

---

# Monitoring POPs in Selected Biota in the Philippines

---

Evangeline C. Santiago, Ph.D.

Charita S. Kwan, MSc.

Research and Analytical Services Laboratory, Natural  
Sciences Research Institute

University of the Philippines

Diliman, Quezon City, Philippines

---

# Introduction

- The United Nations University, in cooperation with Shimadzu Corporation of Japan, has been undertaking the project **Monitoring of Organochlorine Pesticides in the Asian Hydrosphere** with participation of twelve countries in Asia since 1999.
-

---

# Introduction

- Since 2006, the project has focused on the investigation of water and biota in river and coastal environments.
  - The pesticides were investigated in freshwater shrimps from a river system (2006), and in fish (2007) and in squid (2008) from coastal areas in the Philippines.
-

---

# POPs Monitoring in Biota the Philippines

## ■ Target OCPs

- POPs pesticides covered by the Stockholm Convention
    - Hexachlorobenzene, Heptachlor, Aldrin, trans-chlordane, cis-Chlordane, Dieldrin, Endrin, o,p' DDE, p,p' DDE, o,p' DDD, p,p' DDD, o,p' DDT, p,p' DDT and Mirex
  - OCPs not covered by the Stockholm Convention but may still be in use in the Philippines
    - alpha BHC, gamma BHC, Endosulfan1, Methoxychlor, trans Nonachlor, and cis Nonachlor
-

---

# UNU- Prescribed Methodology

- sampling frequency
    - rainy season and dry season
  - analytical methodology
    - solvent extraction using homogenizer (biota), liquid/liquid extraction (biota and water), clean-up with florisil, and silica, and C<sub>18</sub> columns and GCMS SIM analysis, quantitation by internal standard method
-

---

# UNU- Prescribed Methodology

- quality assurance protocols
    - duplicate analysis
    - use of method blank
    - recovery of surrogate
    - determination of quantitative limits,
    - use of OCP- spiked samples
      - 25 ng in 5 g for shrimps
      - 100 ng in 5 g for fish
      - 100 ng in 2 g for squid
      - 25 ng in 1 L for water)
-

---

# UNU- Prescribed Methodology

- treatment of biota samples
    - classified according to size/weight
    - analyzed as composite samples
  - reporting
    - report results above Quantitation Limit (QL)
    - $QL = 10 \times \text{std deviation of repeated injection of } 10 \text{ ppb std / wt or vol of sample}$
-

# Sampling Sites

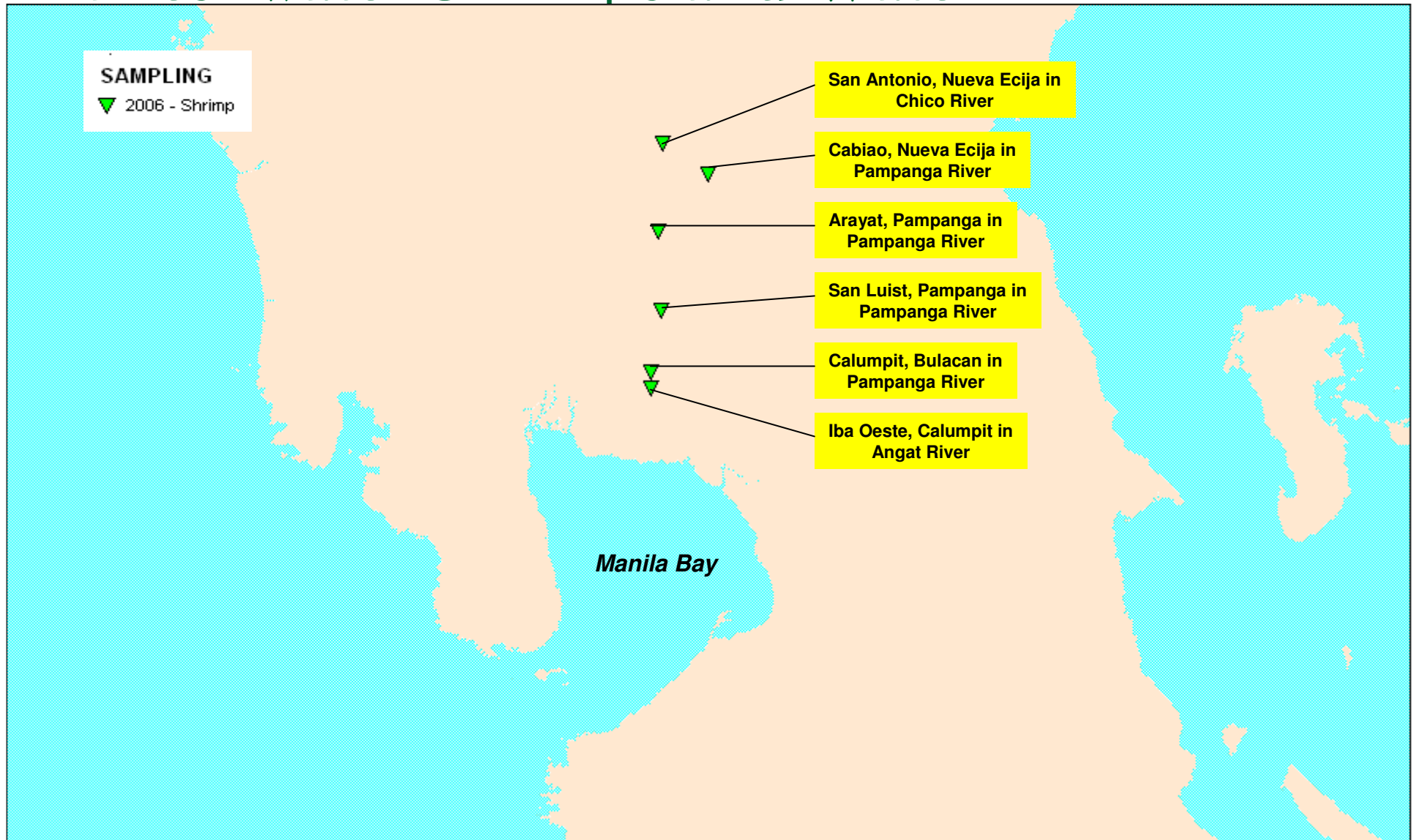
## 2006-2008





# 2006 Sampling Site

## Freshwater Shrimps and Water



# 2006 Monitoring

## Freshwater Shrimps and Water Samples



*Macrobrachium rosenbergii*

*Shrimp Species Collected*

*Macrobrachium rosenbergii rosenbergii*

*Macrobrachium rosenbergii daquete*

*Macrobrachium idella*

*Metapenaeus ensis*



*Shrimp collecting gadget*

# 2006 Results of Monitoring

## Freshwater Shrimp Muscles and Water Samples

### ■ Quality Assurance

Quantitative Limit	Shrimps	Water
	ng/g wet wt	µg/L
Hexachlorobenzene	1	0.005
Heptachlor	2	0.008
Aldrin	1	0.006
trans-chlordane	1	0.007
o,p' -DDE	1	0.007
cis-chlordane	1	0.007
Dieldrin	1	0.006
p,p' -DDE	2	0.008
o,p' -DDD	2	0.009
Endrin	3	0.01
p,p' -DDD	2	0.008
o,p' -DDT	1	0.006
p,p' -DDT	2	0.008
Mirex	2	0.009
alpha-BHC	2	0.009
gamma-BHC	2	0.01
Endosulfan I	3	0.01
trans-nonachlor	1	0.006
cis- nonachlor	2	0.008
Methoxychlor	1	0.006

% Recovery of Spiked Sample	Shrimp	Water
HCB	80	107
Heptachlor	125	94
Aldrin	107	104
trans chlordane	86	85
o,p'-DDE	119	96
cis-chlordane	90	98
Dieldrin	103	99
p,p'-DDE	121	105
o,p'-DDD	107	47
Endrin	184	105
p,p'-DDD	97	91
o,p'- DDT	80	74
p,p'-DDT	139	90
Mirex	74	93
alpha-BHC	108	91
gamma-BHC	128	68
Endosulfan I	93	80
trans-nonachlor	72	96
cis-nonachlor	69	85
Methoxychlor	100	61
p,p'-DDT-13C12	102	113

## 2006 Results of Monitoring Freshwater Shrimp Muscles and Water Samples

Sampling Site	Shrimp Samples		Water Samples	
	Dry Season (Mar-Apr, 2006)	Rainy Season (Jul-Sep, 2006)	Dry Season (Mar-Apr, 2006)	Rainy Season (Jul-Sep, 2006)
Calumpit, Pampanga River	All OCPs either non detected or less than QL in muscles	Methoxychlor (1.11 ng/g)	p,p' DDT (0.02 µg/L)	Methoxychlor (0.15, 0.029µg/L)
	In head of shrimps p,p' DDT (7.68 ng/g) transnonachlor (2.34 ng/g) Methoxychlor (2.90-13.4 ng.g)	No head sample analyzed		
San Luis, Pampanga River	All OCPs either non detected or less than QL in muscles	All OCPs either non detected or less than QL in muscles	All OCPs either non detected or less than QL	Trans-chlordane (0.0080 µg/L)
	In head of shrimps p,p' DDT (1.64-6.85 ng/g) transnonachlor (3.41 ng/g) Methoxychlor (10.4 ng.g) Endosulfan (1.13 ng/g)	No head sample analyzed		

Note: Concentration of biota in ng/g wet weight

## 2006 Results of Monitoring Freshwater Shrimp Muscles and Water Samples

Sampling Site	Shrimp Samples		Water Samples	
	Dry Season (Mar-Apr, 2006)	Rainy Season (Jul-Sep, 2006)	Dry Season (Mar-Apr, 2006)	Rainy Season (Jul-Sep, 2006)
Arayat, Pampanga River	All OCPs either non detected or less than QL in muscles	In muscles Endosulfan 1 (3.96 ng/g)	p,p' DDT (0.03 µg/L)	Trans-chlordane (0.0090 µg/L)
	In head p,p' DDE (4.83 ng/g) p,p' DDT(20.3, 15.7, 23.2 ng/g) trans nonaclor( 1.22, 1.24 ng/g) Methoxychlor ( 7.88 ng/g)	No head sample analyzed		p,p' DDT (0.030 µg/L)  Endosulfan 1 (0.029 µg/L)
Cabiao, Pampanga River	All OCPs either non detected or less than QL in muscles	In muscles Endosulfan 1 (4.81 ng/g) Trans-chlordane (1.11 ng/g, 2.55 ng/g)	p,p' DDT (0.017 µg/L)	Trans-chlordane (0.0080 µg/L)
		In Head Cis chlordane(1.02 ng/g) P,p' DDT (7.38 ng/g) Endosulfan1 (9.23 ng/g) Methoxychlor( 7.23 ng/g)		

## 2006 Results of Monitoring Freshwater Shrimp Muscles and Water Samples

Sampling Site	Shrimp Samples		Water Samples	
	Dry Season (Mar-Apr, 2006)	Rainy Season (Jul-Sep, 2006)	Dry Season (Mar-Apr, 2006)	Rainy Season (Jul-Sep, 2006)
San Antonio, Chico River	In muscles Trans-chlordane (1.98 ng/g 1.88 ng/g) p,p' DDT (2.55 ng/g , 2.16 ng/g)	All OCPs either non detected or less than QL in muscle	Trans-chlordane (0.0074 µg/L)  p,p' DDT (0.013 µg/L)	All OCPs either non detected or less than QL
	In head Dieldrin (1.97 ng/g) p,p' DDE (2.93 ng/g) p,p' DDT (7.72 ng/g) Endosulfan1 (17.7 ng/g) Trans nonachlor (1.04 ng/g) Methoxychlor (5.73 ng/g)	In head p,p' DDT (19.1 ng/g) endrin (6.54 ng/g) trans nonachlor (1.44 ng/g) methoxychlor ( 12.1 ng/g)		

---

## 2006 Results of Monitoring

### Freshwater Shrimp Muscles and Water Samples

- During the dry season
    - The shrimp muscle sample and the water sample from the site in San Antonio in Chico River showed contamination of trans chlordane and p,p' DDT.
    - The water sample from the most sites of Pampanga river showed contamination of p,p' DDT but this pesticide was not detected in the shrimp muscle samples.
  - During the rainy season
    - The shrimp muscle samples in the upstream sites (Cabiao) and in (Arayat) of Pampanga River showed contamination of trans chlordane and endosulfan1
    - The water samples in most sites in Pampanga River showed the presence of trans chlordane while p,p' DDT and endosulfan 1 were detected in Arayat, the confluence of Chico and Pampanga Rivers.
    - The source of Methoxychlor detected in Calumpit may have come from nearby polluting sources.
  - During dry and rainy seasons
    - All head samples analyzed showed varying concentrations of p,p' DDT, trans nonachlor, and methoxychlor. Some head samples showed p,p' DDD, alpha BHC and endosulfan
-

# 2007 Sampling Sites Sea Bass and Water





---

# Samples

**Fish Samples (*Lates Calcarifer*) from Tagkawayan, Quezon**



**Ave Weight: 60.3 grams**

---

# 2007 Results of Monitoring Sea Bass Fillet and Water Samples

## ■ Quality Assurance

Quantitative Limits	Fish	Water
	ng/g, wet wt	µg/L
HCB	0.4	0.002
Heptachlor	0.4	0.002
Aldrin	0.6	0.003
trans chlordane	1	0.007
cis chlordane	1	0.006
Dieldrin	2	0.008
Endrin	1	0.005
o,p' DDE	0.9	0.005
p,p' DDE	0.7	0.004
o, p' DDD	0.5	0.003
p, p' DDD	0.8	0.004
o, p' DDT	0.8	0.004
p, p' DDT	0.5	0.003
Mirex	0.4	0.002
alpha BHC	0.9	0.004
beta BHC	2	0.008
gamma BHC	1	0.005
delta BHC	1	0.007
Heptachlor epoxide	2	0.009
Endosulfan I	3	0.01
trans nonachlor	2	0.008
cis nonachlor	2	0.008
Methoxychlor	1	0.005

% Recovery of spiked samples	Fish, n=4	Water, n=3
HCB	64	54
Heptachlor	86	76
Aldrin	91	73
trans chlordane	100	95
cis chlordane	100	98
Dieldrin	97	96
Endrin	112	98
o,p' DDE	100	101
p,p' DDE	100	101
o, p' DDD	100	102
p, p' DDD	103	105
o, p' DDT	105	103
p, p' DDT	108	101
Mirex	100	96
alpha BHC	50	89
beta BHC	57	110
gamma BHC	101	86
delta BHC	19	83
Heptachlor epoxide	96	99
Endosulfan I	92	95
trans nonachlor	98	100
cis nonachlor	99	102
Methoxychlor	100	38
p,p' DDT 13C12	104	97

## 2007 Results of Monitoring Sea Bass Fillet and Water Samples

Sampling Site	Fish Samples		Water Samples	
	Rainy season (Jul-Sep, 2007)	Dry Season (Dec, 2007-Feb, 2008)	Rainy season (Jul-Sep, 2007)	Dry season (Dec, 2007-Feb, 2008)
Tagkawayan, Quezon, Ragay Gulf	All OCPs either non detected or less than QL	Trans chlordane in six samples (1.02-1.86 ng/g) p,p' DDT in one sample (0.65 ng/g) o,p' DDT in two samples (0.84-0.94 ng/g) p,p' DDD in two samples (0.91-0.96 ng/g) gamma BHC in one sample (3.0 ng/g) beta BHC in one sample (9.5 ng/g )	All OCPs either non detected or less than QL	Trans chlordane (0.0094 µg/L)
Estancia, Iloilo, Visayan Sea	All OCPs either non detected or less than QL	No Samples	All OCPs either non detected or less than QL	No Sample
Barra, Roxas City, Mouth of Pnay River	All OCPs either non detected or less than QL	No Samples	All OCPs either non detected or less than QL	No Sample

Note: Concentration of biota in ng/g wet weight

---

## 2007 Results of Monitoring Sea Bass Fillet and Water Samples

### ■ Observations

- Wild Sea Bass is not commonly found in coastal areas in the Philippines. The project had difficulty in getting wild Sea Bass samples.
  - During the rainy season
    - The fish muscle and water samples from all the three sampling sites did not indicate contamination with OCPs.
  - During the dry season
    - The only fish samples collected during the season which came from Tagkawayan in Ragay Gulf showed contamination with trans chlordane, DDTs and BHCs.
    - Trans Chlordane was also detected in the water in Ragay Gulf.
-

# 2008 Sampling Sites

## Squid and Water



---

2008 Monitoring  
Squid Samples



*Sepoteuthis lessolanina*



A and B - Male, C- Female

---

## 2008 Results of Monitoring Squid Liver and Muscle and Water Samples

Quantitative Limit	Squid	Water	% Recovery	Squid Liver	Squid Muscle	Water
	ng/g wet wt	µg/L		n=1	n=2	n=3
HCB	0.7	0.002	HCB	69	58	72
Heptachlor	2	0.004	Heptachlor	84	72	99
Aldrin	2	0.004	Aldrin	251	78	103
trans-Chlordane	2	0.004	trans-Chlordane	103	97	91
cis-Chlordane	3	0.005	cis-Chlordane	99	95	100
Dieldrin	6	0.02	Dieldrin	95	94	89
Endrin	4	0.008	Endrin	101	123	76
o,p'-DDE	2	0.003	o,p'-DDE	95	86	111
p,p'-DDE	3	0.005	p,p'-DDE	96	92	104
o,p'-DDD	3	0.005	o,p'-DDD	98	98	109
p,p'-DDD	1	0.002	p,p'-DDD	103	99	104
o,p'-DDT	2	0.004	o,p'-DDT	87	74	74
p,p'-DDT	3	0.006	p,p'-DDT	89	90	90
Mirex	3	0.005	Mirex	70	79	79
alpha BHC	2	0.004	alpha BHC	72	58	75
beta BHC	2	0.004	beta BHC	94	65	95
gamma BHC	2	0.005	gamma BHC	101	106	78
delta BHC	2	0.003	delta BHC	71	82	95
Heptachlor epoxide	2	0.003	heptachlor epoxide	97	94	100
Endosulfan I	3	0.006	Endosulfan I	103	48	100
trans nonachlor	2	0.005	trans nonachlor	94	93	110
cis nonachlor	3	0.006	cis nonachlor	86	90	88
Methoxychlor	3	0.006	Methoxychlor	89	106	78
			p,p'-DDT-13C12	98	95	93

## 2008 Results of Monitoring

### Squid Liver and Muscle and Water Samples

<b>Sampling Site</b>	<b>Squid Liver Samples Dry Season May-June, 2008</b>	<b>Squid Muscle Dry Season May-June, 2008</b>	<b>Water Sample Dry Season May-June, 2008</b>
San Fernando, La Union South China Sea	<p>in large sample (240-346 g) o,p' DDT (220 ng/g) trans nonachlor (13.5 ng/g)</p> <p>in medium and small samples (147-197 g, 141-162 g) delta BHC (47.8 , 61.8 ng/g)</p> <p>in all three samples beta BHC (11.5, 7.01, 7.92 ng/g) gamma BHC (17.9, 35.8, 36.2ng/g)</p>	<p>in large sample p,p' DDD (1.98 ng/g)</p> <p>in large and medium samples gamma BHC (17.2, 13.0 ng/g)</p> <p>in all three samples Beta BHC (27.8, 26, 52.7 ng/g)</p>	<p>Heptachlor epoxide in one of two trials (0.008 µg/L)</p>

Note: Concentration of biota in ng/g wet weight



## 2008 Results of Monitoring Squid Liver and Muscle and Water Samples

<b>Sampling Site</b>	<b>Squid Liver Samples Dry Season May-June, 2008</b>	<b>Squid Muscle Dry Season May-June, 2008</b>	<b>Water Sample Dry Season May-June, 2008</b>
Naic, Cavite Manila Bay	<p>in large sample (456-550 g) HCB (1.13 ng/g)</p> <p>in medium sample (252-425g) trans nonachlor (4.0 ng/g)</p> <p>in small sample (86-190 g) delta BHC (42 ng/g)</p> <p>in all three samples beta BHC (14.8, 1.25, 3.88 ng/g) Methoxychlor (3.19, 3.81, 3.46 ng/g)</p>	<p>in largest sample p,p' DDD (1.98 ng/g)</p> <p>in small sample delta BHC (28.5 ng/g)</p> <p>in all samples beta BHC (34.7, 50.2, 53.5 ng/g)</p>	Endrin in one of two trials (0.010 µg/L)

## 2008 Results of Monitoring Squid Liver and Muscle and Water Samples

<b>Sampling Site</b>	<b>Squid Liver Samples Dry Season May-June, 2008</b>	<b>Squid Muscle Dry Season May-June, 2008</b>	<b>Water Sample Dry Season May-June, 2008</b>
Lucena City Tayabas Bay	<p>in large sample (163-240 g) gamma BHC (35.5 ng/g) trans nonachlor (2.54 ng/g) Methoxychlor (3.33 ng/g)</p> <p>in medium sample (95-127 g) p,p' DDD (3.49 ng/g)</p> <p>in small sample (85-95 g) o,p' DDT (3.43 ng/g)</p> <p>in all three samples beta BHC (8.31, 7.91, 6.42 ng/g) delta BHC (47.2, 45.8, 80.2 ng/g)</p>	<p>in all three samples p,p' DDD (2.13, 1.69, 1.64 ng/g) beta BHC (54, 65, 64.5 ng/g)</p>	<p>p,p' DDD in one of two trials (0.0027 µg/L) cis nonachlor in one of two trials (0.006 µg/L )</p>

## 2008 Results of Monitoring Squid Liver and Muscle and Water Samples

<b>Sampling Site</b>	<b>Squid Liver Samples Dry Season May-June, 2008</b>	<b>Squid Muscle Dry Season May-June, 2008</b>	<b>Water Sample Dry Season May-June, 2008</b>
Gumaca, Quezon Lamon Bay	<p>in large sample (146-599 g) Dieldrin (2.11 ng/g)</p> <p>in medium and small samples ( 102-123 g and 61-79 g) p,p' DDD (4.50 and 4.14 ng/g)</p> <p>in large and small samples delta BHC (58.2 and 82.3 ng/g)</p> <p>in medium sample Methoxychlor (3.11 ng/g)</p> <p>in all samples beta BHC (2.84, 26.9 22.9 ng/g)</p>	<p>in medium and small samples p, p' DDD (1.43 and 1.61 ng/g)</p> <p>in all samples beta BHC (64.7, 58.1, 60.5 ng/g)</p>	<p>Endrin in one of two trials (0.010 µg/L) Heptachlor Epoxide in one of two trials (0.0075 µg/L)</p>

## 2008 Results of Monitoring Squid Liver and Muscle and Water Samples

	<b>Squid Liver Samples</b>	<b>Squid Muscle</b>	<b>Water Sample</b>
<b>Sampling Site</b>	<b>Dry Season May-June, 2008</b>	<b>Dry Season May-June, 2008</b>	<b>Dry Season May-June, 2008</b>
Banate, Iloilo Iloilo Strait	in all samples, large (266-500 g), medium (121-125g) and small (79-89 g) beta BHC (12.2, 45.9, 9.09 ng/g) delta BHC (56.1, 55.7, 50.5 ng/g) Methoxychlor (3.05, 3.04, 3.28 ng/g)	in medium sample p,p' DDE (25.9 ng/g ww)  in large and medium samples p,p' DDD (1.32, 1.31 ng/g)  in all samples Beta BHC (62.8, 66.5, 61.6 ng/g)	Endrin in one of two trials (0.0086 µg/L) p,p' DDD in one of two trials (0.0026 µg/L)

---

## 2008 Results of Monitoring

### Squid Liver and Muscle and Water Samples

- **OCPs were detected in liver and muscle tissues in all squid samples.**
  - **$\delta$  BHC and  $\beta$  BHC and Methoxychlor were detected in all liver samples.**
  - **p,p'DDD and  $\beta$  BHC were detected in all muscle samples.**
  - **Depending on the site, other OCPs were detected:**
    - **in the liver:**
      - **p,p'DDT, o,p'DDT (La Union)**
      - **HCB and transnonachlor (Cavite),**
      - **$\gamma$  BHC, transnonachlor, p,p' DDD, o,p' DDT (Lucena)**
      - **p,p' DDD and dieldrin (Gumaca)**
    - **In the muscle**
      - **$\gamma$  BHC (La Union)**
      - **p,p' DDE (Gumaca)**
  - **$\beta$  BHC were detected in higher concentrations in muscles.**
  - **The concentrations of OCPs detected in water are very near the Quantitation Limit**
-

---

# Conclusion

- OCPs not detected in water samples can be detected in biota.
  - Some OCPs detected in the water can be detected in the biota.
  - Shrimps, Fish and Squid can be used as biological indicators of OCP contamination in natural waters.
  - Studies on the metabolism of OCPs in biota are necessary to be able to relate OCPs detected in the biota to the contamination in the environment.
-

---

# Acknowledgment

- To UNU for organizing the regional monitoring program, the funding and guidance on the project implementation
  - To Shimadzu Japan for providing the GCMS, the training on the analytical methodology and for other logistical support to the project
  - To the University of the Philippines for the laboratory facilities and staff
  - To the Bureau of Fisheries and Aquatic Resources of the Philippines and the various local governments for the support in sampling
-