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Review Article

The use of H4IIE Cells to Detect the Polychlorinated Biphenyls in House Dust

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Abstract

In the present study, settled house dust samples were collected form Pearl River Delta, China for PCBs analyses. Total PCBs concentrations in house dust ranged from 50.9 to 1066 ng/g, with a median of 181 ng/g. PCB77, 114, 118, 157, 153, and 194 were found as the dominant congeners. TEQ_{bio} of all dust samples derived from biological assays ranged from 56.7 to 865 pg TEQ/g, which was mostly contributed (average 54.6%) by TEQ_{PCB} derived from chemical analyses. In addition, a significant correlation was obtained between TEQ_{PCB} and TEQ_{bio} ($r = 0.89$, $p< 0.01$, $n=23$). These results suggested that dioxin-like PCBs may be the dominant AhR agonists contained in the dust samples. Risk assessment indicated that indoor dust is an important environmental medium of children exposure to dioxin-like PCBs.

Key words: PCBs; AhR agonists; Risk assessment

1. Introduction

Most of studies related to environmental pollution focused on tracking the sources of pollutants and human health risk assessment that occurred in outdoor environment. Nowadays, people are likely to spend more than 90% of their time indoors [1, 2]. Therefore, the potential health risks posed by chemical contaminants in the indoor environment are of great concern.

Polychlorinated biphenyls (PCBs) were mainly used in electrical equipment and fluorescent lighting fixtures prior to 1977, and can also be found in other products such as surface coatings, sealing materials, plasticizers, inks, flame retardants, pesticide extenders, carbonless duplicating paper, paints and caulking materials [3]. Several non-ortho and mono-ortho substituted polychlorinated biphenyls (PCBs) assume a planar configuration similar to prototype

AhR (aryl hydrocarbon receptor)-inducer, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). These show patterns of toxicity in laboratory animals resembling those of 2, 3, 7, 8-TCDD including teratogenicity, endocrine disorders and adverse effects on skin and immune system [4]. The rat hepatoma (H4IIE), human breast carcinoma (MCF-7), human hepatoma (HepG2), and desert topminnow (Poeciliopsis lucida) hepatoma (PLHC-1) cells have been employed to determine the relative potency of dioxin-like compounds from the induction of EROD activity *in vitro* system [5, 6]. The EROD assay was also employed to investigate the induction potency of environmental extracts containing AhR agonist [7, 8].

Although the majority of non-occupational exposure to PCBs has been widely considered to occur via the diet [9], there have been increasing indications that indoor air remains contaminated by PCBs due to that PCBs escape from indoor equipments or furniture, and enter into the indoor environment [10]. As a result, the inhalation of indoor air and ingestion of indoor dust are potential important pathways of human exposure to PCBs especially for children,

The objectives of this research were to: (1) quantify the levels of PCBs in settled house dust from the residents of Pearl River Delta (PRD), China; (2) examine the EROD induction potency of the house dust; (3) identify the typical AhR agonist in sampled house dust; and (4) perform risk assessment of preschool children exposure to dioxin-like PCBs in home dust.

2. Materials and Methods

2.1 Collection of dust samples

Settled home dust samples were collected from house floor by 23 volunteers using vacuum cleaners. These homes were located in Guangzhou (n = 5), Shenzhen (n = 5) and Hong Kong (n = 13) of PRD. The volunteers were recruited through internet or random visits. About 20% of all people contacted agreed to dust sampling (n=23). A questionnaire was designed to collect household information that might affect chemical loadings of the households when interviewing owners of theses households. The data covered year of house construction, floor area, the number of windows, the number of hours with windows open, furnitures likely to contain foam, the number of electronic appliances, the number of hours each week the electronic appliances were left on, recent home renovation, and carpet coverage on the floor.

2.2 Chemical analyses

All dust samples were filtered through a stainless-steel sieve (<100 μm) onto solvent-rinsed aluminum foil. 1-1.5 g of the mixed sample was extracted with 100 ml dichloromethane/n-hexane (1:1, v/v) in a Soxhlet apparatus for 18 h. The extracts were concentrated to 2 ml and treated with 3 ml of concentrated sulfuric acid by two times. Afterwards, organic extract was cleaned up using an activated copper/sodium sulfate anhydrous/florisil column and eluted with 100 ml n-hexane [11]. The eluant was concentrated to 0.5 ml. 2,4,5,6-tetrachloro-mxylene (TCmX) was added into all extracts to the concentration of 320 ng/g prior to instrumental analysis for quantifications. PCBs were quantitatively analyzed by GC–MS (Agilent 6890 GC coupled with a 5973 MS selective detector), with a fused silica capillary column (5% phenyl, 95% methyl silicone, 30 m ×0.25 mm×0.25 μm). Thirty-seven congeners (PCB 18, 28, 37, 44, 49, 52, 70, 74, 77, 81, 87, 99, 101, 105, 114, 118, 119, 123, 126, 128, 138, 151, 153, 156, 157,158, 167, 168, 169, 170, 177, 180, 183, 187, 189, 194 and 199) were detected. PCBs were confirmed by three criteria: 1) GC retention times matched (±0.05 min) those of standard compounds; 2) qualifier to target ratios (±20%) matched those of standard compounds; and 3) signal to noise ratio was greater than 3.

2.3 QA/QC

A method blank, a Standard Reference Material (SRM 2585, house dust, NIST, USA), and a sample duplicate were processed and analyzed in parallel with each batch of 10-12 dust samples. The variation coefficient of PCBs concentrations between duplicate samples was less than 12%. SRM 2585 was analyzed for selected PCBs congeners, and the recovery percentages ranged from 73±3.1 to 115±6.8%. LOD of PCBs analyzed in dust samples was 0.5 ng/g. Concentrations below the LOQ were assigned a value of 1/2 LOQ for statistical analysis.

2.4 Cell culture and EROD Assay

Rat hepatoma H4IIE cells were grown as a continuous cell line in Eagle's Minimum Essential Medium (ATCC, USA), supplemented with 10% fetal bovine serum (ATCC, USA). Stock culture cells were grown in T-75 (75 cm^2) flask (Nunc, Denmark) at 37 ºC in a humidified air/carbon dioxide (95/5%) atmosphere. EROD assay has been described elsewhere [8, 12]. Briefly, the cells were seeded in 96-well at a density of 1×10^4 cells/100 µl/well. After 24 h, the cell culture medium was removed and replaced by 100 µl culture media containing 2,3,7,8-TCDD standard solution (TCDD final concentrations: 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, 0.019 ng/l). The soxhlet extraction of dust samples without spike of PCB-116-d5 were treated with sulfuric acid and solvent-changed to DMSO. Nine concentrations of sample extracts prepared by 2-fold dilution ranged from 3.9×10^{-2} to 10 g/l. In each well, the final concentration of DMSO was limited to 0.5%. A 0.5% DMSO solution was used as a negative control. There were triplicates for each sample. Protein concentrations were determined as described by Bradford [13]. EROD assay was expressed as mean picomoles of resorufin produced per minute per milligram of microsomal protein (pmol/min/mg protein).

2.5 Risk assessment of dioxin-like PCBs in indoor dust

The following equations with slight modification from USEPA [14] were used to estimate the human health risks via non-dietary ingestion of dioxin-like PCBs in home dust for preschool children.

$$\mathrm{ADD}_{\mathrm{ingest}} = \frac{C_{dust} \times IngR \times F}{BW}$$

IngR = ingestion rate of indoor dust (g/day). The high dust ingestion rate and the moderate dust ingestion rate for preschool children were assumed as 0.05 mg/day and 0.2 g/day, respectively [15]. BW is the average body weight (kg). The standard values of 15 kg for preschool children were used [15]. F is the fraction of time spent at home in a day. A value of 33% was used for time spent in the home for preschool children.

2.6 Data analyses

∑PCBs was defined as the sum of 37 PCB congeners, and ∑i-PCBs (indicator PCBs) as the sum of PCB 28, 52, 101, 138, 153 and 180. The TEQ_{cal} of 12 dioxin-like PCBs ($TEQ_{dl\text{-}PCBs}$) were calculated by multiplying the measured concentration of dioxin-like PCBs (PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) by the corresponding TEF provided by World Health Organization [16]. For calculating $TEQ_{dl\text{-}PCBs}$ and ∑i-PCBs, the PCB-138 and PCB-167 concentrations were both supposed to be the half concentration of PCB-138/167, due to that the peaks of PCB-138 and PCB-167 were extremely close and difficult to be distinguished plus their minor contribution to the $TEQ_{dl\text{-}PCBs}$ and ∑i-PCBs. The calibration dose-response curve for 2, 3, 7, 8-TCDD standard was fit for sigmoid nonlinear curve-fitting module. The mathematics mode is a four-parameter equation: $Y=(A-D)/[1+(EC_{50}/X)^S]+D$, where Y= the measured EROD activity, X=concentration, A= the maximum EROD activity, D= the minimum EROD activity, EC_{50}= the concentration of TCDD eliciting 50% of the maximal inducible EROD activity, and S= curve slope. After correction for background activity (DMSO control), dioxin-like activities (measured in

house dusts) that exhibited responses between 625 and 2500 pg/L 2,3,7,8-TCDD were interpolated onto the fitted 2,3,7,8-TCDD calibration curve to calculate the biological TCDD equivalent (TEQ_{bio}) per gram of sample. All the statistical tests were performed with SPSS 16.0 software. Normality of the data was checked by Shapiro-wilk test. Spearman correlation was used to investigate the relationships between PCBs concentration in home dust and household attributes. The probability value of $p<0.05$ was set as the level for statistical significance.

3 Results and Discussion

3.1 PCBs in house dust and their relationship with household attributes.

Table 1 summarizes the concentrations of PCB congeners, indicator PCBs and total PCBs in house dust samples. The ∑PCBs concentrations ranged from 50.9 to 989 ng/g, with a median of 181 ng/g. ∑i-PCBs concentrations ranged from 9.82 to 284 ng/g, with a median of 43.6 ng/g. The ∑PCBs concentrations were similar in these three cities, Guangzhou (median: 182 ng/g), Shenzhen (median: 121 ng/g), and Hong Kong (median: 181ng/g).

Regardless of sampling location, the median concentration found in the present study was similar to that observed in the house dust from Amarillo/Austin, TX, USA (200 ng/g) and Toronto, Canada (260 ng/g) [9], 4-30 times higher than that found in the house dust from Wellington, New Zealand (46 ng/g) [9] and Sigpore (5.6 ng/g) [17], but are well below that reported in earlier study of house dust from Boston, USA (710 ng/g) [18].

The 40-year-old house contained the highest ∑PCBs (1066 ng/g), which may be due to that PCBs were extensively used in electrical equipment and fluorescent lighting fixtures prior to 1977 [3]. In addition, a weak correlation ($r = 0.48$, $p<.05$, $n=18$) was observed between ∑PCB and house age. This suggested that PCBs was accumulated in dust with time. Once indoors where they are protected from outdoor environmental degradation such as sunlight and bacteria, PCBs associated with dust always persist for long periods. No significant correlation was observed between ∑PCB and other household attributes such as the number of inhabitants and the number of electronic appliances.

3.2 TEQ_{bio} in house dust calculated from EROD Assay

Calculated EC_{50} values of the 2, 3, 7, 8-TCDD standard for the EROD assay were 1.22 ± 0.06 ng/L, with a r^2 of the standard curve greater than 0.99 ($P<0.01$) (Fig. 2). All dust extracts could induce a remarkable EROD response in the H4IIE cells. Figure 4 shows the examples of dose-response curve derived from house dust. According to the 2, 3, 7, 8-TCDD standard curve, the calculated TEQ_{bio} of all samples ranged from 56.7 to 865 pg TEQ/g (Table 1). The TEQ_{bio} of dust samples found in present study were similar to that of dust samples collected from house floor in Japan, ranging from 38 to 900 pg TEQ/g, with a median 110 pg TEQ/g, but were relatively higher than those of various sediments from other countries [20-22].

TEQ_{bio} obtained in present study provided the information on integrated response of the potential AhR agonists and the potency of samples for CYP1A1 gene induction. However, *in vitro* assay could not substitute for *in vivo* assay to accurately evaluate the toxicity of contaminants, due to that the complexity of pharmacokinetic properties *in vivo* including absorption, distribution, and metabolism can affect the outcomes of toxicity [6]. Further *in vivo* investigation is needed to evaluate the integrated toxicity of dust more comprehensively.

3.3 Relationship between TEQ from dioxin-like PCBs and TEQ_{bio}

Table 1. PCBs in house dust (ng/g)

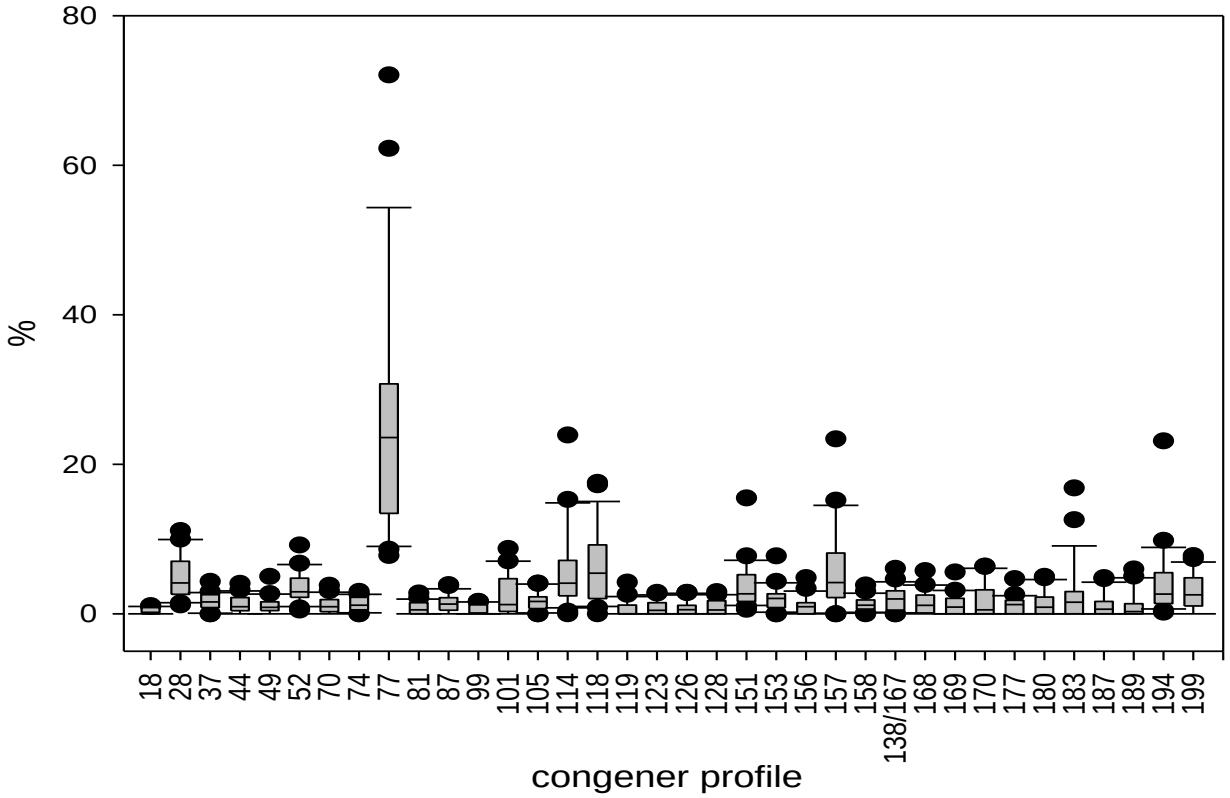
congener	min	median	max	Detection feq %
PCB18	0.00	0.72	3.34	65
PCB28	1.50	9.06	36.1	100
PCB37	0.00	2.43	12.3	91
PCB44	0.00	1.66	8.60	87
PCB49	0.00	1.23	12.5	83
PCB52	2.52	4.69	23.7	100
PCB70	0.00	1.76	9.34	87
PCB74	0.00	2.18	10.8	91
PCB77	10.5	37.2	476	100
PCB81	0.00	0.94	10.4	57
PCB87	0.00	1.73	18.7	87
PCB99	0.00	1.09	5.25	52
PCB101	0.00	2.30	32.8	78
PCB105	0.00	2.34	18.3	91
PCB114	0.00	6.73	70.3	96
PCB118	0.00	7.51	68.9	96
PCB119	0.00	0.00	7.68	39
PCB123	0.00	1.46	3.00	65
PCB126	0.00	1.22	5.20	61
PCB128	0.00	2.65	28.4	91
PCB151	0.00	1.67	18.7	70
PCB153	1.17	4.86	165	100
PCB156	0.00	1.57	12.5	74
PCB157	0.00	6.58	68.8	91
PCB158	0.00	2.08	5.53	96
PCB138/167	0.00	3.24	60	91
PCB168	0.00	1.92	19.6	78
PCB169	0.00	1.65	19.0	74
PCB170	0.00	1.38	67.5	65
PCB177	0.00	1.56	50.0	70
PCB180	0.00	1.65	51.3	70
PCB183	0.00	2.56	134	61
PCB187	0.00	1.60	37.5	65
PCB189	0.00	1.18	9.14	52
PCB194	1.14	3.56	55.7	100
PCB199	0.00	5.15	26.9	83
∑PCBs	50.9	181	989	/
∑i-PCBs	9.82	43.6	284	/
[a]$TEQ_{dl\text{-}PCBs}$	1.41	185	700	/
[b]TEQ_{bio}	56.7	321.5	865	/

Table 1. PCBs in house dust (ng/g)
[a,b]The unit of $TEQ_{dl\text{-}PCBs}$ and TEQ_{bio} is pg/g.

The TEQ_{PCB} in house dust ranged from 1.51 to 700 TEQ pg/g, with a median of 185 pg TEQ /g (Table 1). The TEQ of PCBs in present study was relatively higher than those found in houses dust collected by a high volume small surface sampler (9.70-199 pg TEQ/g; particle size: unknown) in Michigan, USA [23]. The presence of PCBs in indoor dust is possibly due to the release of PCBs contained in building materials and subsequent deposition to indoor dust. In addition, the indoor paints with PCB-imbedded materials are likely to generate indoor dust with high levels of PCBs [24].

Marked EROD response was indicated upon both in vitro bioassays of the sulfuric acid-treated extracts. It has been noted that sulfuric acid treatment decomposes nonhalogenated compounds such as PAHs (polycyclic aromatic hydrocarbons), but not halogenated compounds such as dioxins and their phenolic derivatives [25]. Therefore, the activities probably origniated from halogenated compounds of AhR agonist such as PCDD/Fs, PCBs, and polybrominated dibenzo-p-dioxins/furans (PBDD/Fs) which showed EROD activity [16]. TEQ_{PCB} in the present study contributed to average 54.6% of the TEQ_{bio} in all the house dust samples. In addition, a significant correlation was obtained between TEQ_{PCB} and TEQ_{bio} ($r = 0.89$, $p< 0.01$, $n=23$). These results revealed that dioxin-like PCBs may be the dominant AhR agonists contained in the dust samples.

Some studies were able to attribute the potency to typical AhR agonists in the soil or freshwater sediment [7, 26]. Such cause and effect relationship in indoor dust were seldom reported in indoor dust. Present study provides the first evidence of identification of typical AhR agonist in indoor dust. It should be noted that our extraction techniques were only able to detect the integrated effects of all potential AhR agonists and the calculation of TEQ_{bio} in present study may be contributed by the other potential AhR agonist not measured in present study



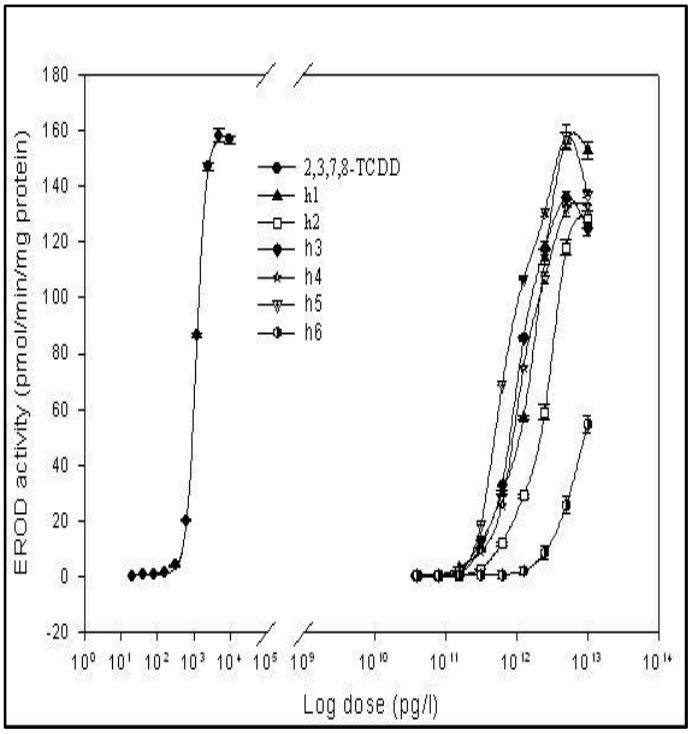


Fig. 2. Dose-response curve of EROD assay derived from 2,3,7,8-TCDD and six house dusts (pg/l).

The error bars represent the standard deviation of three independent EROD experiments.

inhibitory effects may exist in different organic pollutants contained in the indoor dust. The mutual effects should be investigated to modify the induction potency of individual agonist to calculate the potency of "pollutant mixture". However, the result of present study indicated that the bioassay could be regarded as a tool to monitor the cumulative effects of the known AhR agonists in indoor dust.

3.4 Risk assessment of preschool children exposure to dioxin-like PCBs in home dust

When the moderate dust ingestion rate was considered for preschool children, average daily dose of dioxin-like PCBs via indoor dust ranged from 0.002 to 0.77 pg TEQ /kg bw/day, with a median of 0.2 pg TEQ /kg bw/day. These are much lower than the TDI of dioxins (2.3 pg TEQ/kg-bw/day) established by Joint FAO/WHO Expert Committee on Food Additives (JECFA) [27]. When the high dust ingestion rate was considered, average daily dose of dioxin-like PCBs via

indoor dust ranged from 0.01 to 3.08 pg TEQ /kg bw/day, with a median of 0.79 pg TEQ /kg bw/day. 17% of dust sample would lead to the ADDs higher than TDI of dioxins (2.3 pg TEQ/kg-bw/day). On the other hand, the daily intake of dioxins via food ingestion in PRD for preschool children was 1.94 pg/kg bw/day, derived from the investigation of dioxins in food by Centre for Food Safety in Hong Kong [28] and based on that dietary intake of children was estimated at 57% of adult [17]. Although the estimation of dioxins intake via food including the Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like PCBs, the ADDs of dioxin-like PCBs via indoor dust were comparable to that of PCDD/Fs and dioxin-like PCBs via food for preschool children, when high dust ingestion rate was considered. Dietary has been demonstrated as the most important pathway of human exposure to dioxins [25]. However, the present results suggested that indoor dust was an important environmental medium of children exposure to dioxin-like PCBs.

4 Conclusion

Total PCBs concentrations in settled house dust ranged from 50.9 to 1066 ng/g, with a median of 181 ng/g. TEQ_{bio} of all dust samples derived from biological assays ranged from 56.7 to 865 pg TEQ/g, which was significantly correlated ($r = 0.89$, $p < 0.01$, $n=23$) with TEQ_{PCB} derived from chemical analyses. It indicated that dioxin-like PCBs may be the dominant AhR agonists contained in the dust samples. Risk assessment indicated that indoor dust was an important environmental medium of children exposure to dioxin-like PCBs.

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