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Appendices

Appendix I

Chemical reagents

I. Solvents and Chemicals for Organochlorine Residue Analysis

1. Acetone (CH_3COCH_3), (pesticide grade, PR and analytical grade, AR)
2. Dichloromethane (DCM) (CH_2Cl_2), (PR)
3. Diethyl ether ($(C_2H_5)_2O$), (PR)
4. Hexane ($CH_3(CH_2)_4CH_3$), (PR)
5. Petroleum ether, (PR)
6. Ottawa sand from Applied Separation
7. Granular anhydrous sodium sulfate (Na_2SO_4), (PR)
8. Copper powder, (AR)
9. Hydrochloric acid (HCl), (AR)
10. Distilled water

II. Solution for protein extraction and analysis

1. Extraction buffers

Vitellogenin extraction buffer

(0.05 M Tris-HCl, 0.2 M NaCl and Protease Inhibitor Cocktail, pH 7.5)

- Trizma 7.88 g
- NaCl 11.688 g
- dH_2O to make 1,000 mL (final volume)
- Protease Inhibitor Cocktail

GST extraction buffer

(0.05 M Tris-HCl, 0.15 M KCl, pH 7.4)

- Trizma 7.88 g
- KCl 11.184 g
- dH_2O to make 1,000 mL (final volume)

2. Buffer solution for total protein assay

(Phophate buffered saline (PBS), pH 7.2)

- NaCl 8.0 g
- KCl 0.2 g
- Na_2HPO_4 1.44 g
- KH_2PO_4 0.24 g
- dH_2O to make 1,000 ml (final volume)

3. Buffer solutions for vitellogenin ELISA

Buffer solution for standard and sample

(50 mM carbonate-bicarbonate buffer, pH 9.6)

- Na_2CO_3 1.59 g
- $NaHCO_3$ 2.93 g
- dH2O to make 1,000 ml (final volume)

Phosphate buffered saline (PBS), pH 7.2

- NaCl	8.0 g
- KCl	0.2 g
- Na_2HPO_4	1.44 g
- KH_2PO_4	0.24 g
- dH2O to make 1,000 ml (final volume)	

Diethanolamine buffer, pH 9.8

- Diethanolamine	48.5 mL
- $MgCl_2$-$6H_2O$	50.0 mg
- NaN_3	10 mg
- dH2O to make 500 ml (final volume)	0.24 g

0.02 % Tween-20 in PBS (PBS-Tween 20)

- Tween-20	200 µl
- PBS	1,000 mL

2 % Gelatin solution in PBS

- Gelatin (ELISA grade)	1 g
- PBS	50 mL

p-nitrophenolphosphate solution (freshly made before each use)

- p-NPP	3 tablets
- Diethanolamine buffer	15 mL

4. Buffer solution for GST activity

(1 mM 1,2 chlorodinitrobenzene (CNDB) in 100 mM Tris-HCl, pH 7.4)

- Trizma	15.76 g
- 1,2 chlorodinitrobenzene	0.1013 g
- dH_2O to make final volume	1,000 mL

Appendix II

Quantitative control data of organochlorine pesticides

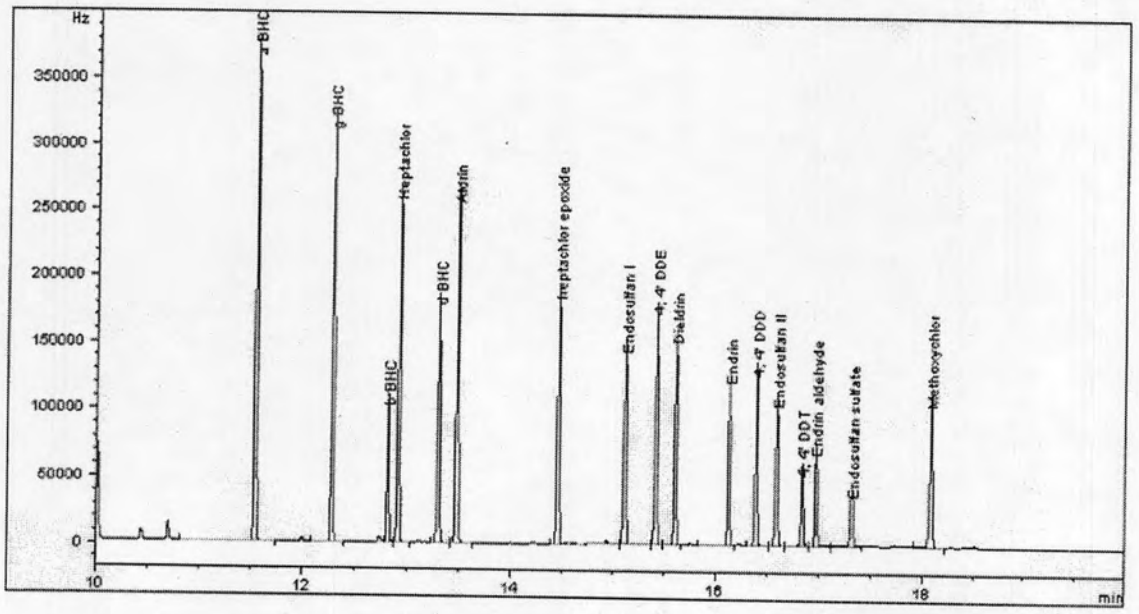

Figure A.1 Standadrd curve of 17 mixed organochlorine pesticides, 100 ng/L in hexane included α-, β-, δ- and γ-HCH, aldrin, heptachlor, heptachlor epoxide, endosulfan I, endosulfan II, endosulfan sulfate, 4,4'-DDD, 4,4'-DDE, 4,4'DDT, endrin, endrin aldehyde, dieldrin, and methoxychlor were obtained from Supelco (Beollefonte, PA, USA).

Table A.1 Limit of detection (LOD), limit of quanitation (LOQ), method detection limit (MDL), spike recovery and relative standard deviation (% RSD) of organochlorine pesticides standard solution in sediment and freshwater mussels

OCPs	LOD	LOQ	MDL		Matrices spike recovery (%)		(% RSD)	
	µg/kg	µg/kg	Sediment	Freshwater mussels	Sediment	Freshwater mussels	Sediment	Freshwater mussels
a BHC	0.03	0.09	3.38	0.56	74.87	72.62	4.93	0.72
g BHC	0.05	0.20	2.26	5.93	77.62	68.83	3.17	8.08
b BHC	0.01	0.05	2.72	4.07	76.85	84.17	3.32	4.33
d BHC	0.01	0.02	1.64	1.68	77.34	85.26	2.31	1.85
Heptachlor &	0.02	0.10	2.11	5.60	84.08	70.82	2.73	5.97
Heptachlor epoxide	0.02	0.05	5.38	14.27	83.02	70.76	7.07	0.53
Aldrin &	0.02	0.07	6.49	4.10	79.99	86.84	9.47	4.20
Dieldrin	0.03	0.20	7.49	1.15	89.30	104.85	9.70	1.03
Endrin &	0.00	0.07	11.44	3.81	85.48	111.55	12.88	3.20
Endril aldehyde	0.00	0.07	9.920	2.17	90.61	70.24	14.60	2.89
Endosulfate I	0.02	0.01	10.38	9.72	90.03	86.40	12.58	10.55
Endosulfate II	0.00	0.09	10.19	2.65	88.87	116.60	12.51	2.13
Endosunfan sulfate	0.01	0.08	14.87	12.93	106.86	94.85		
4,4' DDD	0.02	0.10	9.10	9.72	85.82	104.54	11.58	7.84
4,4' DDE	0.03	0.07	3.98	1.61	86.15	106.24	5.42	1.42
4,4' DDT	0.05	0.01	4.46	6.39	92.51	78.58	5.26	8.38
Methoxychlor	0.02	0.60	10.61	7.94	93.55	72.19	12.20	10.45

Appendix III

Pearson's correlation coefficients tables

Table A.2 Pearson's correlation coefficients relating organochlorine pesticide residue in sediment, OCP residue in *Uniandra contradens ascia*, levels of vitellogenin in gonad, and specific activity of glutathione s-transferase in hepatopancreas of mussel. Shaded cells indicate significant correlation ($p < 0.05$).

	Hept-S	AlDi-S	Endr-S	DDT-S	Endo-S	Met-S	HCH-U	Hept-U	AlDi-U	Endr-U	DDT-U	Endo-U	Met-U	Vtg-U	GST-U
HCH-s	0.997 (0.00)	0.681 (0.32)	0.946 (0.05)	0.937 (0.06)	0.888 (0.11)	0.938 (0.06)	0.79 (0.21)	0.77 (0.23)	0.07 (0.94)	0.53 (0.47)	0.99 (0.01)	1.00 (0.00)	0.57 (0.43)	0.75 (0.25)	0.85 (0.15)
Hept-s		0.733 (0.27)	0.955 (0.05)	0.914 (0.09)	0.853 (0.15)	0.956 (0.04)	0.83 (0.17)	0.82 (0.19)	0.11 (0.89)	0.59 (0.41)	0.98 (0.02)	1.00 (0.00)	0.51 (0.49)	0.71 (0.30)	0.87 (0.13)
AlDi-s			0.765 (0.24)	0.423 (0.58)	0.272 (0.73)	0.843 (0.16)	0.98 (0.02)	0.95 (0.05)	0.53 (0.47)	0.96 (0.04)	0.59 (0.41)	0.73 (0.27)	-0.19 (0.81)	0.09 (0.91)	0.81 (0.19)
EnDr-s				0.786 (0.21)	0.784 (0.22)	0.875 (0.13)	0.80 (0.20)	0.76 (0.24)	-0.04 (0.96)	0.57 (0.43)	0.92 (0.08)	0.95 (0.05)	0.35 (0.65)	0.52 (0.48)	0.73 (0.27)
DDT-s					0.953 (0.05)	0.836 (0.16)	0.59 (0.41)	0.60 (0.40)	0.01 (0.99)	0.31 (0.69)	0.97 (0.03)	0.92 (0.08)	0.81 (0.19)	0.93 (0.07)	0.78 (0.22)
Endo-s						0.692 (0.31)	0.42 (0.58)	0.41 (0.59)	-0.29 (0.71)	0.09 (0.91)	0.94 (0.06)	0.85 (0.15)	0.86 (0.15)	0.90 (0.10)	0.58 (0.42)
Met-s							0.93 (0.07)	0.94 (0.06)	0.40 (0.60)	0.78 (0.23)	0.90 (0.10)	0.96 (0.04)	0.36 (0.64)	0.61 (0.39)	0.97 (0.03)
HCH-U								0.99 (0.01)	0.45 (0.55)	0.06 (0.94)	0.29 (0.71)	0.17 (0.83)	1.00 (0.00)	0.71 (0.29)	0.09 (0.91)
Hept-U									0.62 (0.38)	0.94 (0.06)	0.70 (0.30)	0.82 (0.18)	0.03 (0.97)	0.33 (0.67)	0.95 (0.05)
AlDi-U										0.75 (0.25)	-0.01 (0.99)	0.12 (0.88)	-0.33 (0.67)	-0.07 (0.93)	0.58 (0.42)
Endr-U											0.43 (0.57)	0.59 (0.41)	-0.29 (0.71)	0.02 (0.98)	0.81 (0.19)
DDT-U												0.98 (0.02)	0.66 (0.34)	0.81 (0.19)	0.81 (0.19)
Endo-U													0.51 (0.49)	0.71 (0.29)	0.87 (0.13)
Met-U														0.95 (0.05)	0.32 (0.68)
Vtg-U															0.60 (0.40)

Note: 1)HCH-S = ΣHCH in sediment; Hept-S = Σheptachlor in sediment; AlDi-S = aldrin & dieldrin in sediment; Endr-S = Σendrin in sediment; DDT-S = ΣDDT in sediment; Endo-S = Σendosulfans in sediment; Met-S = methoxychlor in sediment; HCH-U = ΣHCH in mussel; Hept-U = Σheptachlor in mussel; AlDi-U = aldrin & dieldrin in mussel; Endr-U = Σendrin in mussel; DDT-U = ΣDDT in mussel; Endo-U = Σendosulfans in mussel; Met-U = methoxychlor in mussel; Vtg-U = vitellogenin in mussel gonad; GST-U = GST activity in mussel hepatopancreas
2) indicate significant correlation ($p < 0.05$).

Table A.3 Pearson's correlation coefficients relating organochlorine pesticide residue in sediment, OCP residue in *Pilsbryoconcha exilis exilis*, levels of vitellogenin in gonad, and specific activity of glutathione s-transferase in hepatopancreas of mussel. Shaded cells indicate significant correlation ($p < 0.05$).

	Hept-S	AlDi-S	Endr-S	DDT-S	Endo-S	Met-S	HCH-P	Hept-P	AlDi-P	Endr-P	DDT-P	Endo-P	Met-P	Vtg-P	GST-P
HCH-s	0.997 (0.00)	0.681 (0.32)	0.946 (0.05)	0.937 (0.06)	0.888 (0.11)	0.938 (0.06)	0.86 (0.35)	0.92 (0.26)	0.98 (0.12)	0.95 0.21	0.99 (0.11)	0.92 (0.26)	0.97 (0.15)	0.88 (0.31)	1.00 (0.06)
Hept-s		0.733 (0.27)	0.955 (0.05)	0.914 (0.09)	0.853 (0.15)	0.956 (0.04)	0.89 (0.30)	0.95 (0.21)	0.99 (0.07)	0.97 (0.16)	1.00 (0.06)	0.95 (0.21)	0.95 (0.20)	0.84 (0.36)	1.00 (0.01)
Aldi-s			0.765 (0.24)	0.423 (0.58)	0.272 (0.73)	0.843 (0.16)	0.90 (0.29)	0.83 (0.37)	0.69 (0.51)	0.79 (0.42)	0.68 (0.53)	0.83 (0.38)	0.34 (0.78)	0.09 (0.95)	0.62 (0.58)
EnDr-s				0.786 (0.21)	0.784 (0.22)	0.875 (0.13)	0.87 (0.33)	0.93 (0.25)	0.99 (0.11)	0.95 (0.20)	0.99 (0.10)	0.93 (0.25)	0.97 (0.16)	0.87 (0.33)	1.00 (0.04)
DDT-s					0.953 (0.05)	0.836 (0.16)	0.75 (0.46)	0.84 (0.37)	0.94 (0.23)	0.87 (0.32)	0.94 (0.22)	0.84 (0.37)	1.00 (0.04)	0.95 (0.20)	0.97 (0.17)
Endo-s						0.692 (0.31)	0.53 (0.65)	0.64 (0.56)	0.79 (0.42)	0.69 (0.51)	0.80 (0.41)	0.64 (0.56)	0.97 0.15 0	1.00 (0.01)	0.85 (0.36)
Met-s							0.99 (0.10)	1.00 (0.01)	0.98 (0.13)	1.00 (0.04)	0.98 (0.14)	1.00 (0.01)	0.81 (0.40)	0.64 (0.56)	0.96 (0.19)
HCH-P								0.99 (0.09)	0.23 (0.94)	0.13 (0.98)	0.24 (0.93)	0.09 (0.99)	0.49 (0.71)	0.66 (0.51)	0.29 (0.90)
Hept-P									0.98 (0.14)	1.00 (0.05)	0.97 (0.15)	1.00 (0.00)	0.80 (0.41)	0.62 (0.57)	0.95 (0.20)
AlDi-P										0.99 (0.09)	1.00 (0.01)	0.98 (0.14)	0.91 (0.27)	0.78 (0.43)	1.00 (0.06)
Endr-P											0.99 (0.10)	1.00 (0.05)	0.84 (0.36)	0.68 (0.52)	0.97 (0.15)
DDT-P												0.97 (0.15)	0.92 (0.26)	0.79 (0.42)	1.00 (0.05)
Endo-P													0.80 (0.41)	0.62 (0.57)	0.95 (0.20)
Met-P														0.97 (0.16)	0.95 (0.21)
Vtg-P															0.84 (0.37)

Note: 1)HCH-S = ΣHCH in sediment; Hept-S = Σheptachlor in sediment; AlDi-S = aldrin & dieldrin in sediment; Endr-S = Σendrin in sediment; DDT-S = ΣDDT in sediment; Endo-S = Σendosulfans in sediment; Met-S = methoxychlor in sediment; HCH-P = ΣHCH in mussel; Hept-P = Σheptachlor in mussel; AlDi-P = aldrin & dieldrin in mussel; Endr-P = Σendrin in mussel; DDT-P = ΣDDT in mussel; Endo-P = Σendosulfans in mussel; Met-P = methoxychlor in mussel; Vtg-P = vitellogenin in mussel gonad; GST-P = GST activity in mussel hepatopancreas 2) indicate significant correlation ($p < 0.05$).

Table A.4 Pearson's correlation coefficients relating organochlorine pesticide residue in sediment, OCP residue in *Hyriopsis (Limnoscapha) desowitzi*, levels of vitellogenin in gonad, and specific activity of glutathione s-transferase in hepatopancreas of mussel. Shaded cells indicate significant correlation ($p < 0.05$).

	Hept-S	AlDi-S	Endr-S	DDT-S	Endo-S	Met-S	HCH-H	Hept-H	AlDi-H	Endr-H	DDT-H	Endo-H	Met-H	Vtg-H	GST-H
HCH-s	0.997 (0.00)	0.681 (0.32)	0.946 (0.05)	0.937 (0.06)	0.888 (0.11)	0.938 (0.06)	0.97 (0.03)	0.77 (0.23)	0.97 (0.03)	0.99 (0.01)	0.97 (0.03)	0.90 (0.10)	0.86 (0.14)	0.85 (0.15)	0.75 (0.25)
Hept-s		0.733 (0.27)	0.955 (0.05)	0.914 (0.09)	0.853 (0.15)	0.956 (0.04)	0.96 (0.04)	0.74 (0.27)	0.98 (0.03)	0.99 (0.01)	0.96 (0.05)	0.88 (0.12)	0.83 (0.17)	0.85 (0.15)	0.71 (0.29)
Aldi-s			0.765 (0.24)	0.423 (0.58)	0.272 (0.73)	0.843 (0.16)	0.61 (0.39)	0.18 (0.82)	0.77 (0.23)	0.72 (0.28)	0.56 (0.45)	0.43 (0.57)	0.24 (0.76)	0.61 (0.39)	0.05 (0.95)
EnDr-s				0.786 (0.21)	0.784 (0.22)	0.875 (0.13)	0.84 (0.16)	0.53 (0.47)	0.88 (0.12)	0.90 (0.10)	0.83 (0.17)	0.70 (0.30)	0.69 (0.31)	0.67 (0.34)	0.56 (0.44)
DDT-s					0.953 (0.05)	0.836 (0.16)	0.97 (0.03)	0.94 (0.06)	0.90 (0.10)	0.93 (0.07)	0.99 (0.01)	0.98 (0.02)	0.98 (0.02)	0.88 (0.12)	0.92 (0.08)
Endo-s						0.692 (0.31)	0.88 ()0.12	0.87 (0.13)	0.77 (0.23)	0.84 (0.16)	0.91 (0.09)	0.88 (0.12)	0.97 (0.03)	0.70 (0.30)	0.95 (0.05)
Met-s							0.94 (0.06)	0.68 (0.32)	0.99 (0.01)	0.97 (0.03)	0.91 (0.09)	0.85 (0.15)	0.72 (0.28)	0.92 (0.08)	0.56 (0.44)
HCH-H								0.12 ()0.88	0.97 (0.03)	0.99 (0.01)	1.00 (0.00)	0.97 (0.03)	0.91 (0.09)	0.95 (0.05)	0.82 (0.18)
Hept-H									0.76 (0.24)	0.79 (0.21)	0.90 (0.10)	0.96 ()0.04	0.97 (0.03)	0.86 (0.14)	0.95 (0.05)
AlDi-H										0.99 (0.01)	0.96 (0.05)	0.90 (0.10)	0.80 (0.20)	0.94 (0.06)	0.66 (0.34)
Endr-H											0.98 (0.02)	0.92 (0.08)	0.85 (0.15)	0.92 (0.08)	0.73 (0.27)
DDT-H												0.98 (0.02)	0.94 (0.06)	0.93 (0.07)	0.85 (0.15)
Endo-H													0.96 (0.04)	0.95 (0.05)	0.89 (0.11)
Vtg-H														0.81 ()0.19	0.98 (0.02)
GST-H															0.70 (0.30)

Note: 1)HCH-S = ΣHCH in sediment; Hept-S = Σheptachlor in sediment; AlDi-S = aldrin & dieldrin in sediment; Endr-S = Σendrin in sediment; DDT-S = ΣDDT in sediment; Endo-S = Σendosulfans in sediment; Met-S = methoxychlor in sediment; HCH-H = ΣHCH in mussel; Hept-H = Σheptachlor in mussel; AlDi-H = aldrin & dieldrin in mussel; Endr-H = Σendrin in mussel; DDT-H = ΣDDT in mussel; Endo-H = Σendosulfans in mussel; Met-H = methoxychlor in mussel; Vtg-H = vitellogenin in mussel gonad; GST-H = GST activity in mussel hepatopancreas 2) indicate significant correlation ($p < 0.05$).

BIOGRAPHY

Miss Chayathorn Boonlue was born on the 11th of September, 1976, in Udonthani Province. She graduated her Bachelor of Science degree in Biotechnology in 1998 from the Department of Biotechnology, Faculty of Technology, Khon Kaen University. After her graduation, she worked as Project coordinator of *Gurney's pitta* conservation project in the Bird Conservation society of Thailand and the Royal Society for the Protection of Bird of UK (RSPB). She continued her study for a master's degree program in environmental science (Interdisciplinary program) at the Graduate School, Chulalongkorn University in 2004 and completed the program in 2008.

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