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Distribution and Potential Human Risk of Organochlorine Pesticides in Market Mollusks from Dalian, China

Hongliang Jia · Yaqing Chang · Yeqing Sun · Degao Wang · Xianjie Liu · Meng Yang · Diandou Xu · Bo Meng · Yi-Fan Li

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Abstract Levels of organochlorine pesticides (OCPs) were analyzed in mollusk samples collected from markets in Dalian, China. Among 14 OCPs screened, chlordane (Chls), dichlorodiphenyltrichloroethane (DDT) and 1,2,3,4,5,6-hexachlorocyclohexane (HCH) were dominant compounds with the medians of 30.2, 5.31 and 2.03 ng/g wet weight (ww), respectively. Source analysis showed that, much higher concentrations of Chls in mollusk samples were the result of usage of the chemical in the city, HCH in samples were mainly due to historical technical HCH usage, and a small amount of fresh use of DDT might exist, causing high portion of *p*,*p*′-DDT in samples. Cumulative distribution functions for the concentrations of the selected OCPs were compared to several threshold criteria, indicating that concentrations in most mollusk samples were lower than the safe lines. The concentration data of selected OCPs were used for risk assessment, and human exposure levels were compared to noncancer and cancer benchmarks.

Keywords Mollusks · OCPs · Human exposure · Risk assessment · China · POPs

H. Jia · D. Wang · X. Liu · M. Yang · Y.-F. Li (✉)
International Joint Research Center for Persistent Toxic Substances (IJRC-PTS), Dalian Maritime University, 1 Linghai Road, 116026 Dalian, China
e-mail: ijrc_pts_paper@yahoo.com

Y. Chang
Key Laboratory of Mariculture, Ministry of Agriculture, Dalian Fishery University, 52 Heishijiao Street, 116023 Dalian, China

Y. Sun
Environmental System Biology Institute, Dalian Maritime University, 1 Linghai Road, 116026 Dalian, China

D. Xu
IJRC-PTS, Institute of High Energy Physics, Chinese Academy of Sciences, 19 Yuquan Road, 100049 Beijing, China

B. Meng · Y.-F. Li
IJRC-PTS, State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, 202 Haihe Road, 150090 Harbin, China

Y.-F. Li
Science and Technology Branch, Environment Canada, 4905, Dufferin Street, Toronto, ON M3H 5T4, Canada

Toxic chemicals released to the environment from point sources such as industrial and municipal discharges and from nonpoint sources such as agricultural runoff and atmospheric deposition have contaminated surface waters and their sediments across China. Many chemical pollutants concentrate in fish and shellfish by accumulating in fatty tissues or selectively binding to fish muscle tissue. Even extremely low concentrations of bioaccumulative pollutants detected in water or bottom sediments may result in accumulation in fish and shellfish. Lipophilic contaminants, particularly certain organochlorine compounds, tend to accumulate in the fatty tissues of fish and shellfish. Thus, aquatic organisms are commonly used for environmental monitoring of pollutants such as heavy metals, organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs). Aquatic mollusks occupy a particular food niche, such as filter feeding of suspended matter and/or bottom sediments are highly tolerant to many pollutants and exhibit high accumulation property (Bervotes et al. 2005; Kim et al. 2002). These features make mollusks suitable sentinel organisms for monitoring the fraction of bioavailable

pollutants in the aquatic environment, and have been widely used to monitor the quality of water ecosystems.

Mollusks are heavily consumed in people's daily life, even in inshore places, like the study area Dalian. Potential human risk exists universally because of the consumption of seafood contaminated by persistent pollutants. Some studies on persistent pollutant contamination in fish tissues associated with human risk assessment have been carried out in China and other countries (Binelli and Provini 2004; Jiang et al. 2005; Yang et al. 2006; Li et al. 2008). With the banning of production and use of persistent pollutants, the residual levels in foodstuffs have decreased significantly, but potential risk still exists. In China, there are not any national or regional regulations for the maximum allowable highest residue levels in seafood (especially for mollusks) for persistent compounds except for DDT and HCH. This paper is the first comprehensive study reporting on concentrations of a wide selection of OCPs in 14 species of aquatic edible mollusks (n = 229), and also the first study of risk assessment for the consumption of these mollusks in Dalian region, China.

Materials and Methods

A total of 229 aquatic mollusk samples, belong to 14 species, were purchased from 4 seafood markets in the city of Dalian in February, 2008, at least 15 individuals were purchased for each species (see Table 1 for detail). The samples were sent to the laboratory of the International Joint Research Center for Persistent Toxic Pollutants (IJRC-PTS), Dalian Maritime University, Dalian, China for analysis. A clean and acetone rinsed bistoury was used to take off edible parts from shells, and the edible parts were packed in a solvent-rinsed glass bottle with Teflon-lined cap, and stored at −20°C until extraction.

Samples were treated, extracted, and analyzed according to the methods established at the National Laboratory for Environmental Testing (NLET), Environment Canada. All solvents used were of pesticide grade purity (J.T. Baker, USA). A mixture of 14 OCP standards, including α-HCH, β-HCH, γ-HCH, *p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, α-chlordane (*cis*-chlordane, CC), γ-chlordane (*trans*-chlordane, TC), *trans*-nonachlor (TN), α-endosulfan (α-Endo), β-endosulfan (β-Endo), endosulfan sulfate (EndoS), heptachlor epoxide (isomer β, β-HE), were purchased from the Supelco, Inc. (Supelco, USA).

Ten g of homogenized sample and 10 g anhydrous sodium sulfate were accurately measured into a pre-cleaned extraction thimble, spiked with a surrogate standard mixture of D_8-*p*,*p*′-DDT and 1,3,5-Tribromobenzene(TBB) (purchased from Supelco, USA). After sufficient mixture, the samples were then Soxhlet extracted for 24 h with 100 mL of solvent mixture (dichloromethane/acetone, 1:1 v/v). Among whole extract, 10% was used to determine lipid content gravimetrically, 30% was archived for future use. The remaining extract (60%) was added to a separatory funnel, treated 3 times with 98% H_2SO_4, collected, and rotary-evaporated to 1 mL. The extract was passed through 5.5 g silica gel column (Silica 60, Merck, Germany), after pre-rinsing with 25 mL of hexane, eluted with 25 mL of hexane for PCBs and 25 mL of hexane/dichloromethane mixture (1:1, v/v) for OCPs. The elution was rotary-evaporated to 2 mL and then reduced to 1 mL under a gentle nitrogen gas flow. All OCPs were quantified with GC-ECD (Varian CP-3800). DB-5 MS of 0.25 mm ID

Table 1 General information for the collected mollusks

Species	Abbreviation	Number of sample	Collection location	Lipid content (%)
Crassostrea gigas	CRG	17	Market 1	1.31 ± 0.38
Meretrix meretrix	MEM	19	Market 2	1.26 ± 0.19
Mytilus edulis	MYE	15	Market 2	1.87 ± 0.48
Scapharca subcrenata	SCS	15	Market 1	0.72 ± 0.11
Mactra veneriformis	MAV	26	Market 1	0.77 ± 0.34
Hemifusus tuba	HET	15	Market 2	2.12 ± 0.40
Patinopecten yessoensis	PAY	15	Market 3	1.33 ± 0.35
Chlamys farreri	CHF	17	Market 3	1.36 ± 0.46
Ruditapes philippinarum	RUP	15	Market 3	0.63 ± 0.12
Sinonovacula constricta	SIC	15	Market 1	1.53 ± 0.28
Solen dunkerianus	SOD	15	Market 1	0.71 ± 0.02
Saxidomus purpuratus	SAP	15	Market 4	1.48 ± 0.10
Mya arenaria	MYA	15	Market 4	0.89 ± 0.03
Mactra chinensis	MAC	15	Market 1	0.75 ± 0.06

and 30 m length was used for analysis of OCPs. The column oven temperature was programmed at an increasing rate of 15°C/min from an initial temperature of 90°C to a temperature of 160°C, and then 3°C/min to 280°C (5-min hold). Injector and ECD temperatures were 250°C and 300°C, respectively.

All samples were spiked with a labeled recovery standard containing D_8-*p*,*p*′-DDT and TBB prior to extraction. The surrogate standard recoveries were 113 ± 32% and 103 ± 20% for TBB and D_8-*p*,*p*′-DDT, respectively. Spike and blank samples were included at a rate of one for every 10 samples extracted, and the average spike recoveries were from 70 to 102% for all 14 OCPs except for *p*,*p*′-DDE (38%), EndoS (40%), and *β*-endosulfan (recoveries of this compound were under 5% from all spike samples, so only other 13 OCPs were reported in this study). The instrument detection limits (IDLs) were determined by assessing the injection amount that corresponded to a signal-to-noise value of 5:1. For α-HCH, *β*-HCH, *γ*-HCH, *β*-HE, CC, TC, α-Endo, TN and *p*,*p*′-DDE, the IDL values were 1 pg, and the values of *p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDD and EndoS were 5 pg. Results were reported only if the signal exceeded five times the baseline noise. Blank samples were included at a rate of one for every 10 samples extracted, and measurements for blanks were all bellow the IDL.

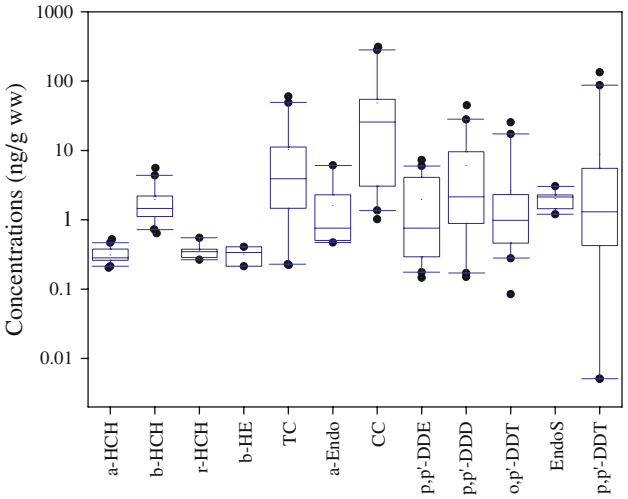

Fig. 1 Box-Plot (log) concentrations (ng/g wet weight) of OCPs in mollusk samples collected from Dalian. The horizontal lines represent 5th, 50th, and 95th percentiles, and the boxes represent 25th and 75th percentiles. Outliers are shown as individual points. Median concentration and range values (ng/g) for OCPs were: 0.28 and ND-0.53 for α-HCH; 1.45 and ND-5.62 for *β*-HCH; 0.32 and ND-0.55 for *γ*-HCH; 21.13 and 1.02–312.70 for α-chlordane; 3.16 and 0.22–60.21 for *γ*-chlordane; 1.44 and 0.01–134.78 for *p*,*p*′-DDT; 0.89 and ND-25.51 for *o*,*p*′-DDT; 0.77 and 0.15–7.31 for *p*,*p*′-DDE; 2.37 and ND-45.11 for *p*,*p*′-DDD; 0.76 and ND-6.14 for α-endosulfan; 1.80 and ND-3.06 for endosulfan sulfate; 0.34 and ND-0.41 for heptachlor epoxide. (*ND* not detected)

Results and Discussion

The detected compounds included HCHs (α-, *β*- and *γ*- isomers), DDT (*p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD), chlordane (CC, TC, TN), α-Endo, EndoS, *β*-HE. The occurrence frequencies were 74% for HCHs, 100% for DDT and chlordane (Chls), 7% for *β*-HE, 26% for α-Endo, and 14% for EndoS. The 50th percentile (median) concentrations were used for comparison and assessments. Box-Plot (log) concentrations of OCPs in mollusks were given in Fig. 1, which showed that the OCP with the highest residue level found in mollusks was Chls with a median of 30.25 ng/g wet weight (ww) (all the concentration numbers were normalized to wet weight except where indicated) followed by DDT (5.31 ng/g) and HCHs (2.03 ng/g).

It is notable from Fig. 1 that the concentrations for the most detected OCPs varied widely among mollusk samples, especially for *p*,*p*′-DDT. In mollusk species, the highest residual levels of *p*,*p*′-DDT were found in CRG (135 ng/g), and the lowest were found in MEM (only 0.005 ng/g), 5 orders of magnitude lower. Two or three orders variations were found in species for α-Chlordane, *γ*-Chlordane, *o*,*p*′-DDT, *p*,*p*′-DDE, and *p*,*p*′-DDD. These results were similar to those found in former studies (Yang et al. 2006; Meng et al. 2007; Li et al. 2008), which suggested that bioaccumulation of OCPs in mollusks were highly species dependent, probably due to different ecological characteristics for different aquatic species such as feeding habits and habitats (Yang et al. 2006).

DDT is still being produced in China for use in malaria control and is also present in dicofol as impurity, an insecticides currently used in China (Qiu et al. 2005; Zhao 2005). After the ban of technical DDT usage in China from 1983 (Li et al. 1999), 50–100 t of DDT were released to environment in Dalian region from 1984 to 2003 (Zhao 2005) due to different purposes, mainly the use of dicofol, which could enter the waterways and accumulate in freshwater, estuarine, and marine environments of China (Wu et al. 1999; Yuan et al. 2001). DDT is known to biodegrade to DDE under aerobic conditions and to DDD under anaerobic conditions, and the values of DDT/(DDE + DDD) over 1 can be used as an indicator of possible new sources. In present study, the values of DDT/(DDE + DDD) were from BDL (below detection limit) to 5.91, with a median of 1, and 52% of which were more than 1. The results showed that potential new sources of DDT probably exist.

HCHs were ubiquitous compounds in environment, and also commonly existed in tissues of mollusks, but were lower than DDT (Fig. 1). Differently from other OCPs, HCH levels were less variable among mollusk samples,

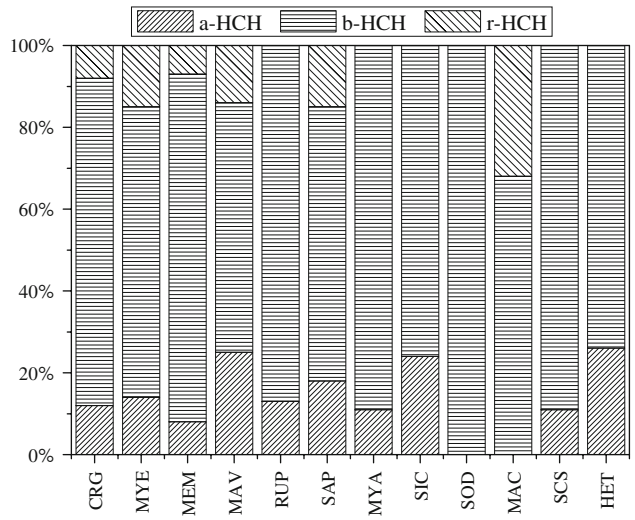

Fig. 2 Composition of α-, *β*- and *γ*-HCHs in 12 species of mollusks. Median of concentration for each species was used in composition calculation. There were no HCHs detected in PAY and CHF

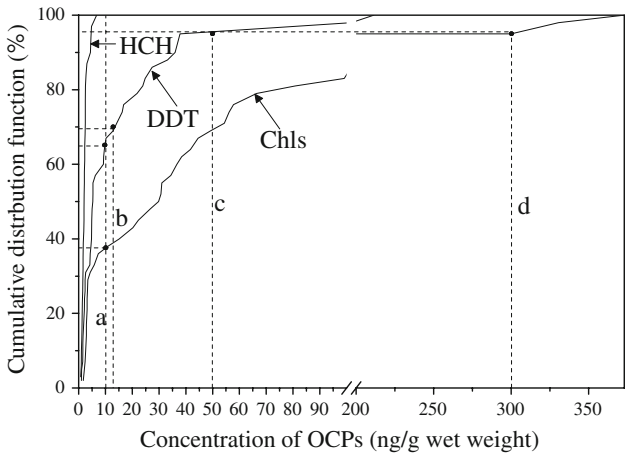

Fig. 3 Cumulative distribution function for OCPs versus mean concentration of different OCPs in all mollusk samples. *Four vertical dot lines* in the figure represent four different assessment standards: (*a*) The most stringent standard (10 ng/g wet weight) of the *marine biological quality* on shellfishes enacted by the Chinese government (GB 18421-2001) for DDT; (*b*) Tolerances for DDT and Chls with EPA's recommended *screening values* (SVs, 14 ng/g wet weight); (*c*) maximum admissible concentration (50 ng/g wet weight for DDT) established by the European Union on *the basis of the lipid percentage of seafood*; (*d*) US *Food and Drug Administration* (FDA) action levels for Chls (300 ng/g wet weight). For HCHs, lower concentration are obvious, for DDT and Chls, some restrict are marked

with a range of 0.78–6.70 ng/g and with median of 2.03 ng/g. Historically, the usage of technical HCHs was much higher than that of DDT in China, and the data reached to 4,500 kt for the former and 270 kt for the later from 1952 to 1984, respectively (Li et al. 1998, 1999, 2001). However, the result of the present study indicated that the residual levels of HCHs in mollusks were lower than those of DDT. There could be two reasons. Firstly, HCH has higher biodegradability and lower lipophilicity than DDT (Guo et al. 2007), and secondly, DDT has still been released to environment since 1983 due to the use of dicifol and other purposes. Relatively low HCH concentration levels in mollusks were also reported by other research groups (Wang et al. 2007; Ramu et al. 2007; Guo et al. 2007).

Technical HCH produced in China contains 65–70% α-HCH, 5–6% *β*-HCH, 13% *γ*-HCH and 6% *δ*-HCH (Cai et al. 1992; Li et al. 1998). Figure 2 presents composition of HCH isomers in 12 species of mollusks, indicating the dominant portion of *β*-HCH in all mollusk species, which was expected. After study of source, fate and pathway of HCHs into the arctic, Li et al. (2003) reported that due to more volatile property, long-range air transport was a primary pathway for α- and *γ*-HCH isomers to enter Arctic, but due to much lower Henry's law constant (about 1/20 of the value of α-HCH), *β*-HCH preferred to stay locally and bioaccumulate in aquicolous biota.

Chlordane was the OCP with the highest residue levels detected in mollusk samples. Its median concentration was 30.25 ng/g, 6 times higher than DDT (5.31 ng/g) and 15 times higher than HCHs (2.03 ng/g). A large variability of concentrations of α-Chlordane was found not only among species, but also among individuals in the same species. For example, the concentration ranges were from 2.03 to 134 ng/g in CRG samples, a variation of 2 orders of magnitude.

In present study, Chls was found in all samples, which indicated that the contamination of Chls was prevailing in mollusks in Dalian. This result was different from some former studies (Yang et al. 2006; Wang et al. 2007; Ramu et al. 2007). The use of technical chlordane was started in the 1970s, and the insecticide has been applied to control termites for construction since 1988 in China. Production of chlordane was approximately 250 t per year from 2000. It was estimated that the total usage of chlordane reached 3 t in Dalian from 1988 to 2004 (Zhao 2005). Some former studies reported that the half life of chlordane was about 4–20 years or even longer (Agency for toxic substances and disease registry 1989, 1994; Ontario ministry of environment and energy 1993), and more than 10% of chlordane residue was found in soil 9 years after the use of this insecticide (Bennett et al. 1974; Steward and Chisholm 1971; Steward and Fox 1971). The local use of Chls is probably the major source of Chls in mollusks in Dalian region.

In order to describe the contamination levels of DDT among all samples, Guo et al. (2007) introduced a cumulative distribution function (CDF), which is defined as

$$\mathrm{CDF}_{<C} = \frac{N_{<C}}{N} \quad (1)$$

Table 2 Human exposures and benchmark concentrations for OCPs in mollusks

	Median of concentration (ng/g ww)	EDI (ng/kg body weight/day)	Noncancer benchmark concentration (ng/kg body weight/day)[a]	Cancer benchmark concentration (ng/kg body weight/day)[b]
∑HCH	2.03	1.16	300[c]	1.50[c]
∑DDT	5.31	3.03	500	1.02
∑Chls	30.3	17.2	500	6.04
α-Endosulfan	0.76	0.43	6,000[d]	–[e]
Heptachlor epoxide	0.34	0.19	13	1.76

∑HCH = α-HCH + β-HCH + γ-HCH, ∑DDT = *p,p′*-DDT + *o,p′*-DDT + *p,p′*-DDE + *p,p′*-DDD, ∑Chls = α-chlordane + γ-chlordane

[a] Risk values derived from chronic RfD, recommending by USEPA

[b] Benchmark concentration for carcinogenic effects equals 10^{-6} divided by the cancer slope factor, represents the exposure concentration at which lifetime cancer risk is one in one million

[c] The value listed as γ-HCH

[d] The value listed as α- and β-endosulfan

[e] No data available

where $N_{<C}$ is the sample number with concentration smaller than C (ng/g), and N is the total number of the samples. CDFs were calculated for HCHs, DDT and Chls in our study and the results presented in Fig. 3. Several threshold residue standards for seafood or shellfishes were collected from different sources, and used here for risk assessment. For HCHs, all of the samples were far below the *standard of marine biological quality* (20 ng/g ww) on shellfish enacted by the Chinese government (Chinese government 2002), and 10 ng/g ww established by the European Union on *the basis of the lipid percentage of seafood* (Binelli and Provini 2003). There were three different residue standards for DDT. The first was the most stringent *standard of marine biological quality* (10 ng/g) on shellfishes enacted by the Chinese government (Chinese government 2002), the second was *screening values* (SVs, 14 ng/g) recommended by the USEPA's (2000), and the third was the *basis of the lipid percentage of seafood* (50 ng/g) established by the European Union (Binelli and Provini 2003). The CDF for DDT were 64%, 69%, and 95% according to the first, the second, and the third standards, respectively. For Chls, two different standards were used in this study. The first one was 14 ng/g, recommended by the USEPA's SVs (USEPA 2000), and the second one was 300 ng/g, established by the US *Food and Drug Administration* (FDA) (USEPA 2000), and the CDF were 38% and 95% according to the two standards. Though the concentration was very low, HCHs was widely detected in mollusk samples in Dalian. Low residual levels of HCHs suggest that HCHs was not a significant organic contaminant in mollusks, but the widespread distribution of HCHs still deserved further monitoring efforts to ensure the long-term safety of consumers with consumption of seafood products. Being different from other studies, high Chls concentrations were unexpected. It was suggested that although the use of Chls in the region was not high (Zhao 2005), the local use of this chemical could still affect aqueous ecosystem seriously, and leads to a high concentration of these contaminants in seafood.

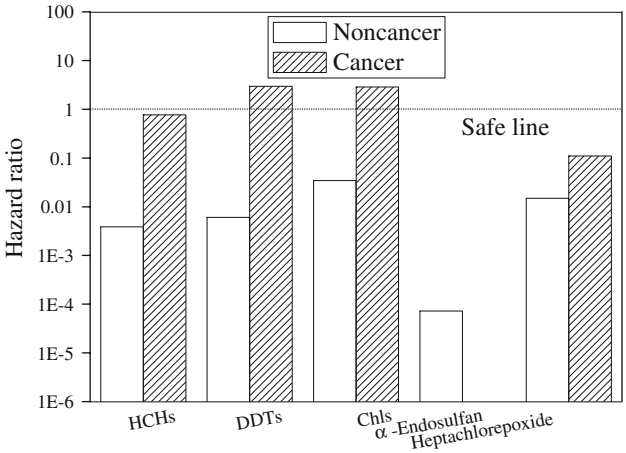

Fig. 4 Noncancer and cancer hazard ratios to human exposure to organochlorines in mollusks. For α-endosulfan, no correlative carcinogens benchmark concentrations are available

Acceptable daily intake (ADI) recommended by the Food and Agriculture Organization and World Health Organization (FAO/WHO) is usually used to assess human exposure to target contaminants without considering different eating habits and consumption rates. For estimated individual exposure assessment for mollusks, we calculated the estimated daily intake (EDI, ng/kg body weight/day) of OCPs by the equation.

$$\mathrm{EDI} = \frac{\mathrm{MC} \times \mathrm{CC}}{\mathrm{BW}} \quad (2)$$

where MC is mollusks daily consumption with a value of 34.2 g d^{-1} (Meng et al. 2007), CC is the median of contaminant concentration of OCPs (Table 2), and BW is body weight (60 kg for adults). To screen for the potential public health significance of estimated exposures, EDI was compared to benchmark concentrations for each contaminant in this paper by using hazard ratios (HR), which was calculated by dividing the EDI by the benchmark concentrations. The values of HR more than one indicate that the exposure level exceeds the benchmark concentration. The USEPA Reference Dose (RfD) values were used as benchmark concentrations for noncarcinogenic effects, and the USEPA cancer slope factors were for carcinogenic effects and represent exposure concentrations at which lifetime cancer risk is one in one million. The benchmark concentrations are listed in Table 2 and HR results displayed in Fig. 4 shows that EDIs of DDTs and Chls were above the benchmark for carcinogenic effects, suggesting existence of potential cancer risk.

Some limitations associated with the exposure can lead to uncertainty of total risk. For example, our risk analysis did not consider different ages, and as childhood exposures may have greater probability of producing tumors than exposures in adulthood. The calculation of exposure to a given chemical may ignore background sources of that chemical. For chemicals that exhibit health effects based on a threshold level, the combination of background contaminant concentration and mollusks consumption exposure may exceed the threshold. The use of median mollusks contaminant concentrations to estimate exposure is another limitation that could underestimate risk if an individual regularly consumes mollusks from a contaminated waterbody. Otherwise, the average mollusks consumption, human body weight, possible interactions among different toxic chemicals could also lead to uncertainties. Our study, however, has provided a clear picture on the distribution of OCPs in mollusks and risk assessment of human exposure.

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