.

Substance	Type of Food	Maximum Residues Limit (ppm)
Aldrin	Fish (edible portion)	0.2
DDT (including DDD and DDE)	Fat of Meat	5
Dieldrin	Fat of Meat	0.2
Endrin	Fat of Meat	0.1
Heptachlor (including its epoxide)	Fat of Meat	0.2
Lindane (γ-HCH)	Fish	1

*extracted only for organochlorines

Where it is not provided in these Regulations, the pesticide residues contained in any food shall not exceed the limits as recommended by the Codex Alimentarius Commission.

EU Guidelines extracted from Informal coordination of MRLs established in Directives 76/895/EEC, 86/362/EEC, 86/363/EEC, and 90/642/EEC, 5058/VI/98, 3 November 2004.

Crop Group	Substance	MRL	Directive			Remarks
Meat and edible offal	Aldrin (see Dieldrin)					
Meat and edible offal	DDT	1	93	57	EEC	
Meat and edible offal	Dieldrin	0.2	93	57	EEC	Singly or combined with Aldrin, expressed as Dieldrin
Meat and edible offal	Endrin	0.05	93	57	EEC	
Meat and edible offal	HCH	0.3	93	57	EEC	the MRL for EC is the sum of 0.2 for alpha & 0.1 for beta isomers
Meat and edible offal	Heptachlor	0.2	93	57	EEC	
Meat and edible offal	Lindane	0.02	02	66	EC	Based on monitoring data

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results (ug/kg)	True value (ug/kg)	z-score	Remarks
2007 (August-September)	FAPAS® Proficiency Test 0555 Pesticide Residues in Minced Chicken	(ß-HCH)	N.A.	57.3	N.A.	Not analysed.
		(γ-HCH)	36.39	59.7	-1.8	Passed. Within z±2
		p,p'-DDE	23.49	39.2	-1.8	
		p,p'-DDD	30.52	40.4	-1.1	
		o,p'-DDT	17.82	27.4	-1.6	
		p,p'-DDT	44.91	72.7	-1.7	

b. Survey Results and Discussion

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Spanish Mackerel (Tenggiri Batang) (Dried)	*Scomberomorus commerson* (Lacepede)	7	N.D (2)	<LOQ (5)	<LOQ	106.04	52.83
	β-HCH				N.D/ (4)	0.0204 (1)	<LOQ	115.44	
	γ-HCH				N.D (2)	<LOQ (5)	<LOQ	108.57	
	δ-HCH				N.D (6)	<LOQ (1)	<LOQ	105.97	
	HCB				N.D (2)	<LOQ (5)	<LOQ	71.06	
	Heptachlor				N.D. (1)	0.0104 (1)	<LOQ	87.61	
	o,p'-DDE				N.D (7)	N.D	N.D	127.73	
	p,p'-DDE				N.D (3)	0.0212 (1)	<LOQ	152.68	
	o,p'-DDD				N.D (6)	0.0396 (1)	<LOQ	127.41	
	p,p'-DDD				N.D (3)	0.1201 (1)	<LOQ	144.57	
	o,p'-DDT				N.D (4)	0.0923 (1)	<LOQ	137.67	
	p,p'-DDT				N.D (2)	0.2252 (1)	<LOQ	163.28	
	Aldrin				N.D (7)	N.D	N.D	89.53	
	Dieldrin				N.D (7)	N.D	N.D	79.13	
	Endrin				0.0057	0.0079	0.0066 (4)	111.63	

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Snakeskin Gouramy (Ikan Sepat) (Dried)	*Trichogaster pectoralis*	7	N.D (5)	0.0079 (1)	<LOQ	114.91	45.14
	β-HCH				N.D (7)	N.D	N.D	114.81	
	γ-HCH				N.D (6)	0.1829 (1)	<LOQ	108.82	
	δ-HCH				N.D (6)	0.2260 (1)	<LOQ	114.11	
	HCB				N.D (6)	<LOQ (1)	<LOQ	104.68	
	Heptachlor				N.D (3)	0.0700 (1)	<LOQ	112.48	
	o,p'-DDE				N.D (7)	N.D	N.D	115.34	
	p,p'-DDE				N.D (7)	N.D	N.D	120.65	
	o,p'-DDD				N.D (7)	N.D	N.D	123.33	
	p,p'-DDD				N.D (7)	N.D	N.D	127.83	
	o,p'-DDT				N.D (5)	<LOQ (2)	<LOQ	112.39	
	p,p'-DDT				N.D (5)	0.0087 (1)	<LOQ	120.49	
	Aldrin				N.D (7)	N.D	N.D	109.17	
	Dieldrin				0.0086	0.0126	0.0100 (3)	99.79	
	Endrin				N.D (2)	<LOQ (5)	<LOQ	117.76	
2005, Singapore	α-HCH	Mei Ren Yu (Dried)		7	N.D (7)	N.D	N.D	118.22	20.99
	β-HCH				0.0066	0.0259	0.0162 (2)	74.94	
	γ-HCH				N.D (5)	<LOQ (2)	<LOQ	105.95	
	δ-HCH				N.D (7)	N.D	N.D	109.43	
	HCB				N.D (2)	<LOQ (5)	<LOQ	75.52	
	Heptachlor				N.D (7)	N.D	N.D	94.43	
	o,p'-DDE				N.D (3)	<LOQ (4)	<LOQ	122.35	
	p,p'-DDE				N.D (7)	N.D	N.D	127.31	
	o,p'-DDD				N.D (7)	N.D	N.D	140.33	
	p,p'-DDD				N.D (7)	N.D	N.D	151.08	
	o,p'-DDT				N.D (7)	N.D	N.D	136.39	
	p,p'-DDT				N.D (7)	N.D	N.D	147.92	
	Aldrin				N.D (2)	<LOQ (5)	<LOQ	90.77	
	Dieldrin				N.D (2)	0.0059 (1)	<LOQ	90.08	
	Endrin				N.D (7)	N.D	N.D	105.90	

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Sand Whiting (Dried)	*Sillago sihama*	7	N.D (7)	N.D	N.D	70.09	25.44
	β-HCH				N.D (6)	0.0096 (1)	<LOQ	53.94	
	γ-HCH				N.D (5)	0.0110 (1)	<LOQ	61.16	
	δ-HCH				N.D (7)	N.D	N.D	65.79	
	HCB				N.D (7)	N.D	N.D	43.63	
	Heptachlor				N.D (7)	N.D	N.D	41.62	
	o,p'-DDE				N.D (7)	N.D	N.D	78.69	
	p,p'-DDE				N.D (7)	N.D	N.D	85.64	
	o,p'-DDD				N.D (7)	N.D	N.D	83.97	
	p,p'-DDD				N.D (7)	N.D	N.D	89.85	
	o,p'-DDT				N.D (7)	N.D	N.D	72.36	
	p,p'-DDT				N.D (7)	N.D	N.D	78.66	
	Aldrin				N.D (7)	N.D	N.D	45.61	
	Dieldrin				N.D (7)	N.D	N.D	51.76	
	Endrin				N.D (6)	<LOQ (1)	<LOQ	61.94	
2005, Singapore	α-HCH	Yellow-banded Scad (Selar Kuning) (Dried)	*Selaroides leptolepis* (Valenciennes)	7	N.D (7)	N.D	N.D	65.80	47.78
	β-HCH				N.D (5)	0.0241 (1)	<LOQ	64.05	
	γ-HCH				N.D (2)	0.0121 (1)	<LOQ	66.71	
	δ-HCH				N.D (6)	<LOQ (1)	<LOQ	71.06	
	HCB				N.D (7)	N.D	N.D	42.48	
	Heptachlor				N.D (3)	<LOQ (4)	<LOQ	53.44	
	o,p'-DDE				N.D (7)	N.D	N.D	61.80	
	p,p'-DDE				N.D (7)	N.D	N.D	71.18	
	o,p'-DDD				N.D (7)	N.D	N.D	71.11	
	p,p'-DDD				N.D (7)	N.D	N.D	74.04	
	o,p'-DDT				N.D (7)	N.D	N.D	61.69	
	p,p'-DDT				N.D (5)	<LOQ (2)	<LOQ	61.28	
	Aldrin				N.D (7)	N.D	N.D	51.45	
	Dieldrin				N.D (7)	N.D	N.D	70.85	
	Endrin				N.D (7)	N.D	N.D	59.27	

Year of analysis & Sampling location	Analyte	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Average Moisture Content (%)
		Common name	Scientific name						
2005, Singapore	α-HCH	Threadfin (Ikan Kurau) (Dried)	*Polynemus indus* Shaw	7	N.D (7)	N.D	N.D	77.52	43.07
	β-HCH				0.0086	0.0122	0.0107 (7)	56.70	
	γ-HCH				N.D (7)	N.D	N.D	72.14	
	δ-HCH				N.D (7)	N.D	N.D	84.05	
	HCB				N.D (7)	N.D	N.D	46.18	
	Heptachlor				N.D (7)	N.D	N.D	62.06	
	o,p'-DDE				N.D (7)	N.D	N.D	64.25	
	p,p'-DDE				N.D (7)	N.D	N.D	70.53	
	o,p'-DDD				N.D (7)	N.D	N.D	68.06	
	p,p'-DDD				N.D (7)	N.D	N.D	69.49	
	o,p'-DDT				N.D (6)	0.0196 (1)	<LOQ	48.81	
	p,p'-DDT				N.D (7)	N.D	N.D	63.50	
	Aldrin				N.D (7)	N.D	N.D	60.00	
	Dieldrin				N.D (6)	0.0298 (1)	<LOQ	65.27	
	Endrin				Not tested				
2003, Singapore	α-HCH	Indian Mackerel (Kembong) (Dried)	*Rastrelliger kanagurta*	7	N.D (7)	N.D	N.D	44.85	49.42
	β-HCH				N.D (7)	N.D	N.D	49.12	
	γ-HCH				0.0241	0.0546	0.0413 (4)	47.61	
	δ-HCH				N.D (7)	N.D	N.D	51.46	
	HCB				N.D (7)	N.D	N.D	31.37	
	Heptachlor				N.D (6)	<LOQ (1)	<LOQ	39.25	
	o,p'-DDE				N.D (7)	N.D	N.D	54.36	
	p,p'-DDE				N.D (7)	N.D	N.D	56.28	
	o,p'-DDD				N.D (3)	<LOQ (4)	<LOQ	56.29	
	p,p'-DDD				N.D (7)	N.D	N.D	59.85	
	o,p'-DDT				N.D (7)	N.D	N.D	62.18	
	p,p'-DDT				N.D (7)	N.D	N.D	69.62	
	Aldrin				N.D (7)	N.D	N.D	41.54	
	Dieldrin				N.D (6)	<LOQ	N.D	42.35	
	Endrin				0.0102	0.0522	0.0209 (6)	90.42	

The above results were reported in wet weight basis. All results obtained were within Singapore regulatory's limit of 0.1ppm.

c. Corrective Actions

No regulatory action was taken as the results were within Singapore's regulatory limits.

5. Problems and Challenges Encountered

In this preliminary survey, only seven types of species were analysed and a total of 49 samples were collected. Due to the small number of samples collected, it may not be truly representative of the fishes available in Singapore.

6. Recommendations and Suggestions for Future Follow up Action

It would be good to replace the extraction method with a more rapid method.