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Article

Historically Polluted Area Increases Human Exposure Risks to Polychlorinated Naphthalenes through Waterfowl Egg Consumption

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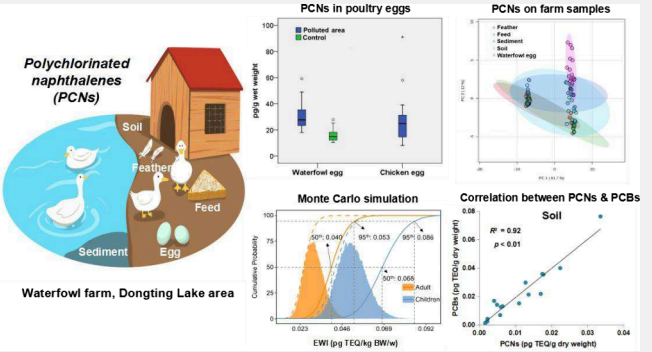
ABSTRACT: Polychlorinated naphthalenes (PCNs), which are ubiquitous in the environment, are listed as persistent organic pollutants under the Stockholm Convention. Poultry can be exposed to PCNs via feed and breeding environments, leading to PCNs accumulation in eggs. However, information on PCNs in eggs from waterfowl raised in contaminated regions is scarce. In this study, waterfowl and chicken eggs were collected from a historically polluted area in Hunan Province, China. In addition, waterfowl eggs were collected from Guangxi Province as a control. The mean concentrations of Σ_{75}PCNs in waterfowl (30.8 pg/g wet weight [ww]) and chicken eggs (26.1 pg/g ww) from Hunan were significantly higher than in waterfowl eggs (15.6 pg/g ww) from Guangxi. The PCN homologue profiles in poultry eggs from the two regions varied, but both were dominated by lower chlorinated CNs. Correlation analysis showed that breeding environment made a greater contribution to PCNs in waterfowl eggs. Interestingly, PCNs and polychlorinated biphenyls (PCBs) showed a significant positive correlation in samples from Hunan, but not in those from Guangxi. Human exposure to PCNs and PCBs was higher through consumption of poultry eggs from historically contaminated areas.



KEYWORDS: *Polychlorinated naphthalene, Waterfowl eggs, Distribution, Source analysis, Dietary intake risk*

1. INTRODUCTION

Polychlorinated naphthalenes (PCNs) are commonly classified as dioxin-like compounds because of their similar structural and toxicity characteristics to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and biphenyls (PCBs). Approximately 400,000 t of PCNs have been produced worldwide since they were first produced in the 1910s.[1] To respond to their adverse impact on the environment and human health, PCNs (with the exception of two mono-CN congeners) have been listed in Annexes A and C of the Stockholm Convention as persistent organic pollutants.[2] The production and use of PCNs as technical chemicals ceased in many countries in the 1980s;[1] however, historically produced and used PCNs can be continuously released into the environment throughout their entire lifecycle, including during recycling and after disposal. Moreover, PCNs are still cogenerated with PCDD/Fs and PCBs as byproducts of industrial thermal processes.[3]

Once released to the environment, PCNs can enter into the food chain and accumulate in foodstuffs, and PCNs have been found to be ubiquitous in eggs, fish, meat and milk.[4−8] Poultry eggs serve as an important animal-protein source; however, environmental pollutants such as PCDD/Fs, PCBs[9] and PCNs[10] tend to accumulate in eggs. Dioxin-like compounds can cause diseases such as dyspnea, wobbly and unsteady gait, stunting, subcutaneous edema, and sudden death in broiler and laying chickens.[11] Moreover, there have been several dioxin-like compound related food safety incidents that were related to poultry eggs. For example, in Belgium in 1999, PCB contaminated recycled mineral oil introduced into animal feed caused high levels of dioxins and PCBs in chickens and eggs, resulting in significant economic losses.[9] In 2004, PCDD/Fs in duck eggs on the market in Taiwan, China were found to exceed the EU limits by 10−15 times as a result of contamination by airborne ash from a nearby electric arc furnace dust treatment plant.[12] Additionally, Fernandes et al. (2010) reported higher concentrations of Σ_{12}PCNs in duck

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eggs (4.33 pg/g wet weight [ww]) than chicken eggs (1.69 pg/g ww) from the United Kingdom. Overall, these findings suggest that attention to PCN contamination in housed waterfowl eggs is needed due to their potential exposure to PCNs through feed and farm environment.

Housed waterfowl is popular in Asian countries and China is the largest producer of duck eggs worldwide, contributing more than 80% of the global supply.[13] Although waterfowl farming has become increasingly important, the farming practices and standards employed by this industry are not well established. Unlike cage-raised poultry such as chickens, housed waterfowl are seldom raised in cages, and they have a larger activity area than chickens. As a result, they may walk on land and swim in ponds that are in direct contact with the farming environment. Soil is considered an important source of PCDD/Fs and PCBs in eggs from free-range laying hens.[14] Housed waterfowl have the potential to be exposed to hazardous environmental pollutants from the breeding environment such as soil and sediment, which may enhance their exposure to dioxin-like compounds.

Although there has been no known historic production of PCNs in China, there are currently unintentional emissions of PCNs from industrial thermal sources such as waste incineration and metallurgical sources.[15] PCN emissions from secondary copper smelters can cause contamination in surrounding agricultural areas, and PCN concentrations have been found to be higher in poultry eggs produced at farms located near secondary copper smelters than in control regions.[16] The sixth Chinese total dietary study found that Σ_{75}PCN concentrations in eggs were second highest among eight studied categories of foodstuffs, and meat has the highest PCN content.[7] However, these studies focused on pooled poultry eggs, while information on PCNs in housed waterfowl eggs is limited.

Dongting Lake, which is the second largest freshwater lake in Hunan Province, China, is an important drinking water source in the Yangtze River Basin. PCDD/Fs and PCBs have been detected at high levels in various environmental matrices from this area because of the historic use of sodium pentachlorophenate between the 1960s and 1980s to control the spread of snail-borne schistosomiasis, and PCDD/Fs and PCBs as byproducts for sodium pentachlorophenate production.[17,18] However, the occurrence of PCNs in agro-products in this area is unclear. Poultry eggs act as an essential sink for poultry exposure to dioxin-like compounds and housed waterfowl are more affected by the farming environment than cage raised poultry. Therefore, this study was conducted to (1) investigate the concentrations and profiles of PCNs in housed waterfowl eggs from the Dongting Lake area and a control region, (2) compare PCNs in waterfowl and chicken eggs from the Dongting Lake area in Hunan, (3) evaluate the distribution and sources of PCNs in waterfowl farm samples, (4) study the relationship between PCNs and PCBs in waterfowl farm samples, (5) identify human dietary risks associated with exposure to PCNs through poultry egg consumption.

2. MATERIALS AND METHODS

2.1. Sampling and Preparation

A total of 87 egg, feather, feed, soil and sediment samples were collected from 20 waterfowl poultry farms (17 duck farms and 3 goose farms) in the Dongting Lake area of Hunan Province, China, in 2023. Overall, 15 waterfowl eggs (12 duck eggs and 3 goose eggs) were collected along with 20 feather, 20 feed, 18 soil and 14 sediment samples. Ducks or geese were raised free range on these farms, they roamed on farmland and swam in water. Detailed information on the samples collected at each farm is provided in Table S1. All specimens were collected and treated according to the Chinese Standard methods for sampling.[19,20] Another 30 chicken egg samples were collected from 30 farms in the same area with waterfowl eggs. To identify the effects of the environment and feed on free-ranged waterfowl, another 74 samples were collected from 28 waterfowl farms in Guangxi Province in 2022 and 2023 as controls, including 28 duck eggs, 25 feed samples and 21 soil samples (Table S2). Waterfowl on these farms were all raised on land, and no sediment samples were collected. At least five eggs that were randomly collected from different birds on the same farm were mixed and analyzed as one egg sample of each farm. Surface soil samples were collected from a depth of 0−10 cm in fields at the waterfowl farms where the ducks were allowed to roam. Sediment samples were collected from the water in which the waterfowl swam. After the samples were transferred to the laboratory, the egg, soil and sediment samples were freeze-dried. The soil and sediment samples were then sieved (100 mesh). The feather samples were first washed with water and *n*-hexane, after which they were dried and ground using an oscillating mill (MM 400; Retsch, Haan, Germany). After processing, samples were wrapped in aluminum foil and stored at < − 40 °C until required for analysis.

2.2. Extraction and Cleanup Procedures

Approximately 4 g of eggs (dry weight [dw]), 2 g of feathers, 5 g of feed, 5 g of soil (dw), and 5 g of sediment (dw) were analyzed. Each specimen was mixed with 10 g of anhydrous sodium sulfate and spiked with a $^{13}C_{10}$-labeled PCN internal standard (ECN 5102, Cambridge Isotope Laboratories, Inc., MA, USA) and a $^{13}C_{12}$-labeled PCB internal standard (68C-LCS, Wellington Laboratories Inc., Canada). The mixture was then extracted at 120 °C using an accelerated solvent extraction apparatus (ASE 350, Thermo Fisher Scientific, USA) with *n*-hexane and dichloromethane (1:1, v/v) as the extraction solvent. The lipid content of egg samples was measured by gravimetric analysis before the subsequent cleanup procedures. The *n*-hexane redissolved egg samples and extracts of other samples were first cleaned with acidic silica gel (44% mass fraction) to remove interfering compounds from the samples. Next, samples were cleaned with an acidic silica gel column and a multilayer silica column successively. Prior to instrumental analysis, samples were spiked with a $^{13}C_{10}$-labeled PCN internal standard (ECN 5260, Cambridge Isotope Laboratories, Inc., MA, USA) and a $^{13}C_{12}$-labeled PCB internal standard (68C-IS, Wellington Laboratories Inc., Canada) for recovery calculations. Detailed analytical procedures were preformed in the Supporting Information.

2.3. Instrumental Analysis of PCNs and PCBs

Mono- to octa-CNs were analyzed using a high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) method as described in our previous study.[21] Both indicator PCBs (IN-PCBs, including CB 28, 52, 101, 138, 153 and 180) and dioxin-like PCBs (dl-PCBs, including CB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189) were analyzed using the isotope dilution-HRGC/HRMS method referring to the US EPA 1668 method. Analyses were conducted with an electron ionization source using an HRGC/HMRS instrument (DFS, Thermo Fisher Scientific, USA). PCN and PCB congener separation was conducted with a fused silica capillary column (TR-DIOXIN-5MS, 60 m × 0.25 mm i.d. × 0.25 μm, Thermo Fisher Scientific, USA). The temperature program for PCNs was an initial temperature of 80 °C for 2 min, followed by an increase to 180 °C at 20 °C/min, which was then held for 1 min. This was followed by an increase to 280 °C at a rate of 2.5 °C/min, which was held for 2 min, after which the temperature was increased to 290 °C at a rate of 10 °C/min and then held for 5 min. The temperature program for PCBs comprised an initial 1 min hold at 140 °C, followed by a 20 °C/min increase to 200 °C, which was held for 1 min. This was followed by a 5 °C/min increase to 220 °C, which was held for 16 min. Samples were then increased to 235 °C at 5 °C/min and held at this temperature for 7 min. Finally, samples were increased to 310 °C at a rate of 5 °C/min and then held for 10 min.

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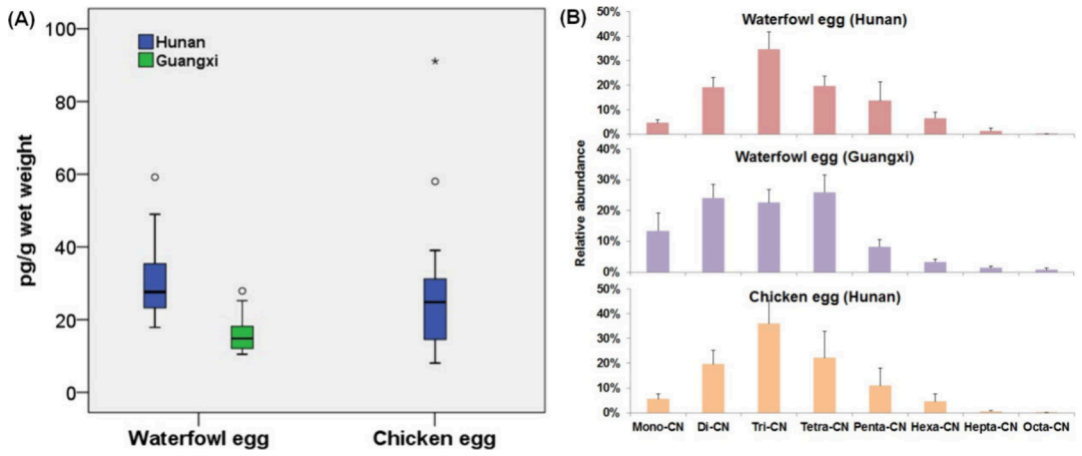

Figure 1. (A) Concentrations and (B) homologue profiles of PCNs in waterfowl and chicken eggs from Hunan Province and Guangxi Province. In the box plot, the circle means the value exceeds 1.5 times the interquartile range (IQR); the asterisk means the value exceeds 3 times the IQR. In the histogram, the column means the average value; the error bar means the standard deviation.

The injection temperature was set at 260 °C and the interface and ion source temperatures were both set at 280 °C. The carrier gas was helium (>99.999%) applied at a flow rate of 1.0 mL/min. The mass spectrometer was operated in selected ion monitoring mode with a resolution of approximately 10,000 for sample analysis.

2.4. Quality Assurance and Quality Control

All glassware was washed with *n*-hexane or dichloromethane three times before use. Prior to use, the ASE instrument cells were extracted following the same procedure that was employed for sample extraction. A laboratory blank without matrix added was analyzed with each batch to monitor the background conditions. CN 5/7, 24/14, and CB 28 and 52 were detected in the blanks, but their concentrations were below 15% of those in the blanks. Thus, there is no blank correction for result calculation. The limits of detection (LODs, a signal-to-noise ratio of 3) of the PCN congeners in the egg, feather, feed, soil and sediment samples were 0.003−0.017 pg/g ww, 0.005−0.08 pg/g, 0.004−0.05 pg/g, 0.005−0.07 pg/g dw, and 0.008−0.10 pg/g dw, respectively. The LOD values of PCB congeners in the egg, feather, feed, soil and sediment samples were 0.007−0.01 pg/g ww, 0.01−0.2 pg/g, 0.01−0.2 pg/g, 0.02−0.08 pg/g dw, and 0.05−0.1 pg/g dw, respectively. The recovery ranges of the $^{13}C_{10}$-labeled PCN and $^{13}C_{12}$-labeled PCB internal standards in the samples were 52%−108% and 67%−112%, respectively.

2.5. Statistical Analysis

PCN congeners in samples with concentrations below the LODs were assigned the value of the LOD for statistical analyses. The quantities of PCN congeners and their LOD values were obtained using the DFS mass spectrometer data processing software Target-Quan (ver. 3.0.0.1336). Both principal component analysis and hierarchical clustering analysis were conducted using R to explore the relationship of PCNs in different samples. One-way Analysis of Variance (ANOVA) was used to compare the mean values between two variables. Spearman correlation analysis was conducted using GraphPad Prism 9.5 to evaluate the relationships between different variables. Probability density distributions with 10,000 simulations were performed with R using Monte Carlo simulations (MCS) to evaluate exposure of Chinese adults and children to PCNs through poultry egg consumption.

3. RESULTS AND DISCUSSION

3.1. PCN Concentrations and Homologue Profiles in Poultry Eggs

3.1.1. PCN Concentrations. The concentrations of Σ_{75}PCNs in domestic waterfowl eggs from Hunan and Guangxi Provinces ranged from 17.9 to 59.2 pg/g ww (mean: 30.8, median: 27.6) and 10.5 to 27.9 pg/g ww (mean: 15.6, median: 14.8), respectively (Figure 1A). The mean PCN concentration in waterfowl eggs from Hunan (30.8 pg/g ww) was significantly higher than that in waterfowl eggs from Guangxi (15.6 pg/g ww) ($p < 0.01$). The concentrations of Σ_{75}PCNs in chicken eggs from Hunan ranged from 8.0 to 91.1 pg/g ww (mean: 26.1, median: 24.8). The mean concentration of PCNs in waterfowl eggs (30.8 pg/g ww) was higher than that in chicken eggs (26.1 pg/g ww) from the same areas in Hunan, but this difference was not significant ($p = 0.32$). Lipid content in waterfowl eggs (mean: 12.4%) was significantly higher than in chicken eggs (mean: 9.5%) ($p < 0.05$). Based on lipid weight, the mean concentrations of Σ_{75}PCNs in domestic waterfowl and chicken eggs from Hunan were 267 and 274 pg/g lipid weight (lw), respectively, which were both significantly higher than that in waterfowl eggs from Guangxi (122 pg/g lw).

As shown in Table S3, not all published studies have reported 75 PCN congeners in poultry eggs. The mean values of Σ_{75}PCNs in domestic waterfowl (30.8 pg/g ww) and chicken eggs (26.1 pg/g ww) from Hunan were comparable with that of cooked eggs (31.4 pg/g ww) collected from the sixth Chinese Total Diet Study,[7] but higher than that of poultry eggs (24.0 pg/g ww) collected from areas surrounding metal smelters in China.[16] However, the mean value of Σ_{75}PCNs in domestic waterfowl eggs (15.6 pg/g ww) from Guangxi in this study was lower than findings in both studies.[7,16] Prior studies only determined certain PCN congeners in poultry eggs. The mean concentrations of tetra- to octa-CNs in waterfowl eggs from Hunan (12.8 pg/g ww) and Guangxi (6.2 pg/g ww) as well as in chicken eggs from Hunan (10.1 pg/g ww) were lower than that in eggs from Spain in 2000 (23 pg/g ww),[4] but higher than that in eggs from Spain in 2006 (4.3 pg/g ww)[22] and in eggs from Latvia (1.1 pg/g ww).[6] Fernandes et al. (2010, 2011) investigated 12 PCN congeners (CN 52/60, 53, 66/67, 68, 69, 71/72, 73, 74 and 75) in poultry eggs from the United Kingdom and Ireland.[5,23] They found that the mean concentration of Σ_{12}PCNs in duck eggs from the United Kingdom (4.3 pg/g ww)[5] was higher than those found in waterfowl eggs from Hunan (3.9 pg/g ww) and Guangxi (1.3 pg/g ww) in the present study, but that Σ_{12}PCN mean value in chicken eggs from the United Kingdom (1.7 pg/g ww) was lower than that

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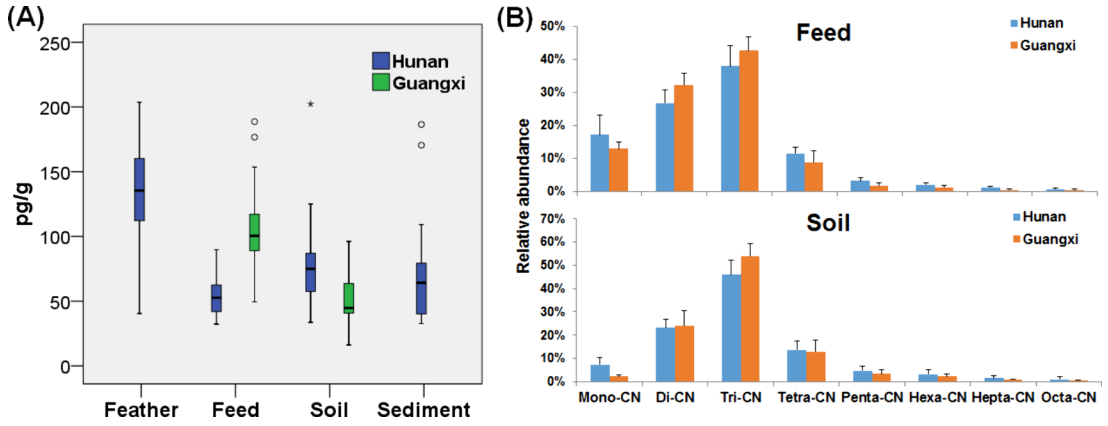

Figure 2. (A) Concentrations and (B) homologue profiles of PCNs in feather, feed, soil and sediment samples from waterfowl farms in Hunan Province and Guangxi Province. In the box plot, the circle means the value exceeds 1.5 times the interquartile range (IQR); the asterisk means the value exceeds 3 times the IQR. In the histogram, the column means the average value; the error bar means the standard deviation.

in chicken eggs from Hunan (2.5 pg/g ww). The mean concentration of Σ_{12}PCNs in eggs from Ireland (0.62 pg/g ww)[23] was much lower than that determined in poultry eggs in this study. A recent study reported that the mean concentration of Σ_{70}PCNs (CN 1, 2, 9 and 48/35 were not included) in chicken eggs from France (12 pg/g ww)[8] was comparable with that found in waterfowl eggs from Guangxi (13.2 pg/g ww), but much lower than those in waterfowl (29.4 pg/g ww) and chicken eggs (24.4 pg/g ww) from Hunan. Overall, the results presented herein indicate that the PCN concentration in poultry eggs from the Dongting Lake area of Hunan Province was rather high and should receive increased attention.

3.1.2. PCN Homologue Profiles. The homologue profiles of PCNs in waterfowl and chicken eggs from Hunan were similar, with tri-CNs (~35%) being the predominant contributor, followed by tetra- (~20%) and di-CNs (~20%) (Figure 1B). The proportions of di- (24.2%), tri- (22.6%) and tetra-CNs (25.9%) in waterfowl eggs from Guangxi were similar, which was quite different than the results observed for samples collected from Hunan. Moreover, the contribution of mono-CNs to total PCNs in waterfowl eggs from Guangxi (13.4%) was higher than in waterfowl (4.6%) and chicken eggs (5.6%) from Hunan. The homologue profiles of PCNs in poultry eggs from Hunan were similar to those in cooked eggs from Hunan in the sixth Chinese Total Diet Study,[7] as well as in poultry eggs from surrounding metal smelters in China.[16] However, the homologue profiles of PCNs in waterfowl eggs from Guangxi in the present study were different from those reported in cooked eggs from Guangxi in the sixth Chinese Total Diet Study, in which tri-CNs were the predominant homologue.[7]

The congener profiles of PCNs in poultry eggs also showed geographic differences. Specifically, the proportions of CN 1, 2, 4 and 5/7 were higher in waterfowl eggs from Guangxi than in poultry eggs from Hunan (Figure S1). Principal component analysis showed that the PCNs in waterfowl eggs from Hunan differed from those collected in Guangxi (Figure S2A), indicating that PCNs in waterfowl eggs from these regions had different sources. CN 1, 2 and 6/12 were the primary contributors to the differences in PCN profiles in waterfowl eggs from Hunan and Guangxi (Figure S2B). In general, CN 24/14 and 45/36 were the predominant congeners in all poultry eggs from Hunan and Guangxi. CN 24/14 were also the predominant congeners in eggs from the sixth Chinese Total Diet Study[7] as well as from surrounding metal smelters in China.[16]

3.2. Distribution and Correlation of PCNs in Waterfowl Farms

3.2.1. PCNs in feather, Feed, Soil, and Sediment Samples. The concentrations of Σ_{75}PCNs in domestic waterfowl feathers from Hunan ranged from 40.4 to 204 pg/g (mean: 134, median: 136). To the best of our knowledge, this is the first study to report PCNs in feathers. The median PCN concentration in feathers (134 pg/g) from Hunan was much lower than those of SCCPs (2460 ng/g) and MCCPs (992 ng/g) in duck feathers from Guangxi.[24] PCN concentrations in waterfowl feed samples from Hunan ranged from 32.2 to 89.8 pg/g (mean: 53.6, median: 52.7), which were lower than those from Guangxi (mean: 108, median: 100 pg/g) (Figure 2A). Poultry feed used in Hunan and Guangxi may not be produced locally, in which case it could have been less affected by the local environment. The mean concentrations of PCNs in poultry feed from Hunan (53.6 pg/g) were comparable to those in animal compound feed samples collected within 10 km (51.5 pg/g ww) and 20−30 km (61.2 pg/g ww) away from secondary copper smelters in China.[16] The mean PCN concentrations in poultry feed (108 pg/g) from Guangxi and in crab feed (89.5 pg/g ww) from Anhui and Shanghai[25] were relatively high.

The concentrations of Σ_{75}PCNs in waterfowl farm soil from Hunan ranged from 33.6 to 202 pg/g dw (mean: 82.2, median: 74.9), which were higher than those from Guangxi (mean: 49.6, median:44.7 pg/g dw). The mean PCN concentration (82.2 pg/g dw) in waterfowl farm soil from Hunan was lower than that in animal farm soil (171 pg/g dw) within 10 km of secondary copper smelters[16] and in dairy cow farm soil (309 pg/g dw) within 10 km of an iron smelting plant in China,[26] but higher than that in animal farm soil (51.2 pg/g dw) 20−30 km away from secondary copper smelters in China.[16] However, the mean PCN concentration (49.6 pg/g dw) in waterfowl farm soil from Guangxi was comparable to that in animal farm soil (51.2 pg/g dw) 20−30 km away from secondary copper smelters in China.[16] PCN concentrations in sediment samples from human waterfowl farms ranged from 32.7 to 187 pg/g dw (mean: 76.4, median: 64.2). The mean value (76.4 pg/g dw) was much lower than that of sediment samples (379 pg/g dw) collected within 10 km of secondary

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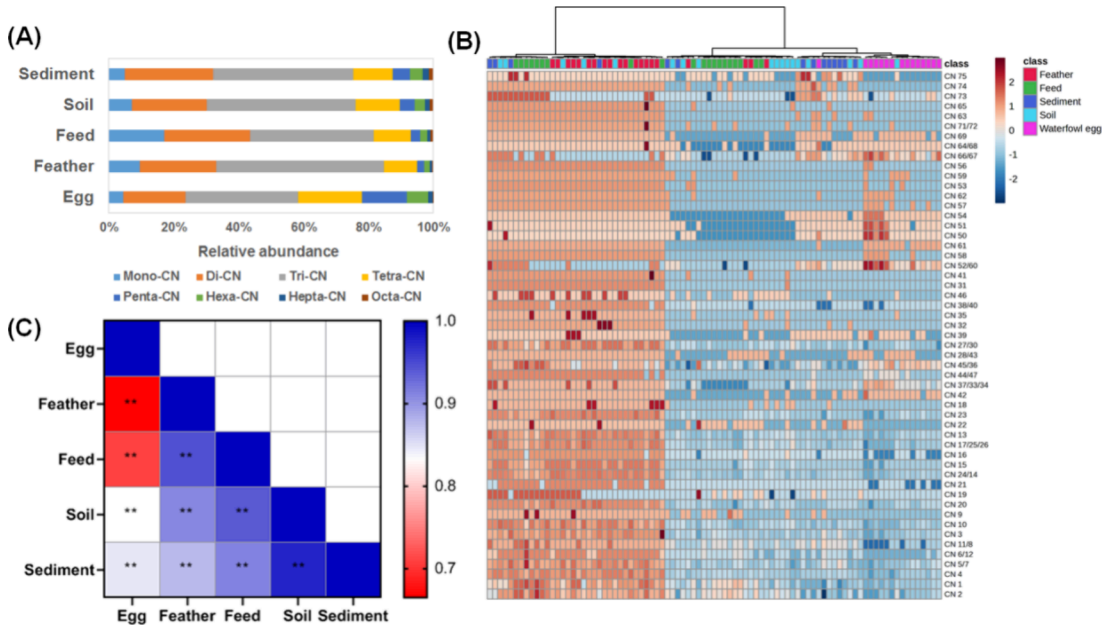

Figure 3. (A) Homologue profiles, (B) hierarchical cluster analysis, and (C) Spearman's correlation analysis (**$p < 0.01$, two-tailed) of PCNs in waterfowl egg, feather, feed, soil and sediment samples from Hunan.

copper smelters, but higher than those in sediment samples (68.0 pg/g dw) 20−30 km away from secondary copper smelters in China[16] and in sediment samples from carb farms in Anhui (15.9 pg/g dw) and Shanghai (37.2 pg/g dw).[25] These findings indicated that PCN contamination in the aquatic environment of Dongting Lake should receive increased attention.

In general, the concentrations of Σ_{75}PCNs in waterfowl farm samples from Hunan decreased in the order: feather > soil > sediment > feed > eggs, while those in samples from Guangxi decreased in the order: feed > soil > eggs. PCN concentrations in feed from Guangxi were twice as high as those from Hunan. Considering the relatively lower concentrations of PCNs in eggs and soil from Guangxi than Hunan, farm environment acts as a more important influencing factor than feed for domestic waterfowl exposure to PCNs.

3.2.2. Relationship between PCNs in Waterfowl Farm Samples. Although the homologue profiles of PCNs in poultry eggs from Hunan and Guangxi varied, similar PCN patterns were identified in feed and soil samples from both provinces (Figure 2B). Tri-CNs were the predominant homologue of PCNs in feed and soil, followed by di-CNs. The relative abundance of tri-CNs in soil (~50%) was higher than in feed (~40%). In general, the homologue profiles of PCNs in waterfowl eggs, feathers, feed, soil and sediment from Hunan were similar (Figure 3A). Specifically, the proportion of mono-CNs in feed was higher than in eggs, feathers, soil and sediment and exceeded 10% of the total PCNs. Tetra- to hexa-CNs were found in higher proportions in waterfowl eggs than in feathers, feed, soil and sediment. This might have occurred because these PCNs are more likely to accumulate in eggs.[10] Compared with previous studies on PCNs in animal farms, the homologue profiles of PCNs in poultry feed samples in the present study were similar to those in animal compound feed samples collected within 10 km and 20−30 km away from secondary copper smelters in China,[16] but different from those in crab feed from Anhui and Shanghai[25] in which the di-CNs contribution was higher. The homologue profiles of PCNs in waterfowl farm soil and sediment samples were similar to those in animal farm samples collected 20−30 km away from secondary copper smelters in China,[16] but different from those in samples collected within 10 km of secondary copper smelters[16] and in crab feed from Anhui and Shanghai,[25] for which tri-CNs were not the predominant homologue. Analysis of congener profiles of PCNs in feathers, feed, soil and sediments from Hunan and Guangxi revealed that CN 24/14 were the most abundant congeners in these samples (Figure S3).

Hierarchical cluster analysis of PCN congeners in samples from Hunan showed that most feather and feed samples clustered together (Figure 3B). This indicated a close relationship between PCNs in feathers and feed, which was consistent with the results observed for CPs in duck feathers and feed from Guangxi.[24] However, PCNs in most waterfowl egg, soil and sediment samples clustered together. The mean concentrations of PCN congeners in waterfowl egg, feather, feed, soil and sediment samples were used for Spearman's correlation analysis (Figure 3C). The results showed a close correlation of PCNs in waterfowl egg, feather, feed, soil and sediment samples. The correlation between waterfowl eggs and farm environment samples (sediment and soil) was closer than that between feathers and feed, which was consistent with the hierarchical cluster analysis. These results indicated that farm environment might be a primary source of PCNs in waterfowl eggs from Hunan. Moreover, there was a closer correlation between PCNs in soil and sediment samples from Hunan, indicating a similar source of PCNs in these samples. Spearman's correlation analysis also showed close relationships between PCNs in waterfowl eggs, feed and soil from Guangxi (Figure S4). Unlike the findings from Hunan, the correlation of PCNs in eggs and feed from Guangxi was closer than that in eggs and soil, and a closer relationship was identified between PCNs in feed and soil.

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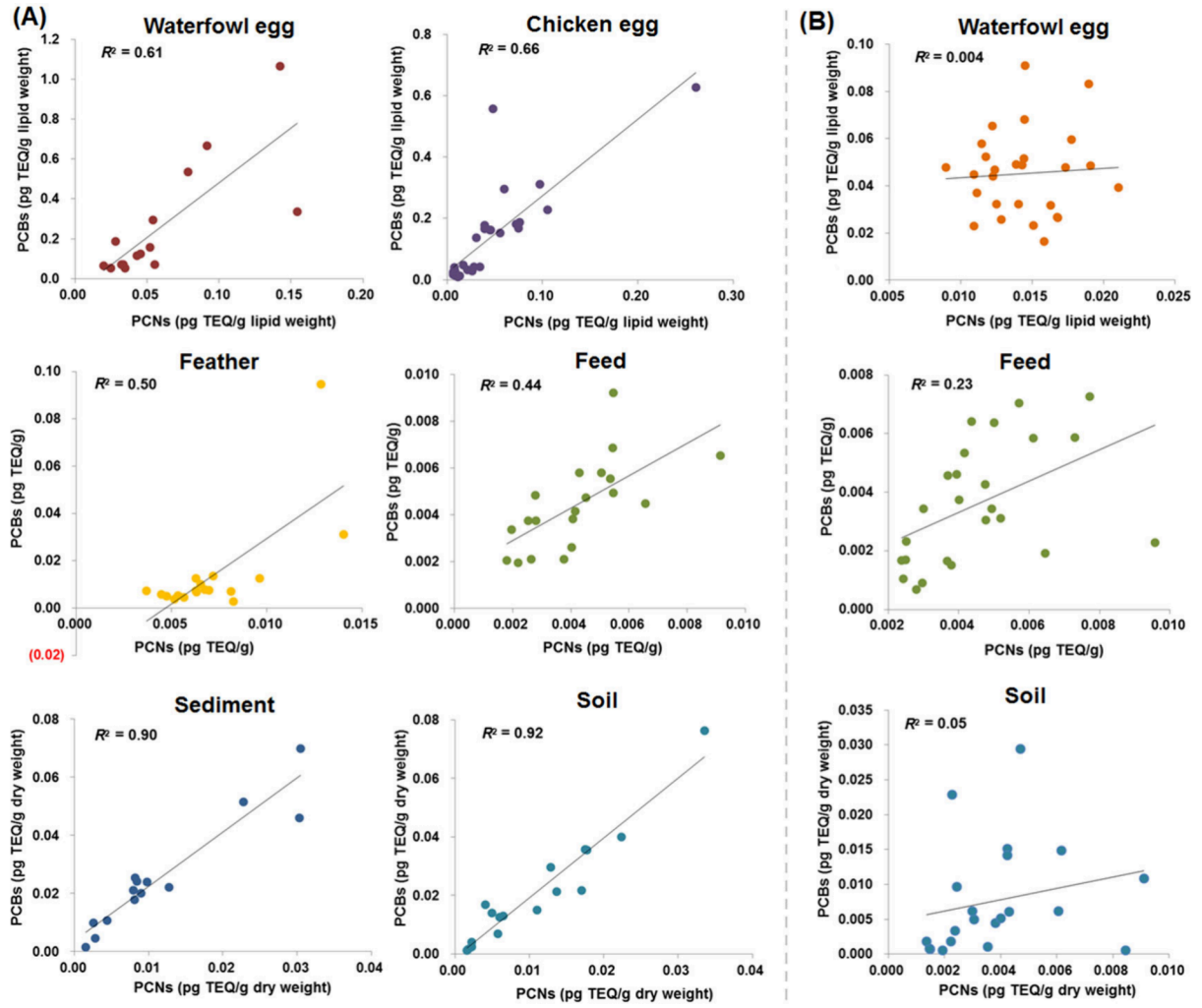

Figure 4. Correlation analysis of PCNs and PCBs in poultry eggs and waterfowl farm samples from (A) Hunan Province and (B) Guangxi Province.

3.3. Correlation between PCNs and PCBs in Poultry and Waterfowl Farm Samples

PCNs and PCBs, which are both coproduced unintentionally during industrial thermal processes, showed similar chemical structures and toxicity.[27] The concentrations of IN-PCBs in poultry eggs and waterfowl farm samples from Hunan and Guangxi were all higher than those of the corresponding dl-PCBs (Table S4). The mean concentrations of IN-PCBs and dl-PCBs in waterfowl eggs, feed and soil from Hunan (31.2 and 13.3 pg/g ww, 17.1 and 4.0 pg/g, and 17.3 and 9.0 pg/g dw) were higher than those in waterfowl eggs, feed, and soil from Guangxi (15.6 and 5.3 pg/g ww, 14.0 and 3.4 pg/g, and 11.6 and 3.4 pg/g dw), respectively. These findings are consistent with those observed for PCNs and indicated that the studied region in Hunan was more contaminated with both PCNs and PCBs than Guangxi. Chicken eggs were less contaminated with PCBs than waterfowl eggs from Hunan. Specifically, both IN-PCBs and dl-PCBs in chicken eggs from Hunan (11.6 and 4.2 pg/g ww) were lower than those in waterfowl eggs from Hunan (31.2 and 13.3 pg/g ww) and Guangxi (15.6 and 5.3 pg/g ww). The concentrations of IN-PCBs and dl-PCBs in poultry eggs in the present study were both lower than the EU limits for eggs.

CB 28 and 118 were the most abundant congeners of IN-PCBs and dl-PCBs in all samples, respectively. Principal component analysis showed that PCB congeners in most waterfowl egg samples from Hunan were different from those in samples from Guangxi (Figure S5A), but that PCBs in most waterfowl and chicken eggs from Hunan overlapped (Figure S5B), indicating that there are different sources of PCBs in poultry eggs from Hunan and Guangxi. Similarly, Spearman's correlation analysis showed that PCBs in eggs were more closely related to farm environment than feed (Figure S6A). Moreover, a close relationship was identified between PCBs in feathers and feed using hierarchical clustering analysis (Figure S6B). These results indicated that waterfowl eggs can act as a bioindicator for PCNs and PCBs in the farm environment, which is consistent with previous findings on dioxins that showed chicken eggs could be a bioindicator for PCDD/Fs and PCBs in soil.[14] The results of the present study also showed that feathers can be used as biomarkers for PCN and PCB monitoring on poultry farms.

Both PCNs and PCBs are dioxin-like compounds. The toxic equivalent (TEQ) values of PCNs were calculated using the upper-bound relative potency factors (RPFs) of dioxin-like PCNs (dl-PCNs) provided by Falandysz et al. (2014).[28] The TEQ values of PCBs were calculated using the toxic

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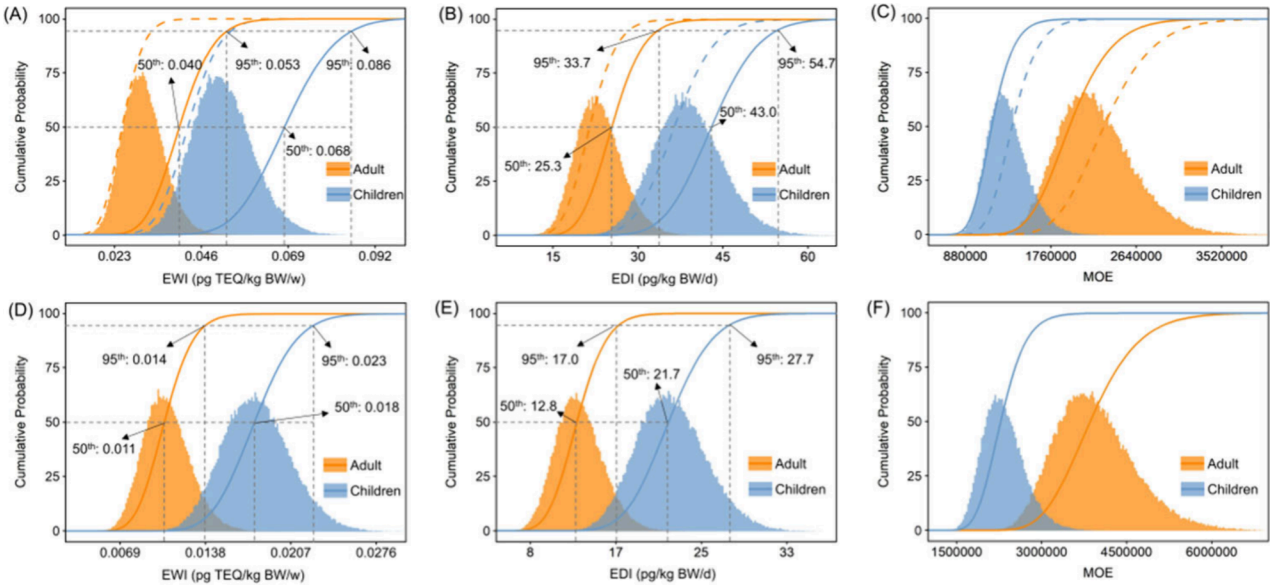

Figure 5. Risk assessment of resident's exposure to PCNs through poultry egg consumption. Probability density distributions of estimated weekly intake (EWI) of PCN TEQs for adults and children in (A) Hunan Province and (D) Guangxi Province (solid line curve for waterfowl eggs; dashed line for chicken eggs). Probability density distributions of estimated daily intake (EDI) of PCNs for adults and children in (B) Hunan Province and (E) Guangxi Province. Probability density distributions of margin of exposure (MOE) of PCNs for adults and children in (C) Hunan Province and (F) Guangxi Province.

equivalency factors (TEFs) provided by the World Health Organization (WHO) in 2022.[29] The PCN TEQs in poultry eggs from Hunan and Guangxi were lower than the PCB TEQs (Figure S7). The mean PCN and PCB TEQs values in waterfowl eggs from Hunan (0.007 and 0.030 pg TEQ/g ww) were higher than those in chicken eggs (0.004 and 0.013 pg TEQ/g ww), but both were higher than those in waterfowl eggs from Guangxi (0.002 and 0.006 pg TEQ/g ww). The TEQ concentrations of PCBs in feather, soil and sediment samples in Hunan and Guangxi were all higher than those of PCNs, with the exception of feed (Figure S8). PCN TEQs in feed from Guangxi (0.005 pg TEQ/g) were comparable with PCB TEQs (0.004 pg TEQ/g), and PCN and PCB TEQs in feed from Hunan were also comparable (both 0.004 pg TEQ/g).

Linear correlations between TEQ values of PCNs and PCBs in different samples from Hunan (Figure 4A) and Guangxi (Figure 4B) were also analyzed. PCN and PCB TEQs in waterfowl and chicken eggs from Hunan showed a moderate correlation (R^2 > 0.6). The linear correlation coefficients of PCN and PCB TEQs in feathers (R^2 = 0.50) and feed (R^2 = 0.44) from Hunan were lower than those in poultry eggs. High correlations were identified between PCN and PCB TEQs in soil and sediment samples from Hunan (R^2 > 0.9). Poor correlations were found between PCN and PCB TEQs in waterfowl eggs, feed and soil from Guangxi. Taken together, these findings indicate that PCNs and PCBs in samples from Hunan might have similar sources, while those in samples from Guangxi might have different sources. Linear correlations between mass concentrations values of dl-PCNs and dl-PCBs in different samples from Hunan and Guangxi were shown in Figure S9. Yet, their correlations were poorer than the TEQ values of PCNs and PCBs.

3.4. Dietary Risk Assessment

Risk assessment for human exposure to PCNs through poultry egg consumption from Hunan and Guangxi was also conducted. The estimated weekly intake (EWI) values of Chinese adults and children to PCNs were calculated using the equation below:

$$\mathrm{EWI} = \frac{C_{\mathrm{PCNTEQ}} \times M}{BW} \quad (1)$$

where $C_{\mathrm{PCN\ TEQ}}$ is the TEQ concentration of PCNs in poultry eggs (pg TEQ/g ww); M is the weekly egg consumption mass (g/week(w)); and BW is the average body weight for Chinese adults (63 kg) and Children (33 kg). Daily intake of one poultry egg (50 g) for one person was recommend by the Chinese Dietary Guidelines, and weekly intake of poultry eggs per person was calculated as 350 g.[30] The mean EWI values for exposure of adults and children to PCNs through consumption of waterfowl eggs (0.039 and 0.074 pg TEQ/kg BW/w) and chicken eggs (0.024 and 0.046 pg TEQ/kg BW/w) from Hunan were higher than those for waterfowl eggs (0.010 and 0.019 pg TEQ/kg BW/w) from Guangxi (Table S5). As shown in Figures 5A and 5D, the EWIs for the 95th percentile of dietary exposures of PCNs ranged from 0.014 pg TEQ/kg BW/w in adults to 0.086 pg TEQ/kg BW/w in children, which are far below the tolerable weekly intake (TWI) value (2 pg TEQ/kg BW/w) proposed by the European Food Safety Authority for dioxins.[31] The EWIs for the 95th percentile of dietary exposure to PCBs in children was 0.37 pg TEQ/kg BW/w, which was also below the TWI value (Figures S10). Thus, the current high level of egg consumption does not raise a health concern.

The estimated daily intake (EDI) values for exposure of Chinese adults and children to PCNs through poultry egg consumption were calculated using the following equation:

$$\mathrm{EDI} = \frac{C_{\mathrm{PCNs}} \times M}{BW} \quad (2)$$

where C_{PCNs} is the concentration of Σ_{75}PCNs in poultry eggs (pg/g ww); M is the daily egg consumption mass (g/day(d));

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and BW is the average body weight of Chinese adults (63 kg) and children (33 kg). Daily intake of one poultry egg (50 g) for one person was recommended by the Chinese Dietary Guidelines.[30] The mean EDI values for adults and children exposed to PCNs through consumption of waterfowl eggs (24.5 and 46.7 pg/kg BW/d) and chicken eggs (20.7 and 39.5 pg/kg BW/d) from Hunan were higher than those for waterfowl eggs (12.3 and 23.6 pg/kg BW/d) from Guangxi (Table S5). The EDIs for the 95th percentile of dietary exposures ranged from 17.0 pg/kg BW/d in adults to 54.7 pg/kg BW/d in children (Figures 5B and 5E).

The margin of exposure (MOE) method was calculated to assess human health risks of exposure to PCNs via poultry consumption as follows:

$$\mathrm{MOE} = \frac{\mathrm{BMDL}_{20}}{\mathrm{EDI}} \quad (3)$$

where BMDL_{20} is the benchmark dose lower bound of 5.0×10^7 pg/kg BW/d for hexa-CNs[32] and was used here for MOE calculation. MOE values ≥2000 are considered to indicate that the current dietary exposure to PCNs does not raise a health concern.[32] The MOE values for the 95th percentile of dietary exposure in this study were far above 2000 (Figures 5C and 5F), indicating that the high dietary exposure to PCNs does not raise a health concern for the studied population groups. Here we should mention that, due to the limited BMDL20 values available for PCN congeners, the BMDL20 value for hexa-CNs was used for MOE calculation in this study. This might introduce uncertainty for the MOE calculation.

In general, human exposure risks to PCNs through poultry egg consumption from the studied region in Hunan were higher than those from Guangxi. The current high consumption of eggs does not raise a health concern. However, it should be noted that there were certain uncertainties associated with this study that might influence the risk assessment results. First, there are no TEFs of PCNs proposed by the WHO or other authorities. Second, the BMDL_{20} used for the MOE calculation in the present study was for hexa-CNs, not all PCNs. Parameters used for the estimated cancer risk and chronic hazard index value calculation for dietary intake of PCNs in previous studies was for other dioxin-like compounds, not PCN congeners.[7] Thus, a comprehensive toxicity investigation of all PCN congeners is needed to enable a more robust exposure assessment.

4. CONCLUSIONS

All 75 PCN congeners were evaluated in poultry egg and waterfowl farm samples from a contaminated area in Hunan and an unpolluted area in Guangxi as a control. PCN contents in both poultry egg and environmental samples from Hunan were higher than those in Guangxi. However, the concentrations of PCNs in feed from Guangxi were higher than those in Hunan. Although the homologue profiles of PCNs in poultry eggs from Hunan were different from those from Guangxi, similar PCN homologue profiles were identified in feed and soil samples from both areas. A close relationship was found between PCNs in waterfowl eggs and the breeding environment, indicating that waterfowl eggs can act as a bioindicator for PCNs in the environment. PCBs concentrations were higher in waterfowl egg and farm samples from Hunan than Guangxi. Additionally, a significant correlation was found between PCNs and PCBs in poultry egg and waterfowl farm samples from Hunan, indicating similar sources of PCNs and PCBs. Risk assessment showed that the risk of human exposure was higher for the consumption of poultry eggs from Hunan than Guangxi. This study, which is the first to investigate PCNs in domestic waterfowl farms, provides useful information on PCNs in waterfowl eggs and provides insight into PCNs dietary exposure risks posed to humans. The results presented herein will help improve waterfowl breeding practices and prevent human exposure to PCNs through poultry eggs. Both PCNs and PCBs are persistent in the environment and can enter the food chain from historically polluted areas; therefore, they should be monitored in the agricultural environment to prevent their contamination in agro-products..

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00134.

Additional experimental details and methods as well as figures as mentioned in the text (PDF)

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Notes

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