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Coexposure of Dioxin-like Polychlorinated Biphenyls and Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Free-Range Hens and Implications Derived from Congener Profile Analysis

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ABSTRACT: The consumption of free-range eggs is becoming more popular worldwide. We analyzed the levels of 12 dioxin-like polychlorinated biphenyls (dl-PCBs) and their congener profiles from 6 free-range and 12 caged egg samples. The mean levels of dl-PCBs in the free-range samples were 5.4 times higher than those in caged eggs. All egg samples exhibited at least two characteristic dl-PCB congener patterns, which reflected distinctive contamination sources. Additionally, for the first time, we demonstrated that the dl-PCB levels in the free-range eggs were highly correlated with elevated levels of 17 polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) (r = 0.986; p < 0.001), indicating a coexposure scenario in free-range hens. Cluster analysis of congener patterns implied that this coexposure scenario could be attributed to distinct dl-PCB and PCDD/F sources. This congener profile information provides insights from a different perspective for further identifying potential dl-PCB and PCDD/F sources in the polluted free-range eggs.

KEYWORDS: *free-range egg, PCB, PCDD/F, congener profile, coexposure*

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are three classes of chemically and structurally related polyhalogenated aromatic hydrocarbons originated from industrial activities and wastes. These ubiquitous compounds can be persistent in soils and sediments as well as in waste repositories for decades to centuries. For example, although the manufacture and use of PCBs were banned in the 1970s, PCB contamination is still a continuing concern due to the long half-lives of these compounds and their production through certain industrial activities.[1] Acute and long-term exposure to PCDD/Fs and PCBs can be hazardous to health. Exposure to PCDD/Fs can lead to carcinogenic concerns,[2] developmental defects,[3] and hormonal disruption,[4] while exposure to PCBs has been reported to be associated with chloracne and related dermal lesion,[5] cancer risk,[6,7] disruption of the nervous function,[8−10] and immune system dysfunction.[11] The toxic effects of these substances are mainly attributed to a subset of 17 PCDD/F congeners and 12 dioxin-like PCB (dl-PCBs) congeners. Because these pollutants occur as a mixture of congeners in the contaminated environments, human subjects normally take in dozens of congeners upon exposure. For example, different congener patterns of highly toxic PCDD/Fs and dl-PCBs can be found in the tissues, blood, and milk of exposed subjects.[12,13] Therefore, rather than analyzing only one or a few congeners, risk assessment of human exposure always includes analysis of a mixture of PCDD/F or dl-PCB congener profiles.

Chicken eggs are one of the most important nutrition sources in many areas including Taiwan. Because eggs contain almost 10% fat, dioxins are likely to accumulate in the fat of the yolk.[14] It has been estimated that the contribution of eggs to the daily dioxin intake of humans is approximately 4% in many European countries.[14] In a Spanish county near a hazardous waste incinerator, the contribution percentage was as high as 17%.[15] Thus, it is important to monitor the levels of PCDD/Fs and dl-PCBs in eggs. For example, one of the strategies implemented by the Commissions of the European Communities (EC) to reduce the health risks associated with PCDD/Fs or dl-PCBs exposure is to regulate the maximum concentration of PCDD/Fs to 3 pg World Health Organization Toxic Equivalents (WHO_{1998}-TEQ)/g lipid and the sum of dioxin and dl-PCBs to 6 pg WHO_{1998}-TEQ/g lipid in eggs.[16]

Hen eggs have been estimated to contribute as much as 7% of monthly intake of PCDD/Fs and dl-PCBs in both male and female Taiwanese individuals.[17] Traditionally, the majority of consumed eggs are produced by caged hens. In recent years, the consumption of eggs produced by free-range hens or by organic farming has gradually increased in the pursue of natural and better nutritional qualities in eggs.[18] The demand for free-range or organic eggs is continuing to grow in Taiwan. Nevertheless,

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free-ranging or organic farming near urbanized or industrialized areas is expected to expose hens to potential PCDD/F and dl-PCB contaminations and possibly render contaminated chicken eggs, as postulated by Schoeters and Hoogenboom.[18] Therefore, analyzing the PCDD/F and dl-PCB congener levels in eggs from different production methods in different districts represents a suitable way to monitor egg contamination and addresses important health issues.

Although there are a few surveys on the PCDD/F levels in potentially contaminated chicken eggs in Taiwan,[17,19,20] investigations focusing on dl-PCB levels in eggs are scarce. A previous study by Hsu et al.[20] from our group showed significantly elevated PCDD/F levels in six free-range egg samples (0.538−5.16 pg WHO_{1998}-TEQ/g lipid), and the mean value was 5.7 times higher than those of caged eggs (mean 0.314 ± 0.073 pg WHO_{1998}-TEQ/g lipid) in Taiwan. The study also demonstrated distinctive PCDD/F profiles among free-range and caged eggs. In this study, we conducted a survey on the levels of dl-PCBs in free-range and caged egg samples and analyzed their PCB congener profiles. In addition, we compared the concentrations and congener profiles of PCBs and PCDD/Fs between free-range and caged eggs. Our finding implies a dl-PCBs and PCDD/Fs coexposure event in free-range eggs and provides a different aspect for delineating potential contamination sources for dl-PCBs and PCDD/Fs.

MATERIALS AND METHODS

Sample Collection. The egg, soil, and feed samples used in this study were the same samples as collected in a previous study reported by Hsu et al.[20] Egg samples were purchased from 6 private farms and 12 stores throughout Taiwan, as depicted in Figure 1. Briefly, the free-range egg samples were produced by free-range hens raised on six private farms (denoted as F1−F6 in Figure 1 and thereafter) located at different regions in southern Taiwan. The egg samples collected from each farm consisted of 10 individual eggs. As shown in Figure 1, farms F1−F4 were located in Tainan County, F5 in Chiayi County, and F6 in Changhua County. Because the majority of commercialized eggs are produced by caged hens in Taiwan, the caged egg samples used in this study were purchased from 12 stores (denoted as C1−C12 in Figure 1 and thereafter) in distinct geographic areas: samples C1−C9 were obtained from the populated western part of Taiwan and C10−C12 from the eastern region of the island. Each caged egg sample also consisted of 10 individual eggs. All of the egg samples were stored at 4 °C and analyzed within 2 months after collection.

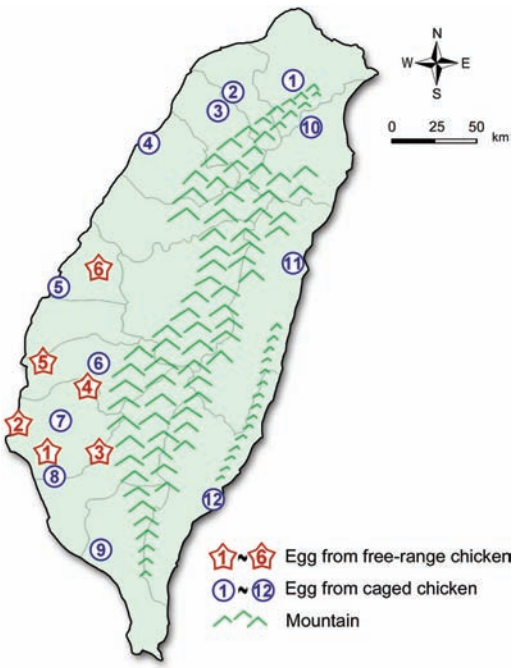

Figure 1. Geographical locations of the egg collection sites in Taiwan. Stars represent farms where eggs were collected from free-range hens at six different sites (sites 1−6 were, respectively, denoted as F1−F6 in the text), and circles represent caged eggs collected from different locations across the island (sites 1−12 were, respectively, denoted as C1−C12 in the text.).

Approximately 50 g of feed was collected from each of farms F1 and F2 for PCB analysis. For soil sampling, the area of the farm on which the free-range hens were raised was divided into nine subdivisions. From each subdivision of a farm, approximately 20 g of soil was shoveled and sampled from the surface sediment (<15 cm). Soil samples from the nine subdivisions of one farm were pooled together and regarded as one soil sample for that farm. The soil samples were kept at 4 °C and analyzed within 2 months after sampling. All sample containers were verified to have no detectable levels of PCB contamination before feed and soil sampling.

PCBs Analysis. The concentrations of 12 dl-PCBs in the egg, feed, and soil samples were analyzed by the Analytical Laboratory for Trace Environmental Pollutant at National Cheng Kung University (ALTEP, NCKU) in Taiwan. Determination of the levels of the 12 dl-PCB congeners was performed by the isotope dilution high resolution gas chromatography−high resolution mass spectrometry (HRGC−HRMS) method. The analytical procedures for sample analysis used in this study were adopted from USEPA method 1668.[21]

For PCB analysis, 30 g of 10 pooled whole egg sample from each individual collection was homogenized in 50 mL of ethanol and 100 mL of acetone/hexane (1/1, v/v), followed by the addition of an internal standard mixture containing 12 $^{13}C_{12}$-labeled PCBs standards (10 μL of 50 ng/mL, isotope purity: 99%, Inc. of Cambridge Isotope Laboratories, MA). The homogenized sample was then extracted with hexane, and the lipid content was determined gravimetrically. After extraction, the sample was treated with concentrated sulfuric acid and then underwent three solid-phase extraction cleanup procedures (acid silica, acid alumina, and Florisil cartridges) before the analysis of 12 dl-PCBs by HRGC−HRMS. Each analytical run included a method blank, a quality control, and eight unknown samples. The samples were analyzed using an Agilent 6890N GC (Agilent Technologies Inc., Santa Clara, CA) and a Micromass AutoSpec Ultima EBE trisector mass spectrometer (Fisons Instruments, Manchester, UK) in the HRGC−HRMS analysis. Samples were fractionated on a J&W DB-5MS capillary fused-silica GC column (60 m, 0.25 mm ID, 0.25 μm film thickness; Agilent technologies Co., Palo Alto, CA). The HRMS was operated in electron impact ionization mode. Selected ion monitoring (SIM) was used to acquire $M/(M+2)$ or $(M+2)/(M+4)$ PCB ions, as defined in USEPA method 1668[21] for identification. The detailed HRGC/HRMS chromatographic procedures used for the determination of PCB levels were as described by Hsu et al.[12]

For the feed samples, 30 g of each sample was Soxhlet-extracted for 24 h by acetone/hexane (1/1, v/v). The extracts were then spiked with a mixture containing 12 $^{13}C_{12}$-labeled PCBs standards, followed by three solid-phase extraction cleanup procedures and analysis of 12 dl-PCBs using HRGC−HRMS. The sample cleanup procedures and instrumental analytical methods for the feed samples were the same as described for the egg samples.

Approximately 10 g of the freeze-dried soil samples was Soxhlet-extracted for 24 h using toluene. After extraction, the sample was spiked with a mixture containing 12 $^{13}C_{12}$-labeled PCB standards followed by three solid-phase extraction cleanup procedures and analysis of 12 dl-PCBs using HRGC−HRMS. The sample cleanup procedures and instrumental analytical methods for the soil samples were the same as described for the egg samples.

Table 1. Dioxin-like PCB Levels in Egg Samples from Free-Range and Caged Hens

	concentration in egg samples, unit: pg/g lipid						
	free-range eggs[e] (N = 6)						caged eggs (N = 12)
analyst	F1	F2	F3	F4	F5	F6	mean[f] ± SD
3,3′,4,4′-TeCB (77)[a]	21.5	9.35	4.99	4.81	18.8	19.1	6.25 ± 1.79
3,4,4′,5-TeCB (81)	4.26	1.74	0.419	0.535	3.24	1.44	0.634 ± 0.263
3,3′,4,4′,5-PeCB (126)	15.7	5.14	0.431	0.748	7.30	2.69	0.926 ± 0.305
3,3′,4,4′,5,5′-HxCB (169)	4.88	1.55	0.329	0.249	1.77	1.18	0.436 ± 0.220
2,3,3′,4,4′-PeCB (105)	217	81	42	37	198	116	32.4 ± 13.6
2,3,4,4′,5-PeCB (114)	13.1	6.87	2.45	2.22	14.3	8.20	2.33 ± 0.833
2,3′,4,4′,5-PeCB (118)	441	165	97.4	82.9	504	276	86.9 ± 41.1
2′,3,4,4′,5-PeCB (123)	12.2	3.95	1.60	1.77	11.3	5.28	1.54 ± 0.577
2,3,3′,4,4′,5-HxCB (156)	99.3	30.9	13.7	9.69	80.6	34.3	11.2 ± 5.20
2,3,3′,4,4′,5′-HxCB (157)	27.2	7.70	3.47	2.31	19.1	8.52	2.78 ± 1.42
2,3′,4,4′,5,5′-HxCB (167)	58.2	14.5	5.68	3.92	40.0	18.1	4.46 ± 1.98
2,3,3′,4,4′,5,5′-HpCB (189)	31.5	6.15	0.771	0.784	11.1	4.76	1.23 ± 0.631
12 dl-PCBs[b]	1.76	0.579	0.071	0.097	0.880	0.349	0.118 ± 0.034
12 dl-PCBs[c]	1.74	0.572	0.059	0.087	0.812	0.321	0.111 ± 0.035
17 PCDD/Fs[b,d]	5.16	1.48	0.604	0.538	2.38	0.598	0.314 ± 0.073
17 PCDD/Fs[c,d]	4.29	1.27	0.533	0.469	2.09	0.507	0.274 ± 0.063
17 PCDD/Fs and 12 dl-PCBs[b,d]	6.91	2.06	0.675	0.635	3.26	0.947	0.432 ± 0.092
17 PCDD/Fs and 12 dl-PCBs[c,d]	6.034	1.837	0.592	0.556	2.897	0.828	0.385 ± 0.081

[a]The number in parentheses are the PCB congener name, which follows the nomenclature rules adopted by the International Union of Pure and Applied Chemistry (IUPAC) and Ballschmiter and Zell (1980).[44,45] The IUPAC numbers at the beginning of the name specify the sites where chlorines are attached to the two phenyl rings. Tetra-, penta-, hexa-, and heptachlorinated biphenyl are expressed as TeCB, PeCB, HxCB, and HpCB, respectively. The number in the parentheses indicated the nomenclature of the systemic PCB numbers by Ballschmiter and Zell.[44] For example, the IUPAC name for PCB 77 is 3,3′,4,4′-tetrachlorobiphenyl, abbreviated as 3,3′,4,4′-TeCB (77). [b]Unit: pg WHO_{1998}-TEQ/g lipid.[25] [c]Unit: pg WHO_{2005}-TEQ/g lipid.[26] [d]The PCDD/F TEQ values were cited from Hsu et al.[20] [e]F1−F6 represented six different farms, as depicted in Figure 1, where free-range egg samples were collected. The free-range egg samples from each farm with a pool of 10 eggs were measured once as described in the Materials and Methods, and the detected value for each PCB congener was shown. [f]The mean dl-PCB levels of the 12 caged egg samples were reported.

Statistical Analysis. The Shapiro−Wilk normality test was used to examine whether a random sampling of the PCB levels in free-range and caged eggs followed a normal distribution. The PCB congener profiles of all egg samples were analyzed and classified by the principal component analysis (PCA).[22,23] These data were arranged into a matrix with n subjects and p variables (12 dl-PCBs). The congener patterns of 12 dl-PCBs were analyzed in all of the egg samples in the PCA model, and all values under the detection limit were regarded as one-half of the limit.[24] In addition, cluster analysis was performed to further cluster the egg samples according to their PCB and PCDD/F congener profiles. The statistical analysis was performed using the Statistica software system (version 6.0, StatSoft Inc., OK).

RESULTS

Concentrations of PCBs in Free-Range and Caged Egg Samples. Six samples of free-range eggs and 12 samples of caged egg were collected from the same geographic sites (Figure 1) and at the same time as in a previous study.[20] Sites of free-range farms 1−6 in Figure 1 were denoted as F1−F6, respectively, and the 12 sites of caged eggs collection were represented as C1−C12, respectively, in the following description. The levels of 12 dl-PCB congeners, consisting of four non-ortho and eight mono-ortho PCB congeners, were analyzed and are summarized in Table 1 and Figure 2. The PCB levels were calculated as toxic equivalency quotient (TEQ) values according to the 1998 and 2005 toxic equivalency factor regulated by World Health Organization (WHO-TEFs) system.[25,26] All of the following TEQ values discussed were calculated on the basis of the 2005 WHO-TEF values. Statistical analysis using the Shapiro−Wilk test revealed that the dl-PCB levels in the six free-range egg samples were normally distributed (p = 0.23). Among these six free-range egg samples, the dl-PCB levels showed regional variations. The egg sample from farm F1 contained the highest concentrations of the 12 dl-PCB (945 pg/g lipid; 1.74 pg WHO_{2005}-TEQ/g lipid), while the eggs from farm F3 exhibited the lowest total PCB concentrations (173 pg/g lipid; 0.059 pg WHO_{2005}-TEQ/

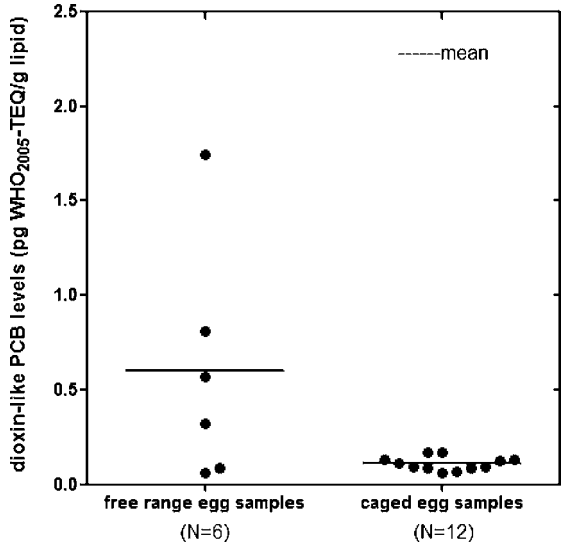

Figure 2. Distribution of 12 dl-PCB levels in the 6 free-range and 12 caged egg samples plotted. The "●" indicates the dl-PCB levels in egg samples. The lines indicate the mean values for all free-range egg samples and caged egg samples.

g lipid). The mean TEQ value for these six farms was 0.599 ± 0.629 pg WHO_{2005}-TEQ/g lipid (mean ± S.D.).

The dl-PCB levels in the 12 caged egg samples were also normally distributed ($p = 0.52$) by the Shapiro–Wilk test. Their dl-PCB concentrations varied from 94.1 to 321 pg/g lipid, and the mean value was 151 ± 64.9 pg/g lipid (mean ± S.D.). The TEQ values of the 12 dl-PCBs ranged from 0.060 to 0.169 pg WHO_{2005}-TEQ/g lipid with a mean value of 0.111 ± 0.035 pg WHO_{2005}-TEQ/g lipid. The concentrations of each specific PCB congener in the free-range and caged eggs are summarized in Table 1.

Concentrations of PCBs in Soil and Feed. The dl-PCB levels in the soil and feed samples from farms F1 and F2 were also measured. As shown in Table 2, the dl-PCB levels in soil samples were 0.081 pg WHO_{2005}-TEQ/g (dry weight) for the farm F1 sample and 0.066 pg WHO_{2005}-TEQ/g (dry weight) for the farm F2 sample. For the feed samples, the concentrations of the 12 dl-PCBs in the two feed samples were calculated as 0.005 pg WHO_{2005}-TEQ/g sample and 0.004 pg WHO_{2005}-TEQ/g sample, respectively, for F1 and F2 samples, both with a 12% water content. The concentrations of each specific PCB congener in the soil and feed samples are summarized in Table 2.

Table 2. Dioxin-like PCB Levels in Soil and Feed Samples

	soil[d] (unit: pg/g dry sample)		feed[d] (unit: pg/g sample 12% w.c.)	
analyst	F1	F2	F1	F2
3,3′,4,4′-TeCB (77)[a]	3.49	1.84	0.872	0.398
3,4,4′,5-TeCB (81)	0.196	0.192	0.055	0.031
3,3′,4,4′,5-PeCB (126)	0.743	0.603	0.044	0.040
3,3′,4,4′,5,5′-HxCB (169)	0.202	0.150	0.009	0.010
2,3,3′,4,4′-PeCB (105)	5.99	5.63	1.04	0.761
2,3,4,4′,5-PeCB (114)	0.292	0.303	0.061	0.054
2,3′,4,4′,5-PeCB (118)	10.4	9.60	2.40	1.68
2′,3,4,4′,5-PeCB (123)	0.271	0.322	0.075	0.042
2,3,3′,4,4′,5-HxCB (156)	3.00	2.42	0.383	0.146
2,3,3′,4,4′,5′-HxCB (157)	1.01	0.701	0.060	0.042
2,3′,4,4′,5,5′-HxCB (167)	1.58	1.22	0.191	0.069
2,3,3′,4,4′,5,5′-HpCB (189)	1.01	1.47	0.041	0.022
12 dl-PCBs[b]	0.081	0.065	0.005	0.005
12 dl-PCBs[c]	0.081	0.066	0.005	0.004

[a]The nomenclature of PCB congeners is described in Table 1. [b]Unit: pg WHO_{1998}-TEQ/g dry sample for soil, pg WHO_{1998}-TEQ/g sample 12% w.c. for feed.[25] [c]Unit: pg WHO_{2005}-TEQ/g lipid.[26] [d]F1 and F2 represented two free-range farms, as depicted in Figure 1, where samples were collected. The sampling of soil and feed samples was described in the Materials and Methods. The samples from farms F1 and F2 were each measured once, and the detected value for each PCB congener was shown.

Principal Component Analysis for PCB Congener Profiles in Eggs. Principal component analysis (PCA) was used to analyze the congener profiles of dl-PCBs in the free-range and caged eggs. The PCA decomposed the data set for the 12 dl-PCBs into two principal components (PCs). The first factor, which accounted for 40.57% of the variables of the data, was positively influenced by PCB-118 (loading factor of factor 1 > 0.7). The second factor, which accounted for 31.37% of the variables of the data, was positively influenced by PCB-77 (loading factor of factor 2 > 0.7; data not shown). The PCA classified all 18 samples into two groups plus one outliner C9 (Figure 3A). Group I consists of the free-range egg samples collected from farms F1 and F2, while group II includes the samples from farms F3, F4, F5, and F6 and all caged egg samples other than C9. Groups I and II represented two characteristic patterns of PCB congener profiles in these egg samples. We will discuss the implications of these patterns in a later section.

Correlation between PCB and PCDD/F Levels in Free-Range and Caged Eggs. Because the free-range egg samples collected in Taiwan showed elevated concentrations of both PCDD/Fs in a previous study[20] and dl-PCBs in this study (Table 1), it was of interest to assess whether there were any correlations between the levels of the 12 dl-PCBs and 17 PCDD/Fs in the free-range and caged eggs (the PCDD/F data in Table 1 and Figure 4 were adopted from Hsu et al.[20]). As shown in Figure 4A, the six free-range egg samples that exhibited elevated dl-PCB concentrations appeared to have higher PCDD/F contents, and there was a linear correlation between the PCB and PCDD/F levels ($r = 0.987$; $p < 0.001$). However, for the 12 caged egg samples, the PCB and PCDD/F concentrations were poorly correlated ($r = 0.296$; $p = 0.35$).

Cluster Analysis for PCB and PCDD/F Congener Profiles in Eggs. The cluster analysis classified the free-range and caged egg samples into groups according to the characteristics of the dl-PCB and PCDD/F congener profile from each sample. With respect to the congener profiles of PCBs in the eggs (as shown in Figure 5A), the cluster analysis revealed that the 18 egg samples were divided into two groups. This indicated that at least two characteristic patterns of PCB congener profiles were observed among these egg samples with the exception of the C9 sample. Group I consists of the free-range egg samples collected from farms F1 and F2 (see Figure 1 for locations), while group II includes the eggs from farms F3–F6, and all of the caged egg samples except the C9 sample. One distinct characteristic of the grouping was the mean contribution of the 4 and 5 Cl-PCB congers to all 12-dl PCBs. As shown in Figure 5C, group I contained 79.2% 4 and 5 Cl-PCBs and 20.8% 6 and 7 Cl-PCBs, while group II presented 86.5% 4 and 5 Cl-PCBs and 13.5% 6 and 7 Cl-PCBs.

The congener profiles of the PCDD/Fs in the free-range and caged egg samples were also classified by the cluster analysis as well (the PCDD/F data in Table 1 and Figure 5 were adopted from Hsu et al.[20]). The analysis divided the 18 samples except for the C10 sample into three groups (Figure 5B and D). The egg samples from C1 and farms F1 and F2 fell into group I (54% PCDD and 46% PCDFs), and samples from farms F3 and F4 fell into group II (41.2% PCDD and 58.8% PCDFs). The rest of the caged egg samples except for those from C1 and C10 were clustered into group III, which presented 77.5% PCDDs and 22.5% PCDFs (Figure 5D).

DISCUSSION

PCBs Levels in Free-Range and Caged Eggs. This study surveyed the dl-PCB levels and congener profiles among free-range and caged eggs in Taiwan. Although the toxicity of dl-PCBs is not as potent as that of PCDD/Fs, the Commissions of the European Communities (EC) does not overlook the adverse health effects of dl-PCBs and specifies regulatory guideline values of 3 pg WHO_{1998}-TEQ/g lipid for 17 PCDD/Fs and 6 pg WHO_{1998}-TEQ/g lipid for the total WHO-TEQ (17 PCDD/Fs and 12 dl-PCBs) in hen eggs.[27] Any contaminated food exceeding these guideline values would be considered unsuitable for human consumption.

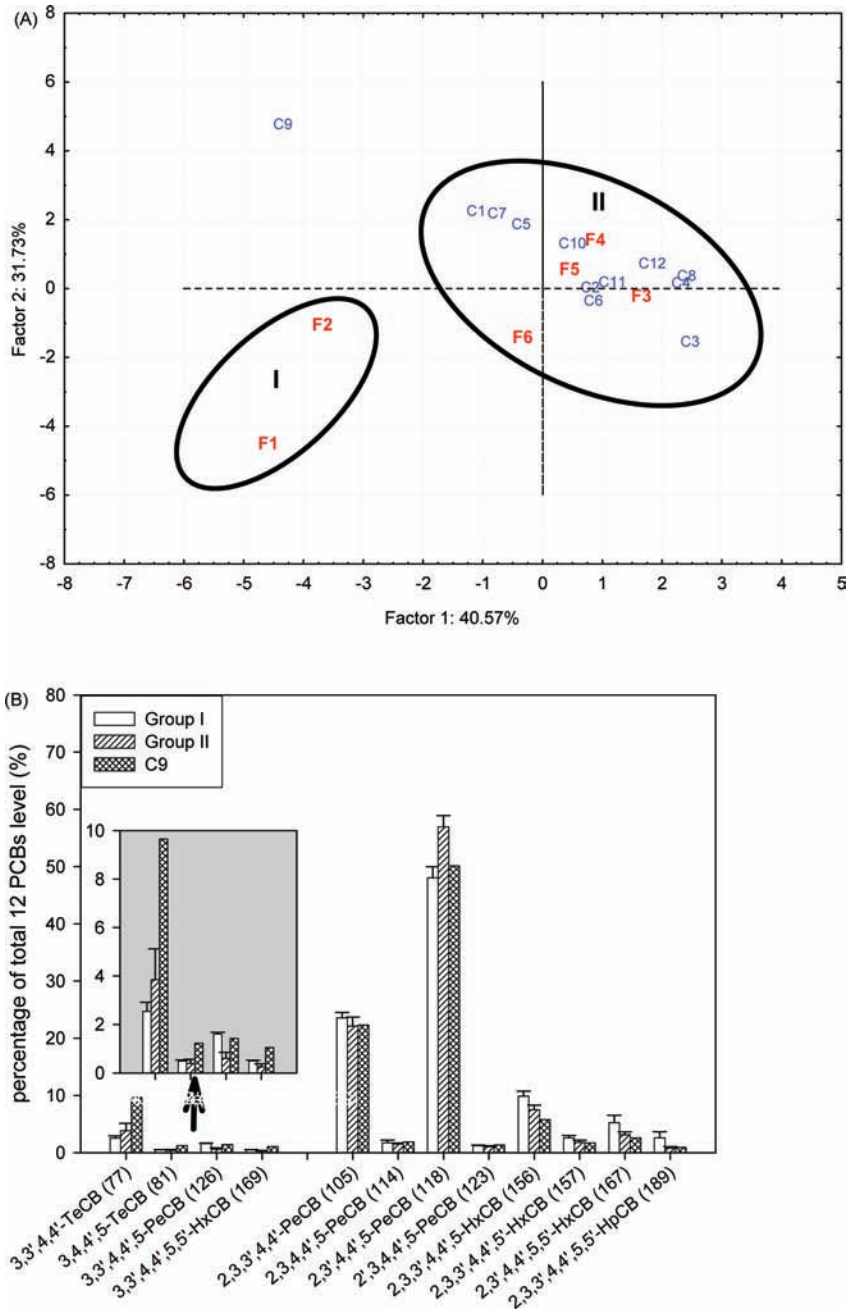

Figure 3. Principal component analysis (PCA) of the 12 dl-PCB congener profiles of all of the free-range and caged egg samples. (A) The score plot of factor 1 against factor 2 according to the congener profiles of the analyzed 18 egg samples (F1−F6 and C1−C12 denoted as in Figure 1). (B) The characteristic dl-PCB congener patterns for groups I,II and for the C9 sample.

The data in Table 1 demonstrate that a major fraction (4 out of 6) of the free-range egg samples exhibited substantially higher PCB levels than the caged eggs. The detected mean value of the 12 dl-PCBs from the free-range egg samples was 5.4 times (0.599/0.111) higher than that of the 12 caged egg samples. One out of the six free-range samples (17%; F1) exhibited a total value for the 12 dl-PCBs + 17 PCDD/Fs of 6.034 pg WHO_{2005}-TEQ/g lipid, which did not comply with the guideline limit of 6.00 pg WHO_{1998}-TEQ/g lipid set by the EC.[27] In addition, the 12 dl-PCB concentrations from the egg samples of farms F2, F5, and F6 were at 3 times or much higher than the mean value presented by the caged eggs. All six free-range egg samples, for which the total WHO-TEQ values (17 PCDD/Fs and 12 dl-PCBs) ranged from 0.556 pg WHO_{2005}-TEQ/g lipid to 6.034 pg WHO_{2005}-TEQ/g lipid, contained substantially higher levels of pollutants than the 12 caged egg samples. In contrast, all of the caged egg samples exhibited total WHO-TEQ values far below the maximum guideline level of 6.00 pg WHO_{1998}-TEQ/g lipid (mean value 0.423 pg WHO_{1998}-TEQ/g lipid). Similar observations have been reported in many European countries, indicating that the average levels of total dl-PCBs and PCDD/Fs are increased in free-range or organic eggs and 10−15% of egg samples do not meet the guideline limit.[18] Although the majority fraction of the free-range eggs in these reports did not necessarily exceed the guideline limits, the authors raised the issue of controlling possible contamination sources for free-range or home-produced eggs. This preliminary study on dl-PCB levels also

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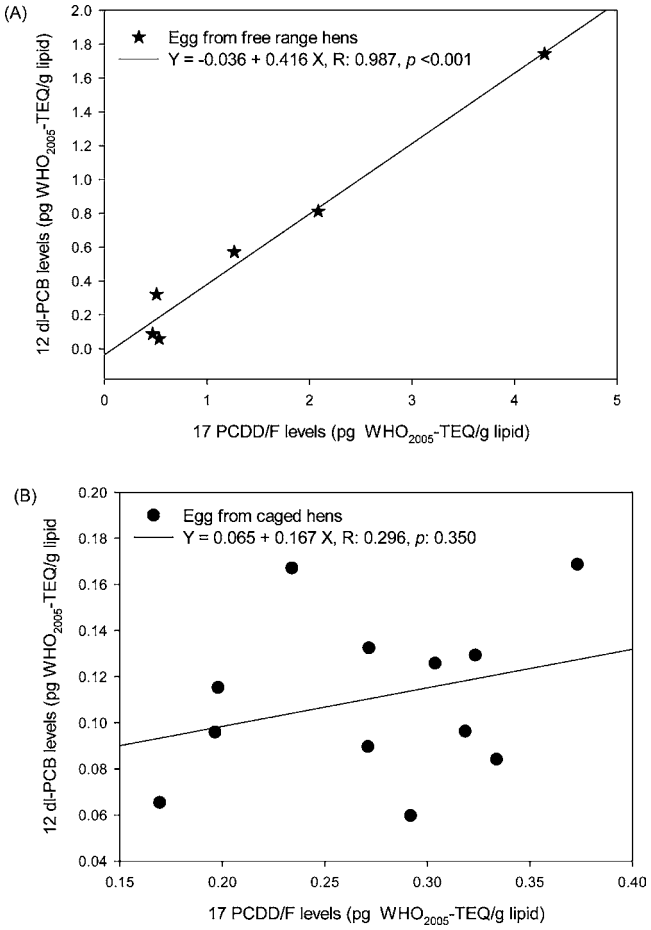

Figure 4. Correlation between the levels of the 12 dl-PCBs and the 17 PCDD/Fs in the analyzed six free-range egg samples (A) and 12 caged egg samples analyzed (B).

gives rise to issues regarding the safety of the consumption of free-range eggs and the importance of monitoring potential contamination sources in Taiwan.

Hens are considered to be the major source of dl-PCBs in eggs. Hens may ingest dl-PCBs through polluted feed, soil, or vegetation and eventually transfer dl-PCBs to their eggs.[28] Battery hens that produce caged eggs have very little contact with polluted outdoor sources. In contrast, free-range hens might have continuous access to open-air runs and to contaminated sources, such as feed, soil, plants, worms, and insects and other sources. Some reports have indicated that the environment is the most important contributor to elevated dl-PCB levels in free-range eggs.[18] Thus, the increases in dl-PCBs could be attributed to the nature of the free ranging system.[14,18]

Different PCB Congener Profiles in Free-Range Eggs and Their Implications. All 18 egg samples except the C9 sample could be divided into two discriminative groups, each presenting a characteristic PCBs congener profile pattern (Figure 3). Group I consisted only of two free-range samples (F1 and F2). The rest of the free-range eggs (F3−F6), along with the other 11 caged eggs samples (C1−C8 and C10−C12), were categorized into group II. For both groups I and II, the most prevalent congener was mono-ortho PCB-118, followed by congeners 105 and 156 (Figure 3B). Group II presented a significantly higher percentage of PCB-118 among the 12 dl-PCBs than group I. In contrast, the free-range eggs in group I contained significantly higher percentages of 6 and 7 Cl-PCBs congeners 156, 157, 167, and 189, as well as the high toxicity congener PCB-126 among the 12 dl-PCBs.

The discriminative congener profiles implied an association with exposure to particular media.[20] The samples from groups I and II might have been subjected to different respective PCB sources. With respect to group I, both farms F1 and F2 located close to the costal industrial area in southern Taiwan (Figure 1). It is reasonable to postulate that samples from F1 and F2 might have been exposed to the same PCB sources and that particular PCB sources could be unique to this geographic area. As the group II samples were collected from free-range hens (F3−F6) and caged hens (C1,C2), the similar PCB congener profiles of these egg samples implied the similar contamination sources of PCBs for these egg samples. It could be a general background contamination source or another specific source. However, the higher PCB levels in the free-range egg samples, F5−F6, could be attributed to the higher exposure rate of the free-range hens than the caged hens. The higher exposure rate of the free-range hens than the caged hens may arise from the foraging behaviors of free-range hens, for example, running outsider behavior, which increases the chances of ingestion soil that contains PCBs. More investigations will be required to identify the primary contamination sources.

Despite the existence of different PCB profiles in various food items, including eggs, the most prominent PCB congeners were generally represented by PCBs 118, 105, and 156 as shown in a Spanish survey[29] and an Italian study.[30] We detected a similar trend in our free-range and caged egg samples for the three most abundant congeners. In our soils and feed samples, the most abundant congeners were PCBs 118, 105, 77, and 156 (Table 2). Nevertheless, our data did not provide sufficient evidence to demonstrate whether PCBs in the soils and feed were the primary contributors to the elevated dl-PCBs in the F1 and F2 free-range eggs. One reason for this was that the levels of dl-PCBs in the soils and feed collected from farms F1 and F2 were similar (F1 0.081 vs F2 0.066 pg WHO_{2005}-TEQ/g sample for soil samples; F1 0.005 vs F2 0.004 pg WHO_{2005}-TEQ/g sample for feed samples), but the dl-PCB levels in F1 eggs were 3.04 (1.74/0.572) times higher than those of the F2 eggs. The detected dl-PCB levels from soil and feed samples did not totally reflect that of the F1 and F2 egg samples. In addition, the bioaccumulation and metabolic rates for each PCB congener contamination varied widely and were determined by their differential induction of cytochrome P450 1A (CYP1A). PCB congeners that retain a planar configuration have higher potential to induce CYP1A and exert subsequent biological effects.[31] Therefore, the calculation of carry-over from feed and soil samples to hens and from hens to eggs for each dl-PCB congener could be complicated. More evidence on the congener profile analysis of different pollution sources will be needed to identify the primary contamination sources.

Is it possible that the elevated PCB levels in free-range eggs in this study be partly ascribed to the polluted soil or feed as previously reported.[28] Because the free-range hens have more opportunities to run in the outdoors and ingest soil that contains PCBs when compared to the caged hens, several studies have indicated that the elevated dl-PCBs in free-range eggs may be attributed to the running behavior of free-range hens.[18,32] To address this question, we have adopted a simplified way[20] to estimate the levels of PCB that might be contributed to the free-range eggs through soil and feed ingestion. For F1 soil samples in this study, the calculated dl-PCBs were 0.081 pg WHO_{2005}-TEQ/g samples, and consider-

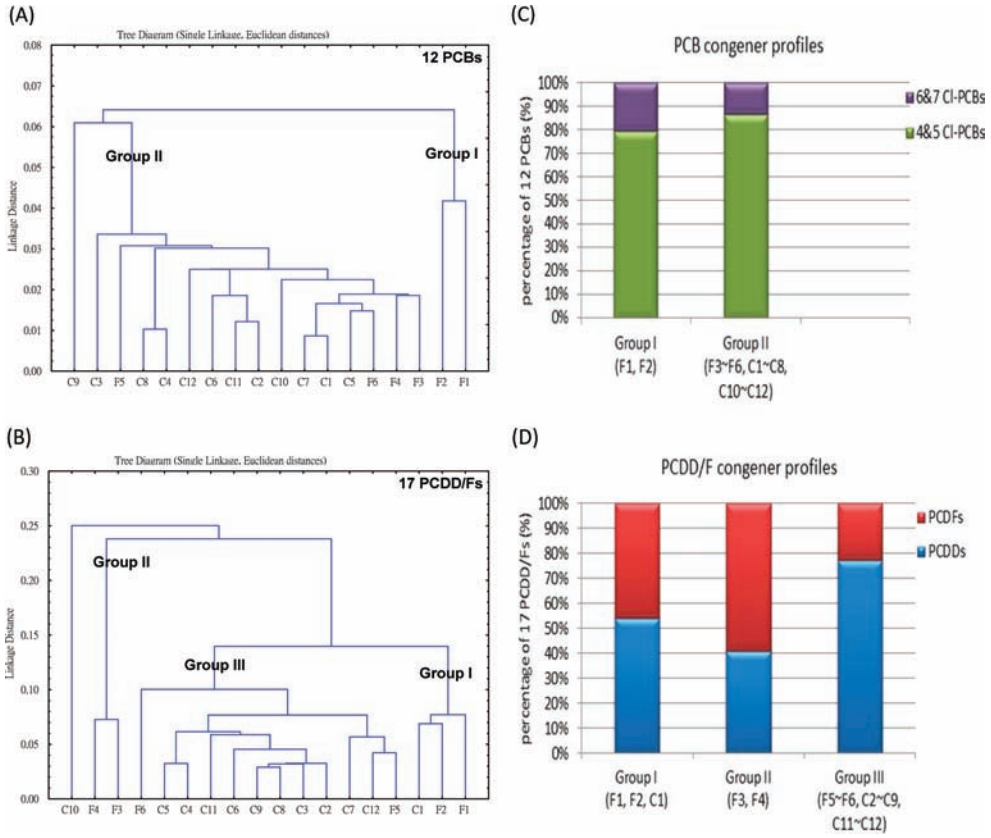

Figure 5. Tree diagrams from the cluster analysis for the 12 dl-PCB levels (A) and 17 PCDD/Fs (B) in the 18 egg samples. (C) The characteristic dl-PCB congener patterns in groups I and II according to the contributions of 4 and 5 and 6 and 7 Cl-PCBs. (D) The characteristic PCDD/F congener patterns in groups I, II, and III according to the contributions of PCDDs and PCDFs to the 17 PCDD/Fs (F1−F6 and C1−C12 denoted as in Figure 1).

ing an average daily intake of soil of 10 g/day by free-range hens,[33] a 40−60% absorption of the dl-PCBs in the soil,[28] and assuming an average of 50% transfer rate for dl-PCB congeners to the eggs,[34] the calculation would result in a contamination level of 0.027−0.041 pg WHO_{2005}-TEQ/g lipid in the eggs (assuming 6 g of lipid/egg). Likewise, soil samples from F2 would contribute 0.022−0.033 pg WHO_{2005}-TEQ/g lipid to the eggs. Similarly, assuming an daily intake of 140 g of feed for a hen,[35] a 40−60% absorption of the dl-PCBs from the feed,[28] and assuming an average of 50% transfer rate for dl-PCB congeners to the eggs,[34] this would give us a daily feed contribution value of 0.023−0.035 pg WHO_{2005}-TEQ/g lipid to the F1 eggs (0.005 pg WHO_{2005}-TEQ/g for F1 feed samples) and 0.019−0.028 for pg WHO_{2005}-TEQ/g lipid to the F2 eggs (0.004 pg WHO_{2005}-TEQ/g for F2 feed samples). Thus, it is likely that ingestion of soil and feed could partly contribute to the elevated dl-PCB levels in the F1 and F2 free-range eggs in this scenario. Again, from our current data, it is difficult to conclude that soils and feed represent the major pollution sources in this scenario. More investigations will be required to identify the primary contamination sources.

Coexposure of Free-Range Hens to PCBs and PCDD/Fs. Because dl-PCBs and PCDD/Fs likely coexist in the environment, reports that evaluate the PCDD/Fs levels in foodstuffs would normally measure the levels of PCBs as well.[29,36] However, little attention has been given to whether there is any correlation between the concentrations of PCDD/Fs and dl-PCBs. The data presented in Figure 4 are the first to demonstrate that the concentrations of dl-PCBs in free-range eggs are highly correlated with those of PCDD/Fs. This concentration correlation indicated the occurrence of coexposure to PCBs and PCDD/Fs associated with free-range farming where hens had access to PCB and PCDD/F sources, with the pollutants subsequently being biotransferred to their eggs. As we discussed in the previous section, air, soils, feed, vegetation, and worms are potential PCBs and PCDD/F sources for free-range eggs.[14,18] Such coexposure incidents were relatively rare in the more controlled caged-egg farming systems (Figure 4B). This phenomenon raised the issue of whether the coexposure to PCBs and PCDD/Fs in free-range eggs arose from the same contamination sources. This question is addressed in the next section.

Distinct Sources of PCBs and PCDD/Fs in Free-Range Eggs. Cluster analysis is a powerful tool that serves to assort the PCB and PCDD/F congener patterns of all 18 egg samples and clusters them into different groups. The congener patterns in any particular cluster resemble each other as much as possible within the cluster and are as distinctive from each other as possible when compared to other clusters. The 18 egg samples were separated into two dissimilar groups based on their PCB congener profiles (Figure 5A and C). The same classification was obtained using PCA as shown in Figure 3A. The characteristic patterns of the PCB congener profiles for the two groups were plotted in Figures 3B and 5C. Each group exhibited a distinctive PCB congener pattern and mean contribution of 4 and 5 Cl-PCBs among all 12 dl-PCBs.

The cluster analysis presented in Figure 5B divided the 18 egg samples into three groups according to their PCDD/F

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congener patterns. A very similar classification was reported using PCA.[20] Considering the six free-range egg samples, group I included the free-range egg samples collected from farms F1 and F2, group II from F3 and F4, and group III from F5 and F6. Each group exhibited a discriminative PCDD/F congener pattern and a mean contribution of PCDDs among all 17 PCDD/Fs.

PCBs and PCDD/Fs are ubiquitous and persistent organic pollutants and can stay for decades or centuries once they are released. Hens are likely to be exposed to mixtures of PCBs and PCDD/Fs from various media (feed, air, soil, vegetation, and worms) in the environment.[14,18,20] Because each medium might contain mixtures of PCBs and PCDD/Fs with unique congener profiles, these congener profiles could be regarded as a signature associated with particular media or sources of contamination.[37] For situations such as environmental pollution and human exposure accidents, this media-specific profile could provide useful information for identification of the source of PCDD/F and PCB contamination.[38,39] Our PCB and PCDD/Fs congener profile data (Figures 3 and 5) indicated that there were two different contamination sources for PCBs and three distinctive sources for PCDD/Fs pollution. The free-range eggs collected from farms F1 and F2 are contaminated by one PCB source, while eggs from farms F3−F6 are contaminated by the other PCB source. As for the contamination of PCDD/Fs, the free-range eggs from farms F1 and F2 are polluted by one source, from farms F3 and F4 by another source, and from farms F5 and F6 by a third source. This implies the coexistence of distinct contamination scenarios for PCBs and PCDD/Fs in the free-range eggs. Whether a broad implication of our finding in free-range egg also applies to other countries still needs more investigation, because the local environment strongly influences contaminant levels in free-range products. Nevertheless, we believe that this coexposure scenario and congener analysis model can be applied to similar studies in other areas to identify possible contamination sources.

It is reasonable to conclude that the free-range eggs addressed in this study were exposed to different PCB and PCDD/F contamination media. Historically, PCBs are man-made chemical products and are released into the environments (soil, water, air) through sewage, incineration emissions, and industrial discharges.[40] After the ban of PCB production in the 1970s, the primary current sources of PCB exposure are generally from accidental leaks from the landfills with incineration emissions becoming increasingly less important.[1] In Taiwan, the manufacture and importation of PCBs products was banned by the Taiwan Environmental Protection Association (EPA) in 1988, but PCBs can still be found in the contaminated environment (e.g., the sediments and water) due to their persistence and long half-lives.[41,42] It is clear that the majority of PCBs found in food or water and ingested by humans come from PCB sources produced as industrial products in the past as surveys reported from Taiwan[41,42] and from the United States.[40] Thus, the PCB exposure sources of free-range hens in this study could be traced back to previous industrial activities in Taiwan.

In contrast, PCDD/Fs have different sources. PCDD/Fs were mainly associated with the chlorine industry and the pulp industry in the past. The majority of PCDD/F contamination might also result from historical industrial activities and their persistence in the environment. At present, combustion (incineration of municipal waste or wood) is the key source of PCDD/Fs pollution.[40,43] Because Taiwan is an industrialized island with 20 municipal waste incinerators and many factories with combustion activities, it is more complicated to attribute the pollution sources for PCDD/Fs.

Coexposure to PCBs and PCDD/Fs occurs frequently in polluted foodstuffs and environments, and, in some cases, it can incorrectly be concluded that these substances come from the same contamination sources. Nevertheless, PCBs and PCDD/Fs do present distinct routes of exposures. As revealed in this study, analysis of dl-PCB and PCDD/F congener profiles provides useful information and demonstrates that there are indeed distinct pollution sources associated with free-range hens.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ALTEP, Taiwan, Analytical Laboratory for Trace Environmental Pollutant at National Cheng Kung University in Taiwan; EC, the Commissions of the European Communities; HRGC−HRMS, high resolution gas chromatography−high resolution mass spectrometry; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; TEQ, the toxic equivalency quotient; WHO-TEFs, World Health Organization toxic equivalency factors

REFERENCES

(1) Kimbrough, R. D.; Krouskas, C. A.; Carson, M. L.; Long, T. F.; Bevan, C.; Tardiff, R. G. Human uptake of persistent chemicals from contaminated soil: PCDD/Fs and PCBs. *Regul. Toxicol. Pharmacol.* **2010**, *57*, 43−54.

(2) Knerr, S.; Schrenk, D. Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in experimental models. *Mol. Nutr. Food Res.* **2006**, *50*, 897−907.

(3) Charnley, G.; Kimbrough, R. D. Overview of exposure, toxicity, and risks to children from current levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds in the USA. *Food Chem. Toxicol.* **2006**, *44*, 601−15.

(4) White, S. S.; Birnbaum, L. S. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.* **2009**, *27*, 197−211.

(5) Guo, Y. L.; Yu, M. L.; Ryan, J. J. Different congeners of PCBs/PCDFs may have contributed to different health outcomes in the Yucheng cohort. *Neurotoxicol. Teratol.* **1996**, *18*, 255−6, Discussion: 271−6.

(6) Pavuk, M.; Cerhan, J. R.; Lynch, C. F.; Schecter, A.; Petrik, J.; Chovancova, J.; Kocan, A. Environmental exposure to PCBs and cancer incidence in eastern Slovakia. *Chemosphere* **2004**, *54*, 1509−20.
(7) Recio-Vega, R.; Velazco-Rodriguez, V.; Ocampo-Gomez, G.; Hernandez-Gonzalez, S.; Ruiz-Flores, P.; Lopez-Marquez, F. Serum levels of polychlorinated biphenyls in Mexican women and breast cancer risk. *J. Appl. Toxicol.* **2011**, *31*, 270−8.
(8) Schantz, S. L.; Gasior, D. M.; Polverejan, E.; McCaffrey, R. J.; Sweeney, A. M.; Humphrey, H. E.; Gardiner, J. C. Impairments of memory and learning in older adults exposed to polychlorinated biphenyls via consumption of Great Lakes fish. *Environ. Health Perspect.* **2001**, *109*, 605−11.
(9) Eubig, P. A.; Aguiar, A.; Schantz, S. L. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ. Health Perspect.* **2010**, *118*, 1654−67.
(10) Bavithra, S.; Selvakumar, K.; Pratheepa Kumari, R.; Krishnamoorthy, G.; Venkataraman, P.; Arunakaran, J. Polychlorinated biphenyl (PCBs)-induced oxidative stress plays a critical role on cerebellar dopaminergic receptor expression: Ameliorative role of quercetin. *Neurotoxic. Res.* **2012**, *21* (2), 149−159.
(11) Noakes, P. S.; Taylor, P.; Wilkinson, S.; Prescott, S. L. The relationship between persistent organic pollutants in maternal and neonatal tissues and immune responses to allergens: A novel exploratory study. *Chemosphere* **2006**, *63*, 1304−11.
(12) Hsu, J. F.; Lee, C. C.; Su, H. J.; Chen, H. L.; Yang, S. Y.; Liao, P. C. Evaluation of background persistent organic pollutant levels in human from Taiwan: Polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls. *Environ. Int.* **2009**, *35*, 33−42.
(13) Shen, H.; Ding, G.; Wu, Y.; Pan, G.; Zhou, X.; Han, J.; Li, J.; Wen, S. Polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in breast milk from Zhejiang, China. *Environ. Int.* **2011**, DOI: 10.1016/j.envint.2011.04.004.
(14) De Vries, M.; Kwakkel, R. P.; Kijistra, A. Dioxins in organic eggs: a review. *NJAS-Wagen. J. Life Sci.* **2006**, *54*, 207−221.
(15) Marti-Cid, R.; Bocio, A.; Domingo, J. L. Dietary exposure to PCDD/PCDFs by individuals living near a hazardous waste incinerator in Catalonia, Spain: temporal trend. *Chemosphere* **2008**, *70*, 1588−95.
(16) European Communities. *European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs* (EC Regulation No. 2375/2001); European Communities: Brussels, 2006.
(17) Hsu, M. S.; Hsu, K. Y.; Wang, S. M.; Chou, U.; Chen, S. Y.; Huang, N. C.; Liao, C. Y.; Yu, T. P.; Ling, Y. C. A total diet study to estimate PCDD/Fs and dioxin-like PCBs intake from food in Taiwan. *Chemosphere* **2007**, *67*, S65−70.
(18) Schoeters, G.; Hoogenboom, R. Contamination of free-range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls. *Mol. Nutr. Food Res.* **2006**, *50*, 908−14.
(19) Wang, I. C.; Wu, Y. L.; Lin, L. F.; Chang-Chien, G. P. Human dietary exposure to polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in Taiwan. *J. Hazard. Mater.* **2009**, *164*, 621−6.
(20) Hsu, J. F.; Chen, C.; Liao, P. C. Elevated PCDD/F levels and distinctive PCDD/F congener profiles in free range eggs. *J. Agric. Food Chem.* **2010**, *58*, 7708−14.
(21) USEPA. *Method 1613: Tetra-through octa-chlorinated dioxin and furans by isotope dilution HRGC/HRMS(Revision B)*; USEPA Office of Water; United States Environmental Protection Agency: Washington, D.C., October, 1994.
(22) Wold, S.; Esbensen, K.; Geladi, P. Principal component analysis. *Chemometr. Intell. Lab.* **1987**, *2*, 37−52.
(23) Hsu, J. F.; Guo, Y. L.; Yang, S. Y.; Liao, P. C. Congener profiles of PCBs and PCDD/Fs in Yucheng victims fifteen years after exposure to toxic rice-bran oils and their implications for epidemiologic studies. *Chemosphere* **2005**, *61*, 1231−1243.
(24) Haas, C. N.; Scheff, P. A. Estimation of averages in truncated samples. *Environ. Sci. Technol.* **1990**, *24*, 912−919.
(25) Van den Berg, M.; Birnbaum, L.; Bosveld, A. T. C.; Brunstrom, B.; Cook, P.; Feeley, M.; Giesy, J. P.; Hanberg, A.; Hasegawa, R.; Kennedy, S. W.; Kubiak, T.; Larsen, J. C.; van Leeuwen, F. X. R.; Liem, A. K. D.; Nolt, C.; Peterson, R. E.; Poellinger, L.; Safe, S.; Schrenk, D.; Tillitt, D.; Tysklind, M.; Younes, M.; Waern, F.; Zacharewski, T. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **1998**, *106*, 775−792.
(26) Van den Berg, M.; Birnbaum, L. S.; Denison, M.; De Vito, M.; Farland, W.; Feeley, M.; Fiedler, H.; Hakansson, H.; Hanberg, A.; Haws, L.; Rose, M.; Safe, S.; Schrenk, D.; Tohyama, C.; Tritscher, A.; Tuomisto, J.; Tysklind, M.; Walker, N.; Peterson, R. E. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* **2006**, *93*, 223−241.
(27) European Communities. *Setting maximum levels for certain contaminants in foodstuffs amending commission regulation* (EC Regulation No. 2375/2001); European Communities: Brussels, 2001.
(28) Van Eijkeren, J. C. H.; Zeilmaker, M. J.; Kan, C. A.; Traag, W. A.; Hoogenboom, L. A. P. A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. *Food Addit. Contam.* **2006**, *23*, 509−517.
(29) Marin, S.; Villalba, P.; Diaz-Ferrero, J.; Font, G.; Yusa, V. Congener profile, occurrence and estimated dietary intake of dioxins and dioxin-like PCBs in foods marketed in the Region of Valencia (Spain). *Chemosphere* **2011**, *82*, 1253−61.
(30) Menotta, S.; D'Antonio, M.; Diegoli, G.; Montella, L.; Raccanelli, S.; Fedrizzi, G. Depletion study of PCDD/Fs and dioxin-like PCBs concentrations in contaminated home-produced eggs: preliminary study. *Anal. Chim. Acta* **2010**, *672*, 50−4.
(31) Koenig, S.; Fernandez, P.; Sole, M. Differences in cytochrome P450 enzyme activities between fish and crustacea: Relationship with the bioaccumulation patterns of polychlorobiphenyls (PCBs). *Aquat. Toxicol.* **2012**, *108*, 11−7.
(32) Kijlstra, A.; Traag, W. A.; Hoogenboom, L. A. Effect of flock size on dioxin levels in eggs from chickens kept outside. *Poult. Sci.* **2007**, *86*, 2042−8.
(33) Stephens, R. D.; Petreas, M. X.; Hayward, D. G. Biotransfer and bioaccumulation of dioxins and furans from soil: chickens as a model for foraging animals. *Sci. Total Environ.* **1995**, *175*, 253−73.
(34) Bargar, T. A.; Scott, G. I.; Cobb, G. P. Maternal transfer of contaminants: case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white Leghorn chickens (Gallus domesticus). *Environ. Toxicol. Chem.* **2001**, *20*, 61−7.
(35) Van der Meulen, J.; Kwakernaak, C.; Kan, C. A. Sand intake by laying hens and its effect on egg production parameters. *J. Anim. Physiol. Anim. Nutr.* **2008**, *92*, 426−431.
(36) Tornkvist, A.; Glynn, A.; Aune, M.; Darnerud, P. O.; Ankarberg, E. H. PCDD/F, PCB, PBDE, HBCD and chlorinated pesticides in a Swedish market basket from 2005--levels and dietary intake estimations. *Chemosphere* **2011**, *83*, 193−9.
(37) Gilpin, R. K. Analytical procedures for measuring safety levels of dioxins, furans and PCBs. *J. Chromatogr. Sci.* **2003**, *41*, 447−8.
(38) DeCaprio, A. P.; Johnson, G. W.; Tarbell, A. M.; Carpenter, D. O.; Chiarenzelli, J. R.; Morse, G. S.; Santiago-Rivera, A. L.; Schymura, M. J. Polychlorinated biphenyl (PCB) exposure assessment by multivariate statistical analysis of serum congener profiles in an adult Native American population. *Environ. Res.* **2005**, *98*, 284−302.
(39) Chen, Y. C.; Tsai, P. J.; Wang, L. C.; Shih, M.; Lee, W. J. An integrated approach for identification of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) pollutant sources based on human blood contents. *Environ. Sci. Pollut. Res.* **2010**, *17*, 759−769.
(40) Weber, R.; Gaus, C.; Tysklind, M.; Johnston, P.; Forter, M.; Hollert, H.; Heinisch, E.; Holoubek, I.; Lloyd-Smith, M.; Masunaga, S.; Moccarelli, P.; Santillo, D.; Seike, N.; Symons, R.; Torres, J. P.; Verta, M.; Varbelow, G.; Vijgen, J.; Watson, A.; Costner, P.; Woelz, J.; Wycisk, P.; Zennegg, M. Dioxin- and POP-contaminated sites--contemporary and future relevance and challenges: overview on

background, aims and scope of the series. *Environ. Sci. Pollut. Res.* **2008**, *15*, 363−93.
(41) Chi, K. H.; Chang, M. B.; Kao, S. J. Historical trends of PCDD/Fs and dioxin-like PCBs in sediments buried in a reservoir in Northern Taiwan. *Chemosphere* **2007**, *68*, 1733−40.
(42) Chi, K. H.; Luo, S.; Hsu, S. C.; Kao, S. J.; Tsai, Y. J.; Chang, M. B. Historical trends of dioxin-like compounds and heavy metals in sediments buried in a reservoir in central Taiwan. *Chemosphere* **2009**, *76*, 286−92.
(43) Alcock, R. E.; Behnisch, P. A.; Jones, K. C.; Hagenmaier, H. Dioxin-like PCBs in the environment-human exposure and the significance of sources. *Chemosphere* **1998**, *37*, 1457−72.
(44) Ballschmiter, K.; Zell, M. Baseline studies of the global pollution. I. Occurrence of organohalogens in pristine European and antarctic aquatic environments. *Int. J. Environ. Anal. Chem.* **1980**, *8*, 15−35.
(45) Mills, S. A. III; Thal, D. I.; Barney, J. A summary of the 209 PCB congener nomenclature. *Chemosphere* **2007**, *68*, 1603−12.