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Original research article

Determination of Organochlorine Pesticide and Polychlorinated Biphenyl as POPs Residues in Freshwater Animals in Thailand during 2017-2018

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ABSTRACT

A rapid multiresidue method for the determination of 22 organochlorine pesticides and 7 polychlorinated biphenyl compounds as POPs contaminant was described. It involved the application of modified QuEChERS procedure followed by gas chromatography - electron capture detector (GC-ECD) analysis. The limits of detection (LOD) and quantification (LOQ) of the developed and validated method in fish tissue were 3 μg/kg and 10 μg/kg, respectively. The following validation parameters were within acceptable range: specificity and selectivity, linearity, accuracy and precision (at levels: 10, 15 and 50 μg/kg, the recovery test values were between 70 and 120% and HorRat ≤2, except hexachlorobenzene and methoxychlor). The application of the method was verified by analyzing a total of 182 freshwater animal samples produced and collected in Thailand during 2017-2018. Detectable POPs residues were found in 1.6% (3 shrimp samples) of the animal samples. One of the positive samples was contaminated with pp'-DDE which was DDT metabolite (<10 μg/kg). Two shrimp samples presented residue of PCB-52 congener (<10 and 30 μg/kg). No sample had contamination higher than the extraneous maximum residue limit (EMRL) set by Ministry of Public Health of Thailand and Codex. Base on the most risky freshwater animal, primary risk assessment using shrimp daily intake of Thai population data has shown that DDT and PCB compounds contain in shrimp are unlikely to pose any health risk to Thai consumers.

Keywords: Freshwater animal; GC-ECD; Organochlorine pesticide; PCB; QuEChERS

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1. Introduction

Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) constitute major groups of synthesized organic compounds of persistent organic pollutants (POPs). Other groups of POPs include by-product substances originating from human activities (ie, dioxins and furans). These hazardous pollutants are released into the environment intentionally or unintentionally. Thus, POPs are resistant to biodegradation because of their persistent property and they can accumulate in adipose tissue due to their highy lipophilicity. These compounds have a strong tendency to bioaccumulate in the food chain and are prone to long-range transport [1]. Humans, as predators on the top of the food chain, can be seriously affected by POP toxicity [2]. Consequently, background levels of POPs can be found in the human body [3]. Moreover, many sources show the high concentration of residues and they were found in human fluid [4-5]. Exposure to certain POPs can cause various effects such as greater susceptibility to disease, and damage to the immune, neurological, and reproductive systems. Furthermore, they cause endocrine disruption, decreased comprehension ability, birth defects, cancer and even death.

Thailand has been one of the signatories of the Stockholm Convention on Persistent Organic Pollutants (the Stockholm Convention) since May 2002 and ratified it in January 2005. The aim of the convention is to protect human health and the environment from POPs. In Thailand, the Ministry of Public Health (MoPH), in cooperation with the Ministry of Natural Resources and Environment (MNRE), has issued procedures to control the impact of pollutants on the health of the population by setting several Public Health Acts since 1992. The MoPH, with the Department of Medical Sciences (DMSc) as a supporting agency, is in charge of enforcement of these national acts and the other related laws. Thus, the DMSc has an accredited laboratory (ISO/IEC 17025:2005) capable of food analysis to ensure the quality and safety of these products based on consumer protection laws and legal purposes. Nevertheless, there are few studies on POPs residues content in food in Thailand. Only environmental contamination surveys were applicable for assessment of bioaccumulative toxicity [6-8]. Environmental contamination by OCPs in natural inland freshwater animals has been a great concern. Based on our previous work in 2015 [9], despite restrictions and bans on the use of many OCPs and PCBs since 1990s, two pesticide metabolites including pp'-DDE and endosulfan sulfate were found in freshwater fish but the concentrations were within EMRLs. Moreover, bioaccumulation of some OCPs and their detection in the egg yolk of birds was continued to be observed and POPs persisted in wild space [10-11].

The appropriate method for animal sample preparation for POPs extraction can be one of a numerous techniques. Traditionally, Soxhlet/pressurized fluid extractors [12], gel permeation chromatography (GPC) [13], and lipid removal using hexane [9] were used. These methods require complex equipment, are time and hazardous solvent consuming, and have low accuracy. That is why routine application of these methods is difficult to handle.

This study was conducted to develop and validate a rapid, easy, safe and accurate analytical method for determination of POPs, including 22 OCPs and 7 PCBs in freshwater animal tissues, together with a wide range of chemical properties, and by doing it in a single extraction per sample and running a chromatographic injection [14]. Furthermore, the project objective is to assess organochlorine pesticides and polychlorinated biphenyls residual level in food products as bioindicators of the pollutants in the food chain in Thailand.

Therefore, our analysis provides both an ecological and a human health risk perspective.

2. Materials and Methods

2.1 Chemicals and standards

Acetonitrile (HPLC), acetone (HPLC), ethyl acetate (HPLC) and glacial acetic acid (AR) were purchased from J.T. Baker, USA. N-hexane (PG) was supplied by RCILabscan, Thailand. Anhydrous sodium sulfate (AR), anhydrous magnesium sulfate (AR) and sodium acetate (AR) were obtained from Fisher Scientific, UK. Dispersive SPE 15ml, Fatty sample, EN: A mixture of 900 mg $MgSO_4$, 150 mg PSA and 150 mg C18EC, Agilent Bond Elut ERM-Lipid tube (p/n 5982-1010) and Agilent Bond Elut Final Polish for Enhanced Matrix Removal-Lipid tubes (p/n 5982-0101) were from Agilent Technologies, USA. Reverse osmosis water was generated by Millipore Milli-Q system, USA. Organochlorine compound and PCB standards were aldrin, BHC-alpha, BHC-gamma, chlordane-alpha, chlordane-gamma, chlordane-oxy, chlorothalonil, dicofol, dieldrin, endosulfan sulfate, endosulfan-alpha, endosulfan-beta, endrin, heptachlor, heptachlor epoxide-cis, heptachlor epoxide-trans, hexachlorobenzene, methoxychlor, pp'-DDD, pp'-DDE, pp'-DDT, tetradifon, PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180. All OCPs and PCBs standards were of high purity grade (>96.0±0.5%) and were purchased from Dr. Ehrenstorfer GmbH, Germany. Individual stock solutions were prepared at about 200 µg/ml in n-hexane and stored at ≤ -10°C in freezer. A mix of intermediate (1 and 10 µg/ml) and working (2, 5, 10, 15, 25, 50 and 100 ng/ml) standard solutions were prepared by serial dilution of stock solution to the appropriate concentrations. The residue concentrations were calculated using the calibration curve generated from the peak area versus the working solution concentrations.

2.2 Sample and sample preparation

Two surveillance programmes were conducted consecutively during two years in 2017 and 2018. A national contaminant monitoring project called "Total Diet Study, TDS" was set up in 2017. A total of 32 animal origin food samples were collected from whole-sale markets at Bangkok, Chonburi, Chiangmai, Phitsanulok, Khon Kaen, Nakhon Ratchasima, Songkla and Trang provinces. Freshwater animal samples were striped snakehead fish (*Channa striata*) (*n*=8), catfish (*Clarias batrachus*) (*n*=8), Nile tilapia (*Oreochromis niloticu*) (*n*=8) and freshwater shrimp (*n*=8). In this project, all foods were purchased on the same day by Regional Medical Sciences Centers (RMSCs) and were sent to the Institute of Food Research and Product Development (IFRPD), Kasetsart University for sample preparation step. Moreover, in 2018, a total of 150 animal samples originating from different zones of production of Department of Fisheries, Ministry of Agriculture and Cooperatives data base were selected for this study. Freshwater animal samples included giant sea perch (*Lates calcarifer*) (*n*=30), catfish (*Clarias batrachus*) (*n*=30), Nile tilapia (*Oreochromis niloticu*) (*n*=30) and freshwater shrimp (*n*=60) shown in Table 1.

Table 1. Number of animal samples collected from 2 surveillance programmes.

Animal samples	Number of samples	
	2017	2018
Snakehead fish (*Channa striata*)	8	0
Catfish (*Clarias batrachus*)	8	30
Nile tilapia (*Oreochromis niloticu*)	8	30
Sea perch (*Lates calcarifer*)	0	30
Freshwater shrimp	8	60
Total	32	150

For the validation method, Pangasius Fillet (*Pangasius Hypophthalmus*) was selected to be the representative matrix of freshwater fish and shrimp. The imported blank sample was purchased from a hypermarket and was tested for the non-chemical contaminant before experimental studies. About 500 g of an edible portion of food was chopped into pieces of 1 cm^3 prior to homogenization by blender. A 15±0.15 g of sample was weighed into a 50 ml polypropylene centrifuge tube. A maximum of 0.2 ml of spiking solution (1 µg/ml for all compounds) was added to the blank sample and was gently evaporated by nitrogen stream before extraction. Fifteen ml of 1% (V/V) acetic acid in acetonitrile were added, and the mixture was shaken vigorously for one minute with the screw cap on. Then 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate were added and the mixture was shaken manually for another minute. The sample contained in the tube was centrifuged for five minutes at 3500-4000 rpm using HERMLE Z366, Germany. The 10 ml of supernatant (equivalent to 10 g of sample) was transferred to a 15 ml PP centrifuge tube containing a mixture of 900 mg $MgSO_4$, 150 mg PSA and 150 mg C18EC, and the tube was shaken in a vortex for 30 seconds. The system was centrifuged for 10 minutes at the same revolutions per minute. Finally, an aliquot of 2 ml of supernatant was transferred to an amber vial, was evaporated under a gentle stream of nitrogen, and was reconstituted for GC-ECD determination with 2 ml of n-hexane. The concentration of the sample represented by the test solution was 1 g/ml. The scheme of the sample preparation is shown in Fig. 1.

2.3 Apparatus

A GC-ECD: Agilent Technologies 6890N (Agilent, USA) with ECD equipped with a Rtx®-CLPesticides2 capillary column (Restex, USA) (30 m x 0.25 mm i.d., 0.2 µm film thickness) and DB-5MS capillary column (Agilent, USA) (30 m x 0.25 mm id, 0.25 µm film thickness) were used for the confirmation. The oven temperature for Rtx®-CLPesticides2 was held at 80°C for one minute, then increased to 190°C by a rate of 20°C/min and then increased to 220°C by a rate of 2.5°C/min and then increased to 240°C by a rate of 1.5°C/min then increased to 270°C by a rate of 8°C/min and held for 8 minutes. By using a rate of 40°C/min, the oven condition was increased to a final temperature of 260°C and was held for 20 minutes. The injection port and detector temperatures were maintained at 220°C and 300°C, respectively. The injection volume was 1 µl in splitless mode with nitrogen make up gas was flowed at 60 ml/min and helium carrier gas flow was set at 1.5 ml/min.

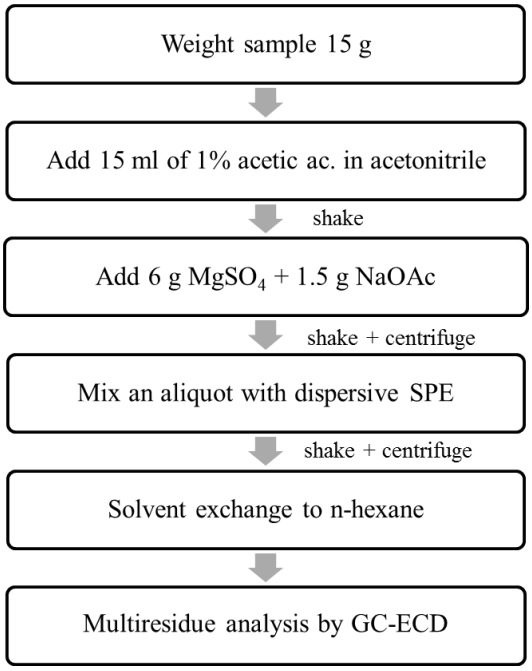

Fig. 1. Scheme of the QuEChERS extraction method used for POP and pesticides residue method validation.

2.4 Method validation

2.4.1 Linearity of calibration curve and working range

Linearity of analytical curves was evaluated by injecting analytical solutions prepared in n-hexane at 2, 5, 10, 15, 25, 50 and 100 ng/ml. According to the US-EPA method (Method 608: Organochlorine Pesticides and PCBs) [15], the gas chromatographic system can be calibrated using the external standard technique by adding volume of stock standards to a volume of organic solvent. It is unnecessary to prepare standards solution on a matrix (matrix matched calibration, MMC) thanks to the GC-ECD technique which did not show the matrix effect. The linearity of working range was tested in three replicates according to the sample preparation described in the sample preparation section. These solutions were injected in ascending concentration order to avoid carry over in the chromatographic system between injections. The criteria for linearity were set using the Pearson correlation coefficient (r) at $r \geq 0.995$ when r^2 is coefficient of determination calculated to give some information about the goodness of fit of a linear model.

2.4.2 Limit of detection (LOD) and limit of quantification (LOQ)

Using data obtained from the linearity study for each analyzed compound, the estimated limit of detection (LOD) as well as estimated limit of quantification (LOQ) were calculated. The calculated LOD was 3 μg/kg for all analytes. To verify the value, eight replicates were tested and it was confirmed that all the analytes were detected for all replicates. The estimated LOQ was evaluated against the target validated LOQ which was 10 μg/kg.

2.4.3 Specificity, accuracy and precision

Seven replicates of the blank sample and method blank were prepared and were injected to the GC system to check interference peak for specificity. In order to evaluate the accuracy and precision of the method, recovery experiments were done. Blank fish tissue samples were spiked with a known amount of the mixture solution (10 μg/ml) to obtain a concentration of all analytes studied at 10, 15 and 50 μg/kg. For each concentration, eight replicates were performed. Accuracy in terms of recovery percentage was calculated. The precision of the analytical method was evaluated in terms of the relative standard deviation (%RSD) and HorRat (Horwitz Ratio) for each concentration. The acceptable range of accuracy and precision were within 70-120% and HorRat ≤ 2 respectively.

2.5. Matrix cleanup assessment

Two different cleanup materials, fatty dSPE (C18/PSA) and ERM-lipid were tested for the matrix cleanup assessment. The fish extract was applied for the experimental comparison between the profile chromatogram of final extracts. The efficiency of matrix cleanup was evaluated by comparing the interference peaks that appeared in chromatographic background. To assess the accuracy of the method, spiked samples at 10 μg/kg were treated using the method described in the sample and sample preparation section followed by the 2 different cleanup procedures.

3. Results and Discussion

QuEChERS (quick, easy, cheap, effective, rugged and safe) method has been reported by Anastassiades *et al*. The original method was chosen for the purpose and has been modified and developed to be the fit method for determination of OC pesticides and PCBs. The official AOAC method was followed in the extraction and partition step. The improvement of the cleanup procedure has been done by testing and choosing the suitable cleanup. The efficiency of the method has to be compromised between the cleanest final extract and the recovery of most of the compounds. Animal origin food does not contain pigment; this is why the negligence of GCB (graphitized carbon

black) was possible. The main obstacle of the fatty food extraction is how to take off the maximum of fat from the sample. The cleanup agent must give the best results of accuracy and fat remover property.

All of the 29 studied compounds in organic solvent showed the Pearson correlation coefficient (*r*) greater than or equal to 0.995. On the other hand, the linearity of working range tested in three replicates at six different levels showed *r* higher than 0.995 for all compounds. The method demonstrated the linearity of the method was between 3 to 100 µg/kg. LODs and LOQs of the analytical method were measured in spiked samples, and calculated by considering a value 3 and 10 times of background noise and by taking in account the linearity of the method, which, for all OC pesticides and PCBs were 3 and 10 µg/kg, respectively. In addition, there is no interference between the components of reagents used for preparation of standard and sample blank extracts. This shows that the method has the specificity.

All of the targeted analytes could be measured by GC-ECD with our chromatographic system set up. The best cleanup agent chosen helped to avoid food matrices which interfered with the calculated results. Although 2 pesticides (dicofol and methoxychlor) had low sensitivity in ECD, the LOD of both pesticides is higher than 3 µg/kg with signal to noise higher than 3 (Fig. 2.).

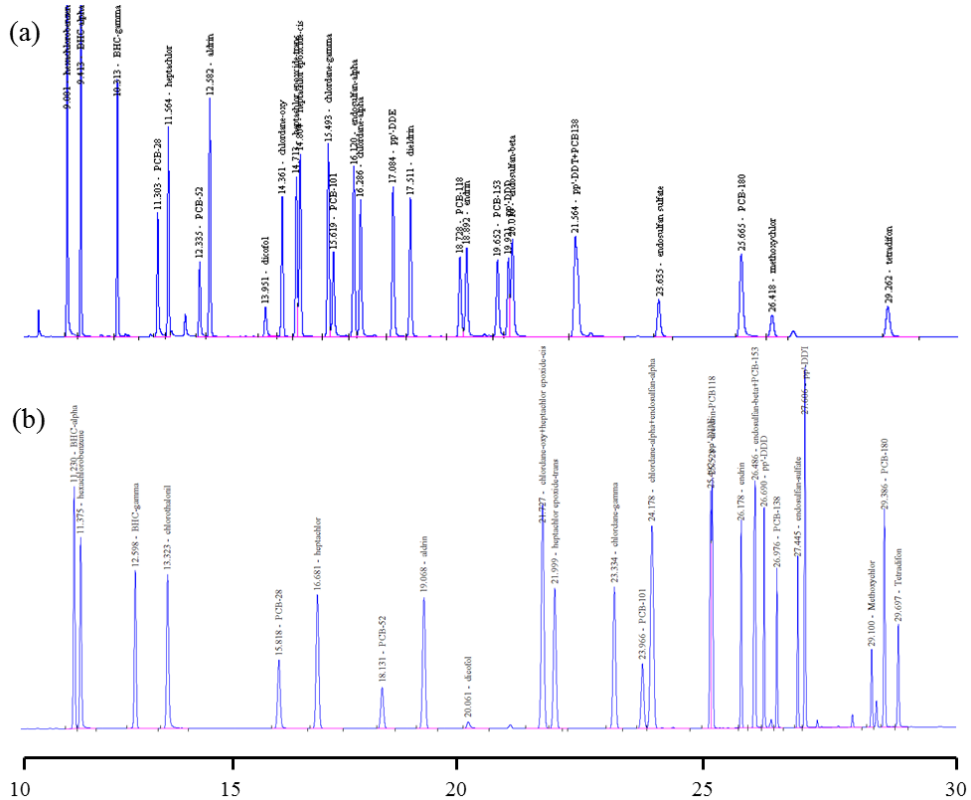

Fig. 2. GC-ECD chromatogram of 22 organochlorine pesticides and 7 PCBs standard solution at a concentration of 100 ng/ml in n-hexane. (a) Rtx®-CLPesticides2 (b) : DB-5MS.

The Rtx-CLPesticides column is specific for organochlorine pesticides and ECD. The excellent separation of analytical peaks was given by the oven temperature optimization. Unfortunately, pp'-DDT and PCB-138 could not be clearly separated by the system. A low polarity phase column, DB-5MS, was alternatively used to identify and quantify the two analytes. The oven temperature conditions in two different chromatographic systems were used for the compound identification and confirmation of the results.

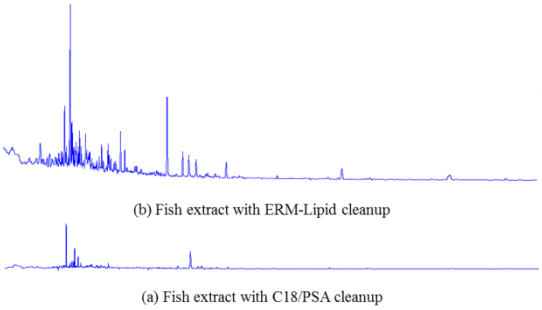

Fig. 3. GC-ECD chromatogram of unspiked fish sample extract (a) with C18/PSA cleanup and (b) with ERM-Lipid cleanup.

The results of sample cleanup assessment are shown in Fig. 3, demonstrating clearly that fatty dSPE (C18/PSA) provides better matrix cleanup efficiency than EMR-lipid dSPE. Because of inefficient matrix lipid removal caused by EMR-lipid dSPE, a high number of interference peaks were observed in chromatographic background. The optimized extraction methods with both cleanups were compared. Fig. 4 shows the statistical recovery comparison results. The C18/PSA protocol provided overall excellent accuracy for most analytes, shows better signal/noise and consistent baseline integration. Only two pesticides were situated out of the 70-120% recovery window (hexachorobenzene 63.8% and methoxychlor 132.9%). Therefore, based on SANCO guidelines [16], they meet acceptable repeatability criteria (HorRat ≤ 2) and the recovery values from experience are all within accuracy criteria. By the way, the accuracy of 12 analytes out of 29 tested has been affected by the ERM-lipid cleanup and shows unsatisfactory recovery. This can be explained by the complication to interpret and to identify the analyte peaks and interference peaks. The matrix background interfered and resulted in higher recovery of most analytes. The selection of C18/PSA to the cleanup step was recommended due to the improvement to make data processing easier and faster. Moreover, this d-SPE gave a higher degree of confidence in the analytical method. Organochlorine pesticides, as well as PCB compounds, are extremely lipophilic and slightly soluble in aqueous phase and they are miscible with lipid component in fatty food such as animal origin products. The challenge for an analytical method is how to extract them with polar solvent, in our case CH_3CN. The loss of recovery will occur if the matrix contains a high amount of fat. To correct this problem, the use of internal standard (ie. isodrin or isotopic compound) calibration will be need for quantification.

Table 2. Linearity data of the target analytes: equation curve and Pearson correlation coefficient (r) of analytical curves obtained from solutions prepared in n-hexane and recovery data for compounds determined by the method.

Analytes	Linearity of calibration curve		Spiking level (μg/kg)					
			10		15		50	
	Equation curve	*r*	Recovery (%)	HorRat	Recovery (%)	HorRat	Recovery (%)	HorRat
aldrin	y = 527x + 822	0.998	112.9	0.1	76.4	0.4	86.8	0.2
BHC-alpha	y = 593x + 140	0.999	105.7	0.6	86.2	0.4	83.4	0.7
BHC-gamma	y = 426x + 226	0.999	103.2	0.4	92.9	0.3	87.3	0.6
chlordane-alpha	y = 369x + 1062	0.999	114.5	0.3	90.8	0.3	82.6	0.4
chlordane-gamma	y = 460x + 1049	0.998	115.6	0.2	88.4	0.4	78.5	0.4
chlordane-oxy	y = 338x + 975	0.998	114.1	0.2	100.6	0.4	82.3	0.4
chlorothalonil	y = 152x + 346	1.000	87.9	1.1	90.1	1.1	87.3	0.4
dicofol	y = 74x + 263	0.998	113.0	0.1	98.5	0.5	86.0	0.5
dieldrin	y = 363x + 991	0.998	108.8	0.4	90.5	0.9	86.2	0.2
endosulfan sulfate	y = 133x + 342	0.998	98.8	0.6	84.1	0.6	79.8	0.5
endosulfan-alpha	y = 412x + 986	0.999	111.6	0.5	86.1	0.4	78.5	0.4
endosulfan-beta	y = 277x + 963	0.997	108.4	0.3	100.7	0.4	90.4	0.5
endrin	y = 200x + 501	0.997	106.4	0.3	114.9	0.3	97.1	0.5
heptachlor	y = 352x + 340	0.999	106.5	0.3	109.0	0.4	91.3	0.6
heptachlor epoxide-cis	y = 468x + 1226	0.998	103.6	0.4	83.7	0.4	80.9	0.4
heptachlor epoxide-trans	y = 396x + 1076	0.998	98.6	0.3	84.3	0.2	79.8	0.4
hexachlorobenzene[a]	y = 611x + 1390	0.998	63.8	0.1	62.2	0.2	61.6	0.5
methoxychlor[a]	y = 51x + 126	1.000	132.9	0.5	141.9	0.4	125.9	0.6
pp'-DDD	y = 208x + 744	0.999	104.0	0.3	88.1	0.3	83.1	0.3
pp'-DDE	y = 334x + 894	0.998	113.7	0.1	90.6	0.9	74.3	0.4
pp'-DDT	y = 352x + 960	0.998	107.1	0.3	95.4	0.7	74.6	0.4
tetradifon	y = 94x + 387	0.998	104.0	0.3	101.3	0.3	95.4	0.5
PCB-28	y = 253x + 755	0.999	102.3	0.1	75.5	0.3	81.7	0.1
PCB-52	y = 180x + 478	1.000	114.5	0.2	87.0	0.5	78.6	0.4
PCB-101	y = 233x + 907	0.998	100.7	0.5	81.4	0.5	72.5	0.6

PCB-118	y = 199x + 744	0.998	100.9	0.4	79.1	0.4	66.7	0.4
PCB-138	y = 352x + 960	0.998	107.1	0.3	95.4	0.7	74.6	0.4
PCB-153	y = 228x + 655	0.999	97.8	0.2	78.4	1.1	60.0	0.3
PCB-180	y = 274x + 684	0.999	91.4	0.2	67.3	0.2	57.8	0.4

[a] Pesticides are out of acceptable range of accuracy (70-120%)

Detailed validation data is shown in Table 2 using C18/PSA as a cleanup agent. The use of dispersive sorbent, including typical C18, is for fat or lipid removal and a primary secondary amine (PSA) could remove polar matrices and soluble fatty acids. The method accuracy and method precision were calculated at 3 levels (10, 15 and 50 µg/kg) of spiked samples (n=8). Recoveries of 20 OC pesticides and PCBs were between 70 and 120%. Nevertheless, in terms of accuracy, two of the OC pesticides (hexachlorobenzene and methoxychlor) were out of acceptable range. The intermediate precision (HorRat) was less than 2 for all analytes at each spiked level. The precision of chlorothalonil was exceptionally high compared to other analytes (HorRat =1.1), which means the analysts might be attentive to this pesticide in routine analysis.

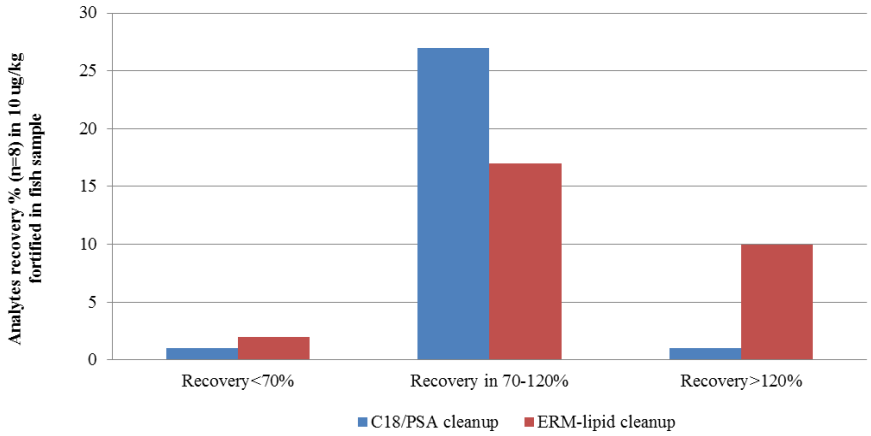

Fig. 4. Number of analytes with % recoveries within the acceptable % recovery range 70-120% for C18/PSA cleanup and ERM-lipid cleanup at 10 µg/kg.

The proposed method shows good selectivity and sensitivity. The excellent recovery and precision allows for a rapid test for screening, as well as confirmative analysis in a regulatory laboratory. A single chemist can prepare 2 dozen homogenized samples within 1 working day (8 hours). The method consumes, per sample, a small volume of non-toxic solvents and needs normal equipment available in every laboratory. It covers specific pesticides and PCBs of interest, and the method is applicable to various animal origin foods such as eggs or processed meat. The proposed method was developed and validated according to the parameters required by ISO/IEC 17025:2005 [17] as a prerequisite for laboratory application to be accredited.

Table 3. Contamination levels detected in freshwater animals collected in 2017 and 2018.

Animal samples	Number of positive samples	
	2017	2018
Snakehead fish (*Channa striata*)	0	0
Catfish (*Clarias batrachus*)	0	0
Nile tilapia (*Oreochromis niloticu*)	0	0
Sea perch (*Lates calcarifer*)	0	0
Freshwater shrimp	0	3

Table 4. Contamination levels detected in positive farmed shrimp collected in 2018.

	Positive samples	Area of collection	POPs	Concentration	
				μg/kg	ng/g fat
1.	Pacific white Shrimp (*Litopenaeus vannamei*)	Bangkok	pp'-DDE	< 10	< 1
2.	Giant freshwater prawn (*Macrobrachium rosenbergii*)	Chiang Rai	PCB-52	30	3
3.	Giant freshwater prawn (*Macrobrachium rosenbergii*)	Udon Thani	PCB-52	< 10	<1

The concentrations of OCPs and PCBs detected in animal tissue samples are given in Table 3. One farmed Pacific white shrimp sample from Bangkok was positive for DDT metabolite with detection of pp'-DDE. Although the usage of such pesticides for agricultural propose has been banned in Thailand since 1983, the trace residue present in food indicates significant current sources of DDT in the central region of the country. It appears that DDT concentration has declined and the concentration found in freshwater product was lower than EMRL. Moreover only one congener of PCB-52 was detected in 2 giant freshwater shrimp from Chiang Rai and Udon Thani with the concentration of 30 and < 10 μg/kg, respectively. PCB-52 is found to be among the more abundant PCB congeners in both animals and in the atmosphere [18-19]. Estimated daily intake of PCB-52 associated with shrimp consumption ranged from < 3 ng/kg/d to 8 ng/kg/d. Results from the present study were comparable to other studies conducted recently and demonstrate that exposure to PCBs from consumption of local farm-raised shrimp from different regions are not likely to pose any health risks.

4. Conclusion

The validated method showed very good performance for most of the studied analytes in terms of sensitivity, accuracy, precision and linearity. except for hexachlorobenzene and methoxychlor. For this reason, the scope of the method was reduced for 27 compounds. After all criteria were evaluated, the method was shown to be appropriate to determine organochlorine pesticide and PCB residues in fish and shrimp matrices. The surveillance study results revealed that OCPs and PBCs were still persistent in the environment and biomagnified to farmed freshwater products. However, there were only 2 POPs (pp'-DDE and PCB-52) contaminated in shrimp which is the most risky freshwater animal. Therefore, low concentrations were found, and estimated daily intake of toxic substances did not exceed the limit

guideline value. Since DDT is one of the most persistent pesticides and needs a certain period of time to biodegrade and PCB-52 is probably generated naturally, the avoidance of this contamination is unlikely to be possible.

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