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Study on Organochlorine Compounds in Cockles, *Anadara granosa* and Mussels, *Perna viridis* from Aquaculture Sites in Peninsular Malaysia

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DDT, BHC and aldrin represent three main groups of organochlorine compounds which were previously used as insecticides. In the 60's they were widely used in public health programs to eradicate pests, for household purposes and agriculture (Matusumura 1985). However in the 1980s, organochlorine compounds were banned in most countries including Malaysia as they were found to be resistant to degradation and could easily enter the food chain because of their chemical stability, low solubility in water and strong lipophilic characteristics (Kenneth 1982). Humans being part of the food chain, are constantly exposed to organochlorine compounds through consumption of contaminated animal, vegetable and liquid foods (Campoy et al. 2001). These compounds have long been taken off the market, yet traces of organochlorine compounds are still detectable in the environment and in organisms (Silvestroni and Palleschi 1999).

Early studies have shown that organochlorine compounds are carcinogenic and act as endocrine disruptor chemicals (Vettorazzi 1981; Matusumura 1985). Heptachlor and chlordane act as tumor promoters, liver toxicants and induce immune cell dysfunction in rats and mice (Rought et al. 1999). Aldrin, dieldrin, lindane, endosulfan, DDT and their metabolites were detected in human milk and had the potential to cause grievous harm to breast-fed babies (Matusumura 1985; Campoy et al. 2001). Its has been suspected that lindane effected sperm count reduction in man (Silvestroni and Palleschi 1999).

Cockles and mussels are marine bivalves (Broom 1985; Vakily 1989), normally found in muddy areas near the river mouth (Pathansali 1963). Cockles occur in the mud layer whilst mussels hold on to the substratum such as poles in the water body. They are sedentary filter feeders, taking in food particulates through their gills when their shells are open (McCoy et al. 1988). These characteristics make them easy targets for contamination with organochlorine compounds. In Malaysia, cockles are mainly cultured along the western coast in the state of Selangor and northwards to Penang whilst mussels, are generally more popularly cultured in the southwest of the peninsular particularly along the coast of Negeri Sembilan, Malacca and Johore. Aquaculture of prawns, fish, cockles and mussels, contributes significantly to the protein requirements of Malaysians. Cockle

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production alone in 1990 totaled closed to 36,000 tonnes and in 1996, increased to almost 72,000 tonnes. Mussel production however was on a smaller scale, reaching slightly over 1,500 tonnes in 1990 and 1,200 tonnes in 1996 (Department of Fisheries Malaysia 1990, 1996). Therefore, the level of organochlorine compounds accumulated in these animals are pertinent with regard to public health. The purpose of this study was to assess the presence (if any) of organochlorine residues, of the banned pesticides in the tissues of cockles and mussels at four farming locations namely Kuala Juru (Pulau Pinang), Kuala Selangor (Selangor), Sebatu (Malacca) and Sungai Danga (Johore).

MATERIALS AND METHODS

Four sites were selected, two for cockles at Kuala Juru, Pulau Pinang and Kuala Selangor, Selangor, and two for mussels at Sebatu, Malacca and Sungai Danga Bay, Johore. Three sampling points were selected randomly, with three replicates at each site. Approximately 20 cockles of size averaging 30-36 mm and 20 mussels measuring 50-80 mm were collected. Samples were wrapped in aluminum foil, stored at 4°C and taken to the laboratory for analysis.

Organochlorine compounds in the tissues were analyzed according to the method outlined by the Association of Official Analytical Chemists, AOAC (1990). Shelled and pooled whole tissues (20 g) were mixed with 40 g of sodium sulfate. For the recovery study, the mussel tissues were spiked with 10, 50 and 100 ppb of organochlorine as shown in Table 1. After 20 min, the mixture was homogenized with 100 mL hexane and the upper layer was carefully transferred into a conical flask. Extraction was repeated twice and the 300 mL of hexane extract was then concentrated in a rotary evaporator at 70°C until the volume was reduced to 25 mL. The extract was then transferred into the Kuderna-Danish apparatus to be concentrated at 90°C whereby the volume was further reduced to 1 mL. The 1 mL sample was purified in 10 g florisil and 8 g sodium sulfate column by eluting with 35 mL hexane: dichloromethane (94:6) and 35 mL hexane: dichloromethane (85:15). The purified sample was again concentrated with the Kuderna-Danish apparatus until the volume reached 1 mL. Ten µL of the sample was injected into the gas chromatograph (Hewlett Packard 5890 Series II) fitted with an ECD detector on a 0.32 mm ID x 30 m quadrex fused silica capillary column of methyl 5% phenyl silicone. Injection and detection temperature was 275°C and 325°C respectively. Nitrogen gas was used as the carrier gas at a flow rate of 1 mL/min.

RESULTS AND DISCUSSION

Table 1 shows the recovery test results on whole tissue for α –BHC, lindane, α – endosulfan, 4, 4-DDE, dieldrin, 2, 4-DDD, β –endosulfan, 4, 4-DDD and DDT. The test ranged from 10 ppb to 100 ppb, the lowest percentage recovery being 73.16% (2,4-DDD at 10 ppb) and the highest 99.95% (lindane at 100 ppb). The results obtained from the samples was well within the recovery test range.

Organochlorine compounds in the whole tissues of both cockles and mussels were

Table 1. Percentage (% ± SD) recovery test on whole tissue samples of mussel.

Organochlorine compounds	10 ppb	50 ppb	100 ppb
α –BHC	98.34 ± 0.5	94.73 ± 2.3	89.54 ± 2.5
Lindane	99.94 ± 0.0	99.74 ± 0.0	99.95 ± 0.0
α -endosulfan	94.74 ± 3.7	98.44 ± 0.8	98.08 ± 1.0
4,4-DDE	84.66 ± 0.2	86.58 ± 0.8	88.74 ± 1.8
Dieldrin	88.83 ± 4.1	94.35 ± 2.3	97.56 ± 0.3
2,4-DDD	73.16 ± 1.5	78.93 ± 3.1	87.29 ± 1.3
β –endosulfan	76.35 ± 1.5	81.26 ± 0.4	88.40 ± 0.5
4,4-DDD	83.17 ± 6.2	88.94 ± 4.6	89.96 ± 1.1
DDT	98.80 ± 0.3	96.67 ±1.8	98.34 ± 0.8

generally low and in some cases were undetectable (Table 2). DDT was only detected in cockles from Kuala Juru, Pulau Pinang. DDT metabolites were however not present in Kuala Juru except for 4, 4-DDD. This metabolite is also present in the cockles from Selangor as well as in mussels collected from both Sebatu and Sungai Danga Bay. Endrin was not detected in any of the samples. This could be because once absorbed by animals, endrin is quickly metabolised to delta-keto and therefore it usually does not accumulate in the tissues as endrin (Vettorazzi 1981).

The highest total organochlorine compounds detected was recorded in cockles from Kuala Juru (173.7 ng/g wet weight), followed by Kuala Selangor (137.6 ng/g wet weight), Sebatu (70.4 ng/g wet weight) and lastly Sungai Danga (57.8 ng/g wet weight). Generally, total organochlorine compounds in mussels are much lower compared to the total organochlorine compounds in cockles. There was a significant difference ($p<0.05$) between total organochlorine compounds in cockles from Kuala Juru and Kuala Selangor. However, when compared with mussel samples from Sebatu and Sungai Danga there was no significant difference ($p>0.05$).

The higher concentration of organochlorine detected in cockles could possibly be due to their habitat and close contact with sediment. Oganochlorine compounds persist and remain bound to sediment for about 2-10 years, depending on the type of organochlorine compounds involved (Brooks 1974). Being lipophilic and hydrophobic, organochlorine compounds also can easily be accumulated in animal adipose tissue (Connell and Miller 1984).

The level of organochlorine compounds detected in cockles and mussels in this study is well within the Maximum Residue Level under the Food Act (1983) Food Regulations 1985, Table 16 (Regulation 41) Insecticide Residue. These levels are much lower than those reported by Supawat et al. (1997) in mussels along the Thai coastline (ranging 0.01-37 ng/g wet weight or that by Ruchaya-Boonyatumanond et al. (2002) (0.78-5.7 ng/g wet weight).

In conclusion, the level of concentration of organochlorine compounds detected in

Table 2. Concentration of organochlorine compounds (ng/g wet weight ±SD) in cockles and mussels from several aquaculture areas.

Organochlorine Compounds	*Maximum Permissible Level (ng/g wet weight)	Cockles, *A. granosa*		Mussels, *P. viridis*	
		Kuala Juru	Kuala Selangor	Sebatu	Sungai Danga
DDT	5000	8.0 ±0.3	ND	ND	ND
4,4-DDE	NA	ND	ND	6.8 ±1.2	ND
2,4-DDD	NA	ND	ND	ND	2.6 ±0.5
4,4-DDD	NA	10.9 ±5.1	14.9 ±10.0	15.1 ±1.4	9.6 ±2.2
Aldrin	200	14.4 ±6.2	10.8 ±3.8	ND	17.1 ±3.5
Dieldrin	200	14.4 ±1.9	19.3 ±1.3	16.6 ±4.0	14.6 ±0.5
Endrin	100	ND	ND	ND	ND
Chlordane	50	19.6 ±2.4	ND	ND	ND
Heptachlor	200	6.6 ±0.3	ND	5.8 ±1.0	ND
α -BHC	NA	14.7 ±1.5	12.7 ±1.0	19.9 ±2.6	13.9 ±0.4
Lindane	NA	75.7 ±1.8	71.7 ±1.0	ND	ND
β -endosulfan	NA	4.2 ±0.2	3.3 ±2.1	6.2 ±3.3	ND
α -endosulfan	NA	5.2 ±1.3	4.9 ±1.2	ND	ND
Total		173.7	137.6	70.4	57.8

ND: Not Detected
NA: Residue level not stated
* Food Act Malaysia 1983

cockle and mussel tissues from several aquaculture sites in peninsular Malaysia is very low, even undetectable in some. Human consumption of these shellfish therefore poses very low risk of organochlorine contamination.

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