CHAPTER IV

RESULTS AND DISCUSSIONS

The current study aims to observe the concentration of 7 groups of organochlorine pesticides (OCP) residues in sediment and tissue of three species of freshwater mussels (*Uniandra contradens ascia*, *Pilsbryoconcha exilis exilis*, and *Hyriopsis (Limnoscapha) desowitzi*) collected from 3 study sites along Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province. In addition, changes in levels of biomarkers (vitellogenin protein and glutathione-s-transferase specific activity) of freshwater mussels were also observed. The study sites were divided according to the flow direction of the canal into 1) upper stream, 2) middle stream, and 3) lower stream sites. The sampling period started from March 2006-2007 and may be divided into period I (March-May 2006), period II (June-August 2006), period III (September-November 2006), and period IV (December 2006-March 2007). The OCPs in sediment and mussel were determined by gas chromatography (GC) with micro-electron capture detector (μECD). Level of vitellogenin, a yolk-protein precursor, in mussel gonad was determined by an enzyme-linked immunosorbent assay (ELISA) and specific activity of glutathione-s-transferase enzyme in hepatopancreas of mussel was measured spectrophotometrically.

4.1 Residues of Organochlorine Pesticide in Sediment

Composite samples of sediment were collected from 3 study sites during 4 sampling periods. The concentrations of organochlorine pesticides (OCPs) in sediment were measured by DB-35 MS column. The mixture of 17 organochlorine pesticide consisting of 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 was used as a standard.

The levels of OCPs concentration were grouped according to their chemical properties into 7 groups as followed

1) ΣHCH	α-, β-, δ- and γ-HCH
2) ΣHeptachlor	Heptachlor, Heptachlor epoxide
3) Aldrin&Dieldrin	Aldrin, Dieldrin
4) ΣEndrin	Endrin, Endrin aldehyde
5) ΣEndosulfans	Endosulfan I, Endosulfan II, Endosulfan sulfate
6) ΣDDT	4,4'-DDD, 4,4'-DDE, 4,4'DDT
7) Methoxychlor	-

The information on quality control of extraction and clean up steps including limit of detection (LOD), limit of quantitation (LOQ), method detection limit (MDL), and the recovery are shown in Table A.1 (Appendix II). The LOD and LOQ were range from 0.0007 to 0.0508 and from 0.0024 to 0.1695, respectively. The OCP concentrations belowed the LOD were described as ND or not detectable. The MDL of 17 mixed OCPs ranged from 1.64 to 14.87 µg/kg in sediment samples and from 0.52 to 14.27 µg/kg in freshwater mussels. The recovery percentages of 17 mixed OCPs ranged from 74.87% to 106.86% in sediment samples and from 68.83% to 111.55% in freshwater mussels.

For each sampling period, statistical analysis was performed to compare concentrations of OCP residues among the three study sites (upper stream, middle stream and lower stream). It was found that there was no significant difference in mean of the OCPs among these study sites (ANOVA, $p>0.05$) at any study period. Therefore, the data from these 3 study sites in each sampling period were combined in further analysis of mean difference between sampling periods. Table 4.1 shows that there were significant differences in mean value of each OCPs group between sampling periods (ANOVA and Student-Newman-

Keul's multiple comparison, $p < 0.05$) with the highest concentration found in sampling period III (September-November 2006).

Table 4.1 The average concentration of OCPs residues in sediment during four sampling periods at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

OCPs	Concentration of OCPs in sediment (µg/kg dry weight) Mean (± S.E.M)			
	Mar-May 2006 (n=9)	June-Aug 2006 (n=9)	Sep-Nov 2006 (n=9)	Dec 2006-Mar 2007 (n=9)
∑HCH	10.67 ^{a} (±0.15)	13.05 ^{b} (±0.09)	15.33 ^{c} (±0.12)	10.85 ^{a} (±0.05)
∑Heptachlor	11.71 ^{a} (±0.07)	13.62 ^{b} (±0.16)	15.02 ^{c} (±0.05)	11.78 ^{a} (±0.08)
Aldrin and Dieldrin	2.84 ^{a} (±0.07)	3.13 ^{b} (±0.04)	2.30 ^{b} (±0.06)	2.79 ^{a} (±0.04)
∑Endrin	0.83 ^{a} (±0.02)	0.90 ^{b} (±0.02)	0.96 ^{b} (±0.03)	0.77 ^{a} (±0.01)
∑Endosulfans	6.93 ^{a} (±0.16)	7.06 ^{a} (±0.06)	8.77 ^{b} (±0.05)	7.05 ^{a} (±0.03)
∑DDT	10.90 ^{a} (±0.15)	12.03 ^{b} (±0.05)	14.05 ^{c} (±0.13)	11.79 ^{b} (±0.14)
Methoxychlor	0.02 ^{a} (±0.16)	0.03 ^{b} (±0.06)	0.03 ^{b} (±0.05)	0.02 ^{a} (±0.03)

Note: Significant difference ($p < 0.05$) of OCPs between sampling periods is indicated by the difference in superscript letter in the same row.

Results on OCP concentration in sediment showed that ∑Heptachlor, ∑HCH, ∑DDT, and ∑Endosulfan were the predominant groups of OCP residues found in sediment at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province. These results are similar to previous studies by Thongkongowm (2005) and Siriwong (2006) which reported the high value of ∑Heptachlor, ∑DDT, ∑HCH, and ∑Endosulfan (14.7±0.5, 12.1±0.3, 9.4±0.3, and 6.4±0.3 µg/kg, respectively) in sediment of Rangsit agricultural area.

Table 4.2 Pearson's correlation coefficients correlating organochlorine pesticide residue in sediment at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province. Shaded cells indicate significant correlation ($p < 0.05$).

	∑Heptachlor	Aldrin& Dieldrin	∑Endrin	∑DDT	∑Endosulfans	Methoxychlor
∑HCH	0.10 (0.00)	0.681 (0.32)	0.95 (0.05)	0.947 (0.06)	0.89 (0.11)	0.99 (0.06)
∑Heptachlor		0.73 (0.27)	0.95 (0.05)	0.91 (0.09)	0.85 (0.15)	0.96 (0.04)
Aldrin & Dieldrin			0.77 (0.24)	0.42 (0.58)	0.27 (0.73)	0.84 (0.16)
∑Endrin				0.79 (0.21)	0.78 (0.22)	0.88 (0.13)
∑DDT					0.95 (0.05)	0.87 (0.16)
∑Endosulfans						0.69 (0.31)

Further analysis on the association of OCPs residues detected in sediment of Khlong 7 canal is shown in Table 4.2. There were significant correlation between levels of ∑HCH v.s. ∑Heptachlor (Pearson's correlation coefficient = 0.10, $p < 0.05$), ∑HCH v.s. ∑Endrin (0.95, $p < 0.05$), ∑Heptachlor v.s. ∑Endrin (0.96, $p < 0.05$), ∑Heptachlor v.s. methoxychlor (0.96, $p < 0.05$) and ∑DDT v.s. ∑Endosulfans (0.95, $p < 0.05$). The association pattern of these pesticides could reflect the pattern of pesticide use in the area, and by inference, indicate the common type of pests found in this area.

4.2 Residues of Organochlorine Pesticide in Freshwater Mussels

Freshwater mussels were collected from 3 study sites during 4 sampling periods. The year-round field surveys showed 3 species of freshwater mussels that were available at high number and could be used in this study including 1) *Uniandra contradens ascia*, 2) *Pilsbryoconcha exilis exilis*, and 3) *Hyriopsis (Limnoscapha) desowitzi*. However, the distribution of these mussels was not homogenous and seemed to be specific to study site. Therefore, the dominant species was used as the representative for each study site as follows: *Uniandra contradens ascia* was collected from the upper stream site; *Pilsbryoconcha exilis exilis* was collected from the middle stream site; and *Hyriopsis* (*Limnoscapha*) *desowitzi* was collected from the lower stream site.

4.2.1 *Uniandra contradens ascia* (upper stream site)

The average concentrations of seven groups of OCPs retained in *Uniandra contradens ascia* collected from the upper stream site were shown in Table 4.3 and Figure 4.1. In the current study period (March 2006-2007), ∑DDT, ∑HCH, ∑endosulfans and ∑heptachlor were major groups of OCPs found in *Uniandra contradens ascia*. The highest level of OCPs found in this mussel was 68.44 ± 0.297 μg/kg of ∑DDT during September-November 2007. Comparison of mean concentrations showed the significant difference among sampling periods (ANOVA and Student-Newman-Keul's multiple comparison, $p < 0.05$). The highest concentrations of ∑HCH, ∑Heptachlor, aldrin & dieldrin, and ∑endrin were found in sampling period II (June-August 2006), while the highest concentrations of ∑Endosulfans, ∑DDT and methoxychor were found in sampling period III (September-November 2007).

Table 4.3 The average concentration of OCP residues in *Uniandra contradens ascia* collected from the upper stream site during four sampling periods at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

OCPs	Average Concentration of OCPs in *Uniandra contradens ascia* (µg/kg wet weight) Mean (±S.E.M.)			
	Mar-May 2006 (n=5)	June-Aug 2006 (n=5)	Sep-Nov 2006 (n=5)	Dec 2006-Mar 2007 (n=5)
ΣHCH	38.60 [a] (± 0.62)	60.12 [c] (± 0.05)	**54.25** [b] (± 0.05)	39.64 [a] (± 0.11)
ΣHeptachlor	20.16 [a] (± 0.62)	26.87 [c] (± 0.051)	**25.07** [b] (± 0.45)	21.33 [a] (±0.11)
Aldrin and Dieldrin	10.42 [a] (± 0.10)	**13.90** [b] (± 0.82)	11.25 [a] (± 0.13)	**12.70** [b] (±0.13)
ΣEndrin	9.05 [a] (± 0.12)	**22.02** [d] (±0.16)	14.32 [c] (±0.24)	10.37 [b] (±0.11)
ΣEndosulfans	29.31 [a] (± 0.83)	38.65 [b] (± 0.80)	**45.34** [c] (± 0.24)	29.89 [a] (± 0.44)
ΣDDT	53.37 [a] (± 0.50)	59.55 [b] (± 0.56)	**68.44** [c] (± 0.30)	54.41 [a] (± 0.12)
Methoxychlor	1.27 [a] (± 0.02)	1.16 [a] (± 0.01)	**1.91** [c] (± 0.04)	1.58 [b] (± 0.07)

Note: Significant difference ($p < 0.05$) of OCPs between sampling periods is indicated by the difference in superscript letter in the same row.

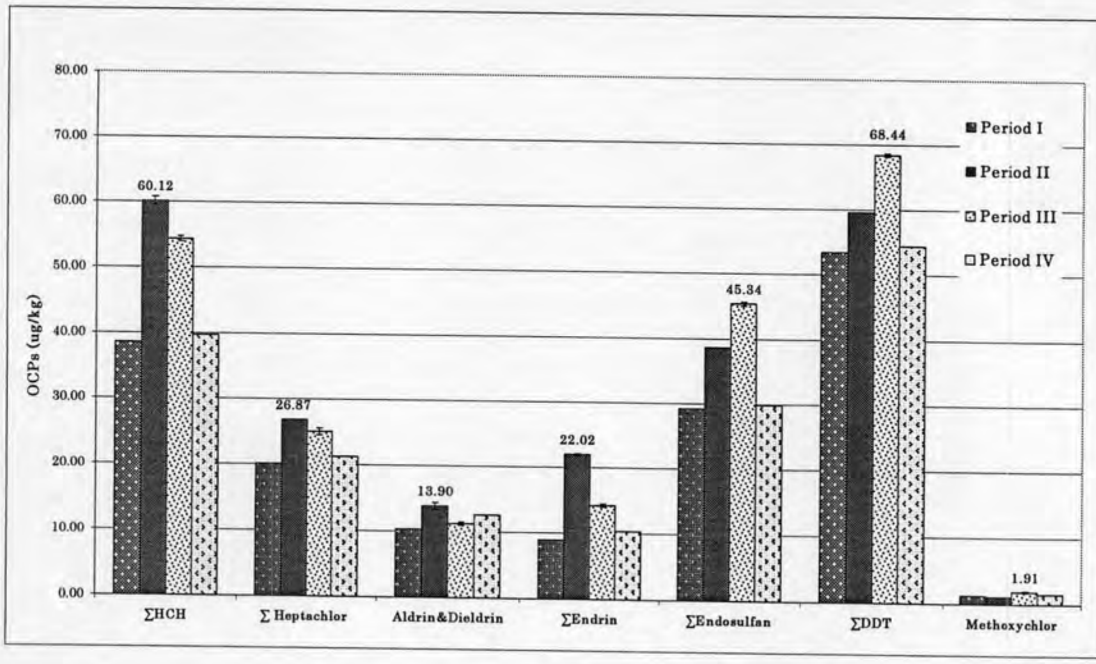

Figure 4.1 The concentration of OCPs residues in *Uniandra contradens ascia* from the upper stream site at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

Association of OCPs residues detected in sediment of Khlong 7 canal and OCP residues detected in *Uniandra contradens ascia* showed several significant correlations (see Appendix III). Presence of ∑HCH or ∑Heptachlor in sediment correlated significantly with levels of ∑DDT and ∑Endosulfans in mussel tissue. Presence of aldrin & dieldrin in sediment markedly correlated with ∑HCH, ∑heptachlor and ∑Endrin in mussels. Presence of ∑Endrin or methoxychlor correlated well with levels of ∑Endosulfans in mussels. It is of interest to note the high significant correlation between levels of ∑DDT in sediment and ∑DDT in mussels.

4.2.2 ***Pilsbryoconcha exilis exilis*** **(middle stream site)**

The concentration of OCPs residues in *Pilsbryoconcha exilis exilis* are shown in Table 4.4 and Figure 4.2. It is of importance to note the lack of *Pilsbryoconcha exilis exilis* sample during the sampling period IV. In the remaining sampling period, ∑DDT, ∑HCH, ∑Endosulfans and ∑heptachlor were major groups of OCPs found in *Pilsbryoconcha exilis exilis*. Comparison of mean concentrations showed the significant difference among sampling periods (ANOVA and Student-Newman-Keul's multiple comparison, $p < 0.05$). The highest concentrations of these OCPs were found in sampling period II (June-August 2006) and sampling period III (September-November 2007). The highest level of OCPs found in this mussel was 61.74 ± 0.10 μg/kg of ∑DDT during September-November 2007.

Table 4.4 The average concentration of OCPs residues in *Pilsbryoconcha exilis exilis* during four sampling periods: Period I (March-May 2006), Period II (June-August 2006), Period III (September- November 2006), and Period IV (December 2006-March 2007), at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

OCPs	Average Concentration of OCPs in *Pilsbryoconcha exilis exilis* (µg/kg; ppb) Mean (± S.E.M.)			
	Mar-May 2006 (n=5)	June-Aug 2006 (n=5)	Sep-Nov 2006 (n=5)	Dec 2006-Mar 2007
∑ HCH	41.53 [a] (± 0.38)	60.12 [b] (± 0.87)	59.49 [b] (± 1.78)	NA
∑ Heptachlor	19.48 [a] (± 0.70)	26.87 [b] (± 0.53)	27.84 [b] (± 0.28)	NA
Aldrin and Dieldrin	10.43 [a] (± 0.34)	13.90 [b] (± 0.82)	15.57 [b] (± 0.20)	NA
∑ Endrin	11.306 [a] (± 0.10)	22.02 [b] (± 0.16)	24.53 [c] (± 0.34)	NA
∑ Endosulfans	33.86 [a] (± 0.40)	38.65 [b] (± 0.80)	39.28 [b] (± 0.11)	NA
∑ DDT	55.32 [a] (± 0.43)	59.55 [b] (± 0.56)	61.74 [c] (± 0.10)	NA
Methoxychlor	1.02 [a] (± 0.04)	1.16 [a] (± 0.01)	1.50 [b] (± 0.03)	NA

Note: 1. Significant difference ($p < 0.05$) of OCPs between sampling periods is indicated by the difference in superscript letter in the same row.

2. NA = not detectable.

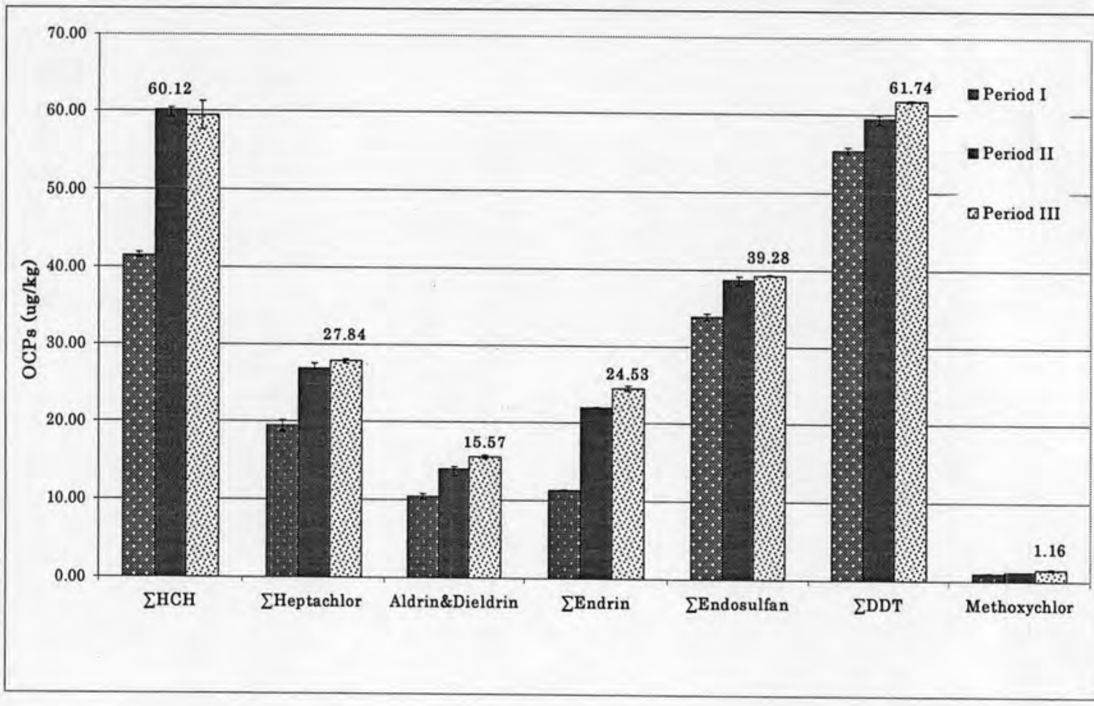

Figure 4.2 The concentrations of OCPs residues in *Pilsbryoconcha exilis exilis* from the middle stream site at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

Association of OCPs residues detected in sediment of Khlong 7 canal and OCP residues detected in *Pilsbryoconcha exilis exilis* showed several significant correlations (Appendix III). Presence of ∑DDT in sediment correlated significantly with levels of methoxychlor in mussel tissue. Presence of methoxychlor in sediment markedly correlated with ∑heptachlor, ∑Endrin and ∑Endosulfans in mussels.

4.2.3 *Hyriopsis* (*Limnoscapha*) *desowitzi* (lower stream site)

The average concentrations of seven groups of OCPs retained in *Hyriopsis (Limnoscapha) desowitzi* collected from the lower stream site were shown in Table 4.5 and Figure 4.3. In the current study period (March 2006-2007), ∑DDT, ∑HCH, ∑endosulfans and ∑heptachlor were major groups of OCPs found in *Hyriopsis (Limnoscapha) desowitzi.* The highest level of OCPs found in this mussel was 66.22 ± 0.158 μg/kg of ∑DDT during September-November 2007. Comparison of mean concentrations showed the significant difference among sampling periods (ANOVA and Student-Newman-Keul's multiple comparison, $p < 0.05$). The highest concentrations of these 7 groups of OCPs were found in sampling period III (September-November 2007).

Table 4.5 The average concentration of OCPs residues in *Hyriopsis* (*Limnoscapha*) *desowitzi* during four sampling periods: Period I (March-May 2006), Period II (June-August 2006), Period III (September- November 2006), and Period IV (December 2006-March 2007), at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

OCPs	Average Concentration of OCPs in *Hyriopsis* (*Limnoscapha*) *desowitzi* (µg/kg; ppb) Mean (± S.E.M)			
	Mar-May 2006 (n=5)	June-Aug 2006 (n=5)	Sep-Nov 2006 (n=5)	Dec 2006-Mar 2007 (n=5)
∑ HCH	41.35 ^{a} (± 0.42)	50.33 ^{b} (± 0.33)	**57.56** ^{c} (± 1.24)	46.09 ^{d} (± 0.52)
∑ Heptachlor	20.84 ^{a} (± 0.56)	22.31 ^{b} (± 0.50)	**25.48** ^{d} (± 0.16)	23.42 ^{c} (± 0.22)
Aldrin and Dieldrin	12.44 ^{a} (± 0.22)	17.82 ^{c} (± 0.34)	**19.69** ^{d} (± 0.48)	14.08 ^{b} (± 0.43)
∑ Endrin	5.41 ^{a} (± 0.60)	8.78 ^{b} (± 0.28)	**10.74** ^{c} (± 0.21)	6.39 ^{b} (± 0.17)
∑ Endosulfans	32.54 ^{a} (± 0.21)	34.92 ^{c} (± 0.28)	**37.56** ^{d} (± 0.45)	34.76 ^{b} (± 0.13)
∑ DDT	56.39 ^{a} (± 0.06)	61.19 ^{c} (± 0.46)	**66.22** ^{d} (± 0.16)	59.12 ^{b} (± 0.19)
Methoxychlor	0.96 ^{a} (± 0.02)	1.15 ^{b} (± 0.17)	**1.87** ^{c} (± 0.06)	1.25 ^{b} (± 0.07)

Note: Significant difference ($p < 0.05$) of OCPs between sampling periods is indicated by the difference in superscript letter in the same row.

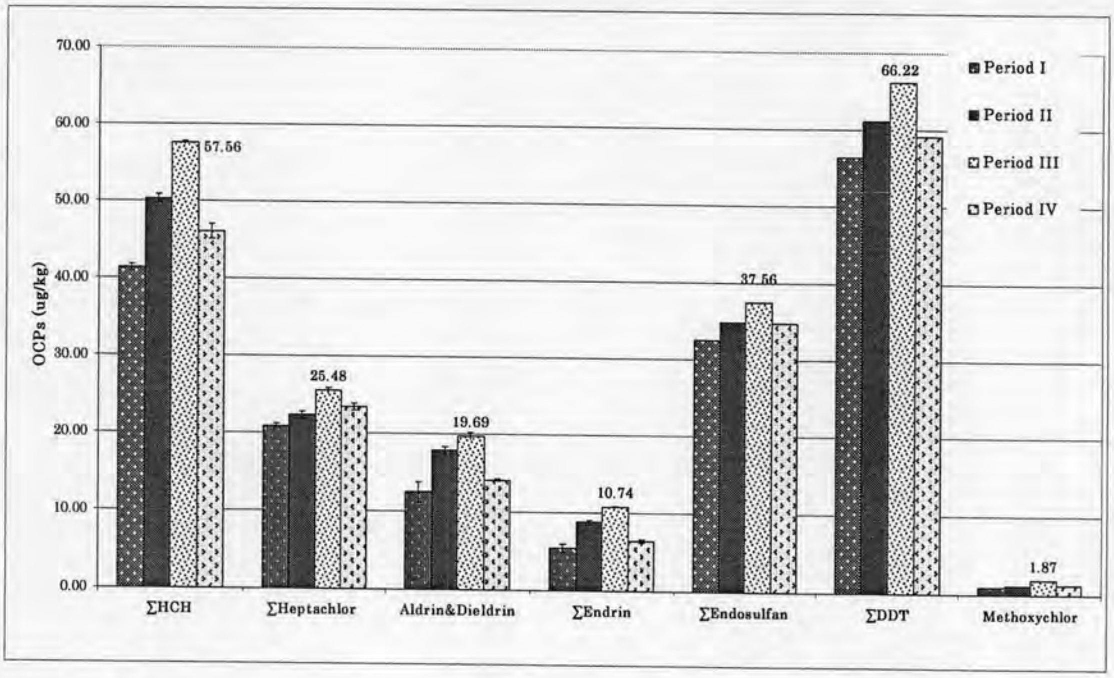

Figure 4.3 The concentrations of OCPs residues in *Hyriopsis* (*Limnoscapha*) *desowitzi* from the lower stream site at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

Association of OCPs residues detected in sediment of Khlong 7 canal and OCP residues detected in *Hyriopsis* (*Limnoscapha*) *desowitzi* showed several significant correlations (Appendix III). Presence of ∑HCH in sediment correlated significantly with levels of ∑HCH, aldrin & dieldrin, ∑endrin and ∑DDT in mussel tissue. Presence of ∑Heptachlor correlated well with levels of aldrin & dieldrin, ∑Endrin and ∑DDT in mussels. Levels of ∑DDT in sediment correlated significantly with levels of ∑HCH, ∑DDT, ∑Endosulfans and methoxychlor in mussels. Presence of ∑Endosulfans markedly correlated with level of methoxychlor in mussels. Presence of methoxychlor in sediment showed significant correlation with levels of aldrin & dieldrin and ∑endrin in mussels.

Overall result of OCP contamination in sediment and freshwater mussels indicated high concentration of OCPs during sampling period II (June-August 2006) and period III (September-November 2006). The high levels of OCPs found in sediment and mussels may be because of the heavy rain and runoff that effectively transferred these compounds into canal during the rainy season.

Although all of these organochlorine pesticides had been banned for few decades, their residues are still found in the soil and other terrestrial environments (Thirakhupt *et al.*, 2006). The current study provides further evidence that, compared to levels found in soil and sediment, OCP residues were detectable at much higher levels in the filter-feeding macroinvertebrates (i.e. freshwater mussels). Remarkably, ∑endosulfans was still illegally used while this study was performing. It was applied into the paddy field for every crop to mainly kill the golden apple snail (*Pomacea* sp.). Likewise, heptachlor, heptachlor epoxide, aldrin, and dieldrin which can be found in the soil around buildings from the termite control schemes may be discharged into the canal as well (Thongkongowm, 2005).

4.3 Vitellogenin

Vitellogenin, the egg yolk protein protein, has become a popular biomarker for measuring exposure of animals to contaminants that can interfere with reproductive and endocrine functions of animals. Vitellogenin was extracted from gonad of 3 species of freshwater mussels (*Uniandra contradens ascia, Pilsbryoconcha exilis exilis* and *Hyriopsis (Limnoscapha) desowitzi*) collected from Khlong 7 canal during 4 sampling periods. Although the ELISA used in this study is based on vitellogenin protein of *Elliptio complanata*, a native mussel species of the USA, the gonad extract of Thai species in this study showed high cross-reactivity with the assay. The levels of vitellogenin in mussel gonad extract were expressed as µg of vitellogenin per mg of total protein.

In every species of freshwater mussels examined, the levels of vitellogenin were relatively high (Table 4.6). There were significant seasonal fluctuations in levels of vitellogenin proteins in gonad of the mussels with the highest levels of vitellogenin detected in sampling period III (September-November 2006). *Uniandra contradens ascia*, *Hyriopsis (Limnoscapha) desowitzi*, and *Pilsbryoconcha exilis exilis* have highest level of vtg at 160.21 (±6.72), 268.78 (±41.55), and 94.14 (±12.83) µg/mL protein, respectively. Since the data on levels of vitellogenin in freshwater mussels in Thailand or other tropical region countries is not available, comparison of the vitellogenin protein level with the current study is thus unlikely. However, the results from this study provided important basis for further study in reproductive biology of the freshwater mussels in Thailand.

Table 4-6 The average concentration of vitellogenin from gonad extract of three species of freshwater mussels collected during four sampling periods: Period I (March-May 2006), Period II (June-August 2006), Period III (September-November 2006), and Period IV (December 2006-March 2007) at Khlong 7 canal, Rangsit agricultural area, Pathum Thani Province.

Freshwater Mussels	Average Concentration of Vitellogenin (µg/mg protein) Mean (±S.E.M)			
	Mar-May 2006 (n=5)	June-Aug 2006 (n=5)	Sep-Nov 2006 (n=5)	Dec 2006-Mar 2007 (n=5)
Uniandra contradens ascia	72.32^{a} (±2.41)	89.31^{a} (±4.12)	160.21^{c} (±6.72)	116.82^{b} (±14.92)
Pilsbryoconcha exilis exilis	64.55^{a} (±3.11)	66.01^{a} (±3.31)	94.14^{b} (±12.83)	NA
Hyriopsis (Limnoscapha) desowitzi	112.53^{ab} (±8.30)	232.42^{c} (±32.77)	268.78^{c} (±41.55)	199.47^{bc} (±30.74)

Note: 1. Significant difference ($p < 0.05$) between sampling periods is indicated by difference in superscript letter in the same row.

2. NA = not detectable.

4.3.1 *Uniandra contradens ascia* (upper stream site)

Additional analysis was performed on the correlation of vitellogenin concentration and OCPs residue in mussel tissue (Appendix III). The vitellogenin concentration of *Uniandra contradens ascia* significantly correlated with methoxychlor concentration in their body (Pearson's correlation coefficient = 0.95, $p < 0.05$). The pattern of change in vtg concentration in relation to levels of OCPs in mussel tissue is shown in Figure 4.4.

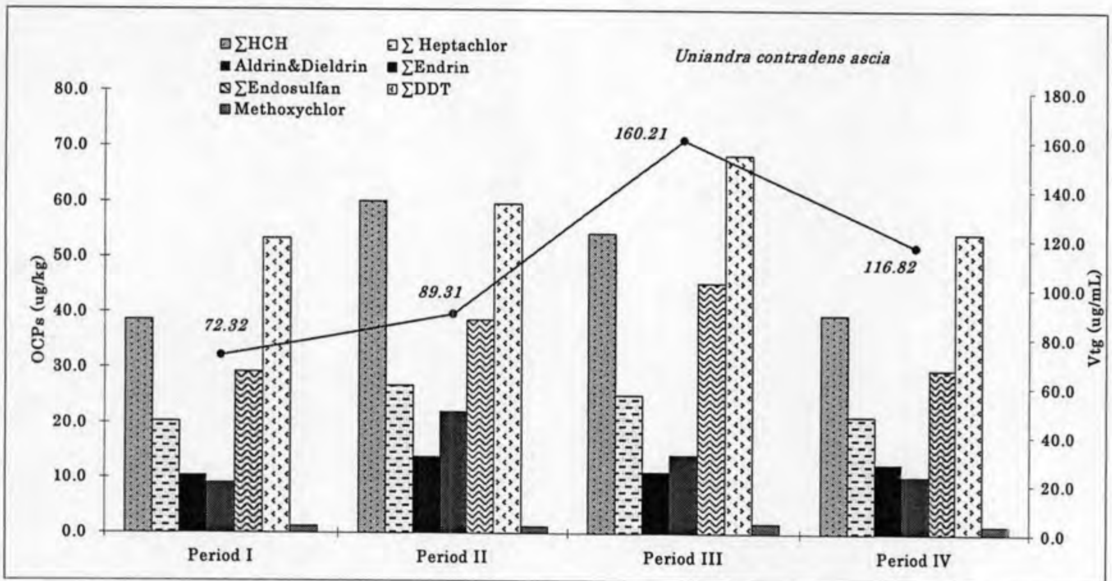

Figure 4.4 The average concentrations of vitellogenin in gonad extract of *Uniandra contradens ascia* and average concentration of OCP residues in the mussels during four sampling periods at Khlong 7, Rangsit agricultural area.

4.3.2 *Pilsbryoconcha exilis exilis* (middle stream site)

In *Pilsbryoconcha exilis exilis*, the vitellogenin concentration markedly correlated with concentration of ∑Endosulfan in sediment at the middle stream site (Pearson's correlation coefficient = 1.00, $p < 0.05$; Appendix III). The general pattern of change in vtg concentration in relation to levels of OCPs in mussel is shown in Figure 4.5.

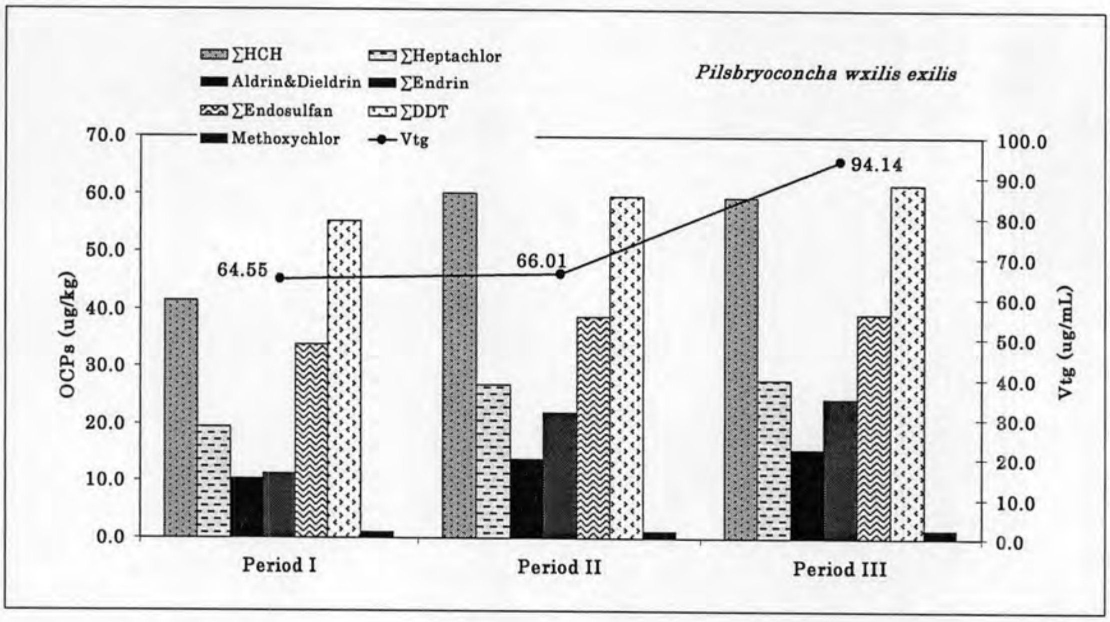

Figure 4.5 The average concentrations of vitellogenin in gonad extract of *Pilsbryoconcha exilis exilis* and average concentration of OCP residues in the mussels during four sampling periods at Khlong 7, Rangsit agricultural area.

4.3.3 *Hyriopsis* (*Limnoscapha*) *desowitzi* (lower stream site)

In *Hyriopsis* (*Limnoscapha*) *desowitzi*, the vitellogenin concentration markedly correlated with concentration of ΣHCH and ΣEndosulfan in their body (Pearson's correlation coefficient = 0.95 and 0.96, $p < 0.05$, respectively; Appendix III). Figure 4.6 shows the general pattern of change in vitellogenin concentration in relation to levels of OCPs found in mussel tissue.

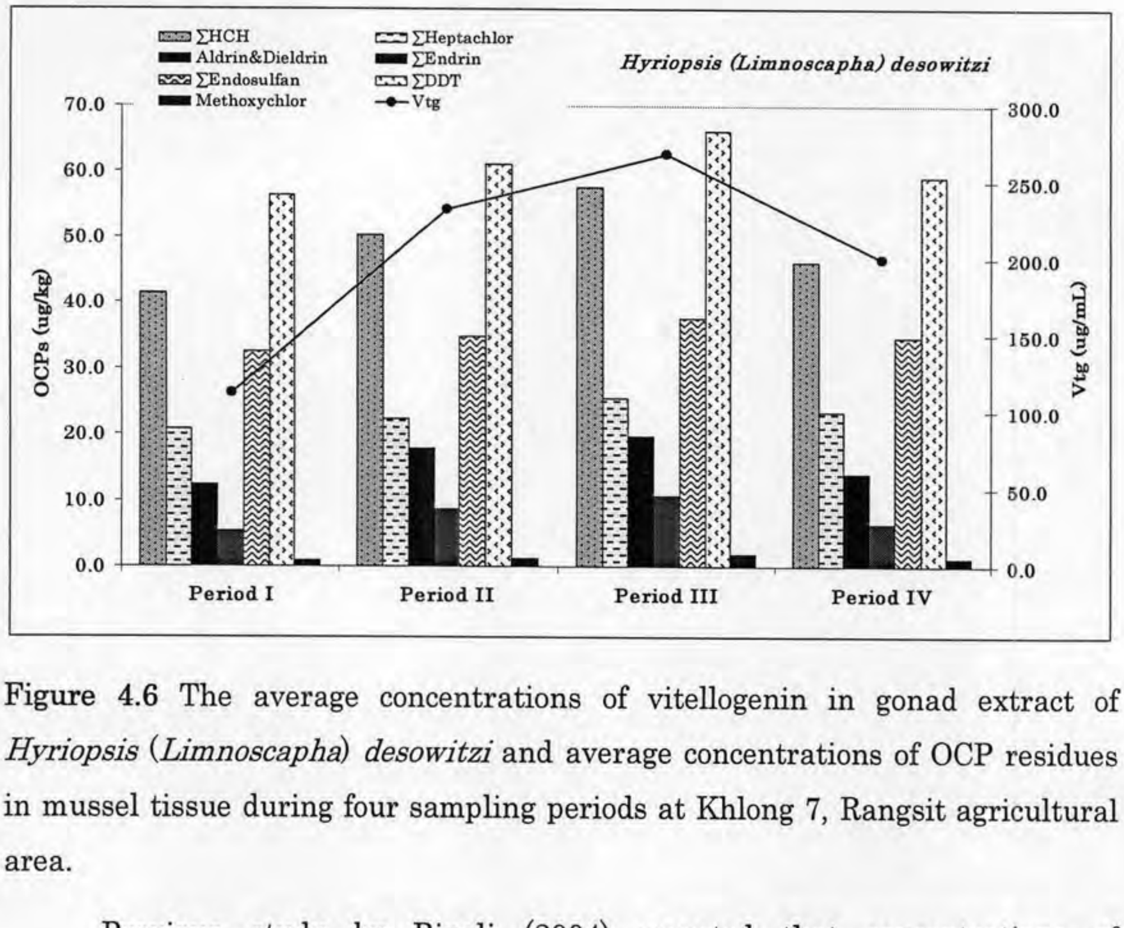

Figure 4.6 The average concentrations of vitellogenin in gonad extract of *Hyriopsis* (*Limnoscapha*) *desowitzi* and average concentrations of OCP residues in mussel tissue during four sampling periods at Khlong 7, Rangsit agricultural area.

Previous study by Bineli (2004) reported that concentrations of organochlorine pesticides such as DDT may affect the endocrine system and reproductive abnormalities of many animals. The significant correlation of the increased vitellogenin levels and the elevated levels of several contaminants found in this study could provide insight into further cause-and-effect study.

4.4 Glutathione S-Transferase

Glutathione S-transferase is the Phase II enzyme activity involved in the process of xenobiotic biotransformation and, potentially, a biomarker for organic contamination in aquatic system. GST was extracted from hepatopancreas of 3 species of freshwater mussels (*Uniandra contradens ascia*, *Pilsbryoconcha exilis exilis* and *Hyriopsis (Limnoscapha) desowitzi*) collected from Khlong 7 canal during 4 sampling periods. GST activity was measured as 1, 2-chlorodinitrobenze conjugation. The results of specific enzyme activity of GST were expressed as µmol/min per mg of total protein.

Since the GST is a biotransformation enzyme, it is usually used as a biomarker. The elevated level of GST is normally associated with exposure to organic pollutants (Won *et al.*, 2005). In this study, specific activity of GST from three freshwater mussels (*Uniandra contradens ascia*, *Pilsbryoconcha exilis exilis*, and *Hyriopsis* (*Limnoscapha*) *desowitzi*) showed significantly fluctuation during this year-round study (Table 4.7). The highest GST activities were found in sampling period II (June-August 2006) in *Uniandra contradens ascia*, and sampling period III (September-November 2006) in *Pilsbryoconcha exilis exilis*, and *Hyriopsis* (*Limnoscapha*) *desowitzi*.

Table 4.7 The average specific activity of GST from 3 species of freshwater mussels collected during four sampling periods: period I (March-May 2006), period II (June-August 2006), period III (September- November 2006), and period IV (December 2006-March 2007) at Khlong 7 canal, Rangsit agricultural srea, Pathum Thani Province.

Freshwater mussels	Average specific activity of GST (μmol/min/mg protein) x 1,000 Mean (±S.E.M)			
	Mar-May 2006 (n=5)	June-Aug 2006 (n=5)	Sep-Nov 2006 (n=5)	Dec 2006-Mar 2007 (n=5)
Uniandra contradens ascia	116.88 [a] (±0.01)	228.96 [b] (±0.03)	232.96 [b] (±0.03)	167.08 [ab] (±0.01)
Pilsbryoconcha exilis exilis	173.61 (±0.02)	193.75 (±0.03)	207.64 (±0.02)	NA
Hyriopsis (Limnoscapha) desowitzi	197.92 (±0.04)	210.42 (±0.03)	247.92 (±0.02)	222.92 (±0.01)

Note: 1. Significant difference ($p < 0.05$) between sampling periods is indicated by difference in superscript letter in the same row.

2. NA = not detectable.

General patterns of change in GST specific activities in hepatopancreas of mussel in relation to levels of OCPs found in mussel tissue are shown in Figure 4.7 (*Uniandra contradens ascia*), Figure 4.8 (*Pilsbryoconcha exilis exilis*) and Figure 4.9 (*Hyriopsis (Limnoscapha) desowitzi*).

4.4.1 *Uniandra contradens ascia* (upper stream site)

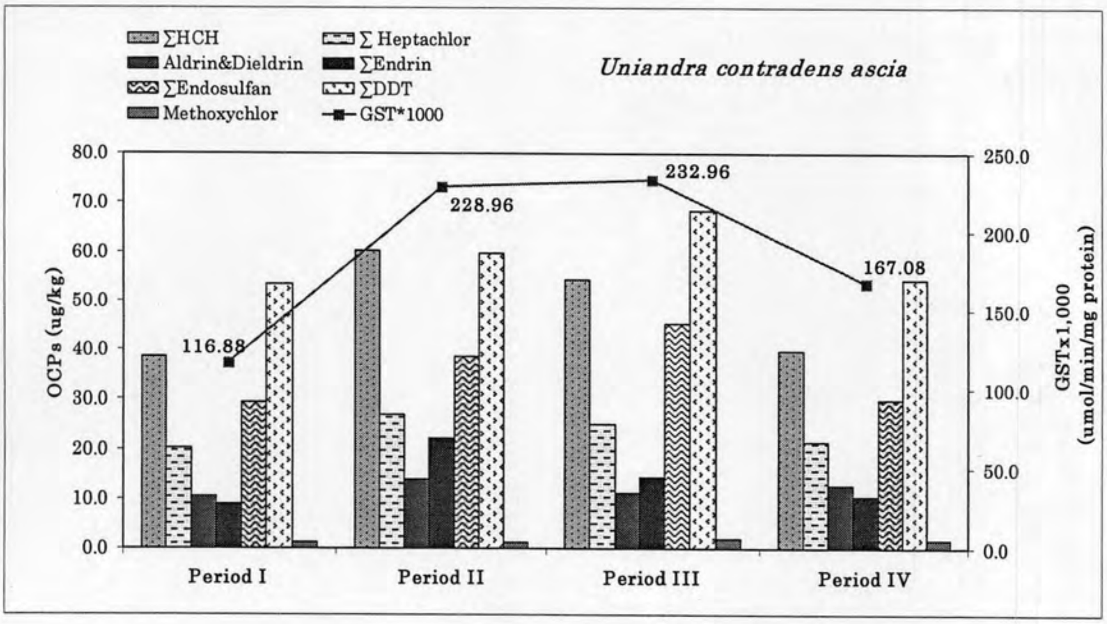

Figure 4.7 The average of specific activity of GST in hepatopancreas extract of *Uniandra contradens ascia* and average of OCP concentrations in the mussels during four sampling periods at Khlong 7, Rangsit agricultural area.

4.4.2 *Pilsbryoconcha exilis exilis* (middle stream site)

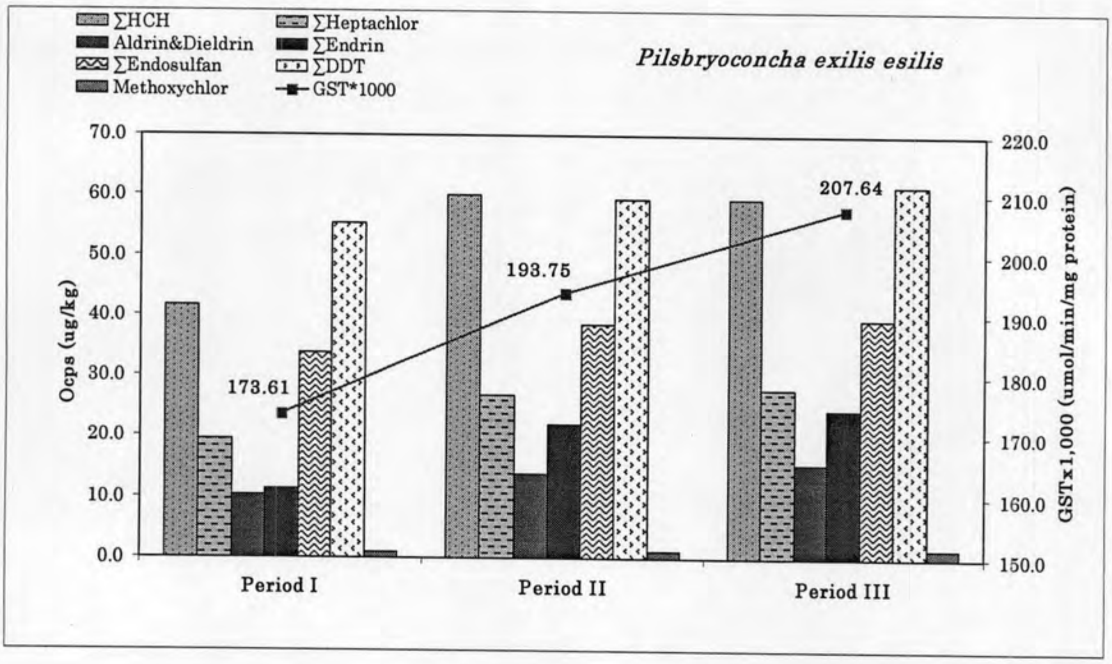

Figure 4.8 The average of specific activity of GST in hepatopancreas extract of *Pilsbryoconcha exilis exilis* and average of the OCP concentrations in mussels during four sampling periods at Khlong 7, Rangsit agricultural area.

4.4.3 *Hyriopsis* (*Limnoscapha*) *desowitzi* (lower stream site)

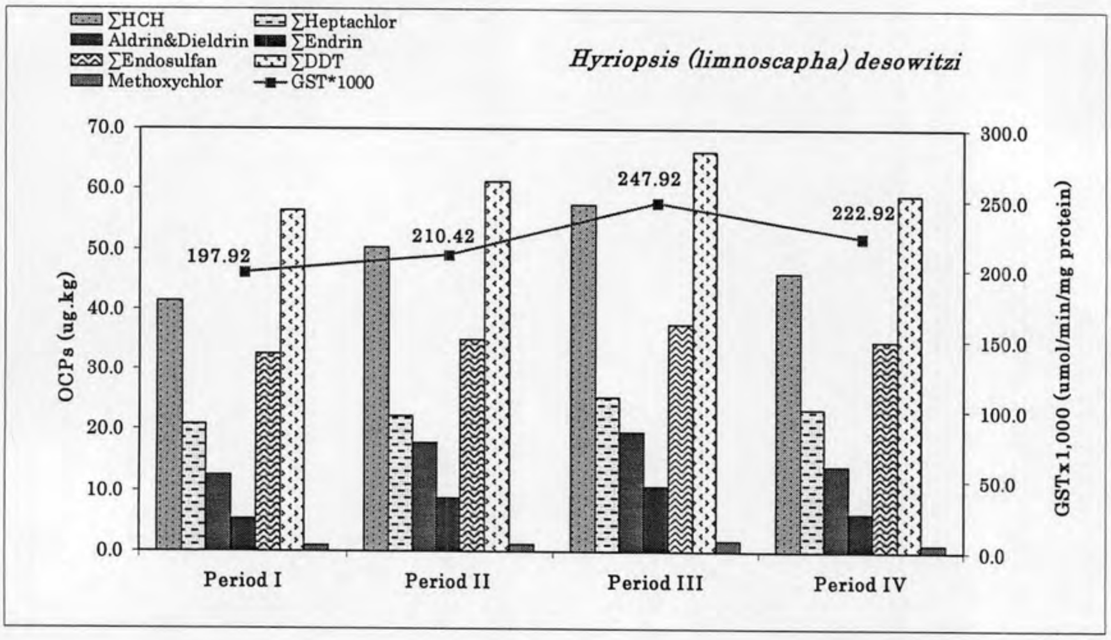

Figure 4.9 The average of specific activity of GST in hepatopancreas extract of *Hyriopsis* (*Limnoscapha*) *desowitzi* and average of the OCP concentrations in the mussels during four sampling periods at Khlong 7, Rangsit agricultural area.

Analysis for association of GST activity in mussel hepatopancreas and OCP residues in sediment and mussel tissue showed several significant correlation (Appendix III). In *Uniandra contradens ascia* significantly correlated with methoxychlor residue in sediment at upper stream site (Pearson's correlation coefficient = 0.97, $p < 0.05$), and ∑HCH residue in their body (Pearson's correlation coefficient = 0.95, $p < 0.05$). In *Pilsbryoconcha exilis exili* significantly correlated with ∑HCH and ∑Endrin residue in sediment at middle stream site (Pearson's correlation coefficient = 1.00, 1.00, $p < 0.05$), and ∑DDT residue in mussel tissue (Pearson's correlation coefficient = 1.00, $p < 0.05$). In *Pilsbryoconcha exilis exilis* significantly correlated with ∑HCH and ∑Endrin residue in sediment at middle stream site (Pearson's correlation coefficient = 1.00, 1.00, $p < 0.05$), and ∑DDT residue in mussel tissue (Pearson's correlation coefficient = 1.00, $p < 0.05$). Since the elevated activity of GST in these mussel species associated with the increased level of OCP contamination, the use of GST as biomarker of OCP exposure should be considered in these species. The results are in accordance with Gagné *et al.* (2004) which reported that the elevated GST activity in freshwater mussel (*Elliptio complanata*) indicated the exposure to organic toxicants in the municipal effluent including pesticides, estrogens, bisphenol A, and polyaromatic hydrocarbons (PAHs) that can potentially harm aquatic biota.

It is of interest to note that in *Hyriopsis* (*Limnoscapha*) *desowitzi*, GST activity showed significant correlation with vitellogenin concentration (Pearson's correlation coefficient = 0.98, $p < 0.05$). This provided further evidence of the link between xenobiotic exposure and reproductive function of animals.