Dietary exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans via fish consumption and dioxin-like activity in fish determined by H4IIE-luc bioassay

Janet Kit Yan Chan a,b, Yu Bon Man a, Guan Hua Xing a,c, Sheng Chun Wu a,d, Margaret B. Murphy e, Ying Xu b, Ming H. Wong a,⁎

⁎ Corresponding author at: Croucher Institute for Environmental Sciences, and Department of Biology, Hong Kong Baptist University, Room SCT701-712, Cha Chi-ming Science Tower, Ho Sin Hang Campus, 224 Waterloo Road, Kowloon Tong, Hong Kong, PR China.
E-mail address: mhwong@hkbu.edu.hk (M.H. Wong).

A B S T R A C T

Dietary exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) via fish consumption in two major electronic (e) waste sites: Guiyu (GY), Guangdong Province and Taizhou (TZ), Zhejiang Province, and dioxin-like activity in fish determined by H4IIE-luc bioassay. In the present study, all fish were below EU's maximum allowable concentration in muscle of fish (4 pg WHO-TEQ/g wet wt), except crucian carp (4.28 pg WHO-TEQ/g wet wt) and silver carps (7.49 pg WHO-TEQ/g wet wt) collected from GY rivers. Moreover, the residual concentration in bighead carp collected from GY (2.15 pg WHO-TEQ/g wet wt) was close to the EU's action level (3 pg WHO-TEQ/g wet wt) which gives "early warning" to the competent authorities and operators to take measures to eliminate contamination. In addition, results indicated that the maximum human intake of PCDD/Fs via freshwater fish consumption in GY was 4.31 pg WHO-TEQ/kg bw/day, which exceeds the higher end of the tolerable daily intake recommended by the WHO, EC-SCF and JECFA (1–4, 2 and 2.3 pg WHO-TEQ/kg bw/day respectively). Furthermore, H4IIE-luc cell bioassay provides a very sensitive and cost-efficient screening tool for assessing the overall dioxin-like toxicity in the study, and is therefore valuable for high-throughput environmental monitoring studies.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are environmental pollutants, which are by-products unintentionally produced from combustion and industrial chemical processes (US EPA, 2004). These groups of compounds (dioxins or PCDD/ Fs) are of a concern because of their highly toxic potential. PCDD/Fs are amongst the chemicals regulated under the Stockholm Convention known as the "dirty dozen". Dioxins are found throughout the world in practically all forms of media. Of all the emission sources of dioxins, waste incinerators (solid and hospital wastes) often release the greatest amounts of dioxins due to incomplete burning (WHO, 2007). PCDD/Fs are typically released as airborne pollutants and often contaminate air, soil, sediments and food (US EPA, 2004). Due to the persistent and lipophilic nature of PCDD/Fs, the higher the trophic level of the animal food chain, the higher the concentration of dioxins found (Nadal et al, 2004). Fish, shellfish, dairy products and meat often contain high concentrations of dioxins whereas very low levels are found in plants, water and air (WHO, 2007).

Humans are exposed to dioxins from contaminated substances via ingestion, inhalation and dermal contact. It has been estimated that more than 90% of total human exposure to dioxins is from food consumption.
(Bocio et al., 2007), particularly food of animal origin (Bilau et al., 2008). Other secondary routes of exposure, such as inhalation of air, drinking water and dermal contact are of a minor concern (Soechitram et al., 2003), as the contribution of air/soil pollution to total dioxin body burden was small even for individuals living in dioxin-polluted areas (Arisawa et al., 2005).

Dioxins have been widely detected in fish in China (Hsu et al., 2007; Zhang et al., 2008). Open burning of electronic waste (e-waste), such as cable wires and circuit boards, for the recovery of metals is a common practice of e-waste processing operations, and is one of the largest non-regulated sources of PCDD/Fs in China (Zheng et al., 2008). Extreme-ly high concentrations of PCDD/Fs have been detected in the environment of Chinese e-waste recycling sites (Leung et al., 2007; Wong et al., 2007), where the intake of fish and shellfish was found to correlate with the body burdens of people living at such locations (Chan et al., 2007).

The application of chemical instruments, for example, high-resolution gas chromatography and mass spectrometry (HR GC/MS) is costly and time-consuming, but also the “gold standard” method for the detection and quantification of dioxin-like compounds (Vanderperren et al., 2004). In the environment, chemicals exist and occur as complex mixtures including different congeners and isomers, with chemical analysis only being able to identify and quantify the chemical for which analytical techniques and standards are available for (Garrison et al., 1996). Instrumental analyses also do not account for interactions amongst the chemicals in complex mixtures and provide minimal information on their biological effects (Vanderperren et al., 2004). Hence, chemical analyses may underestimate the potential risks posed by these chemicals; some toxicologically important compounds may be overlooked. In vitro cell bioassays provide an estimation of overall biological activity of all compounds that act through the same mode of action present in extracts of any environmental media (Hilscherova et al., 2000).

In this study, the stably-transfected rat hepatoma cell line, H4IIE, was to estimate the overall potency of compounds that induce effects through interactions with aryl hydrocarbon receptors (AhR). This bioassay, which measures AhR-mediated chemical-activated luciferase gene expression (CALUX) has been widely adopted to evaluate the effects of dioxin-like compounds in environmental samples, such as biota (Khim et al., 2000), sediment (Khim et al., 1999) and human milk (Nelson et al., 2006). Furthermore, the US EPA approved the CALUX bioassay as an alternate dioxin test (Method 4435) in February 2008 (US EPA, 2008). The results of the bioassay are used to determine relative potencies of single compounds and mixtures by comparing the results of the sample extracts to those of a well-characterized reference compound, e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), for AhR-mediated toxicity. This bioassay is a quick and sensitive screening tool with costs 40%–70% less than the traditional HR GC/MS (US EPA, 2008).

Although PCDD/F concentrations have been measured in various environmental and human samples from China, only a few studies have reported the PCDD/F burdens in fish collected from e-waste recycling sites (Song et al., 2011). Therefore, the objectives of this study were: 1) to measure the concentrations of PCDD/Fs in common species of fish collected from 2 major e-waste recycling sites and 1 reference site in China; 2) to estimate the intake of PCDD/Fs via fish consumption; 3) to assess the potential health risks posed to human consumers as a result of fish consumption in the diet and 4) to investigate the dioxin-like activity in the fish samples by using H4IIE-luc bioassay.

2. Materials and methods

2.1. Sampling sites

Sampling was conducted in 1) Guiyu (GY), Guangdong Province; 2) Taizhou (TZ), Zhejiang Province; and 3) Lin’an (LN), Zhejiang Province. Both GY and TZ are the 2 major e-waste processing sites in China, where the former is located in Guangdong Province, southern China while the latter is in Zhejiang Province, eastern China (Fig. 1, Chan et al., 2007). The reference site chosen was LN, which is situated inland in Zhejiang Province, northern Hangzhou, about 245 km away from TZ and has no history of e-waste recycling activities.

2.2. Collection of socio-demographic and fish consumption data

Socio-demographic data and freshwater fish consumption habits of the study’s residents (125, 100 and 125 residents randomly selected in TZ, GY and LN, respectively) were obtained from face-to-face interviews and semi-quantitative food intake questionnaires, respectively. Details regarding how data was collected can be found in Chan et al. (2007).

2.3. Sampling of fish

Freshwater fish were collected from two rivers, namely Lian Jiang River (N: 23.3121; E: 116.352) and Nanyang River Tributary (N: 23.3215; E: 116.369), in Guiyu (Fig. 1). In TZ, wild fish from rivers were caught and fish were also purchased from the local markets. The sampling of fish in LN was conducted at a market. Details of all the samples are described in Table 1. After sampling, the samples were wrapped with aluminum foil and sealed in clean polystyrene bags and frozen at −20 °C until their chemical analysis.

2.4. Sample preparation

The preparation of the fish samples was conducted according to the US FDA’s Pesticide Analytical Manual Vol. 1 (US FDA, 1999). All fish samples were treated and analyzed as if they had been consumed so as to give more reliable PCDD/F concentration estimations under conditions closely mimicking reality. Briefly, fish samples were prepared as skin-on fillets from which scales, bones, and viscera were removed. The samples were then rinsed with purified water to remove all foreign particles, and patted dry with paper towels. After that, the samples were frozen, freeze-dried, grounded into powder and homogenized. The water content was determined by the weight loss before and after freeze-drying.

2.5. Chemical analysis

Due to the high analytical cost of PCDD/Fs, only composite samples of big head carp (Aristichthys nobilis) (n = 5, 6 and 4) and edible gold fish/crucian carp (Carassius auratus) (n = 8, 13 and 4) collected from TZ, GY and HZ, and sliver carp (Hypophthalmichthys molitrix) (n = 4 and 10) from TZ and GY, respectively were selected for the chemical analyses, yielding 8 composite samples. The chemical analysis on fish samples was conducted by the State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Science, Chinese Academy of Sciences, Beijing, China. Briefly, the PCDD/Fs analysis was performed using the isotope dilution technique based on the US EPA Method 1613B. Before extraction, samples were spiked with a mixture containing 1513C12 labeled 2,3,7,8-substituted PCDD/F internal standards (Wellington Laboratories, Canada) as defined in the US EPA Method 1613B. Extraction was carried out by Soxhlet extraction using 250 ml n-hexane/dichloromethane (1:1) for 24 h, with the resulting extracts reduced by rotary evaporation, then dried with a stream of nitrogen. Lipid contents were calculated with the extract residues. The lipids were dissolved with n-hexane and subjected to sulfuric acid wash, while the eluates were reduced by rotary evaporation, and a multi-step cleanup was performed with adsorption chromatography. A multilayer silica column (from top to bottom: anhydrous sodium sulfate, 1 g silica-gel, 10 g 44% silica-gel–sulfuric acid, 1 g silica-gel, 5 g 33% silica-gel–sodium hydroxide, 1 g silica-gel, 2 g 10% AgNO3 silica-gel, 1 g silica-gel) was used and eluted with 100 ml...
n-hexane. The hexane extracts were further concentrated and passed through a basic alumina column for further purification. Samples were then eluted with 100 ml 5% dichloromethane/hexane and then eluted with 50 ml 50% dichloromethane/hexane; the 50% dichloromethane/hexane elutes were concentrated to about 20 μl by a stream of nitrogen. Prior to injection, a 13C12-labeled injection standard (containing 13C12-labeled 1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD, Wellington Laboratories) was added for calculating the percentage of recovery.

PCDD/Fs were analyzed with an Agilent 6890 gas chromatograph coupled with Micromass Autospec Ultima high-resolution mass spectrometry by tracing the M+, (M + 2)+, or the most intensive ions of the isotope cluster. PCDD/F congeners were analyzed using a 60 m DB5ms-column (60 m × 0.25 mm i.d. × 0.25 μm) and the carrier gas employed was helium at 1.2 ml/min. Injection volume was 1 μl in splitless mode with 60 s splitless period. The MS was operated over 10,000 resolution with EI (38 eV), and data were obtained in the selected ion monitoring (SIM) mode. The limit of detection (LOD) for the 17 PCDD/F potent congeners ranged from 0.01 to 0.37 pg/g wet wt.

Fig. 1. Locations of fish sampling sites in Guiyu, Guangdong Province, China.

Table 1
Fish sampled at the study sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Fish species (scientific name)</th>
<th>Sample size</th>
<th>Sample sizeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taizhou</td>
<td>Big head carp (Aristichthys nobilis)</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Edible gold fish/crucian carp</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(Carassius auratus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silver carp (Hypophthalmichtys molitrix)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Catfish (Psettodes vachelli)</td>
<td>N.S.</td>
<td>4</td>
</tr>
<tr>
<td>Guiyu</td>
<td>Big head carp (A. nobilis)</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Edible gold fish/crucian carp</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(Carassius auratus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Silver carp (Hypophthalmichtys molitrix)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Black carp (Mylopharyngodon piceus)</td>
<td>N.S.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Common carp (Cyprinus carpio)</td>
<td>N.S.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Grass carp (Ctenopharyngodon idellus)</td>
<td>N.S.</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Tilapia (Tilapia mossambica)</td>
<td>N.S.</td>
<td>12</td>
</tr>
<tr>
<td>Lin’an</td>
<td>Big head carp (A. nobilis)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Edible gold fish/crucian carp (C. auratus)</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Sample sizea = sample size of composite fish for chemical analysis of PCDD/Fs, sample sizeb = sample size of individual fish for bioassay derived PCDD/Fs analysis (H4IIE-luc cell bioassay) and N.S. = not suitable.
2.6. Estimated daily intake of PCDD/Fs from residents

The daily intake of freshwater fish by the 125 TZ, 100 GY and 125 LN residents were 24.9 ± 23.9, 25.2 ± 16.2, and 23.6 ± 24.0 g/day, respectively. Therefore, with the results of PCDD/F concentrations in freshwater fish collected from the study sites (Table 2), the estimated daily intake (EDI) can be estimated as follows: Eq. (1)

\[
\text{EDI} = \frac{(C \times M)}{BW}
\]

where,

- **EDI** = estimated daily intake (pg WHO-TEQ/kg bw/day);
- **C** = concentration of PCDD/Fs (pg WHO-TEQ/g wet wt);
- **BW** = consumer body weight (60 kg);
- **M** = daily consumption of freshwater fish (g/day).

2.7. H4IIE-luc cell bioassay

All individual fish samples from TZ, GY and LN were used for H4IIE-luc cell bioassay (Table 1). The fish samples were Soxhlet-extracted according to the US EPA Standard Method 3540C (US EPA 1996) for 18 h with 150 ml of n-hexane/dichloromethane. The lipid content of fish samples was gravimetrically determined from an aliquot of the extract. The extract was purified by a column which contained, from the top to the bottom, 1 g of anhydrous sodium sulfate, 2 g of deactivated silica gel (3.3% reagent water, w/w), 10 g of acidic silica gel (44% concentrated sulfuric acid, w/w), 1 g of deactivated silica gel, 10 g of activated florisor and finally 1 g of anhydrous sodium sulfate. The column was eluted with 180 ml n-hexane, followed by 190 ml n-hexane/dichloromethane (9:1, v/v). The eluate was evaporated until it was completely dry under a gentle stream of nitrogen and redissolved in 200 μl of n-hexane without the addition of internal standards.

The cells employed in the present study, H4IIE-luc, were rat hepatoma cells that were stably transfected with a luciferase reporter gene (Sanderson et al., 1996). The procedures for the in vitro bioassay were similar to those described in Khim et al. (2000, 2001). Briefly, the cells were maintained in Dulbecco's Modified Eagle's Medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum at 37 °C and in a 5% CO₂ atmosphere. They were subcultured every 3–5 days depending on growth rate. For the bioassay, the cells were trypsinized from culture dishes containing more than 80% confluent monolayers and then seeded into 96-well culture plates at a density of approximately 8 × 10^4 cells/ml in 250 μl per well. Cells were incubated overnight before dosing and test wells were dosed with 2.5 μl of the sample extract. Three concentrations were tested for each sample in triplicate. The control wells were dosed with 2.5 μl of n-hexane, while the blank wells received no dosing. A standard curve was constructed by adding different concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) to the standard wells. Three control and blank wells were tested on each plate.

Luciferase assays were conducted after 72 h of exposure, where culture medium was removed from the plate and all wells were washed with phosphate-buffered saline (PBS). Fifty μl of Lucite reagent (PerkinElmer, Waltham, MA) and 100 μl of PBS supplemented with Ca²⁺/Mg²⁺ were added to all wells, and the plate was incubated at room temperature in the dark for 20 min. The plates were scanned with a microplate-scanning Dynatech ML 3000 luminometer (Dynatech Laboratories, Chantilly, VA, USA), with responses to AhR agonists quantified by measuring the relative luminescence units (RLU). The maximum responses of the samples were not equal and the activities of the samples were relatively low when compared to standard activities, which may be justified by the fact that mixtures were compared to a single standard. The assumption of parallelism could also not be tested between the sample and standard dose-response relationships, therefore, the point-of-departure method was used to determine TEQ values (Villeneuve et al., 2000). Dunnett's test (SPSS of Windows, version 11.0; SPSS Inc., Chicago, IL, USA) was used to determine the first sample concentration that was significantly different from zero; this concentration was then compared to the first standard concentration (8 pg/well) that was significantly different from zero in order to calculate TEQs for the samples (Coady et al., 2001).

2.8. Expression of PCDD/F concentrations

Concentrations of PCDD/Fs determined by chemical analysis were indicated as mass concentrations (pg/g wet wt) and toxic equivalency.
(TEQ) of WHO 1998 (pg WHO-TEQ/g wet wt) (Van den Berg et al., 1998), with undetected values treated as zero. The measured values for fish samples collected within a site were pooled to obtain an average value. The TEQ value for the dioxin-like compounds determined by the H4IIE-luc cell bioassay was expressed as pg WHO-TEQ/g wet wt

2.9. Data analysis

The Statistical Package for Social Sciences (SPSS of Windows, version 16.0; SPSS Inc., Chicago, IL, USA) was used for quantitative data analysis. Normality of the data was checked by Shapiro–Wilk test. The differences amongst groups were assessed via the t-test or analysis of variance (ANOVA) (the data was log-transformed to meet the normality assumptions). The significance level was alpha=0.05 and two-tailed with the data presented as means±SD. Spearman correlation was used to investigate the relationships between chemical analysis PCDD/F concentrations based on chemical analyses and bioassay-derived PCDD/F concentrations. The significance level of alpha=0.05 was set as the level for statistical significance.

3. Results and discussion

3.1. Concentrations of PCDD/Fs in fish, and comparison amongst the study sites

Table 2 shows the toxicity equivalents (TEQ) in fish for each site, which were calculated using the TEFs of mammals (Van den Berg et al., 1998). Total TEQ concentrations in TZ fish ranged from 0.62 to 1.34 pg WHO-TEQ/g wet wt (mean: 0.90±0.38 pg WHO-TEQ/g wet wt) while the values for GY fish ranged from 2.15 to 7.49 pg WHO-TEQ/g wet wt (mean: 4.64±2.69 pg WHO-TEQ/g wet wt). Fish collected from the reference site showed that concentrations ranged between 0.07 and 0.08 pg WHO-TEQ/g wet wt, with an average value of 0.08±0.01 pg WHO-TEQ/g wet wt. There were large variations in the concentrations between the sampling sites, where the lowest value was found in LN and the highest in GY. The average TEQ concentration for LN was only 2% of that for GY.

In order to evaluate the pollution levels at different sites, the same species of fish were used for comparison (Fig. 2), as the bioaccumulation of persistent organic pollutants (POPs) in fish is species-specific due to their ecological characteristics such as feeding habits and habitat (Zhou et al., 1999). Fish from GY and TZ were sampled from local rivers and those from LN were sampled at local markets. From amongst the 3 sites, the Crucian carps from GY (4.28 pg WHO-TEQ/g wet wt) were the most contaminated, whereby their concentration exceeded the values for TZ (0.75 pg WHO-TEQ/g wet wt) and LN (0.08 pg WHO-TEQ/g wet wt) by 6 and 54 times, respectively. Regarding the residue levels in bighead carp, the highest concentration was found in GY (2.15 pg WHO-TEQ/g wet wt), which was 3.5 and 31 times higher than TZ (0.62 pg WHO-TEQ/g wet wt) and LN (0.07 pg WHO-TEQ/g wet wt), respectively. Silver carps from GY (7.49 pg WHO-TEQ/g wet wt) were also more contaminated than those from TZ (1.34 pg WHO-TEQ/g wet wt). Furthermore, air (Li et al., 2007) and surface soil (Shen et al., 2007) samples collected from TZ were also less contaminated than those in GY (Leung et al., 2007). Although both TZ and GY are the major e-waste processing sites in China, the latter was more contaminated than the former. This may be possibly due to the fact that open burning of e-waste, which releases significant amounts of PCDD/Fs, may be more common and extensive in GY.

The PCDD/F concentrations in fish species collected from GY and TZ were at least 10-fold higher than those from LN (the reference site). The 2 e-waste areas have been recognized as being contaminated with PCDD/Fs because of the open burning of e-waste, e.g., the recovery of copper. It was found that GY was the most contaminated with PCDD/Fs amongst all dioxin hotspots (e.g. schistosomiasis affected areas, the Pearl River Delta, e-waste recycling area of the Yangtze River Delta, and pentachlorophenol contaminated areas) in China (Zheng et al., 2008). Hence, serious background pollution in GY and TZ has led to high PCDD/F concentrations in fish caught from local rivers.

3.2. Inter-species variations in PCDD/F concentrations

The PCDD/F body burdens in the 3 species of fish collected from the e-waste sites followed the descending order of silver carp > crucian carp > bighead carp. Species type seems to be an important determinant for TEQ concentrations (Matthews et al., 2008) because numerous factors including their interactions can influence the PCDD/F concentrations in fish. These factors include biological aspects (such as fat content, age, dietary absorption efficiencies, elimination rates, metabolism, trophic levels and reproductive status) (Pandelova et al., 2008) and environmental aspects (such as the season, and habitat location and pollution) (Matthews et al., 2008). The extent of exposure to PCDD/Fs in fish collected from either GY or TZ should be similar as they originated from the same site. Normally, the higher the trophic level, the greater the dioxin body burden found in fish (Matthews et al., 2008). Bighead and silver carps are at lower trophic levels as they are filter-feeders, whereas crucian carps are opportunistic omnivores that consume plants, small crustaceans, insects and detritus (Fishbase, 2008). Therefore, the present results showed that habitat pollution and trophic level are probably not the factors which determine the concentrations of PCDD/Fs in fish. However, the fat contents of fish muscle significantly increased with the dioxin body burden of fish from both e-waste sites (TZ: r=0.998, p<0.05; GY: r=0.977, p<0.05), meaning that silver carp had the highest fat content and thus was the most dioxin contaminated (Table 3). The reasons behind the fat content in the muscle of different fish species remain unclear, as the bioaccumulation of dioxins in fish is species-specific (Matthews et al., 2008). Age and harvesting season can also affect the fat contents in sprat (Sprattus sprattus) from the Baltic Sea (Simm et al., 2006). Therefore, further investigations are needed to establish the uptake of this compound group by different fish species living in the same aquatic system.

3.3. Congener concentrations and homologue profiles

The most toxic congener 2,3,7,8-TCDD was present in all samples of the 3 sites of interest. On average, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, and 2,3,4,7,8-PeCDF contributed 10–24%, 10–28%, 15–26% and 26–41%, respectively, to the total TEQs. These 4 congeners proved to be the most dominant which is consistent with previous studies (Chen et al., 2008; Kiviranta et al., 2004), constituting up to 87–95%. The homologue profiles indicate the environmental fate and sources of dioxins (Duarte-Davison et al., 1997). The samples from the e-waste sites showed similar homologue profiles, which have a relatively high

Fig. 2. PCDD/F concentrations in fish species collected from the study sites.
higher than those from Australia (Padula et al., 2008), the Baltic Sea (DeMul et al., 2008). The concentrations in TZ the Pearl River Delta (Zhang et al., 2007), Italy (Taioli et al., 2005), Japan reported by most other studies, with the exception of those from a PCDD/Fs in al., 2007; Martí-Cid et al., 2008). Therefore, the residual levels of comparable concentrations to those from Shenzhen (Zhang et al., 2008), Norway (Knutzen et al., 2003), the Baltic Sea (Shelepchikov et al., 2007; Jiang et al., 2007; Wan et al., 2005; Zhang et al., 2007, 2008), including Ya-Er Lake which had been seriously polluted with 3.4. Comparison with other studies

Table S1 (Supplementary data) compares PCDD/F concentrations in fish collected from GY to be up to 375 times higher than those observed in almost all other regions in China (Han et al., 2007; Hsu et al., 2007; Jiang et al., 2007; Jiang et al., 2007; Wan et al., 2005; Zhang et al., 2007, 2008), including Ya-Er Lake which had been seriously polluted with hexachlorocyclohexane and hexachlorobenzene effluent discharged from a large chemical plant (Wu et al., 2001). The values of GY fish were similar to those of marine fish from the USA (Brown et al., 2006), Norway (Knutzen et al., 2003), the Baltic Sea (Shelepchikov et al., 2008) and Australia (Padula et al., 2008). Fish collected from TZ showed comparable concentrations to those from Shenzhen (Zhang et al., 2008), the Pearl River Delta (Zhang et al., 2007), Italy (Taioli et al., 2005), Japan (Kajiwara et al., 2007), Korea (Moon and Ok, 2006), and the Netherlands (DeMul et al., 2008). The concentrations in TZ fish were up to 134 times higher than those from Australia (Padula et al., 2008), the Baltic Sea (Shelepchikov et al., 2008), Spain (Bocio et al., 2007; Llobet et al., 2008; Martí-Cid et al., 2008), and Sweden (Darnérud et al., 2006). Concentrations in fish from the reference site (LN) were far below the values reported by most other studies, with the exception of those from a fish farm in Taiwan (Lee et al., 2006), and marine fish from Spain (Bocio et al., 2007; Martí-Cid et al., 2008). Therefore, the residual levels of PCDD/Fs in fish from GY, TZ and LN were found to be at the high, moderate, and lowest ends, respectively, of the worldwide range.

3.5. Dietary exposure and health risk assessment of PCDD/Fs in adults

Potential health risks from exposure to PCDD/Fs were assessed by using 3 criteria, with the first being the maximum allowable limits, where the EU’s maximum allowable concentration in muscle of fish was 4 pg WHO-TEQ/g wet wt (EUROPA, 2001). In the present study, all fish were below this limit, except crucian (4.28 pg WHO-TEQ/g wet wt) and silver carp (7.49 pg WHO-TEQ/g wet wt) collected from GY rivers. Moreover, the residual concentration in bighead carp collected from GY (2.15 pg WHO-TEQ/g wet wt) was close to the EU’s action level (3 pg WHO-TEQ/g wet wt) which gives an “early warning” to the competent authorities and operators to take measures to eliminate contamination (EUROPA, 2001).

The second criterion was the screening values (SV) for the PCDD/F concentrations in fishery products, which were set according to the consumers’ diet (FAO, 2006). Regarding the results of the food consumption survey, TZ, GY and LN consumed 75.1, 54.8 and 48.4 g/day of seafood, respectively, which lie in between those of recreational (17.5 g/day) and subsistence consumers (142.2 g/day) defined by the US EPA (US EPA, 2000). A limit of 0.256 pg WHO-TEQ/g wet wt of PCDD/F concentrations in seafood has been set for recreational consumers according to their seafood consumption rates and for subsistence consumers 0.0315 pg WHO-TEQ/g wet wt. The concentrations of PCDD/Fs in the fish samples collected from the 2 e-waste recycling sites ranged from 0.62 to 7.49 pg WHO-TEQ/g wet wt, which exceeds the limits for both recreational and subsistence consumers. In contrast, the concentrations in the fish collected from the reference site were well below both SVs, which indicate that the fish from the e-waste sites may not be suitable for continual human consumption in large amounts and that regulatory attention is needed to focus on this threat.

The last criterion was the standards for human intake of PCDD/Fs and dioxin-like PCBs agreed between the WHO (1998), Scientific Committee on Food of the European Union (EC-SCF, 2001) and Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001) (Table S2 (Supplementary data)). The highest values of dietary exposure to PCDD/Fs from freshwater fish were found in the descending order of GY-TZ-LN (Tukey test, p<0.05) (Table 4). The values for GY ranged between 0.90 and 4.31 pg WHO-TEQ/kg bw/day, meaning that the EDI of almost all GY residents taking part in this study exceeded the lower end of the tolerable daily intake (TDI) recommended by the WHO. Moreover, the intake of PCDD/Fs by a quarter of GY residents were beyond the higher end of this guideline (1–4 pg WHO-TEQ/g wet wt), as well as the standards recommended by the EC-SCF (2001) (2 pg WHO-TEQ/g wet wt) and JECFA (2001) (2.3 pg WHO-TEQ/g wet wt) (Table S2 (Supplementary data)), while the EDIs for TZ and LN residents were below these standards. Although the dietary intake levels found at the e-waste processing sites were within the TDI range of 1–4 pg TEQ/kg bw/day recommended by the WHO, it should be noted that the upper range of the TDI of 4 pg TEQ/kg bw/day is considered as the maximum tolerable intake on a provisional basis and that the ultimate goal is to reduce human intake levels to below 1 pg TEQ/kg bw/day (Tsutsumi et al., 2001). In other studies, the mean intake of PCDFs via fish consumption in regions of China ranged from 0.27 to 1.39 pg WHO-TEQ/kg bw/day (Hsu et al., 2007; Jiang et al., 2007; Song et al., 2011; Wu et al., 2002; Zhang et al., 2007, 2008), with the exception of residents living at a site nearby a PCP factory in Taiwan who normally consume much more PCDD/Fs (0.46–32.5 pg WHO-TEQ/kg bw/day) (Table S3 (Supplementary data)).

The daily intake of PCDD/Fs was assessed to be 0.133 pg WHO-TEQ/kg bw/day in fish in Korea (Lim et al., 2004). In Japan, Sasamoto et al. (2006) estimated PCDD/F dietary intake in fish at 1.28 pg WHO-TEQ/kg bw/day, while Tsutsumi et al. (2001) estimated an intake of 0.60 pg WHO-TEQ/kg bw/day. One market basket study showed a 54 pg WHO-TEQ/person/day intake of PCDD/Fs via fish consumption in Finland (Kiviranta et al., 2004). Darnérud et al. (2006) estimated that the dietary intake of PCDD/Fs via marine fish and shellfish consumption was 12.8 pg WHO-TEQ/person/day for the population in

### Table 3

<table>
<thead>
<tr>
<th>Biological parameters of the fish collected from the study sites.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bighead carp</strong></td>
</tr>
<tr>
<td>Weight (g)</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>GY</td>
</tr>
<tr>
<td>TZ</td>
</tr>
<tr>
<td>LN</td>
</tr>
<tr>
<td><strong>Edible gold fish/crucian carp</strong></td>
</tr>
<tr>
<td>Weight (g)</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>GY</td>
</tr>
<tr>
<td>TZ</td>
</tr>
<tr>
<td>LN</td>
</tr>
<tr>
<td><strong>Silver carp</strong></td>
</tr>
<tr>
<td>Weight (g)</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>GY</td>
</tr>
<tr>
<td>TZ</td>
</tr>
<tr>
<td>LN</td>
</tr>
</tbody>
</table>

Note: “fat weight/dry weight; ”a = not available; GY = Guiyu; LN = Lin’an; TZ = Taizhou.
Sweden. DeMul et al. (2008) reported the value of 0.3 pg WHO-TEQ/kg bw day for residents in the Netherlands. In Egypt, the intake of PCDD/Fs via freshwater fish was estimated to be 0.15–0.21 pg WHO-TEQ/kg bw/day (Loutfy et al., 2006).

In general, the EDIs of PCDD/Fs in fish for the residents at e-waste recycling sites (especially GY) and at PCP-contaminated sites (Lee et al., 2006), were relatively high when compared with the values for the general population in China and other countries. However, it should be noted that the dietary exposure to PCDD/Fs estimated by the present study only included the exposure from freshwater fish. Other important dietary sources, for example, marine fish, shellfish and meat, were not included for estimation due to the high costs which would have been incurred as a result of the chemical analyses. Therefore, the total exposure to PCDD/Fs from diets was underestimated, and people at e-waste recycling sites are potentially threatened by greater risks when compared with the general population.

3.6. Dioxin-like activity determined by H4IIE-luc cell bioassay

The results of the H4IIE-luc cell bioassay performed on freshwater fish from different sampling locations are shown in Fig. 3. The concentrations of dioxin-like compounds in fish collected from TZ rivers ranged from 172 in crucian carp to 414 pg TEQ/g wet wtbio in catfish, while those from GY rivers ranged between 28.0 in black carp and 312 pg TEQ/g wet wtbio in bighead carp. Levels of TEQs in fish from these 2 locations were up to 24 times higher than those collected from markets in TZ and LN, indicating that the fish sampled at TZ markets may not have originated from these rivers.

There was a positive correlation between PCDD/F concentrations based on chemical analyses and bioassay-derived PCDD/F concentrations ($r = 0.500$, at $p > 0.05$). Comparisons of the same species between rivers demonstrated that, in general, the concentrations detected in GY were comparable to those in TZ. These findings disagreed with that of the chemical analysis which showed that the PCDD/F concentrations in GY fish were higher than those in TZ fish. The disagreement between bioassay-derived and instrumentally-based TEQ values suggests that there was the presence of either some form of unidentified and biologically active compounds in a sample or of non-additive interactions between components of the sample (Villeneuve et al., 2000). The H4IIE-luc bioassay was found to be sensitive to dioxin-like compounds such as PAHs and dioxin-like PCBs. For example, the mean concentration of total PCBs in freshwater fish from fish ponds in TZ was 20 times higher than that in the same species collected from GY (Xing et al., 2009, 2010). The TEQ values of PCBs in soil from TZ (Shen et al., 2007) were up to 54,000 times higher than the values for GY (Wong et al., 2007), while the concentrations of PAHs in soil from these two e-waste recycling sites were comparable (Shen et al., 2007; Yu et al., 2006). Furthermore, human milk collected from TZ contained much higher concentrations of total PCBs than those from GY (Xing et al., 2009). In addition, TZ is the largest center for dismantling obsolete transformers and capacitors in China (Zhao et al., 2007). Unwanted electric power electromotors, capacitors, transformers, and their components are common items of e-waste processed in TZ but are scarce in GY (Shen et al., 2007). The PCB-containing transformer oils in e-waste can leak out from scrap transformers and into the environment, leading to bioaccumulation and biomagnification along the local food chain (Zhao et al., 2006). Therefore, the relatively high concentrations of PCBs in TZ together with the high concentrations of PCDD/Fs in GY may well explain the comparable dioxin-like activities observed in fish from the rivers of both locations.

When compared with other studies which also used H4IIE biosay as a toxicity assessment tool, the maximum dioxin-like activity (414 pg TEQ/g wet wtbio or 18,585 pg TEQ/g fatbio in TZ catfish) detected in the present study was greater than the maximum activity...
Duarte-Davison R, Sewart A, Alcock RE, Cousins IT, Jones KC. Exploring the balance between sources, deposition and the environmental burden of PCDD/Fs in the UK terrestrial environment: a study to identifying uncertainties and research needs. Environ Sci Technol 1997;31:1–11.
Lee CC, Lin WT, Liao PC, Su HJ, Chen HL. High average daily intake of PCDD/Fs and serum levels in residents living near a deserted factory producing pentachlorophenol (PCP) in Taiwan: influence of contaminated fish consumption. Environ Pollut 2006;141:381–9.
Llobet JM, Martí-Cid R, Castell V, Domingo JL. Significant decreasing trend in human diet-
ery intake to PCDD/PCDFs and PCBs in Catalonia, Spain. Toxicol Lett 2008;178:

Loufty JF, Faurehammer M, Tundo P, Raccanelli S, Dien AGE, Ahmed MT. Dietary intake of
dioxins and dioxin-like PCBs, due to the consumption of dairy products, fish/seafood
and meat from Ismailia City, Egypt, Sci Total Environ 2006;370:1–8.

Martí-Cid R, Bocic A, Domingo JL. Dietary exposure to PCDD/PCDFs by individuals
living near a hazardous waste incinerator in Catalonia, Spain: temporal trend.
Chemosphere 2008;70:1588–95.

Matthews V, Päpke O, Gaus C. PCDD/FS and PCBs in seafood species from Moreton Bay,

Moon H-B, Ok G. Dietary intake of PCDDs, PCDFs and dioxin-like PCBs, due to the
consumption of various marine organisms from Korea. Chemosphere 2006;62:
1142–52.

Nadal M, Espinosa G, Schuhmacher M, Domingo JL. Patterns of PCDDs and PCDFs in
human milk and food and their characterization by artificial neural networks.

Nelson EAS, Hui LL, Wong TW, Hedley AJ. Demographic and lifestyle factors associated
with dioxin-like activity (CALUX-TEQ) in human breast milk in Hong Kong. Envi-

Padula DJ, Daughtrey BJ, Nowak BF. Dioxins, PCBs, metals, metalloids, pesticides and an-
timicrobial residues in wild and farmed Australian southern bluefin tuna (Thunnus

Pandelova M, Henkelmann B, Roots O, Simm M, Järv L, Benfenati E, et al. Levels of
PCDF/F/D and dioxin-like PCB in Baltic fish of different age and gender. Chemosphere

Ricardo FG, Iria YP, Elean MC, Jesus SG. Feed ingredients mainly contributing to polycyclic
aromatic hydrocarbons and polychlorinated biphenyl residues. Polycyclic Aromat

Sanderson JT, Arts JMJC, Brouwer A, Freese KL, Denison MS, Gieys JP. Comparison of
Ah receptor-mediated lucerase and ethoxyresorufin-O-deethylase induction in
H4IE cells: implications for their use as bioanalytical tools for the detection of

Sasamoto T, Ushio F, Kikutani N, Saitoh Y, Yamaki Y, Hashimoto T, et al. Estimation of
sheepchikov AA, Shenderyuk VV, Brodsky ES, Feshin D, Baholdina LP, Gorogankin SK.
Simm M, Roots O, Kotta J, Lankov A, Henkelmann B, et al. From mother to
child: investigation of prenatal and postnatal exposure to persistent bioaccumulating
toxics using breast milk and placenta biomonitoring. Chemosphere 2007;67:
S256–62.

Simm M, Roots O, Kotta J, Lankov A, Henkelmann B, Shen H, et al. PCDD/Fs in sprat
(Sphyrna spinous) from the Gulf of Finland, the Baltic Sea. Chemosphere

dioxin-like compounds in green-lipped mussels Perna viridis from Hong Kong

Soerchtmann SD, Chan SM, Nelson EAS, Brouwer A, Sauer PJ. Comparison of dioxin and
PCV concentrations in human breast milk samples from Hong Kong and the Nether-

activity in selected mollusks from the coast of the Bohai Sea, China, using the

Song Y, Wu N, Han J, Shen H, Tan Y, Ding G, et al. Levels of PCDD/FS and DL-PCBs in se-
lected foods and estimated dietary intake for the local residents of Luqiao and


Tsutsumi T, Yanagi T, Nakamura M, Kono Y, Ichibe H, Iida T, et al. Update of daily in-
take of PCDDs, PCDFs, and dioxin-like PCBs from food in Japan. Chemosphere

USA: United States Environmental Protection Agency; 1996.

US EPA (U.S. Environment Protection Agency). Guidance for assessing chemical con-
taminant, data for use in fish advisories. Fish sampling and analysis, volume 1.


US EPA (U.S. Environment Protection Agency). Method 4435: method for toxic equiv-
alent (TEQs) determinations for dioxin-like chemical activity with the CALUX®
bioassay. USA: United States Environmental Protection Agency; 2008.


equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.

equivalence factors (TEFs) for PCBs, PCDFs, PDDFs for humans and wildlife. Envi-
ron Health Perspect 1998;106:775–82.