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ARTICLE

Elevated PCDD/F Levels and Distinctive PCDD/F Congener Profiles in Free Range Eggs

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Chicken eggs are one of the most important foods in the human diet all over the world, and the demand for eggs from free range hens has steadily increased. Congener-specific analyses of 17 polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) were performed on 6 free range and 12 caged chicken egg samples collected in Taiwan. The mean level of PCDD/Fs in the free range egg samples was 5.7 (1.79/0.314) times higher than those in the caged egg samples. Principle component analysis revealed that at least three characteristic patterns of PCDD/F congener were observed among the 18 egg samples. The different PCDD/F congener patterns between free range and caged egg samples may reflect distinctive exposure scenarios among the free range and caged hens. We suggest that the differences of PCDD/F levels and congener patterns between free range and caged egg samples give rise to the issues related to the safety of eating free range chicken eggs. The present data may provide useful information for further investigation of the possible PCDD/F sources in the contaminated free range eggs.

KEYWORDS: PCDD/Fs; egg; free range chicken; caged chicken; congener profile

INTRODUCTION

Chicken eggs are one of the most important foods in the human diet all over the world. Previously, eggs from caged hens have been the major production method. Meanwhile, especially in Europe, the demand for eggs from hens other than caged hens has steadily increased due to awareness of animal welfare issues and the healthier nature and better nutritional qualities of eggs. It has been reported that egg yolks from free range hens contain a greater variety of fatty acids than do egg yolks from caged hens (*1*). The term free range hens refers to chickens that have continuous daytime access to open-air runs comprising an area mainly covered by vegetation of not less than 1 m^2 per chicken during at least half their lifetime (*2*). Because the free range hens spend most of their lifetime in an outside environment, they have a better chance of being exposed to contaminants from the environment. Although the free range farming system has gradually gained popularity, it has the potential to lead to chemical or biological contamination of eggs, e.g., from polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls, pesticides such as DDT, dieldrin, hexachlorobenzene, pentachlorophenol, and hexachlorocyclohexanes (*3*), and biological contaminations such as *Salmonella* (*4*). It has been reported that high PCDD/F levels in soil lead to increased PCDD/F levels in eggs from foraging chickens (*5*). Pussemier et al. also showed that PCDD/F levels in eggs can be influenced by the type of farming system used for the chickens (*6*).

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According to a survey of PCDD/F levels in eggs by European Community (EC), data reported by eight European countries revealed that the PCDD/F levels in eggs collected between 1987 and 1999 ranged from 0.46 to 7.32 pg I-TEQ/g fat (*7*). The PCDD/F levels in eggs collected in European countries revealed a large variation. Many factors may influence the PCDD/F levels in eggs including the following: the chicken farming system, the feed, and the sampling strategy. Unfortunately, the data do not accurately note the farming system used for the hens laying eggs. In some previous reports, data indicated that the average PCDD/F levels are increasing in free range chicken eggs compared to those in caged chicken eggs from Germany, The Netherlands, Belgium, and the United Kingdom (*7*−*10*). Moreover, Schoeters and Hoogenboom have stated that "The major question is what are the sources of the contamination and whether these can be removed" (*11*). A congener profile has been regarded as a signature of PCDD/F mixtures associated with particular media or particular sources of exposure. In principle, these source- and/or media-specific profiles could provide useful information in source identification of these compounds (*12*, *13*). However, studies investigating the PCDD/F congener profile in free range eggs and caged chicken eggs are rarely conducted.

Taiwan is an urbanized and industrialized island with many municipal waste incinerators (MWIs). These MWIs, along with other factories with combustive processes, may release unintentional byproduct PCDD/Fs into the environment, and these PCDD/Fs will eventually concentrate in the human body via environmental transport, food chains, and bioaccumulation (*14*, *15*). In Taiwan, chicken eggs are also an important food in the human diet, and the

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annual consumption of eggs is about 300 eggs per person per year (*16*). Caged hens constitute the major production method of eggs in Taiwan; therefore, most commercialized eggs are produced by caged hens. Free range eggs are produced by hens from privately owned farms, and most of these farms are located in southern Taiwan. Therefore, a survey of the PCDD/F levels in caged and free range chicken eggs of Taiwan was conducted. Furthermore, we intended to find potential sources of PCDD/F contaminations in free range eggs by using multivariance analysis methods for PCDD/F congener profile analysis.

MATERIALS AND METHODS

Sample Collection. Free range eggs were produced by hens from privately owned farms located in southern Taiwan. In the present study, free range egg samples from six different regions in southern Taiwan were collected in 2008, and the egg samples were from free range hens from privately owned farms. Four free range egg samples were collected from farms in Tainan County (F1−F4, see **Figure 1** for locations), one from Chiayi County (F5) and one from Changhua County (F6). Each free range egg sample consisted of 10 individual eggs collected from one farm. Caged hens constitute the major production method of eggs in Taiwan; therefore, caged hens were the source of most of the commercialized eggs. The caged egg samples were purchased from stores in 12 geographic areas in Taiwan, including Taipei County (C1, see **Figure 1** for locations), Taoyuan County (C2), Hsinchu County (C3), Miaoli County (C4), Changhua County (C5), Chiayi County (C6), Tainan County (C7), Kaohsiung County (C8), Pingtung County (C9), Yilan County (C10), Hualien County (C11), and Taitung County (C12). Each caged egg sample consisted of 10 individual eggs purchased from one store. The egg samples were kept at 4 °C until the time of analysis.

Feed and soil samples in Farms F1 and F2 were collected (see **Figure 1** for locations). About 50 g of feed for hens was collected at each farm. For the soil sample, the area containing the free range hens was divided into nine parts, and about 20 g of soil was collected from the surface sediment (< 15 cm) using a hand-held shovel in each part of the farm. The nine soil samples from the nine parts of the farm were mixed together to provide one soil sample for each farm. The soil samples were kept at 4 °C until the time of analysis. All sample containers were tested to verify that they contained no detectable levels of PCDD/F contamination.

PCDD/F Analysis. The PCDD/F levels in eggs, feed, and soil samples were measured by Analytical Laboratory for Trace Environmental Pollutant at National Cheng Kung University (ALTEP, NCKU) in Taiwan. This Laboratory has been certified by Taiwan Accreditation Foundational, a member of International Laboratory Accreditation Cooperation Mutual Recognition Arrangement to analyze PCDD/Fs in serum, food, feed, soil, and air samples. The isotope dilution high resolution gas chromatography−high resolution mass spectrometry (HRGC-HRMS) method was used to quantitatively determine the concentrations of the 17 PCDD/Fs. The analytical procedures for all samples were adopted from USEPA Method 1613B (*17*).

Whole egg samples were used in PCDD/F analyses. Each 30 g of egg sample was homogenized in 50 mL of ethanol and 100 mL of acetone/hexane (1/1, v/v). An internal standard mixture containing 15 $^{13}C_{12}$-labeled PCDD/F standards was added to the egg homogenate. The homogenized sample was extracted with hexane, and the lipid content was determined gravimetrically. After extraction, the sample was treated with concentrated sulfuric acid, and three solid-phase extraction cleanup procedures (acid silica, acid alumina, and Florisil cartridges) were carried out. After the cleanup procedures, the sample was used for the analysis of 17 PCDD/Fs using HRGC-HRMS. Each analytical run consisted of a method blank, a quality control, and 8 unknown samples. An Agilent 6890N GC (Agilent Technologies Inc., Santa Clara, CA) and a Micromass AutoSpec Ultima EBE trisector mass spectrometer (Fisons Instruments, Manchester, UK) were used for the HRGC-HRMS analysis. The details of the chromatographic procedures of HRGC-HRMS used for the determination of PCDD/F levels are described elsewhere (*15*).

About 30 g of feed samples were Soxhlet-extracted for 24 h using acetone/hexane (1/1, v/v). The extract sample was spiked with a mixture containing 15 $^{13}C_{12}$-labeled PCDD/F standards. After extraction, the sample was cleaned up by three solid-phase extraction cleanup procedures, followed by the analysis of 17 PCDD/F using HRGC-HRMS. The sample cleanup procedures and instrumental analytical methods for the feed samples were the same as those for the egg samples.

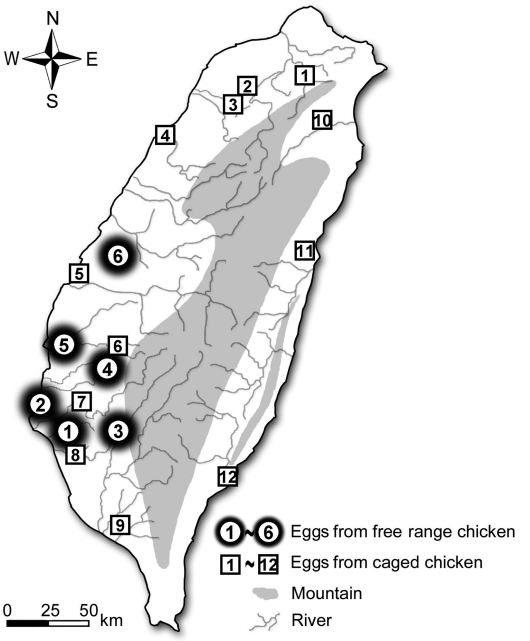

Figure 1. Geographical locations of the areas where egg samples were collected in Taiwan. Circles indicate eggs collected from free range chickens (F1−F6), and squares indicate eggs collected from caged chickens (C1−C12).

For the soil sample, about 10 g of the freeze-dried soil samples were Soxhlet-extracted for 24 h using toluene. The extract sample was spiked with a mixture containing 15 $^{13}C_{12}$-labeled PCDD/F standards. After extraction, the sample was cleaned up by three solid-phase extraction cleanup procedures, followed by the analysis of 17 PCDD/F using HRGC-HRMS. The sample cleanup procedures and instrumental analytical methods for the soil samples were the same as those for the egg samples.

Statistical Analysis. The Shapiro−Wilk normality test was used to determine whether or not a random sample of the levels of PCDD/Fs examined in this study followed a normal distribution. Principal component analysis (PCA) was used to explore and classify the PCDD/F congener profile data (*18*, *19*). The data were organized into a matrix having n subjects and p variables (17 PCDD/Fs) and were normalized to the total concentration of PCDD/Fs by expressing each congener value as a percentage of the sum of the total PCDD/Fs. Seventeen PCDD/F congeners in all egg samples were used in the PCA model, and all values under the detection limit were treated as half of the limit (*20*). The statistical analysis was performed using the Statistica (version 6.0, StatSoft Inc., Tulsa, OK) software system.

RESULTS

PCDD/F Levels in Free Range and Caged Egg Samples. The six free range egg samples were collected from six farms located in different geographic areas in southern Taiwan (**Figure 1**). The 17 PCDD/F levels in these free range egg samples were measured and summarized in **Table 1**. The Shapiro−Wilk test revealed that the PCDD/F levels in the six free range egg samples were statistically described by normal distribution (p = 0.03). The levels of all 17 PCDD/Fs in the 6 free range egg samples ranged from 6.18 to 41.3 pg/g lipid with a mean value of 17.5 ± 14.9 pg/g lipid (mean ± SD). The World Health Organization toxic equivalency factors (WHO-TEFs) system (*21*) was used to calculate toxic equivalency quotient (TEQ) values. The TEQ of the 17 PCDD/Fs ranged from 0.538 to 5.16 pg WHO-TEQ/g lipid with a mean value of 1.79 ± 1.80 pg WHO-TEQ/g lipid.

Table 1. PCDD/F Levels in Egg Samples from Free Range and Caged Hens

	concentration in egg samples (pg/g lipid)							
	free range hens (N = 6)							caged hens (N = 12)
analyst	F1	F2	F3	F4	F5	F6	mean[c] ± SD	mean[c] ± SD
lipid in egg samples	10.5%	11.6%	11.4%	10.0%	11.9%	9.22%	10.8% ± 1.04%	9.16% ± 0.81%
2,3,7,8-TCDF	2.83	1.05	2.63	1.91	1.27	1.07	1.79 ± 0.792	0.266 ± 0.196
1,2,3,7,8-PeCDF	3.30	0.914	0.672	0.631	1.16	0.414	1.18 ± 1.07	0.161 ± 0.060
2,3,4,7,8-PeCDF	4.00	0.993	0.288	0.285	1.39	0.418	1.23 ± 1.43	0.192 ± 0.055
1,2,3,4,7,8-HxCDF	2.75	0.608	0.204	0.249	0.775	0.277	0.810 ± 0.976	0.138 ± 0.042
1,2,3,6,7,8-HxCDF	2.51	0.575	0.096	0.117	0.664	0.238	0.700 ± 0.919	0.110 ± 0.037
2,3,4,6,7,8-HxCDF	2.12	0.468	0.079	0.108	0.669	0.216	0.610 ± 0.774	0.092±0.024
1,2,3,7,8,9-HxCDF	0.191	0.033	0.026	0.024	0.032	0.043	0.058 ± 0.065	0.015[e] ± 0.008
1,2,3,4,6,7,8-HpCDF	2.36	0.517	0.102	0.123	0.608	0.220	0.655 ± 0.860	0.240 ± 0.274
1,2,3,4,7,8,9-HpCDF	0.319	0.058	0.023	0.012[d]	0.079	0.040	0.088 ± 0.115	0.027[f] ± 0.018
1,2,3,4,6,7,8,9-OCDF	1.31	0.352	0.070	0.105	0.479	0.140	0.409 ± 0.469	0.612[g] ± 1.65
2,3,7,8-TCDD	0.288	0.124	0.047	0.027	0.153	0.047	0.114 ± 0.099	0.048 ± 0.018
1,2,3,7,8-PeCDD	1.25	0.429	0.055	0.072	0.891	0.101	0.467 ± 0.502	0.071 ± 0.021
1,2,3,4,7,8-HxCDD	0.710	0.184	0.044	0.048	0.383	0.083	0.242 ± 0.263	0.051[g] ± 0.014
1,2,3,6,7,8-HxCDD	1.91	0.542	0.081	0.111	1.18	0.133	0.660 ± 0.744	0.110 ± 0.029
1,2,3,7,8,9-HxCDD	0.814	0.230	0.045	0.044	0.414	0.074	0.270 ± 0.303	0.057 ± 0.014
1,2,3,4,6,7,8-HpCDD	3.74	1.04	0.294	0.252	3.80	0.475	1.60 ± 1.71	0.464 ± 0.112
1,2,3,4,6,7,8,9-OCDD	10.9	4.00	2.22	2.07	16.9	3.91	6.66 ± 5.96	4.99 ± 3.08
10 PCDFs[a]	3.23	0.822	0.482	0.416	1.10	0.416	1.08 ± 1.09	0.169 ± 0.047
7 PCDDs[a]	1.92	0.660	0.122	0.122	1.28	0.182	0.715 ± 0.745	0.145 ± 0.038
17 PCDD/Fs[a]	5.16	1.48	0.604	0.538	2.38	0.598	1.79 ± 1.80	0.314 ± 0.073
10 PCDFs[b]	2.37	0.605	0.411	0.346	0.800	0.325	0.809 ± 0.784	0.127 ± 0.036
7 PCDDs[b]	1.93	0.660	0.122	0.123	1.29	0.182	0.716 ± 0.746	0.146 ± 0.039
17 PCDD/Fs[b]	4.29	1.27	0.533	0.469	2.09	0.507	1.53 ± 1.50	0.274 ± 0.063

[a] Unit: pg WHO_{1998}-TEQ/g lipid (*33*). [b] Unit: pg WHO_{2005}-TEQ/g lipid (*34*). [c] The mean PCDD/F levels of the 6 free range and 12 caged egg samples were reported. [d] The value under the detection limit was treated as half of this limit. [e] The values of five caged egg samples were under the detection limit and treated as half of the limit and include C5, C6, C9, C10, and C12. [f] The values of three caged egg samples were under the detection limit and treated as half of the limit and include C2, C6, and C9. [g] The value of caged egg sample from Farm C12 was under the detection limit and treated as half of the limit.

In Taiwan, the eggs of commercial products were from caged hens. Twelve caged egg samples were purchased from stores in 12 geographic areas of Taiwan (**Figure 1**). The 17 PCDD/F levels in these caged egg samples were measured. The Shapiro−Wilk test revealed that the PCDD/F levels in the 12 caged egg samples were statistically described by normal distribution (p = 0.69). Levels of all 17 PCDD/Fs in the 12 caged egg samples ranged from 2.85 to 19.8 pg/g lipid, with a mean value of 7.65 ± 4.71 pg/g lipid (mean ± SD). The TEQ of the 17 PCDD/Fs ranged from 0.197 to 0.430 pg WHO-TEQ/g lipid with a mean value of 0.314 ± 0.073 pg WHO-TEQ/g lipid. Concentrations of these 17 PCDD/F congeners in the caged egg samples were summarized in **Table 1** and plotted in **Figure 2**.

PCDD/F Levels in Feed and Soil. Feed and soil samples from two farms (F1 and F2 in **Figure 1**), with egg samples containing higher PCDD/F levels, were collected. The PCDD/F levels in the feed samples from Farm F1 were 0.017 pg WHO-TEQ/g sample with 12% water content and for Farm F2 were 0.020 pg WHO-TEQ/g sample with 12% water content. The PCDD/F levels in the two soil samples were 0.727 and 0.566 pg WHO-TEQ/g sample (dry weight). Concentrations of these 17 PCDD/F congeners in the feed and soil samples are summarized in **Table 2**.

Principal Component Analysis for PCDD/F Congener Profiles in Eggs. Principal component analysis (PCA) was performed to investigate the congener profiles of PCDD/F levels in the free range and caged egg samples. PCA is a multivariate method that can be used to reduce several variables to a few underlying descriptive dimensions that can be used to explain patterns within a set of observed values. It operates with no a priori assumptions about the data structure and readily illuminates the major underlying trends of the data set by grouping variables that covary and samples that have similar compositions. PCA decomposes a data set into a series of matrices or principal components (PCs). PCs are linear combinations of the original variables and are ordered such that the first PC accounts for the greatest fraction of the variance, and the last PC accounts for the least. Each PC is the outer product of a scores vector for the samples and a loadings vector for the variables (*19*).

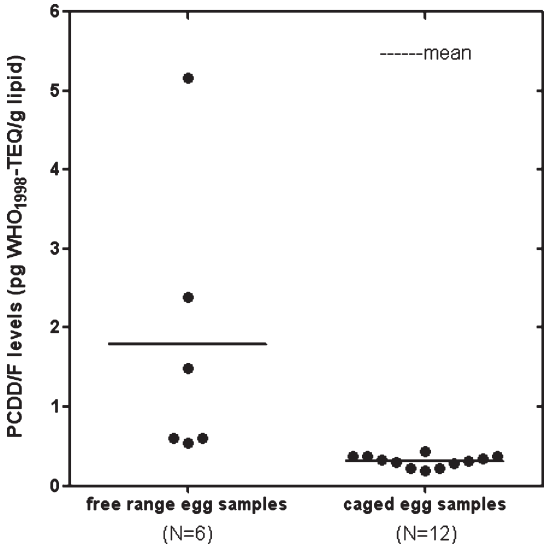

Figure 2. Distribution of 17 PCDD/F levels in the 6 free range and 12 caged egg samples were plotted. The black circles indicate the PCDD/F levels in egg samples. The lines indicate the mean values for all free range egg samples and caged egg samples.

The relative concentrations of 17 PCDD/F congeners (**Table 1**) in 6 free range egg samples and 12 caged egg samples were used as

Table 2. PCDD/F Levels in Soil and Feed Samples

	soil (pg/g dry sample)		feed (pg/g sample 12% w.c.)	
analyst	F1	F2	F1	F2
2,3,7,8-TCDF	0.283	0.315	0.014	0.016
1,2,3,7,8-PeCDF	0.252	0.398	0.007	0.014
2,3,4,7,8-PeCDF	0.310	0.502	0.011	0.013
1,2,3,4,7,8-HxCDF	0.277	0.644	0.010	0.011
1,2,3,6,7,8-HxCDF	0.201	0.521	0.006	0.011
2,3,4,6,7,8-HxCDF	0.257	0.494	0.006	0.008
1,2,3,7,8,9-HxCDF	0.097	0.207	0.002	0.003
1,2,3,4,6,7,8-HpCDF	0.851	2.120	0.021	0.019
1,2,3,4,7,8,9-HpCDF	0.070	0.306	0.004	0.005
1,2,3,4,6,7,8,9-OCDF	1.43	2.56	0.067	0.022
2,3,7,8-TCDD	0.047	0.027	0.001[c]	0.001[c]
1,2,3,7,8-PeCDD	0.169	0.111	0.003	0.005
1,2,3,4,7,8-HxCDD	0.086	0.104	0.006	0.003
1,2,3,6,7,8-HxCDD	0.167	0.195	0.007	0.006
1,2,3,7,8,9-HxCDD	0.181	0.173	0.004	0.003
1,2,3,4,6,7,8-HpCDD	1.59	2.59	0.092	0.033
1,2,3,4,6,7,8,9-OCDD	14.8	22.3	1.20	0.222
10 PCDFs[a]	0.514	0.289	0.010	0.012
7 PCDDs[a]	0.213	0.277	0.007	0.008
17 PCDD/Fs[a]	0.727	0.566	0.017	0.020
10 PCDFs[b]	0.406	0.222	0.007	0.010
7 PCDDs[b]	0.218	0.280	0.007	0.007
17 PCDD/Fs[b]	0.624	0.502	0.014	0.017

[a] Unit: pg WHO_{1998}-TEQ/g lipid (*33*). [b] Unit: pg WHO_{2005}-TEQ/g lipid (*34*). [c] The value under the detection limit was treated as half of this limit.

variables in the PCA. The loading and score plots for the PCDD/F congener profile are shown in **Figure 3**. As indicated in the loading plot (**Figure 3A**), the first factor, which accounted for 54% of the variability of the data set, was mainly negatively influenced by the 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,-6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD, and positively influenced by the 1,2,3,4,6,7,8,9-OCDD (loading factor of Factor 1 > 0.7). The second factor accounted for 16% of the variability and was mainly positively influenced by the 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF (loading factor for Factor 2 > 0.7). In addition, the score plot (**Figure 3B**) in which the first factor is plotted against the second showed that the 18 egg samples could be separated into at least 3 groups. It revealed that at least three characteristic patterns of PCDD/F congener profiles were observed among these egg samples (**Figure 4**). Group I is the free range egg samples collected from Farm F3 and F4 (see **Figure 1** for locations), Group II is from Farms F1 and F2, and Group III is from Farms F5 and F6, and all caged egg samples except egg sample C1. According to the PCDD/F congener patterns of these three groups, the 2,3,7,8-TCDF and 1,2,3,4,6,7,8,9-OCDD congeners were the major congeners for the egg samples in Groups I and II but only 1,2,3,4,6,7,8,9-OCDD for the egg samples in Group III. In addition, the mean contributions of 10 PCDDs to all 17 PCDD/Fs in the 3 groups were different: 41% for Group I, 51% for Group II, and 76% for Group III.

DISCUSSION

PCDD/F Levels in Free Range and Caged Eggs. The PCDD/F levels in free range and caged egg samples are presented in **Table 1**. All of the free range egg samples have higher PCDD/F levels than the caged egg samples. The mean level of PCDD/Fs in the 6 free range egg samples is 5.7 (1.79/0.314) times higher than those in the 12 caged egg samples. In addition, 17% (1/6) of the egg samples from free range hens exceed the maximum guideline value for hen eggs and egg products, 3 pg WHO-TEQ/g fat, set by European Community (EC) Regulation, and 33% (2/6) exceed the action

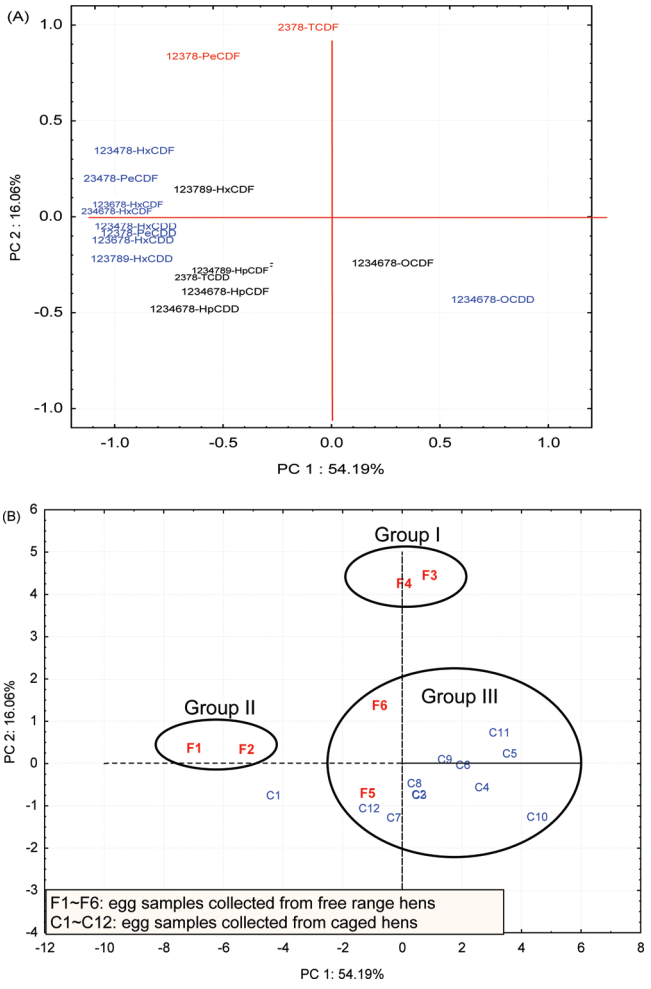

Figure 3. Principal component analysis (PCA) of 17 PCDD/F congener profiles of 18 egg samples. (**A**) Loading plot of PCA for the 17 PCDD/F congeners. The first loading factor was plotted against the second. (**B**) Score plot of Factor 1 against Factor 2 for the 18 egg samples analyzed (F1−F6 and C1−C12 in **Figure 1**).

guideline value, 2 pg WHO-TEQ/g fat (*22*). As for the PCDD/F levels in the caged egg samples, all were far below these two guideline values. According to the recommendation from EC, any food exceeding the maximum guideline value would be considered unsuitable for consumption, and the action guideline value is an early warning value for higher than desirable PCDD/F levels in food, which would trigger investigations to identify and reduce/eliminate the source of contamination. This preliminary study on PCDD/Fs in eggs gives rise to safety issues with the consumption of free range eggs and the sources of PCDD/F contamination in free range eggs in Taiwan. However, it is difficult to evaluate whether the PCDD/F levels in eggs are safe for the human daily diet due to the lack of a guidance value of PCDD/F levels in food that is safe for humans. Since 2001, the EC unveiled a plan to set the target levels of PCDD/Fs in food that would set the ultimate goal of achieving a human exposure below the tolerable weekly intake of 14 pg PCDD/Fs (*22*). In the future, evaluation of the level of PCDD/Fs in food that is safe for human consumption is important.

Numerous reports have indicated that hens may be the source of PCDD/Fs, as hens may transfer PCDD/Fs to their eggs (*5*, *23*, *24*). PCDD/Fs are fat-soluble compounds. Conceivably, PCDD/Fs may enter the eggs following oral intake by the hen and then accumulation in egg fat. The increased PCDD/F levels in free range eggs could be attributed to the characteristics

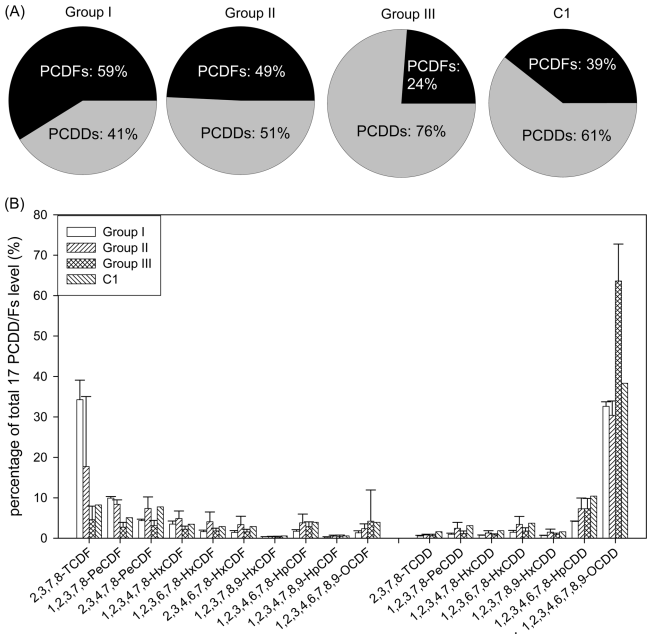

Figure 4. PCDD/F profiles of egg samples. (**A**) Percentage of contribution from 7 PCDDs and 10 PCDFs in the four groups of egg samples. (**B**) Percentage of contribution for 17 PCDD/Fs in the four groups of egg samples.

of the free range farming system, which could have more PCDD/Fs than other systems. The details about how the characteristics of the free range farming system introduce PCDD/F contamination into free range eggs are addressed in the Possible Sources of PCDD/Fs in Free Range Chicken Eggs section.

A survey on PCDD/F levels in eggs from eight European Union (EU) countries revealed that PCDD/F levels were higher in free range eggs (median: 0.85 pg I-TEQ/g fat) than in caged eggs (median: 0.38 pg I-TEQ/g fat). About 10% of free range eggs did not comply with the current limit of the guideline value for hen eggs and egg products (3 pg WHO-TEQ/g fat) set by EC Regulation (*22*), but eggs from caged hens were always far below this limit (*7*). As shown in **Table 1**, we observed a similar phenomenon in our study in that 17% (1/6) of free range egg samples exceeded the EC Regulation of PCDD/F levels (*22*), but all caged egg samples were far below the regulation. In Belgium, the average level of PCDD/F in eggs from privately owned farms of free range hens is 9.9 pg WHO-TEQ/g lipid ($N = 15$) (*6*). Malisch reported that the mean PCDD/F level in eggs from foraging hens raised in fields (4.39 pg I-TEQ/g fat, range 0.49−22.8) was higher than that from hens housed in elevated wire cages (1.28 pg I-TEQ/g fat) or kept on the ground (1.51 pg I-TEQ/g fat) in Germany (*25*). In some European Union (EU) countries, it has been reported that the free range eggs have a higher risk of being contaminated with dioxins (*6*, *7*, *25*). This may be the first report describing data indicating that there are higher levels of PCDD/Fs in free range eggs than in caged eggs in an Asian country. This data may indicate that the issue of contamination in free range eggs could be a global issue, and more research should be done to identify the factors from the external environmental that influence and modify the PCDD/F levels in eggs from free range hens.

Different PCDD/F Congener Profiles in Free Range and Caged Eggs. PCA is a useful tool for identifying classes of similar objects and for studying the systematic variation present in a data matrix (*12*, *13*, *19*). PCA gives an overview of the dominating profiles and major trends in the data by using a projection method, which combines the included variables into a few underlying descriptive dimensions. Two complementary plots, a loading plot and a score plot, can be derived from the PCA. The loading plot shows the extent to which each variable contributes to the sample separation. The score plot indicates the relationship between samples.

As shown in the score plot (**Figure 3B**), in which the first factor is plotted against the second, 18 egg samples were separated into three dissimilar groups. The characteristic patterns of PCDD/F congener profiles for the three groups were plotted in **Figure 4**. Each group exhibited a distinct PCDD/F congener pattern and a mean contribution of 10 PCDDs to all 17 PCDD/Fs. Four free range egg samples (F1, F2, F3, and F4 in **Figure 1**) were grouped into Groups I or II, and the other two free range egg samples (F5 and F6) and all caged egg samples, except egg sample C1, were grouped into Group III. For Groups I and II, both 2,3,7,8-TCDF and 1,2,3,4,6,7,8,9-OCDD congeners were major congeners. For Group III, only 1,2,3,4,6,7,8,9-OCDD congener was the major congener. In addition, the mean contributions of 10 PCDDs to all 17 PCDD/Fs decreased following the order of Groups I, II, and III. These results demonstrated that the PCDD/F congener profiles of free range egg samples collected from Farms F1−F4 were different from those for all caged egg samples. Meanwhile, a congener profile has been regarded as a signature of PCDD/F mixtures associated with particular media or sources of the exposure. The difference of PCDD/F congener profiles in free range and caged egg samples may imply the different sources of the exposure for free range versus caged hens. In addition, this difference of congener pattern may provide useful information to identify the sources that elicit higher PCDD/F levels in free range eggs.

Location Related PCDD/F Congener Profiles in Free Range Eggs. After pointing out the difference between PCDD/F congener profiles in free range and caged egg samples, we considered whether the PCDD/F congener profiles of free range egg samples collected from the different farms were different. As illustrated in **Figures 3B** and **4**, the free range egg samples F1−F4 can be separated into two groups (Groups I and II) with distinct PCDD/F congener patterns. For Group I, the egg samples (F3 and F4) showed a trend of high percentages of PCDFs compared to all 17 PCDD/Fs. For Group II, the egg samples (F1 and F2) showed a trend of low percentages of PCDFs compared to all seventeen PCDD/Fs when compared to those in Group I. This data demonstrated that the PCDD/F congener profiles of free range egg samples collected from different farms were different.

As shown in **Figure 1**, the egg samples of Group I (F3 and F4) were collected from farms close to the mountain area, but the egg samples of Group II (F1 and F2) were collected from farms close to the seaboard area. In Taiwan, the broad coastal plain in the west supports most of the island's population and is the chief industrial zone. Therefore, the free range egg samples collected from farms close to the seaboard area may be influenced by the PCDD/F emitted from incinerators and factories with combustive processes. In the mountain area, there are more farm-related activities, and the uncontrolled burning of grass could be the potential emission source of PCDD/Fs. It has been reported that the PCDD/F congener profiles of air emitted from incinerators and uncontrolled burning of grass are distinct (*26*). So far, we have seen that the PCDD/F congener profiles in free range egg samples can be influenced by the location of farms. But the passage and entry of PCDD/Fs to the free range chickens from incinerators and the uncontrolled burning of grass deserves further investigation.

Possible Sources of PCDD/Fs in Free Range Chicken Eggs. Previous studies reported that eggs from free range hens have a higher risk of being contaminated with PCDD/Fs (*6*, *8*, *10*). Meanwhile, numerous possible sources of PCDD/Fs, leading to

their transfer from the hen to its eggs, have been reported and include feedstuffs, soil, plants, worms, and insects, etc. (*27*, *28*). According to the discussion on PCDD/F congener profiles in free range egg samples, we found that the PCDD/F congener profiles for the egg samples from Farms F1 and F2 were similar but that the PCDD/F levels in these two free range egg samples were different. Therefore, the feed and soil samples from Farms F1 and F2 were collected, and the PCDD/F levels were measured to evaluate whether the elevated PCDD/F levels in the free range egg samples related to that in feed or soil samples.

For the two feed samples, the PCDD/F levels were similar (as shown in **Table 2**, F1, 0.017; and F2, 0.020 pg WHO-TEQ/g sample 12% w.c.) and far below the maximum and action guideline value for feed, 0.75 and 0.5 pg WHO-TEQ/g sample 12% w.c., set by the EC Regulation (*29*). The feed PCDD/F levels below the EC limit in general does not yield the PCDD/F levels in eggs above the EC limit of 3 pg WHO-TEQ/g fat. However, the PCDD/F level in the free range egg sample collected from Farm F1 was above the EC limit of 3 pg WHO-TEQ/g fat. In addition, the PCDD/F levels in the two feed samples collected from Farms F1 and F2 were similar, but the free range egg samples collected from these farms were quite different: the concentration in the F1 egg sample was 3.49 times (5.16/1.48) higher than that in the F2 egg sample. From this data, it follows that the PCDD/F in feed samples may not be the major source of PCDD/F contamination in the free range egg samples.

Previous studies have been reported indicating that the higher PCDD/F levels in free range eggs may be ascribed to the running behavior of free range chickens (*28*). The free range chicken has a greater chance to run in the outside environment and ingest the soil containing PCDD/Fs compared to the caged chickens. The bioavailability of PCDD/Fs from soil has been corroborated by experimental studies involving chickens that received PCDD/F contaminated soil mixed in their diet for six months (*30*). For the two soil samples, the PCDD/F levels were also similar (as shown in **Table 2**, F1, 0.727; and F2, 0.566 pg WHO-TEQ/g dry sample). The PCDD/F levels in the soil were similar to the soil collected in unknown dioxin contaminated areas in Taiwan (*13*). This result was the same as that for feed samples. We do not have evidence to demonstrate that the PCDD/F in the soil samples was the major source of PCDD/F contamination in the free range eggs.

Ingestion of soil could be partly responsible for the observed elevation of PCDD/F levels in free range eggs compared to those in caged eggs. The PCDD/F in free range eggs transferred from the ingestion of soils was calculated on the basis of the PCDD/F levels in the two soil samples (soil samples collected from Farms F1 and F2). The mean PCDD/F level in the two soil samples was 0.65 pg WHO-TEQ/g dry sample (as shown in **Table 2**), and assuming an average daily intake of soil of 10 g/day by free range chickens (average daily feed intake 100 g/day) (*30*), a 40 to 60% absorption of the PCDD/Fs present in the soil based on a physiologically based pharmacokinetic model (*31*), and a 30% transfer into the egg (*11*) would result in a contamination level of 0.13−0.20 pg WHO-TEQ/g lipid in the egg (6 g of lipid/egg). According to the data in **Table 1**, the PCDD/F levels in free range eggs were 0.224 to 4.85 pg WHO-TEQ/g lipid higher compared to the mean PCDD/F level in caged chicken eggs. From this data, it follows that ingestion of soil may be partly responsible for the observed elevation in PCDD/F levels in free range eggs compared to those in caged eggs, but the major sources of PCDD/F contamination in the free range eggs require further investigation.

PCDD/Fs are fat-soluble compounds, and the lipid content in humans having an influence on the PCDD/F levels in humans has been reported (*32*). We suspect that the lipid content in chicken may have a influence on the PCDD/F levels in eggs. The term free range chickens refers to chickens that have continuous daytime access to open-air runs and have more exercise. Therefore, free range chickens may also have slightly less lipids and may have slightly different composition of lipids. The lower lipid levels of chickens may result in less PCDD/Fs stored in the lipid of chickens and more PCDD/Fs transferred into eggs. More PCDD/Fs transfer from free range hens into eggs resulting higher PCDD/F levels in free range eggs compared to those in caged eggs. However, the free range chickens with different lipid composition may have different absorption and/or metabolism of PCDD/Fs in chickens and may result invaried PCDD/F levels and congener profiles in free range eggs.

ABBREVIATIONS USED

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

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LITERATURE CITED

(1) Jones, D. Point of distinction between battery and free range eggs. *Nature* **1968**, *220* (5170), 921.

(2) Commission of the European Communities. Overview of a series of missions carried out in 2004 concerning animal welfare standards on holdings with laying hens. In 2004, Council Directive 1999/74/EC, Commission Regulation 2295/2003/EC, as amended by Commission Regulation 1515/2004/EC.

(3) Huwe, J. K. Dioxins in food: A modern agricultural perspective. *J. Agric. Food Chem.* **2002**, *50* (7), 1739–1750.

(4) Kinde, H.; Read, D. H.; Chin, R. P.; Bickford, A. A.; Walker, R. L.; Ardans, A.; Breitmeyer, R. E.; Willoughby, D.; Little, H. E.; Kerr, D.; Gardner, I. A. *Salmonella enteritidis*, phage type 4 infection in a commercial layer flock in Southern California: Bacteriologic and epidemiologic findings. *Avian Dis.* **1996**, *40* (3), 665–671.

(5) Schuler, F.; Schmid, P.; Schlatter, C. The transfer of polychlorinated dibenzo-p-dioxins and dibenzofurans from soil into eggs of foraging chicken. *Chemosphere* **1997**, *34* (4), 711–718.

(6) Pussemier, L.; Mohimont, L.; Huyghebaert, A.; Goeyens, L. Enhanced levels of dioxins in eggs from free range hens; a fast evaluation approach. *Talanta* **2004**, *63* (5), 1273–1276.

(7) Commission of the European Communities. *Assessment of dietary intake of dioxins and related PCBs by the population of EU Member States*; Directorate-General Health and Consumer Protection: Brussels, Belgium, 2000.

(8) Malisch, R.; Baum, F. PCDD/Fs, dioxin-like PCBs and marker PCBs in eggs of peregrine falcons from Germany. *Chemosphere* **2007**, *67* (9), S1–S15.

(9) Van Overmeire, I.; Waegeneers, N.; Sioen, I.; Bilau, M.; De Henauw, S.; Goeyens, L.; Pussemier, L.; Eppe, G. PCDD/Fs and dioxin-like PCBs in home-produced eggs from Belgium: Levels, contamination sources and health risks. *Sci. Total Environ.* **2009**, *407* (15), 4419–4429.

(10) Little, C.; Walsh, S.; Hucklesby, L.; Surman-Lee, S.; Pathak, K.; Hall, Y.; Pinna, E. D.; Threlfall, E.; Maund, A.; Chan, C.-H. *Survey of Salmonella Contamination of Non-UK Produced Shell Eggs on Retail Sale in the North West of England and London*; Food Standards Agency: London, 2006.

(11) Schoeters, G.; Hoogenboom, R. Contamination of free range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls. *Mol. Nutr. Food Res.* **2006**, *50* (10), 908–914.

(12) 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* (9), 1231–1243.
(13) Lee, C. C.; Guo, Y. L.; Kuei, C. H.; Chang, H. Y.; Hsu, J. F.; Wang, S. T.; Liao, P. C. Human PCDD/PCDF levels near a pentachlorophenol contamination site in Tainan, Taiwan. *Chemosphere* **2006**, *65* (3), 436–448.
(14) Hsu, J. F.; Guo, Y. L.; Liu, C. H.; Hu, S. C.; Wang, J. N.; Liao, P. C. A comparison of PCDD/PCDFs exposure in infants via formula milk or breast milk feeding. *Chemosphere* **2007**, *66* (2), 311–319.
(15) 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* (1), 33–42.
(16) Tzeng, M. S.; Kso, M. D.; Teh, W. T.; Pan, W. H. Food consumption frequency and eating habit among Taiwanese-NAHSIT 1993−1996. *Nutr. Sci. J.* **1999**, *24* (1), 59–80.
(17) 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, DC, October 1994.
(18) 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* (9), 1231–1243.
(19) Wold, S.; Esbensen, K.; Geladi, P. Principal component analysis. *Chemometr. Intell. Lab.* **1987**, *2*, 37–52.
(20) Haas, C. N.; Scheff, P. A. Estimation of averages in truncated samples. *Environ. Sci. Technol.* **1990**, *24* (6), 912–919.
(21) 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* (12), 775–792.
(22) European Communities. *Setting Maximum Levels for Certain Contaminants in Foodstuffs Amending Commission Regulation* (*EC Regulation No. 2375/2001*); European Communities: Brussels, Belgium, 2001.
(23) Chang, R.; Hayward, D.; Goldman, L.; Harnly, M.; Flattery, J.; Stephens, R. Foraging farm-animals as biomonitors for dioxin contamination. *Chemosphere* **1989**, *19* (1−6), 481–486.
(24) Waegeneers, N.; De Steur, H.; De Temmerman, L.; Van Steenwinkel S.; Gellynck, X.; Viaene, J. Transfer of soil contaminants to home-produced eggs and preventive measures to reduce contamination. *Sci. Total Environ.* **2009**, *407* (15), 4438–4446.
(25) Malisch, R. Update of PCDD/PCDF-intake from food in Germany. *Chemosphere* **1998**, *37* (9−12), 1687–1698.
(26) Cleverly, D.; Ferrario, J.; Byrne, C.; Riggs, K.; Joseph, D.; Hartford, P. A general indication of the contemporary background levels of PCDDs, PCDFs, and coplanar PCBs in the ambient air over rural and remote areas of the United States. *Environ. Sci. Technol.* **2007**, *41* (5), 1537–1544.
(27) Hoogenboom, L. A. P.; Kan, C. A.; Zeilmaker, M. J.; Van Eijkeren, J.; Traag, W. A. Carry-over of dioxins and PCBs from feed and soil to eggs at low contamination levels: Influence of mycotoxin binders on the carry-over from feed to eggs. *Food Addit. Contam.* **2006**, *23* (5), 518–527.
(28) Kijlstra, A.; Traag, W. A.; Hoogenboom, L. A. P. Effect of flock size on dioxin levels in eggs from chickens kept outside. *Poult. Sci.* **2007**, *86* (9), 2042–2048.
(29) 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, Belgium, 2006.
(30) 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* (3), 253–273.
(31) 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* (5), 509–517.
(32) Flesch-Janys, D.; Becher, H.; Gurn, P.; Jung, D.; Konietzko, J.; Manz, A.; Papke, O. Elimination of polychlorinated dibenzo-p-dioxins and dibenzofurans in occupationally exposed persons. *J. Toxicol. Environ. Health* **1996**, *47* (4), 363–378.
(33) 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* (12), 775–792.
(34) 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* (2), 223–241.

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