

APPENDIX A

Calibration data of POP standard mixture

Calculate:	Internal standard
Based on:	Peak area
Rel.reference window:	5.00%
Abs.reference window:	0.000 min
Rel.non-ref. Window:	5.00%
Abs.non-ref. Window:	0.000 min
Uncalibration peaks:	not reported
Partial calibration:	Yes, identified peaks are recalibrated
Correct all ret. Times:	No, only for identified peak
Curve type:	Linear
Origin	Included
Weight	Equal
Recalibration settings:	
Average response:	Average all calibrations
Average retention time:	Floating average new 75%

APPENDIX B

Methods for preparation of standard solution of POPs for GC-ECD

Steps of POP standard preparation were as follows:

- (1) Step 1: Critical weight of individual POPs in isooctane (Table B.1)
- (2) Step 2: Preparation of intermediate standard mixture (IM) (Table B.2)
- (3) Step 3: Preparation of intermediate mixture at individual level in 10 ml ethyl acetate (IM₁ – IM₁₀) (Table B.3)
- (4) Step 4: Preparation of working standard mixture in 10 ml isooctane (M₁ - M₁₀) (Table B.4)

Table B.1 Critical weight of individual POPs in isooctane

No	POPs	Critical weight, g in 10 ml isooctane	Stock standard concentration, mg/ml
1	β -HCH	0.0057	0.57
2	HCB	0.0058	0.58
3	γ -HCH	0.0069	0.69
4	Heptachlor	0.0059	0.59
5	Heptachlor epoxide	0.0067	0.67
6	Aldrin (IS)	0.0050	0.50
7	<i>o,p'</i> -DDE	0.0055	0.55
8	<i>p,p'</i> -DDE	0.0051	0.51
9	Dieldrin	0.0053	0.53
10	<i>p,p'</i> -DDD	0.0052	0.52
11	<i>o,p'</i> -DDT	0.0050	0.50
12	<i>p,p'</i> -DDT	0.0053	0.53

Note: IS = internal standard

Table B.2 Preparation of intermediate standard mixture (IM)

No	POPs	Stock standard concentration, mg/ml	Intermediate concentration, $\mu\text{g/ml}$	Preparation in 10 ml EA Pipet stock standard in μl
1	β -HCH	0.57	2.5	43.9
2	HCB	0.58	2.5	43.1
3	γ -HCH	0.69	2.5	36.2
4	Heptachlor	0.59	5	84.7
5	Heptachlor epoxide	0.67	5	74.6
6	Aldrin (IS)	0.50		
7	<i>o,p'</i> -DDE	0.55	7.5	181.8
8	<i>p,p'</i> -DDE	0.51	7.5	147.1
9	Dieldrin	0.53	7.5	141.5
10	<i>p,p'</i> -DDD	0.52	7.5	144.2
11	<i>o,p'</i> -DDT	0.50	7.5	150
12	<i>p,p'</i> -DDT	0.53	12.5	235.8

Table B.3 Preparation of intermediate mixture at individual level in 10 ml ethyl acetate (IM₁ – IM₁₀)

POPs	IM µg/ml	IM ₁		IM ₂		IM ₃		IM ₄		IM ₅		IM ₇		IM ₁₀	
		ml	µg/ml	ml	µg/ml	ml	µg/ml	ml	µg/ml	ml	µg/ml	ml	µg/ml	ml	µg/ml
1	2.5	0.4	0.1	0.8	0.2	1.2	0.3	1.6	0.4	2.0	0.5	2.8	0.7	4	1.0
2	2.5		0.1		0.2		0.3		0.4		0.5		0.7		1.0
3	2.5		0.1		0.2		0.3		0.4		0.5		0.7		1.0
4	5		0.2		0.4		0.6		0.8		1.0		1.4		2.0
5	5		0.2		0.4		0.6		0.8		1.0		1.4		2.0
6															
7	10		0.4		0.8		1.2		1.6		2.0		2.8		4.0
8	7.5		0.3		0.6		0.9		1.2		1.5		2.1		3.0
9	7.5		0.3		0.6		0.9		1.2		1.5		2.1		3.0
10	7.5		0.3		0.6		0.9		1.2		1.5		2.1		3.0
11	7.5		0.3		0.6		0.9		1.2		1.5		2.1		3.0
12	12.5		0.5		1.0		1.5		2.0		2.5		3.5		5.0

APPENDIX C

Preparation of chemicals for estrogenic activity analysis

Table C.1 Preparation of chemicals for estrogenic activity analysis

PBS buffer, pH 7.6	1.38	g	NaH ₂ PO ₄ .H ₂ O
	12.46	g	Na ₂ HPO ₄ .2H ₂ O
	8.50	g	NaCl
	1,000	ml	Distilled water
10X PBS Washing buffer, pH 7.6	11.38	g	NaH ₂ PO ₄ .H ₂ O
	12.46	g	Na ₂ HPO ₄ .2H ₂ O
	8.50	g	NaCl
	5	ml	Tween 20
	1,000	ml	Distilled water
PBSE buffer, pH 7.6	11.38	g	NaH ₂ PO ₄ .H ₂ O
	12.46	g	Na ₂ HPO ₄ .2H ₂ O
	8.50	g	NaCl
	1.86	g	Na ₂ EDTA
	1,000	ml	Distilled water
Coating buffer, pH 9.6	1.59	g	Na ₂ CO ₃
	2.93	g	NaHCO ₃
	1,000	ml	Distilled water
Substrate buffer, pH 3.8	46.04	g	KH ₂ -citrate
	0.1	g	Sorbic acid
	1,000	ml	Distilled water
TMB substrate	0.375	g	3,3',5,5'-tetramethylbenzidine
	5	ml	Dimethylsulfoxid (DMSO)
	20	ml	Methanol
2 Normal sulfuric acid	53.3	ml	H ₂ SO ₄
	946.7	ml	Distilled water
EDTA 0.5 M, pH 8.0	37.22	g	Na ₂ EDTA
	200	ml	Distilled water

APPENDIX D

Acceptable criteria of precision and recovery

(1) Acceptable criteria of precision (AOAC Prev-Verified Method, Nov. 1993)

Table D.1 Acceptable criteria of precision

Concentrations of analytes	%CV
100 ppm	155.3
10 ppm	7.3
1ppm	11
100 ppb	15
10 ppb	21
1 ppb	30

(2) Acceptable criteria of recovery (AOAC Prev-Verified Method, Nov. 1993)

Table D.2 Acceptable criteria of recovery

Concentrations of analytes	% recovery
100 ppm	90 - 107
10 ppm	80 - 110
1ppm	80 - 110
100 ppb	80 - 110
10 ppb	60 - 115
1 ppb	40 - 120

APPENDIX E

Statistical analysis

1. Multiple linear regression analysis of research section 2:

Effect of POPs on reproductive hormone levels in adult men

(1.1) Multiple linear regression analysis for the association of POPs with years of residence and DDT use for farming (Table E1 – E7)

Table E.1 The association of *p,p'*-DDE with years of residence and DDT use for farming

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
			Beta			Lower Bound	Upper Bound
	β	SE					
1 (Constant)	7.275	0.671		10.839	0.000	5.926	8.625
<i>ln</i> years of residence	0.299	0.205	0.206	1.461	0.150	-0.112	0.711
<i>ln</i> years of DDT use for farming	0.37	0.082	0.063	.448	0.657	-0.129	0.203

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln</i> years of residence	.208	.206	.206
<i>ln</i> years of DDT use for farming	.071	.064	.063

Table E.2 The association of p,p' -DDT with years of residence and DDT use for farming

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β		
	β	SE	Beta			Lower Bound	Upper Bound	
	1 (Constant)	4.665	0.683				6.830	0.000
	<i>ln</i> years of residence	0.471	0.208	0.298	2.267	0.028	0.053	0.891
	<i>ln</i> years of DDT use for farming	0.177	0.084	0.278	2.112	0.040	0.008	0.346

Model	Correlations			
	Zero-order	Partial	Part	
	1 (Constant)			
	<i>ln</i> years of residence	0.308	0.311	0.298
	<i>ln</i> years of DDT use for farming	0.289	0.292	0.277

Table E.3 The association of p,p' -DDD with years of residence and DDT use for farming

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β		
	β	SE	Beta			Lower Bound	Upper Bound	
	1 (Constant)	8.912	2.471				3.606	0.069
	<i>ln</i> years of residence	-1.029	0.755	-0.635	-1.363	0.306	-4.278	2.220
	<i>ln</i> years of DDT use for farming	0.283	0.304	0.432	0.929	0.451	-1.026	1.591

Model	Correlations			
	Zero-order	Partial	Part	
	1 (Constant)			
	<i>ln</i> years of residence	-0.618	-0.694	-0.633
	<i>ln</i> years of DDT use for farming	0.408	0.549	0.432

Table E.4 The association of *o,p'*-DDE with years of residence and DDT use for farming

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	4.662	1.519		3.069	0.013	1.226	8.098
<i>ln</i> years of residence	-0.199	0.464	-0.132	-0.429	0.678	-1.248	0.850
<i>ln</i> years of DDT use for farming	0.223	0.187	0.368	1.195	0.263	-0.199	0.646

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln</i> years of residence	-0.118	-0.142	-0.132
<i>ln</i> years of DDT use for farming	0.363	0.370	0.367

Table E.5 The association of *o,p'*-DDT with years of residence and DDT use for farming

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	4.892	2.017		2.425	0.032	0.497	9.288
<i>ln</i> years of residence	-0.012	0.616	-0.005	-0.019	0.985	-1.354	1.330
<i>ln</i> years of DDT use for farming	-0.194	0.248	-0.221	-0.783	0.449	-0.734	0.346

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln</i> years of residence	-0.014	-0.006	-0.005
<i>ln</i> years of DDT use for farming	-0.221	-0.220	-0.220

Table E.6 The association of dieldrin with years of residence and DDT use for farming

Model		Unstandardized		Standardized	t	Sig.	95% CI for β	
		Coefficients		Coefficients			Lower	Upper
		β	SE	Beta				
1	(Constant)	6.000	0.839		7.149	0.000	4.311	7.688
	<i>ln</i> years of residence	-0.303	0.256	-0.165	-1.182	0.243	-0.818	0.212
	<i>ln</i> years of DDT use for farming	-0.167	0.103	-0.226	-1.618	0.112	-0.374	0.041

Model		Correlations		
		Zero-order	Partial	Part
		1	(Constant)	
	<i>ln</i> years of residence	-0.174	-0.170	-0.165
	<i>ln</i> years of DDT use for farming	-0.233	-0.230	-0.226

Table E.7 The association of δ -HCH with years of residence and DDT use for farming

Model		Unstandardized		Standardized	t	Sig.	95% CI for β	
		Coefficients		Coefficients			Lower	Upper
		β	SE	Beta				
1	(Constant)	4.088	0.673		6.079	0.000	2.717	5.460
	<i>ln</i> years of residence	-0.440	0.205	-0.357	-2.146	0.040	-0.858	-0.022
	<i>ln</i> years of DDT use for farming	-0.052	0.083	-0.105	-0.631	0.533	-0.221	0.116

Model		Correlations		
		Zero-order	Partial	Part
		1	(Constant)	
	<i>ln</i> years of residence	-0.361	-0.360	-0.357
	<i>ln</i> years of DDT use for farming	-0.118	-0.113	-0.105

(1.2) Multiple linear regression analysis for the association of plasma p,p' -DDE and o,p' -DDE with E_2 after adjusting for age and BMI (Table E8 – E10)

Table E.8 The association of plasma p,p' -DDE with E_2 , adjusted for age and BMI

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
		β	SE	Beta			Lower Bound	Upper Bound
1	(Constant)	48.785	38.797		1.257	0.212	-28.259	125.829
	$\ln p,p'$ -DDE	-7.093	2.899	-0.245	-2.446	0.016	-12.851	-1.336
	$\ln age$	-4.773	5.676	-0.095	-0.841	0.403	-16.045	6.499
	$\ln BMI$	15.917	11.743	0.153	1.356	0.179	-7.401	39.236

Model		Correlations		
		Zero-order	Partial	Part
1	(Constant)			
	$\ln p,p'$ -DDE	-0.239	-0.246	-0.244
	$\ln age$	-0.042	-0.087	-0.084
	$\ln BMI$	0.087	0.139	0.135

Table E.9 The association of plasma o,p' -DDE with E_2 , adjusted for age and BMI

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
		β	SE	Beta			Lower Bound	Upper Bound
1	(Constant)	-146.689	77.918		-1.883	0.074	-309.222	15.845
	$\ln o,p'$ -DDE	16.381	5.596	0.587	2.927	0.008	4.708	28.054
	$\ln age$	-4.043	10.566	-0.080	-0.383	0.706	-26.083	17.997
	$\ln BMI$	36.110	23.061	0.348	1.566	0.133	-11.995	84.214

Table E.9 The association of plasma *o,p'*-DDE with E₂, adjusted for age and BMI
(continue)

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln o,p'</i> -DDE	0.473	0.548	0.543
<i>ln age</i>	-0.042	-0.085	-0.071
<i>ln BMI</i>	0.085	0.330	0.291

Table E.10 The association of plasma *p,p'*-DDE and *o,p'*-DDE with E₂, adjusted for age and BMI

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	45.025	63.802		0.706	0.489	-88.514	178.563
<i>ln p,p'</i> -DDE	-4.410	4.398	-0.208	-1.003	0.329	-13.615	4.795
<i>ln o,p'</i> -DDE	8.467	4.140	0.425	2.045	0.055	-0.198	17.131
<i>ln age</i>	-8.526	7.898	-0.222	-1.079	0.294	-25.056	8.005
<i>ln BMI</i>	2.338	18.694	0.029	0.125	0.902	-36.788	41.465

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln p,p'</i> -DDE	-0.234	-0.224	-0.191
<i>ln o,p'</i> -DDE	0.473	0.425	0.390
<i>ln age</i>	-0.312	-0.240	-0.206
<i>ln BMI</i>	-0.289	0.029	0.024

2. Multiple linear regression analysis of research section 3:

Effect of POPs on reproductive and thyroid hormone levels in infants

(2.1) Multiple linear regression analysis for the association of cord serum p,p' -DDE with testosterone in infants from Mae Rim District (Table E11)

Table E.11 The association of cord serum p,p' -DDE with testosterone in infants from Mae Rim District

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	6.098	4.342		1.404	0.181	-3.158	15.354
<i>ln</i> cord p,p' -DDE	0.110	0.066	0.371	1.664	0.117	-0.031	0.250
<i>ln</i> birth weight	-0.791	0.522	-0.337	-1.514	0.151	-1.905	0.322

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln</i> cord p,p' -DDE	0.478	0.395	0.351
<i>ln</i> birth weight	-0.456	-0.364	-0.320

(2.2) Multiple linear regression analysis for the association of cord serum POPs with testosterone in infants from Mae Rim District (Table E12 – E14)

Table E.12 The association of cord serum p,p' -DDE with TT_4

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	12.507	2.162		5.785	0.000	8.117	16.896
<i>ln</i> cord p,p' -DDE	-0.743	0.304	-0.372	-2.445	0.020	-1.361	-0.126
<i>ln</i> gender od infants	0.827	0.523	0.240	1.5579	0.123	-0.236	1.889

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln</i> cord p,p' -DDE	-0.366	-0.382	-0.372
<i>ln</i> gender od infants	0.231	0.258	0.240

Table E.13 The association of cord serum p,p' -DDT with TT_4

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	11.177	1.961		5.700	0.000	7.188	15.167
<i>ln</i> cord p,p' -DDT	-0.799	0.390	-0.323	-0.202	0.049	-1.602	0.005
<i>ln</i> gender od infants	0.752	0.551	0.218	1.366	0.181	-0.368	1.872

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln</i> cord p,p' -DDT	-0.332	-0.332	-0.323
<i>ln</i> gender od infants	0.231	0.231	0.218

Table E.14 The association of cord serum *o,p'*-DDE with TT₄

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for β	
	β	SE	Beta			Lower Bound	Upper Bound
1 (Constant)	18.993	4.449		4.269	0.005	8.107	29.878
<i>ln cord o,p'-DDE</i>	-2.693	0.999	-0.745	-2.695	0.036	-5.138	-0.248
<i>ln gender od infants</i>	0.142	0.951	0.041	0.149	0.887	-2.186	2.469

Model	Correlations		
	Zero-order	Partial	Part
1 (Constant)			
<i>ln cord o,p'-DDE</i>	-0.755	-0.740	-0.720
<i>ln gender od infants</i>	0.231	0.061	0.04

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Health System Research Institute (HSRI), Ministry of Public Health, Thailand, 2003.
German Academic Exchange Service (DAAD), Germany, April 2005 – September 2005.

Research Publications:

- (1) Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. Plasma levels of DDT and their association with reproductive hormones in adult men from northern Thailand. *The Science of the Total Environment* 2006; 355: 98 –105.
- (2) Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: a study from northern Thailand. *Environment International* 2006 (Accepted).

Presentations:

- (1) Sreesai S., Asawasinsopon R., Satitvipawee P. Treatment and reuse of swine wastewater, 14th National Annual Conference 2002, Bangkok, Thailand, May 9-10, 2002.
- (2) Asawasinsopon R., Prapamontol T., Prakobvitayakit O., Vaneesorn Y., Mangklabruks A., Bertold Hock. Persistent organic pollutants in adult males from Chiang Mai, RGJ-Ph.D. Congress IV, Chonburi, Thailand, April 25-27, 2003.
- (3) Asawasinsopon R., Prapamontol T., Prakobvitayakit O., Vaneesorn Y., Mangklabruks A., Bertold Hock. Plasma levels of persistent organic pollutants in adult

males residing in Mae Sa Mai Village, Mae Rim District, Chiang Mai Province, International Symposium on Uses and Effects of Pesticides in Southeast Asia: Ecological, Biomedical and Economic, December 11-13, 2003.

- (4) Asawasinsopon R., Prapamontol T., Prakobvitayakit O., Vaneesorn Y., Mangklabruks A., Bertold Hock. Persistent organochlorines in mother-neonate pairs from Chiang Mai Province, 2nd Asian International Conference on Ecotoxicology and Environmental Safety, Songkla, Thailand, September 26-29, 2004.
- (5) Asawasinsopon R. Prenatal exposure to organochlorines in mother-infants pairs from northern Thailand, Academic Meeting of Cell Biology Department, Center of Life and Food Sciences Weihenstephan, Technical University of Munich, Germany, May 31, 2005.
- (6) Asawasinsopon R., Prapamontol T., Bertold Hock, Prakobvitayakit O., Vaneesorn Y., Mangklabruks A. Measurement of estrogenic activities of chemicals in human serum by using enzyme-linked receptor assay (ELRA), 3th National Conference on Science and Technology, Nakorn Ratsima, Thailand, October 18-20, 2005.