

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Qualitative and quantitative analysis of POPs

3.1.1 Retention time

Retention times of individual POPs in standard mixture are presented in Table 3.1.

Table 3.1 Retention times of individual POPs in standard mixture

Retention times (minutes)	POPs
8.792	β -HCH
9.135	HCB
9.328	γ -HCH
11.281	Heptachlor
12.322	Aldrin
13.449	Heptachlor epoxide
14.219	<i>o,p'</i> -DDE
15.126	<i>p,p'</i> -DDE
15.259	Dieldrin
16.366	<i>p,p'</i> -DDD
16.485	<i>o,p'</i> -DDT
17.491	<i>p,p'</i> -DDT

3.1.2 Linearity

Linear regression equations and regression coefficients (R^2) of individual POP standard mixture are presented in Table 3.2.

Table 3.2 Linear regression equations and regression coefficients (R^2) of POP standard mixture

POPs	Linear regression equations	R^2
Aldrin	$Y = 1X + 0$	1.00000
<i>p,p'</i> -DDE	$Y = 0.7732X + 0.0571$	0.99829
<i>p,p'</i> -DDT	$Y = 0.1669X + 0.0003$	0.99645
<i>p,p'</i> -DDD	$Y = 0.7268X + 0.0668$	0.96936
<i>o,p'</i> -DDE	$Y = 0.6417X + 0.0286$	0.99873
<i>o,p'</i> -DDT	$Y = 0.2834X + 0.0013$	0.99399
Heptachlor	$Y = 0.6215X + 0.0175$	0.99841
Heptachlor epoxide	$Y = 0.7497X + 0.0256$	0.99953
Dieldrin	$Y = 0.6329X + 0.0395$	0.99925
HCB	$Y = 0.4605X + 0.00004$	0.99903
β -HCH	$Y = 1.7408X + 0.0433$	0.97635
γ -HCH	$Y = 0.8901X - 0.0275$	0.92604

X: amount ratio of individual POPs to internal standard (amt ratio)

Y: response ratio

R^2 : regression coefficient

3.2 Quality control of POP analysis

3.2.1 Level of detection

LOD and LOQ of individual POPs are presented in Table 3.3. LOD ranged from 0.01 ng/ml for *o,p'*-DDE to 0.24 ng/ml for *p,p'*-DDT. LOQ ranged from 0.03 ng/ml for *o,p'*-DDE to 1.08 ng/ml for *p,p'*-DDT. LOD was applied for all POPs' quantification.

3.2.2 Recovery

Recoveries of individual POPs are presented in Table 3.4 and 3.5. In low spiked levels, % recoveries ranged from 55.2 for β -HCH to 109.6 for HCB. In high spiked levels, % recoveries ranged from 50.4 for β -HCH to 100.6 for HCB. It was found that only the value of % recoveries for β -HCH was lower than acceptable criteria (60-115%).

3.2.3 POP variations of intra- and inter-batches of pooled samples

Variations of intra-batch of pooled pig fat samples for research section 1 are presented in Table 3.6. Five of pooled pig fat samples were analysed for intra-batch and % CV ranged from 13.1% for *p,p'*-DDE to 42.9% for *o,p'*-DDE. Only % CV of *p,p'*-isomers, including *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD, were in acceptable criteria, which were less than 21%.

Table 3.3 Limit of detection (LOD) and limit of quantification (LOQ) of individual POPs

POPs, ng/ml	LOD	LOQ
<i>p,p'</i> -DDE	0.10	0.43
<i>p,p'</i> -DDT	0.24	1.08
<i>p,p'</i> -DDD	0.18	0.61
<i>o,p'</i> -DDE	0.01	0.03
<i>o,p'</i> -DDT	0.20	0.91
Heptachlor	0.13	0.57
Heptachlor epoxide	0.18	0.60
Dieldrin	0.22	0.72
HCB	0.21	0.70
β -HCH	0.18	0.61
γ -HCH	0.13	0.42

Variations of intra- and inter-batches of pooled serum samples are presented in Table 3.7. Five of pooled serum samples were analyzed for intra-batch and 22 of pooled serum samples were analysed for inter-batch. For intra-batch, % CV ranged from 4.0 for *p,p'*-DDT to 8.9 for heptachlor. For inter-batch, % CV ranged from 5.8 for *p,p'*-DDE to 17.9 for *o,p'*-DDT. Both of them were in the acceptable criteria, which were less than 21%.

Table 3.4 Recoveries (% recoveries) of individual POPs for low spiked levels

POPs	Spiked levels (ng/ml)	Recoveries (%)
<i>p,p'</i> -DDE	7.5	81.7
<i>p,p'</i> -DDT	12.5	100.3
<i>p,p'</i> -DDD	7.5	103.6
<i>o,p'</i> -DDE	10	83.2
<i>o,p'</i> -DDT	7.5	69.6
Heptachlor	5.0	89.4
Heptachlor epoxide	5.0	93.6
Dieldrin	7.5	92.3
HCB	2.5	109.6
β -HCH	2.5	55.2
γ -HCH	2.5	79.6

Table 3.5 Recoveries (% recoveries) of individual POPs for high spiked levels

POPs	Spiked levels (ng/ml)	Recoveries (%)
<i>p,p'</i> -DDE	15	80.3
<i>p,p'</i> -DDT	25	91.2
<i>p,p'</i> -DDD	15	96.1
<i>o,p'</i> -DDE	20	76.3
<i>o,p'</i> -DDT	15	70.3
Heptachlor	10	80.7
Heptachlor epoxide	10	85.5
Dieldrin	15	84.7
HCB	5	100.6
β -HCH	5	50.4
γ -HCH	5	78.2

Table 3.6 POP variations of intra-batch of pooled pig fat samples

POPs, ng/g fats	Intra-batch levels (n = 5)	
	AR \pm SD	% CV
<i>p,p'</i> -DDE	506.9 \pm 66.2	13.1
<i>p,p'</i> -DDT	296.2 \pm 65.3	20.1
<i>p,p'</i> -DDD	56.3 \pm 9.2	16.3
<i>o,p'</i> -DDE	14.5 \pm 6.2	42.9
<i>o,p'</i> -DDT	6.0 \pm 2.3	38.8

Table 3.7 POP variations of intra- and inter-batches of pooled serum samples

POPs, ng/ml	Intra-batch (n = 5)		Inter-batch (n = 22)	
	AR \pm SD	% CV	AR \pm SD	% CV
<i>p,p'</i> -DDE	22.81 \pm 1.34	5.9	23.86 \pm 1.38	5.8
<i>p,p'</i> -DDT	7.29 \pm 0.37	4.0	4.23 \pm 0.58	13.7
<i>p,p'</i> -DDD	5.36 \pm 0.25	4.7	6.04 \pm 0.53	8.8
<i>o,p'</i> -DDE	4.46 \pm 0.27	6.1	4.61 \pm 0.28	5.9
<i>o,p'</i> -DDT	3.09 \pm 0.25	8.1	2.65 \pm 0.47	17.9
Heptachlor	2.93 \pm 0.26	8.9	2.88 \pm 0.31	10.6
Heptachlor epoxide	5.26 \pm 0.38	7.3	4.70 \pm 0.59	12.6
Dieldrin	4.54 \pm 0.30	6.6	4.88 \pm 0.44	9.1
HCB	2.05 \pm 0.13	6.6	2.24 \pm 0.24	10.6
β -HCH	0.85 \pm 0.05	5.7	0.87 \pm 0.06	7.2
γ -HCH	1.27 \pm 0.07	5.4	1.33 \pm 0.08	5.8

3.3 Quality control of lipids

Lipid variations of intra- and inter-batches of pooled serum samples are presented in Table 3.8. The intra-batch % CV was 2.2 for cholesterol and 1.7 for triglycerides. The inter-batch % CV was 1.5 for cholesterol and 4.4 for triglycerides.

Table 3.8 Lipid variations of intra- and inter-batches of pooled serum samples

Lipids, mg/dl	Intra-batch (n = 5)		Inter-batch (n = 5)	
	AR \pm SD	% CV	AR \pm SD	% CV
Cholesterol	158 \pm 3.51	2.2	152 \pm 2.35	1.5
Triglycerides	115 \pm 2.00	1.7	117 \pm 5.15	4.4

3.4 Quality control of hormones

Hormone variations of intra- and inter-batches of pooled serum samples are presented in Table 3.9. The intra-batch % CV ranged from 2.1 to FT₄ and 6.0 for TT₄. The inter-batch % CV ranged from 6.4 for TT₄ to 10.9 for testosterone.

Table 3.9 Hormone variations of intra- and inter-batches of pooled serum samples

Hormones	Intra-batch (n = 5)		Inter-batch (n = 5)	
	AR \pm SD	% CV	AR \pm SD	% CV
E ₂ , ng/ml	487 \pm 12.45	2.6	498 \pm 46.10	9.3
Testosterone, ng/ml	2.29 \pm 0.08	3.5	2.20 \pm 0.24	10.9
LH, mIU/ml	55.15 \pm 2.46	4.5	55.54 \pm 5.12	9.2
FSH, mIU/ml	44.49 \pm 1.43	3.2	43.24 \pm 4.45	10.3
TT ₄ , μ g/dl	6.29 \pm 0.37	6.0	0.67 \pm 0.43	6.4
FT ₄ , ng/dl	1.33 \pm 0.07	2.1	1.26 \pm 0.11	8.7
TSH, mIU/l	2.43 \pm 0.13	5.4	2.46 \pm 0.19	8.0

3.5 Quality control of ELRA

The detection limit of ELRA was 0.078 ng/ml E₂ equivalent. Recoveries of E₂ in human serum extracts were 115.5% (Table 3.10)

Table 3.10 Recoveries of spiked E₂ in human serum extracts

Spiked E ₂ levels	Measured E ₂ levels	Recoveries (%)
0.20 ng/ml	0.23 ± 0.04 ng/ml	115.5

3.6 Results and discussion of research section 1:

Distribution of POPs in the environment

POP levels in twelve pig fats are presented in Table 3.11. *p,p'*-DDT was detected in all samples and had the highest levels with a geometric mean of 20.7 ng/g fats. The second highest level was that for *p,p'*-DDD, followed by *p,p'*-DDE. It can be hypothesized that this population has been recently exposed to DDT because *p,p'*-DDT levels were higher than *p,p'*-DDD and *p,p'*-DDE levels. Hence, DDT was probably illegally used in the study site in spite of its banned.

Table 3.11 POP levels (ng/g fats) in 12 pig fats

POPs	N	% detected	GM	AR	S.	Min - Max
<i>p,p'</i> -DDE	10	83.3	10.5	22.8	30.4	1.2 - 94.4
<i>p,p'</i> -DDT	12	100	20.7	28.3	29.7	6.0 - 117
<i>p,p'</i> -DDD	7	58.3	11.0	17.4	18.2	3.3 - 52.4

3.7 Results and discussion of research section 2:

Effect of POPs on reproductive hormone levels in adult men

3.7.1 Characteristics and demographic data of adult men

Characteristics and demographic data of 97 adult men are presented in Table 3.12 and 3.13. A geometric mean age was 28.4 years and all of them (100%) were farmers. The mean times of residence in this village and farm experience were 25.6 and 13 years, respectively. BMI ranged from 18.7 to 37.3 kg/m² with a geometric mean of 23 kg/m². Sixty-two men (63.9%) had used DDT for farming, of whom 55 (88.7%) mixed DDT with plant seed before planting and 7 (11.3%) used it only for spraying. The mean duration of DDT use for farming was 4 years (ranged from 1 to 20 years). By student's t-test analysis, the men who had used DDT for farming had higher mean plasma levels of *p,p'*-DDE, *p,p'*-DDT and *o,p'*-DDE than the men who never used it ($P < 0.05$).

Table 3.12 Characteristics of 97 adult men

Variables	GM	AR	SD	Min - Max
Anthropometric measures				
Age, years.	28.4	29.4	7.5	18.0 – 45.0
Weight, kg	52.2	56.8	8.6	47.0 – 85.0
Height, cm	157	157	4.8	145 – 170
BMI, kg/m ²	23.0	23.2	3.1	18.7 – 37.3
Plasma lipids				
Cholesterol, mg/dl	146	149	27.3	90.0 – 241
Triglycerides, mg/dl	138	161	94.4	40.0 – 492
Total lipids, mg/dl	484	499	128	293 – 963
Others				
Years of residence, years.	25.6	26.8	7.5	10.0 – 41.0
Years of farm experience, years.	13.0	16.0	8.3	1.0 – 35.0
Years of DDT use, years.	4.0	5.3	4.1	1.0 – 20.0

3.7.2 POP levels in adult men

Plasma levels of POPs in 97 adult men are presented in Table 3.14. Plasma *p,p'*-DDE and *p,p'*-DDT were detected in all subjects of the studied population. *p,p'*-DDE had the highest level with a geometric mean of 4,013 ng/g lipids. The second highest level was that for *p,p'*-DDT, followed by *p,p'*-DDD. Heptachlor, heptachlor epoxide, HCB, and β -HCH were not detected in any subjects. Therefore, these compounds were not included.

Table 3.13 Demographic data of 97 adult men

Variables	Frequency (Persons)	Percent (%)
Main occupation		
Farmer	86	88.7
Employee	5	5.2
Government employee	2	2.1
Student	4	4.1
Minor occupation		
Farmer	11	11.3
Seller	18	18.6
Employee	20	20.6
Other	9	9.3
None	39	40.2
Education		
No school education	17	17.5
Primary education	52	53.6
Secondary education	25	25.8
Bachelor degree	3	3.1
DDT usage for farming purposes		
Had used	62	63.9
Not used	26	26.8
Not sure	9	9.3

Table 3.14 Plasma levels (ng/g lipids) of POPs in 97 adult men

POPs	N	% detected	GM	AR	SD	Min – Max
<i>p,p'</i> -DDE	97	100	4,013	4,449	2,173	1,325 – 12,684
<i>p,p'</i> -DDT	97	100	629	717	433	225 – 3,085
<i>p,p'</i> -DDD	10	10.3	391	445	279	212 – 1,162
<i>o,p'</i> -DDE	24	24.7	75.8	85.2	48.2	36.9 – 219
<i>o,p'</i> -DDT	25	25.8	97.8	130	135	42.9 – 633
Dieldrin	96	98.9	120	144	121	35.7 – 1,079
γ -HCH	66	68.0	13.3	14.3	5.5	5.7 – 32.4

The *p,p'*-isomers (i.e. *p,p'*-DDE and *p,p'*-DDT) were detected in all studied samples and the *o,p'*-isomers (i.e. *o,p'*-DDE and *o,p'*-DDT) were also detected in a quarter of the studied samples (Table 3.14). In general, the *p,p'*-isomers were present at much higher levels than the *o,p'*-isomers, because technical grade DDT contains approximately 80% of *p,p'*-DDT and 20% of *o,p'*-DDT (Kelce et al., 1995). The *p,p'*-isomers also have long half lives, which partly accounts for their higher levels in people (Turusov et al., 2002).

3.7.3 Reproductive hormone levels in adult men

Plasma levels of reproductive hormones in 97 adult men are presented in Table 3.15. The median level of plasma E₂, testosterone, LH and FSH was 22.29 pg/ml, 4.67 ng/ml, 3.68 mIU/ml, and 5.53 mIU/ml, respectively. The value at 5th percentile of plasma E₂, testosterone, and LH was below the normal range of the manufacturer, Roche, USA. Therefore, these reference values may be inappropriate for Thai populations.

Table 3.15 Plasma levels of reproductive hormones in 97 adult men

Hormones	Min	Percentile					Max	Normal range ^a (5 th - 95 th)
		5 th	25 th	50 th	75 th	95 th		
E ₂ , pg/ml	5.75	8.72	15.38	22.29	28.23	42.21	114.10	11.00 – 43.90
Testosterone, ng/ml	1.18	2.18	3.45	4.67	5.80	7.99	15.00	2.80 – 8.00
LH, mIU/ml	1.23	1.55	2.59	3.68	5.02	7.02	12.90	1.70 – 8.60
FSH, mIU/ml	2.09	3.27	4.18	5.53	8.57	13.88	46.55	1.50 – 12.40

^a Normal range from manufacturer, Roche, USA

3.7.4 The association of POPs with environmental and occupational exposures

The association of POPs with years of residence and DDT use for farming is presented in Table 3.16. Plasma *p,p'*-DDT levels were positively associated with years of residence ($\beta \pm SE = 0.47 \pm 0.21$, $P = 0.028$) and years of DDT use for farming ($\beta \pm SE = 0.18 \pm 0.08$, $P = 0.040$).

This population might have been exposed to these compounds by a continuing low-level intake, presumably dietary, or an extremely high cumulative exposure in the years preceding the ban. DDT was applied for malaria vector control and farming purposes for 40 years in the studied village. Although the legal use of DDT had stopped for agricultural use more than 15 years before the present data were collected, DDT and its metabolites are still detected in food chain (Stuetz et al., 2001).

In our data, *p,p'*-DDT was associated with years of residence and DDT use for farming. In addition, the men who had reportedly ever used DDT for farming had higher mean plasma levels of *p,p'*-DDE, *p,p'*-DDT and *o,p'*-DDE than the men who never used it ($P < 0.05$). It would seem that durations of environmental exposure and DDT use for farming in the past were determinants of DDT levels. The results therefore suggest that DDT residues in this group were due to both environmental and occupational exposures.

3.7.5 The association of POPs with reproductive hormones

By determining the association of POPs with reproductive hormones using Pearson correlation, plasma E_2 levels were negatively associated with plasma *p,p'*-DDE levels ($r = -0.24$, $P = 0.018$), but positively associated with plasma *o,p'*-DDE levels ($r = 0.47$, $P = 0.020$) (Table 3.17). The associations did not change after adjusting for age and BMI ($\beta \pm SE = -7.09 \pm 2.90$, $P = 0.016$ for the association between plasma E_2 and *p,p'*-DDE and $\beta \pm SE = 16.38 \pm 5.60$, $P = 0.008$ for the association between plasma E_2 and *o,p'*-DDE). No significant association was found when *p,p'*-DDE, *o,p'*-DDE, age and BMI were all once included in multiple linear regression (Table 3.18).

Table 3.16 Multiple linear regression analysis for the association of POPs with years of residence and DDT use for farming

Variables	N	β	SE	Partial r	P
<i>ln p,p'</i> -DDE	97				
<i>ln</i> Years of residence		0.30	0.21	0.21	0.150
<i>ln</i> Years of DDT use for farming		0.04	0.08	0.06	0.657
<i>ln p,p'</i> -DDT	97				
<i>ln</i> Years of residence		0.47	0.21	0.31	0.028*
<i>ln</i> Years of DDT use for farming		0.18	0.08	0.29	0.040*
<i>ln p,p'</i> -DDD	10				
<i>ln</i> Years of residence		-1.03	0.76	-0.69	0.306
<i>ln</i> Years of DDT use for farming		0.28	0.30	0.55	0.451
<i>ln o,p'</i> -DDE	24				
<i>ln</i> Years of residence		-0.20	0.46	-0.14	0.678
<i>ln</i> Years of DDT use for farming		0.22	0.19	0.37	0.263
<i>ln o,p'</i> -DDT	25				
<i>ln</i> Years of residence		-0.01	0.62	-0.01	0.985
<i>ln</i> Years of DDT use for farming		-0.19	0.25	-0.22	0.449
<i>ln</i> Dieldrin	96				
<i>ln</i> Years of residence		-0.30	0.26	-0.17	0.243
<i>ln</i> Years of DDT use for farming		-0.17	0.10	-0.23	0.112
<i>ln γ</i> -HCH	66				
<i>ln</i> Years of residence		-0.44	0.21	-0.36	0.040
<i>ln</i> Years of DDT use for farming		-0.05	0.08	-0.11	0.533

* $P < 0.05$

Table 3.17 Pearson correlation for the association of POPs with reproductive hormones

POPs	N	E ₂		Testosterone		LH		FSH	
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<i>ln p,p'</i> -DDE	97	-0.24	0.018*	-0.18	0.085	-0.13	0.208	-0.12	0.235
<i>ln p,p'</i> -DDT	97	-0.20	0.054	-0.14	0.165	-0.15	0.134	0.07	0.480
<i>ln p,p'</i> -DDD	10	0.37	0.291	0.19	0.608	0.20	0.547	-0.37	0.293
<i>ln o,p'</i> -DDE	24	0.47	0.020*	0.33	0.112	-0.40	0.052	0.18	0.398
<i>ln o,p'</i> -DDT	25	0.01	0.953	-0.16	0.442	-0.08	0.702	0.05	0.831
<i>ln Dieldrin</i>	96	0.10	0.318	0.19	0.071	-0.14	0.182	0.05	0.600
<i>ln γ</i> -HCH	66	-0.02	0.883	0.23	0.064	0.00	0.978	-0.02	0.862

* $P < 0.05$

Table 3.18 Multiple linear regression analysis for the associations of plasma *p,p'*-DDE and *o,p'*-DDE with E₂ after adjusting for age and BMI

Variables	N	β	SE	Partial <i>r</i>	<i>P</i>
Model 1:	97				
E ₂					
<i>ln p,p'</i> -DDE		-7.09	2.90	-0.25	0.016 *
<i>ln Age</i>		-4.77	5.68	-0.09	0.403
<i>ln BMI</i>		15.92	11.74	0.14	0.179
E ₂	24				
<i>ln o,p'</i> -DDE		16.38	5.60	0.55	0.008 **
<i>ln Age</i>		-4.04	10.57	-0.09	0.706
<i>ln BMI</i>		36.11	23.06	0.33	0.133
Model 2:	24				
E ₂					
<i>ln p,p'</i> -DDE		-4.41	4.40	-0.22	0.329
<i>ln o,p'</i> -DDE		8.47	4.14	0.43	0.055
<i>ln Age</i>		-8.52	7.90	-0.24	0.294
<i>ln BMI</i>		2.34	18.69	0.03	0.902

* $P < 0.05$, ** $P < 0.01$

The opposite direction of the associations for *p,p'*-DDE and *o,p'*-DDE may reflect their different mechanisms of hormonal activities, since the *p,p'* isomers act as androgen antagonists whereas the *o,p'* isomers act as estrogen agonists (Bitman et al., 1968; Kelce et al., 1995; Sohoni and Sumpter, 1998). However, these associations were rather weak. It is possible that a mixture of estrogenic and anti-androgenic effects may affect different pathway of hormonal responses. Another possibility is that the association for *o,p'*-DDE may be a chance finding due to multiple comparison, since plasma *o,p'*-DDE in the present study was detected in only 24.7% of the study population and only 24 tests of the association were therefore analysed. Moreover, no significant association was found when *p,p'*-DDE, *o,p'*-DDE, age, and BMI were all once included in multiple comparison.

Levels of DDT in the body might be an important factor affecting hormonal status in men (Martin, Jr. et al., 2002; Cocco et al., 2004). High environmental exposures to DDT might result in an increased binding of DDT with human receptors, although binding of DDT is much less potent than endogenous hormones (Kelce et al., 1995; Chen et al., 1997; Matthews et al., 2000). Higher doses of DDT entering the body would increase bioaccumulation. Hence the levels of DDT at receptors would increase until the estrogen receptors reach a point of saturation with DDT binding (Eaton and Klassen, 1989).

Table 3.19 showed average levels of *p,p'*-DDE and *p,p'*-DDT and their hormonal effects in men from different countries. Average of the *p,p'*-isomers levels in North Carolina farmers, in Latvian and Swedish men, and in Italian men were

approximately 3 – 10 times less than that in the present study, and there was no association with hormonal status (Hagmar et al., 2001a; Martin, Jr. et al., 2002; Cocco et al., 2004). On the other hand, averages of the *p,p'*-isomers levels in adult men from Mexico and male workers from South Africa, where DDT is still used for malaria control, were approximately 16 - 19 times higher than that in the present study (Ayotte et al., 2001; Dalvie et al., 2004a). The study in Mexican men found a negative association of plasma *p,p'*-DDE levels with the ratio of bioavailable to total testosterone, semen volumes, and sperm counts (Ayotte et al., 2001). The study in South African male workers found positive associations of *p,p'*-DDT and *p,p'*-DDD levels with E₂ and testosterone levels; however, they suggested that these associations might be due to chance, since *p,p'*-DDT and *p,p'*-DDD were not known to be a strongly anti-androgenic or estrogenic (Dalvie et al., 2004a, b). Therefore, it could be hypothesized that plasma levels of DDT in the present study might not be high enough to compete completely at the receptor level, or to affect hormonal metabolism and transport (Cocco, 2002). In addition, low levels of exposure in adult might be compensated by normal homeostatic mechanisms and therefore resulted in a small or an insignificant hormonal response.

3.7.6 Estrogenic activities of chemicals in adult men

Estrogenic activities of chemicals were detected in all samples with a geometric mean and a maximum level of 3.85 and 10.84 ng/ml E₂ equivalent, respectively (Table 3.20).

Table 3.19 Average levels of *p,p'*-DDE and *p,p'*-DDT and their hormonal effects in men from different countries

Authors	Study population	Mean or median of <i>p,p'</i> -isomers (ng/g lipids)		Hormonal effects
		<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	
Ayotte et al. (2001)	Mexican men (n = 24)	77,900	n/r	Decreased testosterone
Dalvie et al. (2004)	South African workers (n = 47)	65,000	26,100	Increased E ₂ and testosterone
The present study (2004)	Thai adult men (n = 97)	4,013	628.7	Increase E ₂
Martin, Jr. et al. (2002)	North Carolina Farmers (n = 137)	1,213 (DDE)	n/r	No effect
Hagmar et al. (2001)	Latvian and Swedish men (n = 110)	828	50	No effect
Cocco et al. (2004)	Italian men (n = 107)	396	47	No effect

n/r = not reported

Table 3.20 Estrogenic activities of chemicals in 97 adult male plasma.

	GM	AR	SD	Min	Max
Estrogenic activities, ng/ml E ₂ equivalent	3.85	3.87	2.20	1.03	10.84

3.7.7 The association of POPs with estrogenic activities of chemicals

By determining the association of POPs with estrogenic activities of chemicals in adult male plasma using Pearson correlation, no association was found (Table 3.21).

Estrogenic activities of chemicals detected in adult male plasma were quite high. It can be hypothesized that the male plasma extracts have EDCs that are capable of interacting with estrogen receptors in human bodies. Currently, many chemicals, including POPs, surfactants, polymers, drugs, phytoestrogens, and other pesticides, can be identified as EDCs (Jaga, 2000; Pocar et al., 2003). The results therefore suggest that the studied population might have been exposed to other estrogenic chemicals contaminated in the area.

Although ELRA cannot distinguish between estrogen agonistic and antagonistic effects, this assay is suitable for indicating estrogenic activities of chemicals for a first and/or a large-scale screenings (Baker, 2001; Li et al., 2004). The positive samples should be re-analysed with cellular assays or animal models to specify agonistic or antagonistic effects. In addition, chemical analysis should be applied for identification of receptor-bound substances (Seifert et al. 1998; Baker, 2001).

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Table 3.21 Pearson correlation for the association of POPs with estrogenic activities of chemicals in adult male plasma

POPs	N	<i>ln</i> Estrogenic activities of chemicals	
		<i>r</i>	<i>P</i>
<i>ln p,p'</i> -DDE	97	-0.03	0.802
<i>ln p,p'</i> -DDT	97	0.05	0.612
<i>ln p,p'</i> -DDD	10	0.21	0.591
<i>ln o,p'</i> -DDE	24	-0.27	0.216
<i>ln o,p'</i> -DDT	25	0.27	0.198
<i>ln</i> Dieldrin	96	0.01	0.953
<i>ln</i> γ -HCH	66	0.03	0.815

3.8 Results and discussion of research section 3:

Effect of POPs on reproductive and thyroid hormone levels in infants

3.8.1 Characteristics and demographic data of mother-infant pairs from Mae Rim and Chiang Dao Districts

Characteristics and demographic data of the mother-infant pairs from Mae Rim and Chiang Dao Districts are presented in Table 3.22, 3.23 and 3.24. Within 39 mother-infant pairs from Mae Rim District, thirty-six mothers (92.3%) were farmers, 1 (2.6%) was housewife, 1 (2.6%) was seller, and 1 (2.6%) was employee. Thirteen mothers (33.3%) were nulliparous, 9 (23.1%) were primiparous, and 17 (43.6%) were multiparous. With reference to tribe, all mothers (100%) were Hmong. Eighteen infants (46.2%) were males and 21 (53.8%) were females. Only one infant (2.6%) had low birth weight.

Within 88 mother-infant pairs from Chiang Dao District, seventy-four mothers (84.1%) were farmers, 7 (8%) were housewives, 4 (4.6%) were sellers, and 3 (3.4%) were employees. Twenty-seven mothers (30.7%) were nulliparous, 34 (38.6%) were primiparous, and 27 (30.7%) were multiparous. With reference to tribe, twenty-nine mothers (32.9%) were Lahu, 19 (21.6%) were local people, 15 (17.1%) were Lisu, 9 (10.2%) were Thai Yai, 6 (6.8%) were Keren, and 5 (5.7%) were others. Forty-two infants (47.7%) were males and 46 (52.3%) were females. Six infants (6.8%) had low birth weight.

Table 3.22 Characteristics of 127 mother-infant pairs

Variables	Total (n = 127)			
	GM	AR	SD	Min - Max
Characteristics of mothers				
Maternal age, years.	24.6	25.4	6.3	15.0 - 43.0
Years of residence, years.	15.4	18.8	8.8	5.0 - 43.0
Years of farm experience, years.	9.4	11.5	6.6	1.0 - 30.0
Years of DDT use for farming, years.	4.4	6.5	5.4	1.0 - 20.0
Maternal lipids, g/l	10.9	11.8	4.9	1.5 - 32.0
Characteristics of infants				
Birth weight, g	3,015	3,041	386	2,200 - 3,900
Birth height, cm	51.0	51.1	2.6	35.0 - 57.0
Head circumference, cm	33.3	33.4	2.2	30.0 - 53.0
Cord lipids, g/l	5.6	6.3	3.0	1.0 - 16.8

Table 3.23 Characteristics of mother-infant pairs from Mae Rim and Chiang Dao Districts

Variables	Mae Rim District (n = 39)				Chiang Dao District (n = 88)			
	GM	AR	SD	Min - Max	GM	AR	SD	Min - Max
Characteristics of mothers								
Maternal age, years.	23.8	24.5	6.2	16.0 – 38.0	25.0	25.8	6.4	15.0 – 43.0
Years of residence, years. ^a	12.6	16.3	8.5	5.0 – 37.0	16.8	19.9	8.8	5.0 – 43.0
Years of farm experience, years.	10.2	12.1	7.1	2.0 – 30.0	9.0	11.3	6.4	1.0 – 28.0
Years of DDT use for farming, years.	3.8	4.6	2.6	1.0 – 10.0	4.6	7.3	6.1	1.0 – 20.0
Maternal lipids, g/l	10.1	10.9	4.2	1.5 – 22.5	11.3	12.1	4.6	1.6 – 32.0
Characteristics of infants								
Birth weight, g	3,007	3,024	324	2,200 – 3,750	3,022	3,049	412	2,250 – 3,900
Birth height, cm ^a	50.0	50.0	1.1	47.0 – 52.0	51.5	51.6	2.8	35.0 – 57.0
Head circumference, cm ^a	33.9	34.0	0.9	33.0 – 38.0	33.1	33.1	2.5	30.0 – 53.0
Cord lipids, g/l	6.1	6.7	2.7	1.0 – 12.5	5.5	6.2	3.2	1.5 – 16.8

Table 3.24 Demographic data of mother-infant pairs from Mae Rim and Chiang Dao

Districts

Characteristics	Total (n = 127)		Mae Rim District (n = 39)		Chiang Dao District (n = 88)	
	Frequency (Person)	Percent (%)	Frequency (Person)	Percent (%)	Frequency (Person)	Percent (%)
Tribe						
Hmong	44	34.6	39	100	5	5.7
Local people	19	15.0	-	-	19	21.6
Karen	6	4.7	-	-	6	6.8
Thai Yai	9	7.1	-	-	9	10.2
Lisu	15	11.8	-	-	15	17.1
Lahu	29	22.8	-	-	29	32.9
Others	5	3.9	-	-	5	5.7
Occupation						
Farmer	110	86.6	36	92.3	74	84.1
Seller	5	3.9	1	2.6	4	4.6
Employee	4	3.2	1	2.6	3	3.4
Housewife	8	6.3	1	2.6	7	8.0
Education						
non scholl	67	52.8	19	48.7	48	54.6
education	39	30.7	13	33.3	26	29.6
1 st education	21	16.5	7	18.0	14	15.9
2 nd education						
Parity						
Nulliparous	40	31.5	13	33.3	27	30.7
Primiparous	43	33.9	9	23.1	34	38.6
Multiparous	44	34.7	17	43.6	27	30.7
Delivery history						
Abortion	8	6.3	3	7.7	5	5.7
Preterm birth	4	3.2	-	-	4	4.6
Fetal loss	2	1.6	-	-	2	2.3
Post term birth	3	2.4	-	-	3	3.4
Normal term	110	86.6	36	92.3	74	84.1
Smoking						
Smoke	30	23.6	23	59.0	7	8.0
Not smoke	97	76.4	16	41.0	81	92.0
Alcohol						
Drink	9	7.1	3	7.7	6	6.8
Not drink	118	92.9	36	92.3	82	93.2
DDT for farming						
Spraying	20	47.6	2	20.0	18	56.3
Mixing with seed	14	33.3	6	60.0	8	25.0
Both	8	19.1	2	20.0	6	18.8

3.8.2 POP levels in maternal and cord sera

A total of 127 maternal and cord sera was analysed for 11 pesticides and the results of POP levels, expressed on a lipid basis, are presented in Table 3.25 and 3.26. β -HCH was not detected in any samples, therefore this compound was not included. *p,p'*-DDE was detected in all subjects and had the highest level in both Districts. The second highest level was that for *p,p'*-DDT, followed by *p,p'*-DDD.

POPs were detected in both maternal and cord sera, indicating that these chemicals have an efficient placental transfer (Kanja et al., 1992; Bjerregaard and Hansen, 2000; Waliszewski et al., 2001; Sala et al., 2001a; Covaci et al., 2002; Butler et al., 2003). The placenta acts as a lipid membrane that permits bi-directional transfer of substances between maternal and fetal compartments (Klaassen and Rozman, 1996). Rate of placental transfer of chemicals depends on the characteristics of placental membrane and chemicals, placental blood flow, plasma protein binding of chemicals, and respective pHs of the maternal and fetal circulation (Pratt, 1990). Regarding the characteristics of chemicals, lipophilic and low molecular weight chemicals, including POPs, are fully capable of crossing the placental membrane by passive diffusion (Morgan, 1997). The placental transfer also depends on lipid solubility characteristics of individual chemicals, meaning that more lipid-soluble chemicals attain equilibrium between mother and fetus more rapidly (Klaassen and Rozman, 1996).

The mean levels of *p,p'*-DDE, *p,p'*-DDD, heptachlor, and HCB were higher in the mother-infant pairs from Chiang Dao than Mae Rim Districts with statistically

significant difference ($P < 0.01$). The mean levels of p,p' -DDT, o,p' -DDE, and γ -HCH were also higher in the pairs from Chiang Dao than Mae Rim District, but there were no statistical significance ($P > 0.1$). The mean level of dieldrin was higher in the pairs from Mae Rim than Chiang Dao Districts with statistically significant difference ($P < 0.01$) (Table 3.26). In the present data, years of residence and DDT use for farming of the pairs from Chiang Dao District were higher than those from Mae Rim District. Therefore, durations of environmental exposure and DDT use for farming in the past were determinants of DDT levels.

The results regarding the association between POP levels in maternal and cord sera for a total of 127 mother-infant pairs were found a significant association for individual POPs, except heptachlor epoxide. Considering the association for Mae Rim District, significantly high association was found for p,p' -DDE ($r = 0.86$, $P = 0.000$), p,p' -DDT ($r = 0.77$, $P = 0.000$), and p,p' -DDD ($r = 0.66$, $P = 0.000$) and significantly moderate association was found for dieldrin ($r = 0.60$, $P = 0.000$). By the association for Chiang Dao District, significantly high association was found for p,p' -DDE ($r = 0.82$, $P = 0.000$), p,p' -DDT ($r = 0.66$, $P = 0.000$), and p,p' -DDD ($r = 0.78$, $P = 0.000$). Significantly moderate association was found for o,p' -DDE ($r = 0.40$, $P = 0.005$), heptachlor ($r = 0.41$, $P = 0.000$), dieldrin ($r = 0.36$, $P = 0.003$), and HCB ($r = 0.61$, $P = 0.048$) (Table 3.27).

A high Pearson correlation coefficient between POP levels in maternal and cord sera indicates an equilibrium pattern between these body compartments during pregnancy (Waliszewski et al., 2000; Dorea et al., 2001). In the present study, a high

correlation coefficient was found for *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD in both Mae Rim and Chiang Dao Districts (Table 3.27). It can be concluded that placental transfer of these three compounds reaches a balanced state between mother and fetus. A fetus has low cytochrome P450 enzyme-mediated biotransformation of POPs, resulting in a weak detoxification process. Therefore, POPs are accumulated in body compartments of the fetus, subsequently causing adverse health effects (Waliszewski et al., 2001).

The predominant contaminants in maternal and cord sera were *p,p'*-isomers of DDT, including *p,p'*-DDE and *p,p'*-DDT. To compare the present results for their levels with other populations, the highest level expressed on a lipid basis was found in Mexican blood samples and the lowest level was found in Nicaraguan blood samples (Table 3.28). Expressed on a fresh weight basis, the highest level was found in Thai blood samples and the lowest level was found in Canadian blood samples. The banned year of DDT use might be an important factor affecting DDT levels in serum. DDT use for public health program was banned in 1996 for Mexico, in 1989 for the present study site, and in the 1970s for European countries and Canada (Waliszewski et al., 2001; Turusov et al., 2002; Dallaire et al., 2003). However, it is difficult to compare the results from diverse human studies because data are often collected at different time periods, using different experimental designs, in different racial groups, and under different exposure conditions.

Table 3.25 Levels of POPs (ng/g lipids) in 127 maternal and cord sera

POPs		N	% detected	Total (n = 127)			Min - Max
				GM	AR	SD	
<i>p,p'</i> -DDE	Maternal	127	100	1,581	2,486	3,198	58.3 - 27,169
	Cord	127	100	1,068	1,776	2,637	81.3 - 23,185
<i>p,p'</i> -DDT	Maternal	127	100	138	229	293	11.6 - 2,003
	Cord	116	91.3	93.1	147	241	17.0 - 2,363
<i>p,p'</i> -DDD	Maternal	126	99.2	135	231	337	11.1 - 2,984
	Cord	117	92.1	123	185	232	24.1 - 1,800
<i>o,p'</i> -DDE	Maternal	67	52.8	18.8	44.9	133	0.6 - 1,067
	Cord	62	48.8	35.8	49.3	47.5	5.0 - 280
<i>o,p'</i> -DDT	Maternal	37	29.1	31.9	61.4	105.3	7.0 - 484
	Cord	26	20.5	37.6	70.7	92.7	10.0 - 426
Heptachlor	Maternal	117	92.1	28.1	36.3	32.6	3.8 - 288
	Cord	117	92.2	58.7	84.9	78.5	9.1 - 545
Heptachlor epoxide	Maternal	69	54.3	34.3	41.5	34.3	8.2 - 238
	Cord	67	52.8	49.1	59.7	40.8	15.0 - 234
Dieldrin	Maternal	104	81.9	42.5	59.1	66.6	7.5 - 533
	Cord	115	90.6	75.6	95.0	79.9	18.8 - 684
HCB	Maternal	30	23.6	29.2	42.1	40.2	6.3 - 173
	Cord	15	11.8	70.6	145	174	9.7 - 620
γ -HCH	Maternal	12	9.5	17.9	44.9	96.5	4.0 - 347
	Cord	3	2.4	28.6	32.0	18.4	16.3 - 52.2

Table 3.26 Maternal and cord serum levels of POPs (ng/g lipids) in Mae Rim and Chiang Dao Districts

POPs	Mae Rim District (n = 39)						Chiang Dao District (n = 88)						
	N	% detected	GM	AR	SD	Min - Max	N	% detected	GM	AR	SD	Min - Max	
<i>p,p'</i> -DDE	Maternal ^a	39	100	1,191	1,792	1,760	58.3 - 7,981	88	100	1,793	2,794	3,626	208 - 27,169
	Cord ^a	39	100	742	1,025	866	81.3 - 4,265	88	100	1,255	2,108	3,063	146 - 23,185
<i>p,p'</i> -DDT	Maternal	39	100	123	195	217	18.0 - 1,067	88	100	145	245	321	11.6 - 2,003
	Cord	37	94.9	77.1	103	112	21.5 - 660	79	89.8	102	166.9	281	17.0 - 2,363
<i>p,p'</i> -DDD	Maternal ^a	39	100	104	139	115	16.8 - 527	87	98.9	152	273	392	11.1 - 2,984
	Cord ^a	39	100	89.1	105	63.0	24.1 - 309	78	88.6	145	225	273	28.6 - 1,800
<i>o,p'</i> -DDE	Maternal	8	20.5	21.6	24.8	11.2	5.8 - 37.1	59	67.0	18.5	47.7	142	0.6 - 1,067
	Cord	9	23.1	46.6	51.9	27.4	23.9 - 107	53	60.2	34.2	48.8	50.3	5.0 - 280
<i>o,p'</i> -DDT	Maternal ^a	19	48.7	21.5	26.3	21.1	7.0 - 100	18	20.5	48.5	98.5	142	9.8 - 484
	Cord ^a	10	25.6	17.1	18.1	6.5	10.0 - 27.8	16	18.2	61.7	104	106	10.5 - 426
Heptachlor	Maternal ^a	32	82.1	20.8	27.2	22.6	3.8 - 111	85	96.6	31.4	39.7	35.1	4.1 - 288
	Cord ^a	31	79.5	37.1	64.3	103	9.1 - 545	86	97.7	69.2	92.3	66.8	10.3 - 282
Heptachlor epoxide	Maternal ^a	32	82.0	39.6	45.2	29.0	17.8 - 177	37	42.0	30.2	38.3	38.4	8.2 - 238
	Cord ^a	29	74.4	38.8	45.9	27.3	15.0 - 114	38	43.2	58.7	70.3	46.3	17.3 - 234
Dieldrin	Maternal ^a	35	89.7	68.0	91.9	94.1	15.9 - 533	69	78.4	33.4	42.4	38.2	7.5 - 239
	Cord ^a	37	94.9	94.9	120	116	32.4 - 639	78	88.6	67.9	83.1	52.3	18.8 - 260
HCB	Maternal ^a	14	35.9	17.3	18.9	8.0	6.3 - 38.4	16	18.2	46.2	62.3	46.2	11.5 - 173
	Cord	nd	nd	nd	nd	nd	nd	15	17.0	70.6	145	174	9.7 - 620
γ -HCH	Maternal	8	20.5	16.1	53.0	119	4.0 - 347	4	4.5	22.2	28.9	27.6	14.3 - 70.4
	Cord	2	5.1	29.1	34.2	25.4	16.3 - 52.2	1	1.1	27.5	27.5	0.0	27.5 - 27.5

^a a mean values of statistics in Mae Rim and Chiang Dao Districts were significantly different, nd = not detected

Table 3.27 Pearson correlation between maternal and cord serum POPs levels in mother-infant pairs from Mae Rim and Chiang Dao Districts

Districts	Total (n = 127)			Mae Rim District (n = 39)			Chiang Dao District (n = 88)		
	N	r	P	N	r	P	N	r	P
<i>In</i> Maternal - <i>In</i> Cord <i>p,p'</i> -DDE	127	0.82	0.000 ***	39	0.86	0.000 ***	88	0.82	0.000 ***
<i>In</i> Maternal - <i>In</i> Cord <i>p,p'</i> -DDT	116	0.64	0.000 ***	37	0.77	0.000 ***	79	0.66	0.000 ***
<i>In</i> Maternal - <i>In</i> Cord <i>p,p'</i> -DDD	117	0.74	0.000 ***	39	0.66	0.000 ***	78	0.78	0.000 ***
<i>In</i> Maternal - <i>In</i> Cord <i>o,p'</i> -DDE	56	0.38	0.004 **	8	0.42	0.296	48	0.40	0.005 **
<i>In</i> Maternal - <i>In</i> Cord <i>o,p'</i> -DDT	14	0.82	0.000 ***	9	0.41	0.268	5	0.85	0.069
<i>In</i> Maternal - <i>In</i> Cord Heptachlor	110	0.47	0.000 ***	27	0.34	0.079	83	0.41	0.000 ***
<i>In</i> Maternal - <i>In</i> Cord Heptachlor epoxide	65	0.10	0.420	28	0.31	0.114	37	0.16	0.358
<i>In</i> Maternal - <i>In</i> Cord Dieldrin	101	0.42	0.000 ***	35	0.60	0.000 ***	66	0.36	0.003 **
<i>In</i> Maternal - <i>In</i> Cord HCB	11	0.61	0.048 *	nd	nd	nd	11	0.61	0.048 *

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$, nd = not detected

Table 3.28 Mean levels of *p,p'*-DDE and *p,p'*-DDT in maternal and cord sera from different countries

Countries	No. of samples	<i>p,p'</i> -DDE		<i>p,p'</i> -DDT		References
		Maternal	Cord	Maternal	Cord	
Lipid basis, ng/g lipids						
Mexico	90	4,378	4,676	1,848	2,800	Waliszewski et al., 2000
Kenya	11	1,520	1,260	810	600	Kanja et al., 1992
Thailand	127	1,581	1,068	123	77.1	The present study
Japan	32	89.7	33.0	3.4	-	Fukata et al., 2005
Nicaragua	52	7.1	6.4	n/r	n/r	Dorea et al., 2001
Fresh weight basis, ng/ml						
Thailand	127	26.94	8.56	2.54	0.70	The present study
Republic of Uzbekistan	18	4.71	1.39	0.27	0.08	Ataniyazova et al., 2001
Spain	72	2.24	0.83	n/r	n/r	Sala et al., 2001
Belgium	44	2.16	0.58	n/r	n/r	Covaci et al., 2002
Canada	351	1.05	0.34	0.06	0.03	Butler et al., 2003

n/r = not reported

3.8.3 Reproductive and thyroid hormone levels in cord serum

Cord serum levels of reproductive and thyroid hormones are presented in Table 3.29. The mean levels of E_2 , testosterone, and FT_4 between the infants from Mae Rim and Chiang Dao Districts were not significantly different ($P > 0.1$), but the mean levels of TT_4 and TSH were higher in the infants from Mae Rim than Chiang Dao District with statistically significant difference ($P < 0.05$). Within 39 mother-infant pairs from Mae Rim District, the value at 95th percentile of TT_4 and TSH was higher than the normal range. Within 88 mother-infant pairs from Chiang Dao District, the value at 5th percentile of TT_4 was lower than the normal range.

3.8.4 The association of POPs with reproductive and thyroid hormones in cord serum

Pearson correlation between POPs and hormones in the infants from Mae Rim and Chiang Dao Districts are presented in Table 3.30 and 3.31. Although the mean levels of individual POPs, except dieldrin, were higher in the mother-infant pairs from Chiang Dao than Mae Rim Districts, the association with hormones was found only in the pairs from Mae Rim District. From demographic data (Table 3.24), all of the mothers from Mae Rim District were Hmong, while the mothers from Chiang Dao District were mix tribes, including Lahu, local people, Lisu, Thai Yai, Keren, and others. It is possible that tribes of the studied population were determinants of hormonal effects. However, the hormone levels between tribes were not significantly different based on statistical analysis.

Table 3.29 Reproductive and thyroid hormone levels in cord serum

Hormones	Percentile					Max	Normal range in Thai infants 5 th - 95 th
	Min	5 th	25 th	50 th	75 th		
E2, ng/ml							
Total (n = 127)	1.42	2.97	6.77	9.63	16.64	26.52	43.00
Mae Rim District (n = 39)	1.42	3.42	5.83	8.69	15.51	26.54	43.00
Chiang Dao District (n = 88)	1.58	2.69	7.44	10.73	17.89	30.90	43.00
Testosterone, ng/ml^{a, b}							
Total (n = 60)	0.96	1.03	1.42	1.75	2.12	7.48	7.99
Mae Rim District (n = 18)	0.96	0.96	1.34	1.70	1.98	2.26	2.26
Chiang Dao District (n = 42)	1.03	1.07	1.43	1.77	2.22	7.66	7.99
TT₄, µg/dl^a							
Total (n = 127)	3.30	4.22	6.64	7.98	9.38	11.76	17.65
Mae Rim District (n = 39)	4.00	6.28	7.70	8.65	9.95	13.00	13.00
Chiang Dao District (n = 88)	3.30	4.13	6.09	7.70	9.09	11.76	17.65
FT₄, ng/dl							
Total (n = 127)	0.50	0.80	0.90	1.04	1.20	1.50	1.90
Mae Rim District (n = 39)	0.50	0.80	0.88	1.05	1.30	1.51	1.60
Chiang Dao District (n = 88)	0.66	0.82	0.90	1.04	1.20	1.50	1.90
TSH, mIU/l^a							
Total (n = 127)	1.71	3.14	5.34	8.38	12.20	26.62	56.50
Mae Rim District (n = 39)	2.70	3.76	6.83	9.55	15.50	31.80	33.70
Chiang Dao District (n = 88)	1.71	3.10	5.12	7.57	10.95	22.69	56.50

^a mean value of statistics in Mae Rim and Chiang Dao Districts were significantly different; ^b measured only in male infants; n/r = not reported

Table 3.30 Pearson correlation between cord serum levels of POPs and reproductive hormones in infants from Mae Rim and Chiang Dao Districts

Variables	<i>ln E₂</i>			<i>ln Testosterone^a</i>		
	N	<i>r</i>	<i>P</i>	N	<i>r</i>	<i>P</i>
Total (n = 127)						
<i>ln Cord p,p'-DDE</i>	127	0.12	0.114	60	0.28	0.033 *
<i>ln Cord p,p'-DDT</i>	116	0.03	0.744	53	0.12	0.384
<i>ln Cord p,p'-DDD</i>	117	0.09	0.330	56	0.09	0.497
<i>ln Cord o,p'-DDE</i>	62	-0.15	0.256	30	-0.20	0.302
<i>ln Cord o,p'-DDT</i>	26	0.10	0.635	14	-0.07	0.809
<i>ln Cord heptachlor</i>	117	0.04	0.673	54	0.20	0.149
<i>ln Cord heptachlor epoxide</i>	67	0.19	0.129	32	0.34	0.058
<i>ln Cord dieldrin</i>	115	-0.05	0.565	55	0.02	0.914
<i>ln Cord HCB</i>	15	0.50	0.060	9	0.22	0.572
Mae Rim District (n = 39)						
<i>ln Cord p,p'-DDE</i>	39	0.01	0.930	18	0.48	0.045 *
<i>ln Cord p,p'-DDT</i>	37	-0.22	0.202	18	-0.18	0.479
<i>ln Cord p,p'-DDD</i>	39	-0.17	0.311	18	0.06	0.819
<i>ln Cord o,p'-DDE</i>	9	-0.47	0.201	4	-0.89	0.110
<i>ln Cord o,p'-DDT</i>	10	0.22	0.536	3	0.87	0.329
<i>ln Cord heptachlor</i>	31	-0.57	0.760	13	0.22	0.481
<i>ln Cord heptachlor epoxide</i>	29	0.12	0.538	11	0.60	0.053
<i>ln Cord dieldrin</i>	37	0.00	0.983	16	-0.28	0.293
<i>ln Cord HCB</i>	nd	nd	nd	nd	nd	nd
Chiang Dao District (n = 88)						
<i>ln Cord p,p'-DDE</i>	88	0.17	0.111	42	0.20	0.196
<i>ln Cord p,p'-DDT</i>	79	0.10	0.398	35	0.14	0.418
<i>ln Cord p,p'-DDD</i>	78	0.15	0.205	38	0.06	0.727
<i>ln Cord o,p'-DDE</i>	53	-0.10	0.487	26	-0.36	0.413
<i>ln Cord o,p'-DDT</i>	16	-0.13	0.623	11	-0.17	0.279
<i>ln Cord heptachlor</i>	86	0.05	0.683	41	0.14	0.368
<i>ln Cord heptachlor epoxide</i>	38	0.18	0.268	21	0.17	0.462
<i>ln Cord dieldrin</i>	78	-0.05	0.698	39	0.13	0.438
<i>ln Cord HCB</i>	15	0.50	0.060	9	0.22	0.572

nd = not detected

Table 3.31 Pearson correlation between cord serum levels of POPs and thyroid hormones in infants from Mae Rim and Chiang Dao Districts

Variables	TT ₄			FT ₄			ln TSH		
	N	r	P	N	r	P	N	r	P
Total (n = 127)									
ln Cord <i>p,p'</i> -DDE	127	-0.07	0.432	127	-0.12	0.189	127	0.03	0.774
ln Cord <i>p,p'</i> -DDT	116	0.03	0.728	116	-0.17	0.080	116	0.01	0.938
ln Cord <i>p,p'</i> -DDD	117	-0.10	0.309	117	0.05	0.570	117	-0.03	0.761
ln Cord <i>o,p'</i> -DDE	62	-0.25	0.053	62	0.17	0.199	62	0.07	0.611
ln Cord <i>o,p'</i> -DDT	26	-0.11	0.598	26	-0.13	0.524	26	-0.17	0.418
ln Cord heptachlor	117	0.10	0.315	117	-0.16	0.086	117	-0.06	0.507
ln Cord heptachlor epoxide	67	0.08	0.534	67	-0.11	0.384	67	-0.13	0.314
ln Cord dieldrin	115	0.08	0.409	115	0.02	0.814	115	0.14	0.131
ln Cord HCB	15	0.13	0.651	15	-0.48	0.081	15	0.36	0.216
Mae Rim District (n = 39)									
ln Cord <i>p,p'</i> -DDE	39	-0.37	0.024*	39	-0.07	0.664	39	0.02	0.893
ln Cord <i>p,p'</i> -DDT	37	-0.33	0.048*	37	-0.04	0.807	37	0.06	0.715
ln Cord <i>p,p'</i> -DDD	39	-0.27	0.105	39	0.06	0.740	39	0.16	0.338
ln Cord <i>o,p'</i> -DDE	9	-0.76	0.019*	9	-0.01	0.976	9	0.10	0.796
ln Cord <i>o,p'</i> -DDT	10	-0.54	0.105	10	-0.09	0.808	10	-0.05	0.902
ln Cord heptachlor	31	-0.15	0.444	31	-0.21	0.270	31	-0.23	0.227
ln Cord heptachlor epoxide	29	0.03	0.892	29	-0.24	0.217	29	-0.13	0.523
ln Cord dieldrin	37	-0.17	0.328	37	0.09	0.611	37	-0.03	0.854
ln Cord HCB	nd	nd	nd	nd	nd	nd	nd	nd	nd
Chiang Dao District (n = 88)									
ln Cord <i>p,p'</i> -DDE	88	0.08	0.445	88	-0.14	0.209	88	0.11	0.320
ln Cord <i>p,p'</i> -DDT	79	0.17	0.142	79	-0.22	0.051	79	0.05	0.674
ln Cord <i>p,p'</i> -DDD	78	0.02	0.858	78	0.06	0.602	78	0.01	0.930
ln Cord <i>o,p'</i> -DDE	53	-0.27	0.054	53	0.22	0.122	53	0.05	0.714
ln Cord <i>o,p'</i> -DDT	16	0.12	0.653	16	-0.13	0.622	16	0.04	0.883
ln Cord heptachlor	86	0.27	0.063	86	-0.17	0.118	86	0.06	0.606
ln Cord heptachlor epoxide	38	0.15	0.377	38	-0.06	0.738	38	0.03	0.883
ln Cord dieldrin	78	0.09	0.446	78	-0.00	0.978	78	0.15	0.184
ln Cord HCB	15	0.13	0.651	15	-0.48	0.081	15	0.35	0.216

nd = not detected

Cord serum testosterone levels were positively associated with cord *p,p'*-DDE levels ($r = 0.48$, $P = 0.045$) in male infants from Mae Rim District. Cord serum TT_4 levels were negatively associated with cord serum levels of *p,p'*-DDE ($r = -0.37$, $P = 0.024$), *p,p'*-DDT ($r = -0.33$, $P = 0.048$), and *o,p'*-DDE ($r = -0.76$, $P = 0.019$) (Table 3.31). Cord serum testosterone levels were not associated with cord serum levels of *p,p'*-DDE after adjusting for birth weight ($\beta \pm SE = 0.11 \pm 0.06$, $P = 0.103$). However, their associations with TT_4 did not change after adjusting for gender of infants ($\beta \pm SE = -0.74 \pm 0.30$, $P = 0.02$ for *p,p'*-DDE, $\beta \pm SE = -0.80 \pm 0.39$, $P = 0.049$ for *p,p'*-DDT, and $\beta \pm SE = -2.69 \pm 0.99$, $P = 0.036$ for *o,p'*-DDE) (Table 3.32).

Table 3.32 Multiple linear regression analysis for the association of cord serum levels of POPs with reproductive and thyroid hormones in infants from Mae Rim District

Variables	<i>ln</i> Testosterone ^{a, b}				
	N	β	SE	Partial <i>r</i>	<i>P</i>
<i>ln</i> Cord <i>p,p'</i> -DDE	18	0.11	0.06	0.41	0.103
			TT_4 ^c		
<i>ln</i> Cord <i>p,p'</i> -DDE	39	-0.74	0.30	-0.38	0.02 *
<i>ln</i> Cord <i>p,p'</i> -DDT	37	-0.80	0.39	-0.33	0.049 *
<i>ln</i> Cord <i>o,p'</i> -DDE	9	-2.69	0.99	-0.74	0.036 *

* $P < 0.05$, ** $P < 0.01$

^a adjustment for *ln* birth weight

^b measured in male infants

^c adjustment for gender of infants

Several studies in humans have focused on the effects of HCB, PCBs, and dioxins on thyroid hormone levels. HCB levels were negatively associated with TT_4 levels, but not associated with TSH and FT_4 levels in adult population (Sala et al., 2001b). Prenatal exposure to HCB was not associated with TSH levels in infants (Ribas-Fito et al., 2003b). PCBs and dioxin levels in breast milk were negatively associated with T_4 and T_3 levels, and positively associated with TSH (Koopman- Esseboom et al., 1994; Nagayama et al., 1998). In other studies, PCBs and dioxins levels in breast milk and maternal serum were not associated with thyroid hormone levels, including T_4 , T_3 , and TSH (Fiolet et al., 1997; Longnecker et al., 2000; Steuerwald et al., 2000; Matsuura et al., 2001). However, no studies have considered the association between DDT and thyroid hormones.

Field and laboratory animal studies have reported that DDT has an effect on T_4 and T_3 levels. DDT levels were negatively associated with plasma T_4 levels in male glaucous gulls, and plasma T_3 in gray seals (Verreault et al., 2004; Sormo et al., 2005). In laboratory animal studies, T_4 decreased in flown birds dosed with DDT and T_3 decreased in fasted birds dosed with DDT (Scollon et al., 2004). DDT has been shown to disrupt thyroid hormones by increasing peripheral metabolism of thyroid hormones through an induction of hepatic microsomal P450 enzymes, resulting in increased excretion of plasma thyroxine (Fregly et al., 1968; Bastomsky, 1974; Capen, 1994; Scollon et al., 2004). The study of Bastomsky et al. (1974) found that DDT altered thyroid metabolisms in rats by increasing uridine diphosphate glucuronosyltransferase (UDPGT) activity, causing an increased clearance rate of plasma T_4 . DDT does not bind to the thyroid receptor, indicating that it is unlikely to

disrupt the thyroid axis through a negative feedback mechanism (Cheek et al., 1999). Therefore, it is unlikely to have an effect on TSH secretion.

In the present study, only cord serum TT_4 levels were negatively associated with cord serum levels of p,p' -DDE, p,p' -DDT, and o,p' -DDE (Tables 3.31 and 3.32). However, TT_4 levels of most subjects (92.3%) were within the normal range. It is possible that serum DDT levels might not be high enough to have an obvious effect on hormonal metabolism. Another possibility is that the association for o,p' -DDE may be a chance finding due to multiple comparison, since o,p' -DDE in the present study was detected in only 23.1 % of the studied population, and only 9 tests of association were therefore analysed. In fact, humans are exposed to a variety of the contaminants and the levels of individual contaminants in the body might be rather closely interrelated. It is therefore difficult to assess which compounds have an effect on hormonal status (Longnecker et al., 1997; Hagmar, 2003). The present results therefore suggest that exposure to DDT and its metabolites during fetal development may cause some effects on thyroid hormonal status in infants.

3.8.5 Estrogenic activities of chemicals in maternal and cord sera

Estrogenic activities of chemicals in a total of 127 maternal and cord sera samples were detected in all samples with a geometric mean of 0.85 and 0.52 ng/ml E_2 equivalent, respectively (Table 3.33).

Table 3.33 Estrogenic activities of chemicals in 127 mother-infant pairs from Mae Rim and Chiang Dao Districts

Estrogenic activities of chemicals, ng/ml E ₂ equivalent	GM	AR	S.D.	Min – Max
Maternal serum				
Total (n = 127)	0.85	1.07	0.86	0.15 – 5.69
Mae Rim District (n = 39)	0.98	1.26	1.07	0.20 – 5.69
Chiang Dao District (n = 88)	0.80	0.98	0.74	0.14 – 4.58
Cord serum				
Total (n = 127)	0.52	0.64	0.40	0.09 – 1.94
Mae Rim District (n = 39)	0.57	0.72	0.51	0.12 – 1.94
Chiang Dao District (n = 88)	0.50	0.60	0.33	0.09 – 1.47

3.8.6 The association of POPs with estrogenic activities of chemicals in maternal and cord sera

By determining the association of POPs with estrogenic activities of chemicals in 127 maternal and cord sera using Pearson correlation (Tables 3.3.4 and 3.3.5), only cord serum *p,p'*-DDE levels were positively associated with estrogenic activities of chemicals in the infants from Mae Rim District ($r = 0.37$, $P = 0.032$) (Table 3.3.5). It can be hypothesized that *p,p'*-DDE acts as estrogen disruptor. However, correlation coefficient of this association was rather weak. Many chemicals, including POPs, surfactants, polymers, drugs, phytoestrogens, and other pesticides, can be identified as EDCs (Jaga, 2000; Pocar et al., 2003). It is possible that the studied population might have been exposed to other estrogenic chemicals contaminated in the area.

Table 3.34 Pearson correlation for the association of POPs with estrogenic activities of chemicals in maternal serum

Variables	<i>ln</i> Estrogenic activities of chemicals in maternal serum		
	N	<i>r</i>	<i>P</i>
Total (n = 127)			
<i>ln</i> Maternal <i>p,p'</i> -DDE	127	-0.18	0.074
<i>ln</i> Maternal <i>p,p'</i> -DDT	127	-0.14	0.165
<i>ln</i> Maternal <i>p,p'</i> -DDD	126	-0.11	0.251
<i>ln</i> Maternal <i>o,p'</i> -DDE	67	0.03	0.804
<i>ln</i> Maternal <i>o,p'</i> -DDT	37	-0.28	0.136
<i>ln</i> Maternal heptachlor	117	-0.15	0.143
<i>ln</i> Maternal heptachlor epoxide	69	0.04	0.750
<i>ln</i> Maternal dieldrin	104	0.15	0.173
<i>ln</i> Maternal HCB	30	-.39	0.056
<i>ln</i> Maternal γ -HCH	12	-0.09	0.810
Mae Rim District (n = 39)			
<i>ln</i> Maternal <i>p,p'</i> -DDE	39	-0.03	0.870
<i>ln</i> Maternal <i>p,p'</i> -DDT	39	0.02	0.912
<i>ln</i> Maternal <i>p,p'</i> -DDD	39	-0.06	0.751
<i>ln</i> Maternal <i>o,p'</i> -DDE	8	-0.55	0.162
<i>ln</i> Maternal <i>o,p'</i> -DDT	19	-0.40	0.128
<i>ln</i> Maternal heptachlor	32	-0.16	0.150
<i>ln</i> Maternal heptachlor epoxide	32	-0.03	0.903
<i>ln</i> Maternal dieldrin	35	0.15	0.448
<i>ln</i> Maternal HCB	14	-0.26	0.412
<i>ln</i> Maternal γ -HCH	8	-0.11	0.839
Chiang Dao District (n = 88)			
<i>ln</i> Maternal <i>p,p'</i> -DDE	88	-0.23	0.056
<i>ln</i> Maternal <i>p,p'</i> -DDT	88	-0.21	0.082
<i>ln</i> Maternal <i>p,p'</i> -DDD	87	-0.10	0.387
<i>ln</i> Maternal <i>o,p'</i> -DDE	59	0.11	0.474
<i>ln</i> Maternal <i>o,p'</i> -DDT	18	-0.23	0.445
<i>ln</i> Maternal heptachlor	85	0.05	0.683
<i>ln</i> Maternal heptachlor epoxide	37	0.05	0.786
<i>ln</i> Maternal dieldrin	69	0.06	0.693
<i>ln</i> Maternal HCB	16	-0.22	0.465
<i>ln</i> Maternal γ -HCH	4	-0.58	0.609

Table 3.35 Pearson correlation for the association of POPs with estrogenic activities of chemicals in cord serum

Variables	<i>ln</i> Estrogenic activities of chemicals		
	in cord serum		
	N	<i>r</i>	<i>P</i>
Total (n = 127)			
<i>ln</i> Cord <i>p,p'</i> -DDE	127	0.04	0.728
<i>ln</i> Cord <i>p,p'</i> -DDT	116	-0.01	0.902
<i>ln</i> Cord <i>p,p'</i> -DDD	117	-0.02	0.833
<i>ln</i> Cord <i>o,p'</i> -DDE	62	-0.03	0.824
<i>ln</i> Cord <i>o,p'</i> -DDT	26	-0.38	0.094
<i>ln</i> Cord heptachlor	117	-0.21	0.059
<i>ln</i> Cord heptachlor epoxide	67	-0.24	0.081
<i>ln</i> Cord dieldrin	115	-0.08	0.436
<i>ln</i> Cord HCB	15	-0.33	0.326
Mae Rim District (n = 39)			
<i>ln</i> Cord <i>p,p'</i> -DDE	39	0.37	0.032 *
<i>ln</i> Cord <i>p,p'</i> -DDT	37	0.21	0.252
<i>ln</i> Cord <i>p,p'</i> -DDD	39	0.18	0.320
<i>ln</i> Cord <i>o,p'</i> -DDE	9	-0.41	0.309
<i>ln</i> Cord <i>o,p'</i> -DDT	10	-0.16	0.704
<i>ln</i> Cord heptachlor	31	0.02	0.927
<i>ln</i> Cord heptachlor epoxide	29	-0.01	0.975
<i>ln</i> Cord dieldrin	37	0.00	0.993
<i>ln</i> Cord HCB	0	-	-
Chiang Dao District (n = 88)			
<i>ln</i> Cord <i>p,p'</i> -DDE	88	-0.09	0.475
<i>ln</i> Cord <i>p,p'</i> -DDT	79	-0.11	0.393
<i>ln</i> Cord <i>p,p'</i> -DDD	78	-0.00	0.992
<i>ln</i> Cord <i>o,p'</i> -DDE	53	-0.06	0.702
<i>ln</i> Cord <i>o,p'</i> -DDT	16	-0.36	0.226
<i>ln</i> Cord heptachlor	86	-0.27	0.058
<i>ln</i> Cord heptachlor epoxide	38	-0.36	0.053
<i>ln</i> Cord dieldrin	78	-0.23	0.081
<i>ln</i> Cord HCB	15	-0.14	0.689

nd = not detected