Malaysian Journal of Chemistry, 2009, Vol. 11, No. 1, 014 - 018

Contamination Levels of POPs in Various Food Items from the Southernmost of Thailand: Primary Health Risk Assessment Through Dietary Intake

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ABSTRACT: The objectives of this study were to determine concentrations of OCPs and PCBs in food products and marine food and to assess the potential health risk involved consuming contaminated food through dietary intake. The food samples collected were various brands of processed fish ball, baby food, instant cereal, canned seafood, frying oil and fresh marine food as well as fishes and shellfishes. They were purchased from local main markets in Yala and Pattani cities and the vicinity districts. These markets received food product items from all over Thailand and abroad. Biological samples were purchased from the major domestic markets. Analytical method was modified and validated to suit local conditions in laboratory experiment. The recovery results of several concentration levels of standards were within the acceptable range of 70-130 % as recommended by US-EPA. Solid samples were soxhlet extracted with the solvent mixture of hexane and dichloromethane (in 1:1 ratio). Cooking oil samples were liquid-liquid extracted with acetonitrile using separatory funnel. The extracted solvents were concentrated by rotary evaporator and cleaned up by hot florisil column. The final sample was analyzed by GC-ECD. The linearity of ECD detector responses were in good condition within the calibration ranges. The results showed that the commonly found OCPs in the analysed food samples were p, p'-DDD, p, p'-DDE, endrin aldehyde, endosulfan 1, endosulfan sulfate and some PCB congeners at wide range concentration levels. Health risk assessment methodology and all estimated values involved in this work were based on US-EPA's IRIS databases. The concentrations of OCPs detected in food samples were then converted into average daily exposure per person (ug/kg body weight per day). The ratios of calculated daily intake values against reference dose (RfD) were used for the evaluation of possible health risk effects associated with contaminated food ingestion.

Keywords: OCPs, PCBs, Risk assessment, Food items, Southernmost of Thailand

Introduction

Persistent organic pollutants (POPs), most popular of which are organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) that have been reported their contaminations in various compartments such water, sediments and living organisms [1]. A number of toxicity studies reported that these chemicals had caused various ill-effects to human health [2, 3]. Several international organizations have placed these toxic chemicals in a priority list of concern due to their highly persistence in natural environment, bioacumulative through food web, and toxic to human and aquatic organisms [4]. Under the Stockholm Convention, more than 12 POPs have been selected to be eliminated from use which most of them are OCPs including DDT, dieldrin, Endrin, heptachlor, aldrin, mirex, chlordane, toxaphene and hexachlorobenzene as well as PCBs and also two industrial by-products, namely polychlorinated dibenzodioxins and dibenzofuran [5]. PCBs are the mixtures of 209 difference congeners that have been used extensively since 1930 in a variety of industrial uses [6]. IARC has concluded that PCBs are classified as probable human carcinogens (Group 2A), especially non-ortho coplanar pentachlorobiphenyl (Co-PeCBs) which are the most toxic among the PCB congeners [7]. The exposure of these toxicants by human can occur through various ways in daily activities such as direct contact, skin adsorption, inhalation, ingestion of contaminated food, especially eating contaminated food which is a main route of exposure to these toxic chemicals [8, 9].

Growing dynamic economy and community expansion in the southernmost of Thailand are among the factors influence the standard of living of local society, including changes in dietary habits. For instance, various food products items from all over Thailand and neighboring countries can be found mostly in local markets and widely consumed among local people. However, marine food from local fisheries, particularly fishes and bivalves still dominates the southernmost people diets. It was reported that by the mid-1990s the consumption levels of fishes and other seafood in most of Asian and the Pacific countries exceeded that of world per capita seafood consumption [10]. Thus, the contamination of seafood and other food products, particularly by POPs would pose great risk to human health dietary intake and seafood consumption.

To our knowledge, there are two research studies conducted during 1990-1994 for determining persistent organic pesticide residues in green mussels (*Perna Viridis*) samples from the Pattani bay area, southernmost of Thailand [11,12]. Since the data on

POPs concentrations are very scarce and no current comprehensive research work on POPs contamination in food items have been undertaken, this study would be the first effort to assess the current contaminations of selected OCPs and PCBs in food items sold in local markets of southern border provinces.

Materials and Methods

Chemicals and apparatus

The glassware and chemicals were prepared and maintained properly following the standard laboratory practices as stated in the US-EPA protocol [13]. The organic solvents and chemicals used in this study were of analytical grade (AR) and used without further purification. The TLC standard of 18 OCPs and PCBs (arochlor 1260) were purchased from local supplier of Supelco, and then they were diluted with hexane into a proper stock solution of 200 ng/mL OCPs and 400 µg/mL PCBs. These stock solutions were diluted into several concentration levels of working solutions for the purpose of method validations, instrumental calibrations and quantifications.

Sample collection

Sample collection was carried out between June 2007 and August 2008. Food items collected for this random basket survey were 5 species of marine fish and shellfish and several brands of food products as well as processed fish ball, baby food, instant cereal, canned seafood and frying oil. These food items were purchased from several main markets in Yala and Pattani cities and from the markets in vicinity districts including Raman, Saiburi, and Palas. These markets received various food items from all over Thailand and abroad. Fresh marine food samples selected for this study were 5 species of fish and bivalves such as mullet, catfish, jewfish, green mussels and blood cockle which are popular seafood for the majority of the population in the southernmost of Thailand. They were bought from the major domestic markets for seafood and agricultural products in Yala city and from a supermarket in Pattani province. These biological samples are from small scale-fisheries in the Pattani bay and vicinity area. Then, their weight, size and length were recorded. After returning to analytical laboratory, they were cleaned with distilled water and wrapped individually with aluminium foil and then kept in a freezer at -20 ^{o}C until analysis.

Method validation and sample extraction

The determinative methods used were based on the standard US-EPA protocols and other approved methods [14, 15]. Several modifications of the methods were made to suit local conditions in analytical experiments. The modified methods were then validated by carrying out recovery experiments at three concentration levels of standard mixtures. The efficiency of hot florisil for cleaning up fat and oil from target analytes was determined by spiking pure cod liver oil with the standard mixture of OCPs and PCBs at several concentration levels. In our experiment, the parameters modified were solvent strength, florisil column loading and the volume of eluting solvent used. Overall, percentages of recovery for these multicomponent mixtures were within the target range of 70-130 % as recommended by the standard US-EPA protocol.

For real samples, 10 grams of food samples were homogenized with anhydrous sodium sulfate (left to dry for about 1 hour for tissue sample). The mixtures were then extracted with the solvent mixture of hexane and dichloromethane (1:1 ratio) using soxhlet extractor at the reflux rate of 4-5 cycles/hour for 20 hours. Approximately 17 g (20 mL) of cooking oil samples were liquid-liquid extracted with acetonitrile using separatory funnel. The extracted solvents were concentrated by rotary evaporator at the reduced pressure and adjusted the sample volume to 1-2 mL by blowing with a gentle stream of nitrogen gas. The concentrated samples were cleaned up with hot florisil column by eluting with the first fraction of 80 mL hexane for PCBs analyses, followed by the second fraction of 180 mL hexane: dichloromethane (45:55 ratio) for OCPs analyses. Both fractions were then concentrated into exactly 1 mL before being analysed by GC-ECD. Lipid content in tissue samples were determined by drying the concentrated extracts in pre-weighed concentrator tube.

Instrumental analysis

A Varian 3600 Cx gas chromatograph equipped with ECD was used for quantification of OCPs and PCBs. 1 µL of final sample was injected (splitless for 1 min) by using a 8200 Cx autosampler with sandwich injection mode. The analytes were separated on capillary column SPB-5 (30 m x 0.25 µm film thickness x 0.25 mm i.d.). For OCPs analyse, the column oven was programmed from 120 ^{o}C (maintained for 1 min), increased to 195 ^{o}C at 15.0 ^{o}C/min (held for 0.5 min) and ramped from195 ^{o}C to 240 ^{o}C at 4.9 ^{o}C/min (held for 4.82 min). As for PCBs, the column oven was programmed from 120 ^{o}C (maintained for 0.5 min), increased to 200 ^{o}C at 20.0 ^{o}C/min and from 200 ^{o}C to 230 ^{o}C at 4.7 ^{o}C/min and from 230 ^{o}C to 280 ^{o}C at 3.7 ^{o}C/min and held at the final temperature for 8.59 min. The injection port and detector temperature were set at 250 ^{o}C and 300 ^{o}C, respectively. All data were quantified by external standard of five point calibrations. The correlation coefficients of all calibration curves were greater than 0.99 reflecting that ECD responses were in good condition within the calibration ranges. GC instrument LOD for OCPs was ranging from 0.014 to 0.035 ng/mL.

Quality assurance and quality control measures

All data were subjected to strict quality control procedures. Method blanks were analysed together with each set of real samples. In certain case, recovery results carried out under the validated experimental conditions were within arrow targeted range of 90 %-110 % with standard deviations at the limit warning of 10 % – 15 % as stated in quality

assurance for analytical method [16]. The OCPs and PCBs standards used for calibration purposes were routinely monitored for area counts in order to maintain proper concentration levels during instrumental quantifications.

Risk assessment methodology

Primary risk assessment methods used for evaluating health risk effects associated with dietary intake were based on US-EPA's IRIS databases and other risk assessment report [17, 18]. The average daily exposures of OCPs per person (ug/kg body weight per day) were calculated using the present analytical results. The ratios of calculated daily intake values against reference dose (RfD) were used for the evaluation of possible health risk effects associated with contaminated food ingestion.

Results and Discussion

Table 1 summarizes the concentration ranges of OCPs and total PCBs detected in food product items analysed in the present study. On the average, the analytical results showed that seafood products from various brands were more contaminated with OCPs and PCBs than other food samples. On the other hand, instant cereals samples were contaminated with several OCPs at the lower concentration levels. Among the food samples analysed in this study, baby foods were found to be lower contaminated with total PCBs than other products. α-HCH compound was the major contaminant detected in all analysed food product samples with the highest mean concentration of total HCH isomers found in processed fish ball samples at the concentration of 9.79±10.48 µg/kg total wt. γ-HCH or lindane, the major isomer of HCH compounds was detected in a sample of fish ball at the concentration of up to 13.04 µg/kg wet wt. Among the HCH isomers, α-HCH shows the most carcinogenic activity and has been classified as a probable human carcinogen by the US-EPA [19]. The concentration levels of OCPs and PCBs in fresh marine fish and bivalves samples analysed in the present study are summarized in Table 2. Overall, the concentration levels of total OCPs quantified in fresh seafood samples were varied from species to species with the highest value was found in jewfish samples at the concentration of up to 170.18±19.74 ug/kg lipid wt. However, blood cockle samples collected for this survey recorded the highest mean total PCBs contamination at the concentration of up to 501.67±99.76 ug/kg lipid wt. The frequently found OCP residues in seafood samples were p, p'-DDD, p, p'-DDE, endrin aldehyde, endosulfan 1 and endosulfan sulfate. These OCP residues are classified as agricultural pesticide residues which widely used in the past. p, p'-DDT compound, the most persistent organochlorine pesticide was detected in a few samples at trace concentration levels indicating that DDTs contaminated in seafood samples may come from old use. In this study, we found that OCPs contaminations in green mussel (*Perna Viridis*), especially DDTs were decreasing when compared to those in the two previous studies reported by Ruangwises *et al.* (1994) and Siriwong, *et al.* (1991). In most cases, mullet and jewfish samples were more contaminated with OCP residues than other marine food samples.

Table 1. The concentration ranges of POPs (ug/kg total wt) detected in various food products items

OCPs and CBs	Canned seafood (7 samples)	Fish balls (4 samples)	Baby foods (3 samples)	Instant cereals (3 samples)	cooking oils (4 samples)
ΣHCHs	0.12-15.65 (5.24±3.25)	0.27-23.21 (9.79±10.48)	0.30-1.12 (0.71±0.41)	0.22-1.43 (0.95±0.49)	1.07-6.39 (3.03±1.68)
ΣHeptachlor	0.65-3.83 (1.90±1.15)	0.83-5.55 (2.30±2.19)	0.11-13.81 (5.86±7.11)	1.37	0.49-3.70 (1.84±1.09)
ΣAldrin	0.84-7.35 (3.61±1.52)	ND	0.24-6.89 (2.73±3.34)	ND	0.10-0.23 (0.13±0.08)
ΣEndrin	0.18-3.69 (2.19±1.13)	0.32-2.42 (1.68±0.93)	0.27-3.18 (1.59±2.13)	0.47	0.11-1.40 (0.63±0.39)
ΣEndosulfan	0.20-6.51 (4.14±2.18)	0.18-4.49 (2.09±1.84)	0.12-88.04 (30.35±49.98)	ND	0.30-14.97 (4.05±5.46)
ΣDDT	0.30-21.66 (9.59±6.97)	0.25-0.69 (0.40±0.26)	0.28-2.69 (1.55±1.21)	0.9-1.51 (1.27±0.25)	ND
ΣOCPs	3.97-58.70 (34.02±20.74)	4.80-36.70 (20.62±13.34)	6.50-108.45 (30.56 ±22.23)	1.82-4.78 (3.00±1.19)	4.40-27.75 (11.94±8.40)
ΣPCBs	56.10-210.70 (129.60±58.39)	30.20-170.90 (95.43±58.31)	20.80-86.60 (50.10±36.91)	30.70-95.90 (69.10±34.11)	41.70-140.80 (82.18±42.22)

ΣHCHs : α-HCH+ β-HCH + γ-HCH, **ΣHeptachlor** : heptachlor + heptachlor epoxide, **ΣAldrin**: aldrin + dieldrin, **ΣEndrin** : endrin+endrin aldehyde+endrin ketone, **ΣEndosulfan**: endosulfan 1+ endosulfan 2+ endosulfan sulfate, **ΣDDT**: p, p'-DDT+ p, p'-DDD+ p, p'-DDE, **ΣPCBs**: total active peaks of TetCBs + PenCBs + HexCBs + HepCBs mixtures, (mean ± SD), ND: not detected (below instrument LOD)

Table 2 The concentrations of OCPs and PCBs (ug/kg lipid wt) in tissue samples

OCPs and PCBs	Mullet *Mugil sp* (3 samples)	Catfish *Arius sp.* (2 samples)	Jewfish *Pernahia sp.* (3 samples)	G. mussel *Perna viridis* (3 samples)	B. cockle *Anadara granosa* (3 samples)
ΣHCHs	10.98-63.93 (34.54±26.96)	10.39-43.47 (26.93±3.39)	4.78-57.19 (35.09±27.15)	14.70-32.60 (22.80±9.07)	2.48-17.12 (9.43±7.35)
ΣHeptachlor	0.90-52.04 (23.35±28.83)	1.71-7.26 (4.49±3.92)	14.05-78.56 (50.91±33.23)	11.23-32.66 (19.56±11.48)	5.87-19.33 (12.03±6.80)
ΣAldrin	ND	10.03	59.76	23.48	12.18-30.64 (23.00±9.63)
ΣEndrin	2.72	1.55-2.78 (2.17±0.87)	ND	8.11	7.96-33.89 (22.98±13.45)
ΣEndosulfan	2.66-33.95 (17.57±15.70)	1.52-3.23 (3.14±1.21)	5.49-6.93 (6.17±0.72)	8.65-15.76 (11.77±3.63)	10.77-12.60 (11.42±1.02)
ΣDDT	3.8-52.04 (25.05±24.63)	1.21-25.72 (13.47±17.33)	13.97-51.70 (29.04±19.98)	42.81-78.79 (60.23±18.02)	14.11-76.97 (52.99±33.98)
ΣOCPs	125.56-185.08 (150.85±30.75)	68.76-69.79 (69.28±0.73)	150.48-189.96 (170.18±19.74)	86.05-193.04 (129.80±56.10)	90.78-163.88 (121.85±37.76)
ΣPCBs	298-429 (368.33±66.03)	282-360 (316.67±37.86)	330-389 (359.67±29.50)	339-584 (447.67±125.66)	398-597 (501.67±99.76)

ΣHCHs : α-HCH+ β-HCH + γ-HCH, **ΣHeptachlor** : heptachlor + heptachlor epoxide, **ΣAldrin**: aldrin + dieldrin, **ΣEndrin** : endrin+endrin aldehyde+endrin ketone, **ΣEndosulfan**: endosulfan 1+ endosulfan 2+ endosulfan sulfate, **ΣDDT**: p, p'-DDT+ p, p'-DDD+ p, p'-DDE, **ΣPCBs**: total active peaks of TetCBs + PenCBs + HexCBs + HepCBs mixtures, (mean ± SD), ND: not detected (below instrument LOD)

Table 3 The selected calculation results of average daily intake, HQ ratios, and I(10^{-5}) used for risk assessments

Food samples	The highest OCPs detected	Concentrations (ng/g wet wt)	Calculated intake I (ug/kg body wt-day)	Calculated intake II (ug/kg body wt-day)	Oral RfD (mg/kg-day)	SF (risk per mg/kg-day)	HQ ratio	I(10^{-5})	Added lifetime carcinogenic risk estimate
Mullet	α-HCH	15.13	0.047	0.020	NA	6.3	-	0.32	3.2×10^{-6}
	Endosulfan I	10.45	0.033	0.014	6.0(6.0)*	NA	0.006	-	-
G.mussel	Heptachlor	4.26	0.013	0.006	0.5(0.1)*	4.5	0.026	1.20	1.2×10^{-5}
B. cockle	p, p'-DDD	4.77	0.015	0.006	NA(2.0)*	0.24	0.008	2.50	2.5×10^{-5}
	p, p'-DDT	3.23	0.010	0.004	0.5(2.0)*	0.34	0.020	1.20	1.2×10^{-5}
Fish ball	γ-HCH	13.04	0.041	0.017	0.3(8.0)*	NA	0.130	-	-
Canned food	Endrin aldehyde	4.77	0.015	0.006	0.3	NA	0.012	-	-

Calculated intake I and II (ug/kg body wt-day) are based on non-carcinogenic and carcinogenic risk respectively, ( )* : ADI value recommended by FAO/WHO, SF: cancer slope factor, NA: not available data.

Table 3 shows average daily intake, hazard quotient ratios (HQ) and intake based on 10^{-5} used for risk assessment associated with the consumption of contaminated food. The average daily intake of OCPs per person was calculated based on the highest concentration level of OCP residues determined in each tissue samples (ug/kg wet wt). The HQ and I(10^{-5}) ratios were calculated for non-carcinogenic and carcinogenic risk assessment, respectively. The average daily intakes of selected OCPs calculated in this study were below RfD values as recommended by the US-EPA's IRIS. The analytical results of the present study also showed that the calculated average daily intake of both HCHs and DDTs through seafood eating were below the tolerable daily intake (TDI) of 20 ug/kg-day as proposed by the WHO [20]. HQ ratios of all OCPs were less than 0.1 indicating an unlikely potential for adverse health effects associated with marine food consumption within exposure duration. As shown in Table 3, I(10^{-5}) ratios are in the rage of 0.32-2.50, indicating that added lifetime carcinogenic risk estimates for an adult who continuously consumes fresh marine food contaminated with OCPs were calculated to be in the range of 3.2×10^{-6} to 2.5×10^{-5}. This mean that there is a strong possibility that 3 to 25 additional population out of one million will get cancer when such amount of seafood contaminated with such level of OCPs are to be consumed at the estimate rate.

Conclusion

This study has provided useful information on current contamination of POPs in various food items sold in local markets from southernmost of Thailand. These findings showed that various food items consumed by the majority of population in southern border provinces of Thailand were contaminated with various POP chemicals at wide range of concentration levels. Base on the calculated results, health risk of consumer for long-term hazards posed by these chemical contaminants are estimated to be low.

Acknowledgement

The authors would like to acknowledge Research Center for Southern Border Provinces Development, Yala Rajabhat University for funding this research work. Acknowledgement is also to Assoc. Prof. Dr. Muhamad Sani bin Ibrahim, a former lecturer of the School of Chemical Sciences, UNIVERSITI SAINS MALAYSIA, Pulau Pinang, Malaysia for helpful support on special apparatus and quantifying some PCBs congeners in this study.

References

1. UNEP/GEF (2002). *Regionally Based Assessment of PTS: regional report*. 1-53.
2. Crouch, M.D. and Barker, S. A. (1997). *J. Chromatogr .A.* **774**: 287-309.
3. Ahmed, F.E. (2003). *Trend. Anal. Chem.* **22:** 170-185.
4. Eckley, N. (2001). *Environ.* **43 (7):** 26-36.
5. Stockholm Convention on POPs, [Online], [Accessed 17th April 2008]. http://www.pops.int/documents/signature.htm.
6. Ballschmiter, K. and Zell, M. (1980). *Fres. J. Anal. Chem.* **302:** 20-31.
7. Otaki, Y., Matsuemura, T. and Kurokawa, Y. (1997). *Environ. Pollut.* **96 (1):** 79-88.
8. Bineli, A. and Provini, A. (2004). *Ecotox. Environ. Safe.* **58:** 139-145.
9. Abelson, A., Gibson, B. L. and Sanborn, M. (2002). *J. Am. Med. Commun.* **166(12):** 1549-1554.
10. Adeel, Z. and King, C. (2002). *Conserving Our Coastal aenvironment.* 5-11.
11. Ruangwises, S., Ruangwises, N. and Tabucanon, M. (1994). *Mar.Pollut.Bul.* **28(6):** 351-355.
12. Siriwong, C., Hiroka, H., Onodra, S. and Tabucanon, M. (1991). *Mar.Pollut.Bul.* **22(10):**510-516.
13. US-EPA. (1995). *Method 1664*. 1-24.
14. US-EPA. (1996). *Method number 3510A, 8000B*. Revision 4, 30-44.
15. Tanabe, S. (1999). *ASL laboratories clean up procedure: Trace organics*. 1-12.
16. Hugdahl, M. (1998). *Asian-Canada Cooperative Program on Marine Science-Phase II.* 1-11.
17. US-EPA.*Integrated risk information system (IRIS).* [Online], [Accessed 20th October 2008]. http://www.epa.gov/iris
18. Jiang, Q., Lee, T., Chen, K., Wong, H., Zheng, J., Giesy, J., Lo, K, Yamashita, N. and Lam, P. (2005). *Environ. Pollut.* **136:** 155-165.
19. Walker, K., Vallero, D.A. and Lewis, R. G. (1999). *Environ. Scie. Technol.* **33(24):** 4373-4378.
20. Kunisue, T., Someya, M., Kayama, F., Jin, Y. and Tanabe, S. (2004). *Environ. Pollut.* **131:** 381-392

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