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# Daily intake and human risk assessment of organochlorine pesticides (OCPs) based on Cambodian market basket data

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#### ABSTRACT

To assess organochlorine pesticide (OCP) contaminations and its possible adverse health impacts, different food samples were collected from three areas of Cambodia, one of the poorest countries in the world. The  $\sum$  OCP concentrations in Kampong Cham, Kratie and Kandal provinces ranged from 1.28 to 188 (median 3.11), 1.06 to 25.1 (5.59) and 2.20 to 103 (20.6) ng g<sup>-1</sup>, respectively. The dichlorodiphenyl-trichloroethanes (DDTs) were the predominant OCPs and accounted for 62.2% (median) among all foodstuffs. Congener profile analyses suggested that there were new input sources of DDTs and hexachlorocyclohexanes (HCHs) in Cambodia, particularly in Kandal province. The estimated daily intake of OCPs (330 ng kg<sup>-1</sup> day<sup>-1</sup>) for residents in Kandal province ranked No. 1 among the 13 compared countries or regions. On the basis of 95th percentile concentrations, the carcinogenic hazard ratios (HRs) of most investigated individual OCPs in vegetable and fish in Cambodia exceeding unity. Particularly for  $\alpha$ -HCH in vegetable, the 95th HR was as high as 186. The data revealed that there is a great cancer risk for the local residents with life time consumption of OCPs in Cambodia.

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#### 1. Introduction

Organochlorine pesticides (OCPs) have been widely used since the second world war [1]. Aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT) were listed in the Stockholm Convention under the category of persistent organic pollutants (POPs) of the United Nations Environment Program [2]. OCPs may pose high toxicity even when they were present in low concentrations, because they were highly lipid soluble and thus bioaccumulate to a significant degree in organism tissues [3]. The main adverse health effects for human are difficulty in breathing, headaches, neurological or psychological effects, irritation of skin and mucous membranes, skin disorders, effects on the immune system, cancer, and reproductive effects [4]. Comparing to mutagenicity, tetragenicity, neurotoxicity, reproductive toxicity and chronic toxicity, carcinogenicity of OCPs is the most important concern for public health issue [5,6]. Therefore, chlordecone, DDT, HCB, hexachlorocyclohexane (HCH), lindane, mirex, and toxaphene have been listed as "reasonably anticipated to be a human carcinogen" by the United States National Toxicology Program (USNTP).

Although the production and use of most OCPs were banned or restricted in many countries, their residues/metabolites are still detectable in various environmental samples from different regions [1,7] and continuously induced a significant impact on the environment and biota, including human beings. In general, daily intake represents the greatest source of OCP exposure. It has been estimated that over 90% body load of DDTs in the general population is derived from food, particularly fatty food of animal origin (i.e. meat, fish and dairy products) [8,9]. In order to study the significance of daily intake to OCP exposure, food monitoring programs based on market basket studies or parallel diets have been carried out in different countries, including USA [10], Sweden [11], and Denmark [12]. However, there is a lack of such comprehensive studies in developing countries in Southeast Asia such as Cambodia.

In Cambodia, there is very limited information about the contamination and distribution of OCPs not only in foodstuffs, but also in other environmental samples. Previous studies revealed that

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concentrations of DDTs in human milk collected from Cambodia were notably higher than those collected from developed countries [13]. The usage of DDTs for agricultural and public health purpose in Cambodia will be continued due to their low cost and versatility against various insects [14]. Much less information is available on human exposure to OCP in Cambodia. To our knowledge, there was only one investigation about the OCP distribution in fish muscle with relatively high levels of DDTs detected in some fish [15]. The study was conducted more than ten years ago, due to the lack of monitoring programs affected by a long-term civil war.

Hence, there is an urgent need to identify the total daily intake and key exposure sources of OCPs for the Cambodian population. More specifically, the objectives of the present study were: (1) to measure OCP concentrations and to analyze congener profiles in different food items collected from the Mekong River basin of Cambodia; (2) to evaluate human exposure to OCPs via daily intake and to identify the major food sources of dietary exposure to OCPs at the three studied sites; and (3) to compare the extent of health impacts with respect to different levels of OCP contaminated foods. To our knowledge, this is the first study to investigate the daily intakes of organic pollutants such as OCPs in Cambodia.

#### 2. Materials and methods

#### 2.1. Sampling sites

The design of the present study was a cross-sectional study. Food samples were collected from three areas in the Mekong River Basin of Cambodia. As shown in Fig. 1, the food samples were collected from Kampong Kong commune (Preak Russey & Lvea Toung villages) in Kandal province, Khsarch Andaet commune (Preak Samrong I & II villages) in Kratie province, and Ampil commune (Andoung Chros & Veal Sbov villages) in Kampong Cham province. Kratie and Kampong Cham provinces are located along the Mekong River, upstream of Phnom Penh; whereas Kandal province is located between the Mekong and the Bassac Rivers, downstream of Phnom Penh.

#### 2.2. Food consumption survey and sample collection

A semi-quantitative food frequency questionnaire was used to characterize the food consumption pattern of each participant. In total, 58 types of food items were included. The data of personal information (sex, age, body weight, period of residency) was also included in the questionnaire. The detailed information about the participants in three sampling sties is listed in Table S1. The number of participants from Kampong Cham, Kratie and Kandal was 58, 31 and 69, respectively. The response rate was 100%. Based on the consumption questionnaire, the weekly consumption of the listed food items was obtained and summarized in Table S2. The selection of food items for analysis was based on the occurrence of OCPs in these food groups and local consumption patterns. As a result, six major food groups, namely (1) vegetable, (2) fruit, (3) fish, (4) meat, (5) viscera, and (6) dairy product, comprising of 21 food items in total, were chosen for OCPs analyses. The reasons for choosing these groups were based on previous results showing that food of animal origin contributed the major part of POPs intake from food [8,16], as well as their availability in the local markets or grocery stores. At least three samples were collected for each food category at each sampling site, with 138 samples in total for the present study.

#### 2.3. Chemical analyses

Only the edible parts of food items were analyzed, with inedible parts such as bone and skin removed prior to analyses. Fish samples were analyzed with the skin since it is commonly eaten. All the edible parts of samples were washed, separated, freeze-dried, and then grounded separately. The chemical analyses were processed with our previously described method [17] with slight modification. Briefly, the samples (about 3 g, dry weight, dw) were spiked with surrogate standards (PCB-60 and PCB-137, 10 ng for each sample) and Soxhlet extracted using mixture of acetone, dichloromethane (DCM) and n-hexane (1:1:1, v:v:v, 120 mL) for 18 h at 68 °C. The lipid content was determined by gravimetric method. A series of chromatographic columns were applied for sample cleanup such as Florisil cleanup (EPA Standard Method 3620B) [18] and gel permeation cleanup (EPA Standard Method 3640A) [19]. Deuterated internal standard TCmX was added into all extracts to 100 ng g<sup>-1</sup> prior to instrumental analysis. The final volume for all samples was 200 µL. OCPs were quantitatively analyzed by a Hewlett-Packard (HP) 6890 N gas chromatograph (GC) coupled with a HP-5973 mass selective detector (MSD). The mass spectrometry mode is selected ion monitoring (SIM). The oven temperature was programmed from 100 °C (initial time, 2 min) to 175 °C at a rate of 15 °C min<sup>-1</sup>, then 3 °C min<sup>-1</sup> to 250 °C held for 9 min. The 20 targeted OCP compounds included DDT and metabolites (o, p'-DDD, p, p'-DDD, o, p'-DDE, p, p'-DDE, o, p'-DDT and p, p'-DDT), HCHs ( $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, and  $\delta$ -HCH), CHLs (heptachlor, *trans*-chlordane, cis-chlordane, trans-nonachlor, and cis-nonachlor), DRINs (aldrin, dieldrin, and endrin), mirex and HCB (hexachlorobenzene). The peaks of p, p'-DDD and o, p'-DDT were extremely close and difficult to be distinguished, therefore these two compounds were combined as one. The details about the quantification procedures were described in Supporting Information.

#### 2.4. QA/QC

The surrogate (PCB-60 and PCB-137) and standard reference material (SRM) 2978 (mussel tissue) obtained from the National Institute of Standards and Technology (NIST, USA) were used to determine the recovery rates, which ranged from 86.4 to 112% for all individual compounds. For each set of 20 samples, a procedural blank, a sample duplicate, and a NIST SRM 2978 sample were processed. The limit of detection (LOD) was determined as the concentrations of analytes in a sample that gave rise to a peak with a signal-to-noise ratio (S/N) of 3, which ranged from 0.05 to 0.20 ng mL<sup>-1</sup>. For measurement values below LOD, a proxy value of half the LOD was assigned for statistical analyses.

#### 2.5. Risk assessment

Risks of OCPs to human health via daily food intakes were assessed according to the guidelines recommended by the USEPA. For non-carcinogenic effects, the estimated daily intake was compared with the recommended reference doses (RfD). For the carcinogenic effects, the hazard ratios (HRs) were calculated by the formula (1) below [20]:

$$Hazard ratios(HRs) = \frac{EDI}{CBC}$$
(1)

where EDI is estimated daily intake, CBC (cancer benchmark concentration) calculated using formula (2) [20]:

$$CBC = \frac{(RL/OSF) \times BW}{CR}$$
(2)

where RL is the maximum acceptable risk level  $(1 \times 10^{-6}, \text{dimensionless})$ , OSF is the oral slope factor (mg kg<sup>-1</sup> d<sup>-1</sup>), BW is the body weight (kg) and CR is the consumption rate (g d<sup>-1</sup>). The CBC for carcinogenic effect is derived by setting the risk to one in one million due to a lifetime exposure. The average body weight of Cambodian

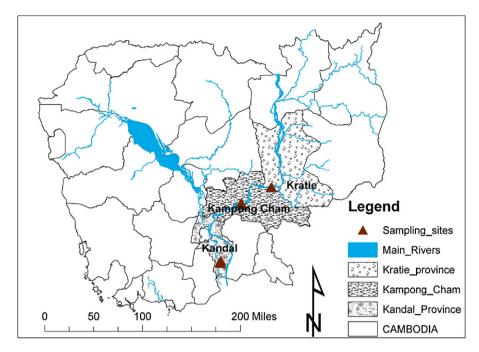


Fig. 1. Map of food sampling sites.

in the present study is based on the results of the survey. The cancer slope factor for individual OCPs is listed in Table 3.

daily intake. Normality was confirmed by the Kolmogorov–Smirnov test. Data of OCP concentrations were analyzed using two independent *t*-tests, Wilcoxon rank sum test, one-way ANOVA and Kruskal–Wallis test as the requirement.

#### 2.6. Data analyses

The OCP concentrations reported in  $ng g^{-1}$ , wet weight (ww) in all samples were not adjusted with the recovery rate due to the adequate recovery. The data analyses were performed using SPSS 17.0 for Windows. The arithmetic mean for each kind of food was used to compare the average OCP concentrations with other studies. However, as there were large variations of OCP levels detected in different types of food items even among the same food group, geometric means were used in the calculation to estimate the dietary

#### 3. Results and discussion

#### 3.1. Food consumption survey

The proportions of different food groups of food baskets at the three study areas are shown in Fig. S1 A–C. The overall average proportion of the three areas is summarized in Fig. S1 D. Among the three study areas, cereals (46.8%), vegetables (23.8%), fruits (13.5%)

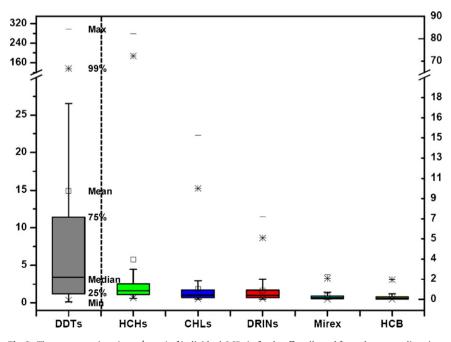


Fig. 2. The concentrations (ng g<sup>-1</sup>, ww) of individual OCPs in foodstuffs collected from three sampling sites.

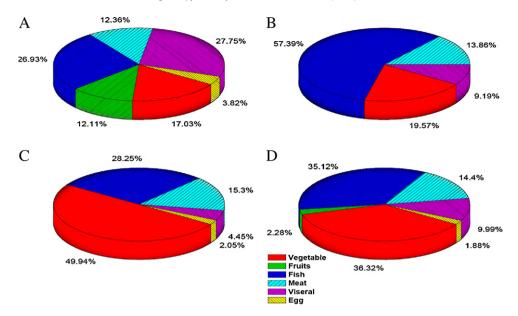


Fig. 3. Contribution from different groups of foodstuffs to the daily intake of  $\sum$  OCPs. (A) Kampong Cham province; (B) Kratie province; (C) Kandal province; and (D) average.

and fish (10.2%) contributed to greater proportions of the total consumption, while foods of other animal origins, such as meat (3.11%) and viscera (1.73%), contributed lesser portion. When compared with the surveys conducted in nearby countries such as China (fish and sea food 3%) [16], Thailand (about 6.6%) [21], Vietnam (about 10%) [22] and European countries such as Spain (33.7%) [23], the residents of Cambodia in general consumed more fish than the Chinese and Thai, comparable with Vietnamese, but less than that of the Spanish. Within Cambodia, the residents in Kandal province consumed significantly (p<0.05) less fish than residents in the other two areas of study.

#### 3.2. OCP levels in foodstuffs

The OCP concentrations in the foodstuffs collected from the three study areas are listed in Table 1. These results were given in fresh weight basis, for estimating OCPs intake from food. Among different food samples, the  $\sum$ OCP concentrations ranged from 1.28 to  $188 \text{ ng g}^{-1}$  in Kampong Cham (median  $3.11 \text{ ng g}^{-1}$ ), 1.06 to  $25.1 \text{ ng g}^{-1}$  in Kratie (median  $5.59 \text{ ng g}^{-1}$ ), and 2.20 to  $103 \text{ ng g}^{-1}$  in Kandal (median 20.6 ng g<sup>-1</sup>). With reference to other countries, the levels of OCP concentrations in the foodstuffs, like vegetable (45.2  $\pm$  36 ng g^{-1}), fish (36.2  $\pm$  9.6 ng g^{-1}) and meat  $(64.0 \pm 41 \text{ ng g}^{-1})$ , collected from Kandal province, were significantly higher than those collected from USA (max  $23.1 \text{ ng g}^{-1}$ in salmon, ww) [10], Sweden (max 16.1 ng g<sup>-1</sup> in fish Gothenb., ww) [11], Romania (max 9.0 ng g<sup>-1</sup> in beef, ww) [24] and China  $(0.67-13 \text{ ng g}^{-1}, \text{ ww})$  [9]. In comparison with the nearby countries, the levels of DDTs in Cambodia foodstuffs were comparable with or higher than that collected from Vietnam and Thailand. For example, the concentrations of DDTs were as much as  $130 \text{ ng g}^{-1}$ (mean of  $48 \text{ ng g}^{-1}$  for meat) in meat and fish collected from Vietnam [25] and 1.21–12.7 ng g<sup>-1</sup> in oyster collected from Thailand [26]. The results further confirmed that the residents in Southeast Asia countries have been exposed to relatively high levels of these contaminants.

The highest and the lowest concentrations of  $\sum$ OCPs were respectively observed in the beef heart  $(189 \pm 98 \text{ ng g}^{-1})$  collected from Kampong Cham and the Chinese Radish  $(1.06 \pm 0.56 \text{ ng g}^{-1})$  collected from Kratie. The OCP concentrations in viscera  $(51.0 \pm 32.1 \text{ ng g}^{-1})$  were significantly (Kruskal–Wallis test,

p < 0.05) higher than other food items collected from all the studied areas. For food items collected from Kampong Cham and Kratie, the OCP concentrations in vegetable  $(2.98 \pm 1.51 \text{ ng s}^{-1})$  were significantly (*t*-test, p < 0.05) lower than that in fish  $(10.8 \pm 6.88 \text{ ng g}^{-1})$ and meat  $(11.5 \pm 3.10 \text{ ng g}^{-1})$ . The results were in line with previous studies which showed that food of animal origin contained higher concentrations of POPs [16,27,28]. However in this study, OCP concentrations in vegetable  $(45.2 \pm 36 \text{ ng g}^{-1})$  were comparable with that in fish  $(36.2 \pm 9.6 \text{ ng g}^{-1})$  and meat  $(64.0 \pm 41 \text{ ng g}^{-1})$ collected from Kandal (Kruskal–Wallis test: p > 0.05). The high OCP concentrations observed in all these foodstuffs collected from Kandal province suggested that there might be recent input sources of OCPs in this area and this will be further discussed below. In addition, the concentrations of  $\sum$ OCPs in the same types of foodstuffs (i.e. cabbage, morning glory, winter melon, fish and beef) collected from Kandal were significantly (paired *t*-test, p < 0.05) higher than those from the other two areas. This was in line with our previous study which suggested that As concentrations in groundwater collected from Kandal (247–1842  $\mu$ g L<sup>-1</sup>, mean 846  $\mu$ gL<sup>-1</sup>) were much higher than those collected from Kratie  $(0.12-141 \,\mu g \, L^{-1})$ , mean of 22.2  $\mu g \, L^{-1})$  and Kampong Cham  $(0.12-2.37 \,\mu g L^{-1}, \text{ mean of } 1.28 \,\mu g L^{-1})$  [29].

#### 3.3. OCP congener profiles and potential sources

As shown in Fig. 2, the concentrations of individual OCPs decreased as follows: DDTs>HCHs>CHLs>DRINs>Mirex>HCB. The DDTs  $(0.14-297 \text{ ng g}^{-1})$ , median 3.47 ng g<sup>-1</sup>) were the predominant OCPs in food items collected from the three areas, accounting for 29.0–97.1% (median 62.2%) of the  $\sum$ OCPs. This observation is in line with the previous studies revealing that DDT contributed most of the OCPs in dietary sources [12,30]. As the second highest concentration of individual OCPs, the HCHs  $(0.09-82.2 \text{ ng g}^{-1})$ , median  $0.79 \text{ ng g}^{-1}$ ) accounted for 0.25-62.5% (median 14.9%) of the  $\sum$ OCPs, which was in consistence with previous studies in Russia [27], Serbia [31] and Denmark [12] where HCHs dominated the OCPs. On the other hand, the concentrations of HCHs were much less than that of other OCPs in market mollusks collected from Dalian, China [32]. The proportions of CHLs, DRINs, mirex, and HCB to  $\sum$ OCPs (median 5.81, 5.66, 2.22 and 1.07%, respectively) were much lower than DDTs and HCHs in foodstuffs collected from

Food Items	Kampon	g Cham						Kratie							Kandal						
	DDTs	HCHs	CHLs	DRINs	Mirex	HCB	$\sum$ OCPs	DDTs	HCHs	CHLs	DRINs	Mirex	HCB	$\sum$ OCPs	DDTs	HCHs	CHLs	DRINs	Mirex	HCB	$\sum$ OCPs
Vegetable																					
Bitter guard	3.61	1.16	0.31	0.13	0.08	0.10	$5.39 \pm 2.28$														
Cabbage	1.09	0.39	0.19	0.08	0.03	0.01	$1.79\pm0.63$	1.19	0.83	0.24	0.18	0.11	0.03	$2.59\pm1.85$	18.8	25.6	1.93	0.70	0.69	0.69	$48.7 \pm 19.7$
Carrot	1.74	0.55	0.08	0.13	0.06	0.03	$2.58\pm0.57$														
Chinese Radish	1.17	0.27	0.44	0.23	0.22	0.03	$2.35\pm0.63$	0.47	0.22	0.16	0.11	0.07	0.02	$1.06\pm0.56$							
Cucumber	2.14	0.31	0.15	0.14	0.07	0.01	$2.82 \pm 1.41$								39.4	30.2	3.80	2.11	0.40	0.69	$76.6\pm29.1$
Eggplant															1.95	1.19	0.51	0.48	0.35	0.03	$4.51 \pm 2.34$
Long Bean	1.35	0.52	0.07	0.19	0.04	0.02	$2.20\pm0.99$	2.75	0.80	0.43	0.50	0.18	0.35	$5.01 \pm 1.16$							
Morning glory	1.49	0.56	0.06	0.13	0.05	0.02	$2.32\pm0.66$	3.75	0.76	0.57	0.34	0.22	0.21	$5.87 \pm 2.13$	58.9	32.20	5.96	3.94	1.16	0.41	$103\pm46.4$
Mustard Green	0.77	0.37	0.03	0.09	0.02	0.01	$1.28\pm0.28$														
Petsai	1.33	0.27	0.24	0.25	0.08	0.02	$2.20\pm0.50$	1.10	0.27	0.19	0.08	0.02	0.07	$1.73 \pm 0.51$							
Sponge gourd	1.49	0.41	0.11	0.07	0.03	0.01	$2.14 \pm 1.29$								0.64	0.66	0.43	0.23	0.13	0.11	$2.20\pm0.61$
Wintermelon	4.13	0.38	0.20	0.15	0.04	0.05	$4.95 \pm 2.86$	2.59	0.93	0.33	0.22	0.09	0.18	$4.33 \pm 2.18$	16.7	16.3	2.12	1.07	0.38	0.38	$37.0 \pm 25.9$
Fruits																					
Banana	1.55	0.47	0.56	0.34	0.13	0.05	$3.09 \pm 1.04$														
Fish	5.47	1.01	0.35	0.51	0.45	0.09	$7.88 \pm 0.81$	9.49	1.86	1.11	0.65	0.31	0.25	$13.7\pm6.63$	23.0	5.72	3.20	2.94	0.35	1.07	$36.3\pm9.56$
Meat																					
Beef	8.81	0.83	0.23	0.58	0.30	0.07	$10.8 \pm 2.59$	8.93	0.94	1.01	0.75	0.13	0.16	$11.9 \pm 0.81$	57.3	2.90	1.43	1.51	0.41	0.48	$64.0 \pm 11.2$
Pork	6.58	1.21	0.19	0.79	0.23	0.14	$9.13 \pm 3.24$	10.9	1.20	0.71	0.85	0.39	0.12	$14.1 \pm 3.91$							
Viscera																					
Beef heart	182	1.70	1.21	1.47	0.78	0.27	$188\pm98.3$														
Beef kidney	7.56	0.95	0.54	0.48	0.22	0.06	$9.81 \pm 2.26$								2.59	0.99	1.36	1.02	0.79	0.02	$6.76 \pm 2.57$
Beef liver	27.3	2.35	1.07	1.11	0.33	0.29	$32.5\pm15.8$								32.4	1.85	4.07	3.76	1.17	1.00	$44.2\pm7.62$
Beef stomach								22.4	1.25	0.52	0.52	0.19	0.21	$25.1 \pm 3.57$							
Diary																					
Egg	22.8	2.84	0.54	0.87	0.00	0.66	$27.8 \pm 13.7$								3.23	1.61	1.06	1.88	1.07	0.14	8.98 ± 1.14

### 1446 Table 2

Estimated exposure to OCPs ( $ng kg^{-1} day^{-1}$ ) via daily intake in different countries/regions.

Country/region	DDTs	HCHs	CHLs	DRINs	Mirex	НСВ	$\sum$ OCPs	References
Cambodiaª								
Kampong Cham	73.4	11.0	5.07	5.68	2.43	0.93	103	The present study
Kratie	88.1	18.1	11.6	7.93	3.46	2.62	139	The present study
Kandal	182	62.7	23.7	19.9	6.54	3.40	330	The present study
Denmark	3.7	2.2	1.5	1.8		1.3		[12]
Spain					2.19			[51]
Australia	0.5-0.6							[52]
Canada	2.44							[53]
USA <sup>b</sup>	3.75	0.142	0.571					[10]
Hong Kong	145							[8]
Sweden <sup>b</sup>	8.93	1.15	1.64		1.62			[11]
Romania	30	21.4						[24]
Serbia	1.28	5.00	0.113	0.066			7.71	[31]
Russia	31	5.8	1.5			3.7		[27]
China <sup>c</sup>	14.7	0.47	0.24		0.19			[41]
South Korea <sup>c</sup>	4.5	0.035	0.217		0.183		4.94	[42]
Reference dose (RfD)	500 <sup>d</sup>	1600 <sup>e</sup>	500 <sup>f</sup>	100 <sup>g</sup>		170 <sup>h</sup>		

<sup>a</sup> The average body weight (kg) based on the food consumption questionnaire for residents of Kampong Cham, Kratie and Kandal was 54, 52, and 51 kg respectively. <sup>b</sup> Estimated body weight 70 kg.

<sup>c</sup> Estimated body weight 60 kg for Asian.

<sup>d</sup> [54].

<sup>e</sup> For β-HCH and γ-HCH.

f [55].

<sup>g</sup> Sum of aldrin and dieldrin [55].

<sup>h</sup> [56].

Cambodia. This observed contamination profile is different from the studies conducted in Russia [27] and Denmark [12] where these individual OCPs had comparable or even higher concentrations than DDTs.

It is well-known that the relative concentrations of DDT and its metabolites could be used to evaluate their input sources [33]. In the present study, the p, p'-DDT was the predominant congener and accounted for 3.76-79.4% (median 28.4%) of  $\sum$ DDTs in food samples. In addition, there were significant (p < 0.01) correlations between the concentrations of *p*, *p*'-DDT and  $\sum$ DDTs in foodstuffs collected from the three areas. The correlation coefficient  $(R^2)$  was 0.645, 0.755 and 0.796 in Kampong Cham, Kratie and Kandal, respectively. The data suggested that there was a recent DDT contamination in the foodstuffs collected from Cambodia [34], especially those collected from Kandal province. It has been shown that if the ratio of total DDE (o, p'-DDE + p, p'-DDE) to total DDTs is less than 0.6, that would suggest a recent input of DDT into the environment [33,35]. The present study showed low ratios (0.05-0.75, median 0.26) for all food items from Cambodia, further confirming the fresh input of DDTs.

For HCHs, the  $\beta$ -HCH was the predominant congener in the foodstuffs collected from Kampong Cham (11.3-61.3%, median 30.3%). It might be due to the fact that the  $\beta$ -HCH is the most resistant to biodegradation among the four HCH isomers [36]. However, the proportions of  $\alpha$ -HCH to  $\sum$ HCHs (10.2–86.5%, median 44.4%) in the foodstuffs collected from Kandal, particularly in vegetables (63.9  $\pm$  26.7%), were much higher than the proportions of  $\beta$ -HCH and  $\gamma$ -HCH (15.2  $\pm$  7.1% and 18.6  $\pm$  3.4%, respectively), suggesting different input sources of HCHs in Kandal from the other two areas. Technical HCH contains 60–70% α-HCH, 5–12% β-HCH, 10–12%  $\gamma$ -HCH and 6–10%  $\delta$ -HCH [37]. Alternatively, the lindane contains more than 99% y-HCH [38]. Therefore, it has been suggested that if  $\alpha$ -/ $\gamma$ -HCH < 1, the main source is lindane. While  $3 < \alpha - /\gamma$ -HCH < 7, it indicates the input of technical HCHs [39,40]. The median ratios of  $\alpha$ -/ $\gamma$ -HCH were found to be 0.54 (0.03–14.1), 1.36 (0.13-23.7) and 2.43 (0.08-46.2) in food items collected from Kampong Cham, Kratie and Kandal, respectively. The results suggested that in Kandal province, the input from technical HCHs is significant [39].

#### 3.4. Estimation of daily intake

The total daily intake levels of individual and total OCPs are listed in Table 2. The calculated daily intakes were obtained by multiplying the amount of food consumed based on our food consumption survey and the corresponding mean concentrations of OCPs detected in each food group [16]. This approach has been previously applied for the investigation of dietary OCPs intake via mixed food categories [31].

The daily intake of OCPs via food consumption was 5.6, 7.3 and 16.8  $\mu$ g day<sup>-1</sup> in Kampong Cham, Kratie and Kandal, respectively. Based on the average body weight of local residents, the estimated daily intake (EDI) for the residents in Kampong Cham, Kratie and Kandal were 103, 139 and 330 ng kg<sup>-1</sup> day<sup>-1</sup>, respectively. Generally, the calculated intake of individual OCPs ranked according to the following trend: DDTs > HCHs > CHLs > DRINs > Mirex > HCB, which was in line with their concentration trends detected in the foodstuffs as mentioned above and in human milk collected from Cambodia [13]. The results suggested that daily intake seems to be the major pathway of human exposure to OCPs in Cambodia, which were consistent with prior studies performed in other regions such as Hong Kong [17] and Sweden [11].

Comparison of daily exposure to OCPs  $(ng kg^{-1} day^{-1})$  via daily intake in Cambodia with other countries or regions is summarized in Table 2. Our data suggested that the EDI of OCPs in Cambodia, particularly for Kandal (330 ng kg^{-1} day^{-1}), was one to two orders of magnitude higher than that of Serbia (7.71 ng kg^{-1} day^{-1})[31], China (15.6 ng kg^{-1} day^{-1})[41] and South Korea (4.94 ng kg^{-1} day^{-1}) [42]. The EDI of all individual OCPs in Kandal ranked No. 1 among the 13 countries and regions listed in Table 2, suggesting that the residents of Cambodia, especially Kandal, may intake much higher levels of OCPs via food than those of other countries or regions. The high daily intake values of OCPs in Cambodia were mainly due to the high concentrations of OCPs in food items, particularly for food items available in Kandal province.

The contributions of OCPs from different food items to the dietary OCP intake are summarized in Fig. 3. Fish consumption contributed the greatest proportion of total OCP intake in Kratie (about 57.4%) and Kampong Cham (about 26.9%). Our results were in line

		lunc noor r																	
Compounds	OSFa	CBCs (n£	CBCs (ng/kg body weight/day)	veight/day)				50th co	50th concentrations HRs	ns HRs				95th con	95th concentrations HRs	ns HRs			
		Veg.	Fru.	Fish	Meat	Vis.	Egg	Veg.	Fru.	Fish	Meat	Vis.	Egg	Veg.	Fru.	Fish	Meat	Vis.	Egg
p, p'-DDE	0.34	43.4	691	44.9	109	158	837	0.07	0.04	0.14	0.01	0.00	0.00	4.62 <sup>b</sup>	0.05	0.55	0.08	0.06	0.01
p, p'-DDT	0.34	43.4	691	44.9	109	158	837	0.17	0.08	0.11	0.02	0.01	0.00	2.52	0.11	0.45	0.11	0.13	0.00
<b>V</b> DDTs	0.34	43.4	691	44.9	109	158	837	0.50	0.16	0.53	0.05	0.04	00.00	11.8	0.22	1.75	0.32	0.30	0.01
α-HCH	6.30	2.34	37.3	2.42	5.91	8.51	45.2	0.51	0.09	0.57	0.02	0.01	0.00	186	0.22	1.57	0.17	0.04	0.01
<b>β-</b> HCH	1.80	8.20	131	8.48	20.7	29.8	158	0.25	0.05	0.13	0.01	0.00	0.00	2.82	0.07	0.79	0.02	0.01	0.01
γ-HCH	1.30	11.4	181	11.7	28.6	41.3	219	0.13	0.04	0.09	0.01	00.00	00.0	2.44	0.05	1.04	0.02	0.01	0.00
Chlordane	0.35	42.2	671	43.6	106	153	813	0.03	0.02	0.01	0.00	00.00	00.0	0.43	0.07	0.03	0.00	00.0	0.00
Dieldrin	16.0	0.92	14.7	0.95	2.33	3.35	17.8	0.65	0.23	0.49	0.03	0.03	0.01	15.6	0.51	5.07	0.17	0.12	0.04
HCB	1.60	9.22	147	9.54	23.3	33.5	178	0.05	0.03	0.07	0.00	0.00	0.00	1.17	0.04	0.39	0.02	0.01	0.00
CBC: cancer benchmark concentrations (ng/kg body weight/day) (the food consumption rate and body weight were on the basis of survey). Veg.: vegetable; Fru.: fruits; vis.: viscera; HRs: hazard ratios.	cer slon fact	centrations	$(ng/kg bod_{1})$	y weight/day	y) (the food c 1 from USEPA	onsumption I Vs Integrated	uption rate and body weight were on t orated Risk Information System (IRIS)	ty weight ation Syst	were on th	ie basis of 5	survey). Ve	g.: vegetab	le; Fru.: fri	uits; vis.: vi	scera; HRs	: hazard ra	itios.		

Table 3

USF (Dial cancer stop factors, (filg Kg <sup>- (d)</sup> - ) <sup>-</sup> Values in bold: hazard ratio is greater than 1. 18

with previous studies showing that fish was the most important exposure pathway for humans exposed to OCPs in Hong Kong [8], Denmark [12] and Sweden [11]. In contrast with previous studies that vegetable only contributed 8% to total PCB daily intake in Taizhou, China [16] and the contribution of vegetable was much less than dairy products and cereals in India [43], the present data revealed that in Cambodia, consumption of vegetables contributed a great proportion (mean 36.3%, Fig. 3D) of the total OCP intake, especially for the residents of Kandal (49.9%, Fig. 3C). Our results were in line with an early study conducted in Vietnam that the OCP daily intake from vegetable contributed about 35% [44]. This was mainly due to the fact that vegetable collected from Kandal contained very high concentrations of  $\sum$ OCPs (mean 45.2 ng g<sup>-1</sup>). For example, the concentrations of  $\sum$ OCPs in cabbage collected from Kandal was 48.7 ng  $g^{-1}$  (ww), which was significantly higher than the other two sites (1.79 and  $2.59 \text{ ng g}^{-1}$  in cabbage collected from Kampong Cham and Kratie, respectively) and the surrounding countries such as India (DDTs, 2.33  $ngg^{-1}$  in cabbage) [45]. As suggested by the results of source apportionments, the high concentrations of OCPs in vegetable collected from the Kandal province main due to the fresh input of DDTs and technical HCHs. Although the cooking processes leading to loss of organochlorines [46] was not taken into the consideration in the present study, our results revealed that the washed vegetable could also contribute largely to the total OCP daily oral intake of people living in Kandal province. The contribution of meat towards OCPs ranged from 12.4 to 15.3% with a mean of 14.4%, which were lower than that in Russia (22% to 48%) [27] and Vietnam (36% for DDTs) [44]. Importantly, the viscera contributed the most (27.8%) in OCPs intake among different food groups in Kampong Cham, which was never reported before. This might be caused by the high concentrations of OCPs  $(36.6 \text{ ng g}^{-1})$ and large amounts of viscera consumption according to the local food habits. Although the general scientific observed suggested that pesticides dissipate fast in tropical/semitropical environment [47,48], the high concentration of residues is attributed to that Cambodia has not adhered to the Stockholm Convention, and the use of organochlorine insecticides is not banned.

#### 3.5. Risk assessment and food safety aspects

The tolerable daily intake (TDI) or acceptable daily intake (ADI) values have been established for the OCPs on a body weight basis. These reference values can be used in risk assessment to evaluate the maximum daily intake of OCPs over a lifetime without causing any noticeable health risk [49]. To estimate the non-carcinogenic risks from dietary OCP exposure in Cambodia, the exposure levels (expressed as daily intakes) were compared with the tolerable daily intake value (reference dose, RfD) for individual OCPs. The estimated daily intakes of these individual OCPs are well below the RfD for non-carcinogenic effects (Table 2). This, however, does not necessarily suggest that there is no potential health risk associated with food consumption in Cambodia. For example, the present results indicated that the levels of DDTs in about 44% of the fish samples in Cambodia were higher than the maximum admissible concentrations suggested by the USEPA  $(14.4 \text{ ng g}^{-1}, \text{ ww})$  [50]. In addition, the RfD value is based on experimental toxicological data of that particular pesticide. Although the levels of individual OCPs in Cambodia food were lower than RfD, these OCPs could accumulate in the organism and exert harmful effects. Furthermore, this approach neglected the possibilities of synergistic effects when two or more pesticides or other pollutants are taken up simultaneously.

The cancer benchmark concentrations (CBCs) for carcinogenic effects were derived using cancer slope factors from the USEPA [9]. HRs was estimated by dividing the average exposure by the CBCs. The HR exceeding 1 indicates that there is potential risk to human health. Two HRs (50th and 90th percentile exposure) were used to estimate the potential risks. As shown in Table 3, the 50th HRs for all individual OCPs were less than one, indicating that there may not be adverse effects for food consumption in Cambodia. However, on the basis of 95th percentile concentrations, most investigated individual OCPs in vegetable and fish had HRs exceeding unity. This was particularly true for  $\alpha$ -HCH in vegetable; the 95th HR was as high as 186, indicating that there was high cancer risk for humans. As it is not sure that cancer risks caused by the combined effects of individual OCPs for the same reason as non-carcinogenic risks, the cancer hazard risks for both 50th and 95th percentile concentrations would be even higher if this is taken into account.

In the present study, some limitations associated with the exposure can lead to uncertainty of total risk assessment. For example, the standard deviations of OCP concentrations were even higher than 50% of the mean values due to the high variability of pollutant concentrations detected in food samples collected. In addition, the cereal samples such as rice, corn and noodle made from wheat were not taken into account for the analyses, which likely to result in a heavy bias. If these and other potential types of uncertainty (such as sample representation and the human errors based on questionnaire survey) are taken into account, the results of EDIs and health risk assessment are more qualitative and should be used with caution. A more refined assessment of the daily intake and subsequently the potential health risk assessment are deemed necessary in the future, when more information becomes available. Our study, however, has provided a clear picture on the high daily intakes of OCPs via food consumption in Cambodia, a developing country with OCPs still being used.

#### 4. Conclusion

The daily intake of OCPs based on consumption survey and food basket analyses in Cambodia was investigated in the present study. The concentrations of  $\sum$ OCPs dominated by DDTs and HCHs in foodstuffs collected from Cambodia were significantly higher than other developing or developed countries. The food of animal origins (meat, fish and viscera) contained higher OCP concentrations than other food items in Kampong Cham and Kratie. However, the OCP concentrations in vegetables were comparable with that in the fish and meat in Kandal due to the recent input of OCPs. It was confirmed by the congener profile analyses of DDTs and HCHs. The estimated daily intakes of OCPs in Cambodia were much higher than other countries, with EDIs of Kandal province (330 ng kg<sup>-1</sup> day<sup>-1</sup>) ranked No. 1 among the 13 investigated regions. Risk characterization on the basis of 95th HR suggested that there is a high cancer risk for residents at Kandal due to OCPs intake through life time consumption of vegetable and fish.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.06.062.

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