Environ Sci Pollut Res (2015) 22:5218–5227
DOI 10.1007/s11356-014-3909-1

RESEARCH ARTICLE

Human health risk assessment of soil dioxin/furans contamination and dioxin-like activity determined by ethoxyresorufin-O-deethylase bioassay

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Received: 12 May 2014 / Accepted: 24 November 2014 / Published online: 10 December 2014

Abstract The major objective of this study was to evaluate the human health risks of agricultural land use conversion to other purposes in Hong Kong, based on the levels of polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran (PCDD/Fs) and determined dioxin-like activity in soil using ethoxyresorufin-O-deethylase (EROD) bioassay. Hazard quotient showed soils of open burning site (OBS) and electronic waste open burning site (EW (OBS)) exert a relatively higher non-cancer risk on adults (50.9 and 8.00) and children (407 and 64.0) via the pathway of accidental ingestion of soil particles than other types of land use. In addition, the levels of 17 PCDD/Fs congeners in OBS and EW (OBS) soils indicated high and moderate (1654 and 260 in one million people) cancer risks through the above pathway. Furthermore, the biologically derived TCDD concentrations (TEQ_{bio}) were also significantly correlated to the chemically derived toxic equivalent concentrations of dioxin-like chemicals (TEQ_{cal} (sum of chemically derived 2,3,7,8-TeCDD toxic equivalent concentrations ($TEQ_{PCDD/F}$) and chemically derived dioxin-like PAHs toxic equivalent concentrations (TEQ_{PAH})) (r= 0.770, p <0.05). PCDD/Fs (95.4 to 99.9 %) were the major stressor to the TEQ_{cal} in the soil samples, indicating higher concentrations of PCDD/Fs derived from chemical

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (doi:10.1007/s11356-014-3909-1) contains supplementary material, which is available to authorized users.

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analyses may reflect a higher potency of inducing EROD activity.

Keyword PCDD/Fs · Health risk assessment · Open burning · EROD assay · Agricultural soil

Introduction

Dioxin is a family of toxic chemicals that includes seven polychlorinated dibenzodioxins (PCDDs), ten polychlorinated dibenzofurans (PCDFs) and twelve polychlorinated biphenyls (PCBs) (US EPA 2010). The most toxic congener of the PCDDs is 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TeCDD), which may be related to skeletal deformities, kidney defects, disruption of sex hormones, reduction of sperm, and increased miscarriage rates in animal tests (ATSDR 1998). Three recent epidemiologic cohort studies also reported that human exposure to 2,3,7,8-TeCDD could be resulted in decreased sperm count and motility in men, and increased thyroid hormone metabolism in neonates (Baccarelli et al. 2008; Mocarelli et al. 2008, 2011).

Though TeCDD has been found to cause cancer in animals in many independent studies since 1977 (US DHHS 2011), there is no conclusively proven evidence that 2,3,7,8-TeCDD is cancer causing in humans. Boffetta et al. (2011) concluded that there is still insufficient evidence of proving that 2,3,7,8-TeCDD is cancer causing in humans, based on limited epidemiologic data. Therefore, evidence for proving a causal link between 2,3,7,8-TeCDD and cancer risks based on epidemiologic studies has remained controversial.

However, there are sufficient evidence for proving (1) 2,3,7,8-TeCDD carcinogenesis by animals tests and (2) mechanism of 2,3,7,8-TeCDD carcinogenesis via initial binding to the aryl hydrocarbon receptor (AhR), leading to changes in gene expression, cell replication and apoptosis in humans and animals, which were sufficiently robust enough for proving 2,3,7,8-TeCDD as a carcinogen (Baan et al. 2009; Nebert et al. 2000). In general, TeCDD's carcinogenicity was defined by three organizations as: "probable human carcinogen" (US EPA), "carcinogenic to humans" (International Agency for Research on Cancer or IARC), and "known human carcinogen" (National Toxicology Program or NTP) (ATSDR 1998).

Since the 1970s, due to the rapid urban development in the rural part of Hong Kong (New Territories), a lot of farmlands have been vanished and the remaining ones were slowly changed to greater profit-making purposes such as car dismantling workshops (CDW) and electronic waste (e-waste) recycling (dismantling and open burning) (Bryant et al. 1982; Lindstrom et al. 1999). Nevertheless, persistent toxic substances such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and PCDD/Fs could be released into the surrounding farm soil and potentially exerted adverse health effects on locals residents (Man et al. 2011; 2013a; 2013b).

In addition, ethoxyresorufin-O-deethylase (EROD) assay is commonly applied as a marker for AhR agonists, such as PCDD/Fs (Gooch et al. 1989; Schmitz et al. 1995). The 2,3,7,8-TeCDD is believed to be the most active inducer of EROD activity. If the sample is related to TeCDD or TeCDD-like substance, it will induce the similar level of EROD activity as the positive control of TeCDD, thus presenting the EROD activity in terms of TeCDD concentrations (Safe 1990). EROD assay can be used not only when chemical analyses of PCDD/Fs are not available but also to detect the overall PCDD/Fs-like substance in an environmental sample matrix (Tsang et al. 2009).

With the above background, it was hypothesized that soils from different types of land use may generate different levels of PCDD/Fs to the surrounding environment, leading to different degrees of non-cancer and cancer risks exerted on locals. The major objective of this study was to assess non-cancer and cancer risks that are attributable to PCDD/Fs in soils of different land use types through three exposure pathways, namely ingestion, dermal contact, and inhalation. Furthermore, the current study was also aimed at evaluating the biologically derived PCDD/Fs level in the soils of different land use types by an EROD assay.

Materials and methods

Sampling and preparation

The Σ PCDD/Fs-World Health Organization toxicity equivalent concentration (PCDD/F WHO-TEQ or $TEQ_{PCDD/F}$) were previously reported in a study by Man et al. (2013b), where its major difference with this study is that it involved PCDD/Fs concentrations used for human health risk evaluation, and EROD assay was used for assessing the overall biologically derived dioxin-like substance in the samples. Briefly, in total, 275 composite soil samples were collected from 55 agricultural areas in Hong Kong. Each site was grouped according to their current land use, leading to 12 identified soil types: (1) organic farm (OF), (2) agricultural (A), (3) abandoned agricultural (Ab), (4) e-waste storage (EW (S)), (5) container storage (CS), (6) metal recycling workshop (MRW), (7) construction waste (CW), (8) petrol station (PS), (9) open burning site (OBS), (10) e-waste dismantling workshop (EW (DW)), (11) e-waste open burning site (EW (OBS)), and (12) car dismantling workshop (CDW) (Fig. 1). Descriptions of each type of land use, and the number of sites investigated are listed in Table 1. For most of the land use types, samples were taken within the agriculture area. However, for some of the land use types (EW (S), CS, MRW, PS, and CDW) where no soils are

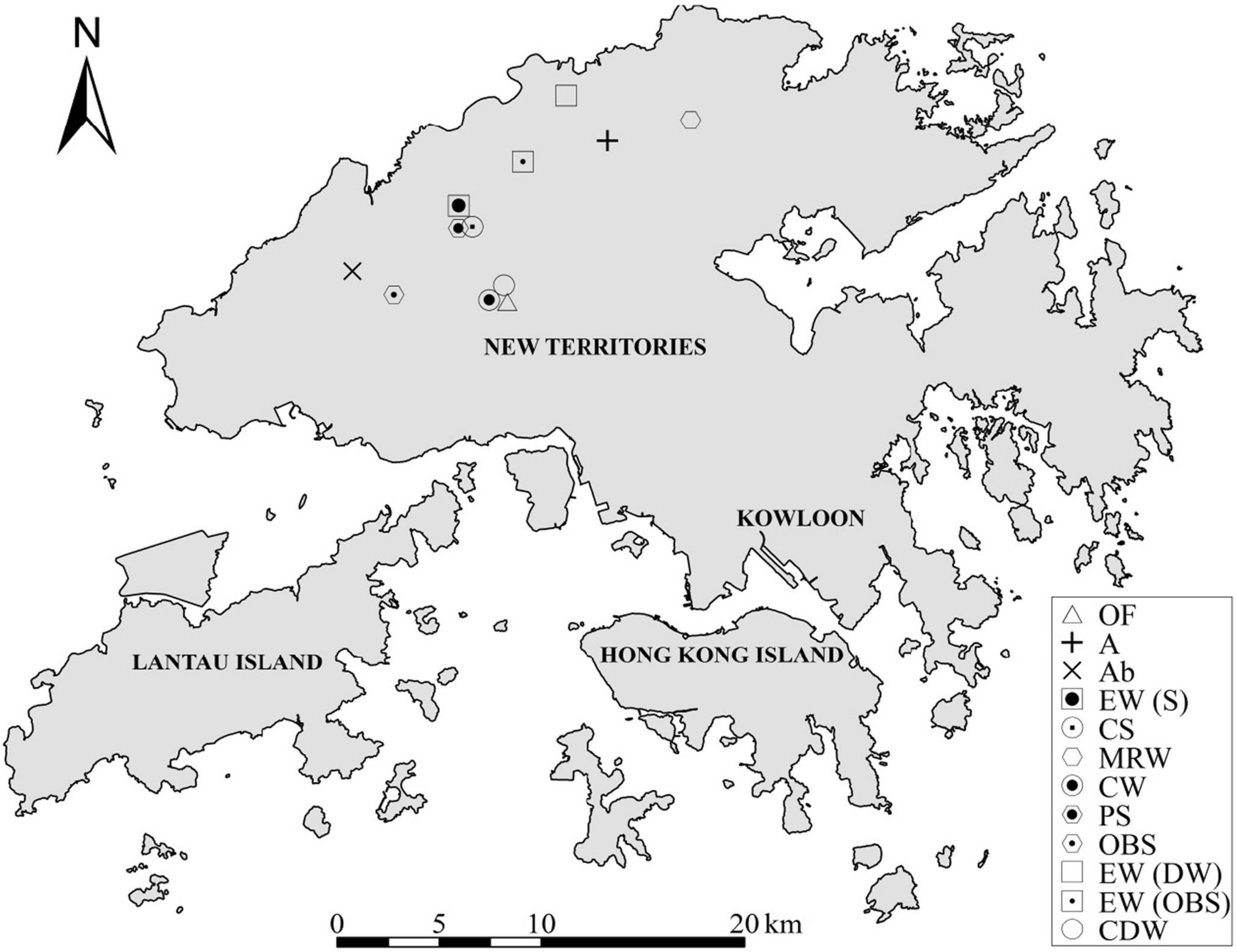

Fig. 1 Soil sampling map of the 12 different types of land use (Man et al. 2013b). *OF* organic farm, *A* agricultural, *Ab* abandoned agricultural, *EW* (*S*) e-waste storage, *CS* container storage, *MRW* metal recycling workshop, *CW* constructions waste, *PS* petrol station, *OBS* open burning site, *EW* (*DW*) e-waste dismantling workshop, *EW* (*OBS*) e-waste open burning site, and *CDW* car dismantling workshop

available within the areas, samples were taken from the periphery of the area, with less than 10 m from the source of activities.

Each of the sampling area was separated into 5 equal soil sampling areas. In each of the 5 subsoil sampling areas, 5 subtop soil samples (0–5 cm) were randomly collected using a stainless steel spade to form an individual composite soil sample (0.5 kg). Hence, there were 275 composite soil samples in total (55 sampling areas×5 composite soil samples in each area=275 composite soil samples) were collected from the 55 sampling areas. All soil samples were freeze-dried for at least 2 weeks, sieved through a 2 mm mesh and stored in at −20 °C in glass container for subsequent analyses.

Criteria for choosing soil samples for the analysis of PCDD/Fs

Man et al (2013a) previously reported the concentrations of 16 PAHs (naphthalene (Nap), acenaphthylene (Any), acenaphthene (Ane), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (IcdP), dibenz(a,h)anthracene (DahA), and benzo(g,h,i)perylene (BghiP)) in the same study area. Burning activities including municipal waste and e-waste can release large amounts of PAHs and PCDD/Fs to the surrounding environment (Chien 2004; Wong et al. 2007). It was necessary to cut down the number of PCDD/Fs samples due to their high analytical cost. It was assumed that open burning activities would generate PAHs and PCDD/Fs simultaneously; and hence, for each type of land use, the soil samples with the maximum total PAHs concentration (sum of 16 individual PAHs concentrations) were selected for PCDD/Fs analysis, yielding 1 (maximum)×12 (land uses)=12 soil samples (Man et al. 2013a) (Table S1).

Table 1 Descriptions of the different assorted agricultural land uses and their respective number of sites under investigation (Man et al. 2013b)

Different types of agricultural land use	Number of sites	Site description
1 Organic farm (OF)	5	Growing vegetable without using chemical fertilizer, pesticide, and recycle nutrients within the cultivated area
2 Agricultural (A)	20	Traditional farming system and legal chemical fertilizer and pesticides are allowed
3 Abandoned agricultural (Ab)	11	Former cultivating area without vegetation or cover with wild grass
4 E-waste storage (EW (S))	1	Electronic waste storage sites with concrete floor and surrounded by concrete wall
5 Container storage (CS)	1	Container storage with concrete floor and surrounded by metal wall, heavy loading of containers by trucks
6 Metal recycling workshop (MRW)	1	Recycling of heavy metals with concrete floor and surrounded by metal wall and with heavy machine press different assorted of bulky metals into smaller volume
7 Construction waste (CW)	3	Dumpling of demolishing building materials including sand, wood, scrap metal, concrete, bamboo, etc..
8 Petrol station (PS)	2	Petrol filling station with concrete floor and surrounded by concrete walls
9 Open burning site (OBS)	2	Existing agricultural land with small areas to burn bulky woody furniture, household waste, and wild grass
10 E-waste dismantling workshop (EW (DW))	3	Breaking down electronic components such as refrigerators, computers, and printers on exciting agricultural land
11 E-waste open burning site (EW (OBS))	1	Burning electronic components such as refrigerators, computers, cables, and printers on existing agricultural land
12 Car dismantling workshop (CDW)	5	Repairing and breaking down cars or vehicles on existing agricultural land

Chemical analysis of PCDD/Fs

Analysis of PCDD/Fs, including 17 2,3,7,8-substituted PCDD and PCDF congeners ((1) 2,3,7,8-TeCDD, (2) 1,2,3,7,8-PeCDD, (3) 1,2,3,4,7,8-HxCDD, (4) 1,2,3,6,7,8-HxCDD, (5) 1,2,3,7,8,9-HxCDD, (6) 1,2,3,4,6,7,8-HpCDD, (7) OCDD, (8) 2,3,7,8-TeCDF, (9) 1,2,3,7,8-PeCDF, (10) 2,3,4,7,8-PeCDF, (11) 1,2,3,4,7,8-HxCDF, (12) 1,2,3,6,7,8-HxCDF, (13) 2,3,4,6,7,8-HxCDF, (14) 1,2,3,7,8,9-HxCDF, (15) 1,2,3,4,6,7,8-HpCDF, (16) 1,2,3,4,7,8,9-HpCDF, and (17) OCDF) measured in the soil samples, were conducted according to the Standard Method 1613 (US EPA 1994) by the Dioxin Analysis Laboratory of the Hong Kong Baptist University. Three grams of each soil sample and blanks were spiked with a mixture of 15 ^{13}C-labeled internal standards and then Soxhlet extracted with toluene for 16 h. After extraction, $^{37}Cl_4$-2,3,7,8-TeCDD certified reference standard (CRS) was spiked, followed by acid/base silica gel, acid alumina, and activate carbon cleanup. A mixture of 17 PCDD/F congeners (10 μl) was then injected into each soil extract for precision and recovery (PAR) and topped up with 2 mL nonane. The extracts were analyzed using a high-resolution gas chromatograph combined with a high-resolution mass spectrometer (HRGC/HRMS) (Waters Co., Ltd., Korea). Only the 12th soil sample was analyzed in duplicate with the analytical blanks included in the batch. No detectable concentration of PCDD/Fs was found in any of the analytical blanks. Mean recoveries of PCDD/Fs ranged from 54±15 % for IS $^{13}C_{12}$-2,3,7,8-TeCDF to 99±18 % for IS $^{13}C_{12}$-OCDD, and the PCDD/Fs clean up was 73±7.7 % $^{37}Cl_4$-2,3,7,8-TeCDD.

The aforementioned 17 2,3,7,8-substituted PCDD and PCDF congeners with their respective toxic equivalency factors (TEFs) were used to calculate the chemically derived 2,3,7,8-TeCDD toxic equivalent concentration (PCDD/F WHO-TEQ or $TEQ_{PCDD/F}$) (Van den Berg et al. 1998). In addition, the date of chemically derived seven dioxin-like PAHs (BaP, BaA, BbF, BkF, Chr, DahA, and IcdP) toxicity equivalent concentration (TEQ_{PAH}) from the 12 soil samples mentioned in the above section were also used to find out the major stressor contributed to TEQ_{cal} (sum of $TEQ_{PCDD/F}$ and TEQ_{PAH}) (Man et al. 2013a).

Exposure scenarios and health risk assessment equations

In this study, potential non-cancer and cancer risks imposed on workers or farmers and children as a result of being in contact with contaminated soil were assumed to occur via three major exposure pathways. These included: accidental ingestion of soil particles, dermal absorption of pollutants via soil particle contact, and inhalation of fugitive soil particle, with potential non-cancer and cancer risks via these means estimated using the equations (A1), (A2) (US EPA 1989), and (A3) (US EPA 2009) listed in the supplementary data. In addition, the details and parameters used in the health risk assessment were also listed in the supplementary data.

Extraction of soil samples for EROD assay

Soxhlet extraction and clean up of soil samples were performed according to Standard Method 3540C (US EPA 1996a) and Standard Method 3620B (US EPA 1996b). Briefly, 2 g of soil samples and 10 g of anhydrous sodium sulfate were added into a cellulose extraction thimble and inserted into a Soxhlet fitted with a 250 mL flask. Subsequently, 150 mL of dichloromethane and acetone (v:v 1:1) was added, and the whole set up was then heated for 18 h in a water bath at 69 °C. The extracts were concentrated to 10 mL by a rotary evaporator and used for subsequent clean up. Florisil column clean up was applied for purification of the concentrated extract. Finally, the elutes were concentrated to less than 1 mL and exchanged to and topped up with 1 mL of dimethyl sulfoxide (DMSO) by a rotary evaporator. Each soil sample from the 12 different types of land use was extracted in triplicates. Therefore, three individual soil samples of each 12 different types of soil were used to calculate the mean and standard deviation.

Cell culturing for EROD assay

The EROD assay has been widely used as a biomarker of exposure to environmental contaminants such as PCDD/Fs. Firstly, H4IIE rat hepatoma cells were cultured in Dulbecco's modified eagle medium (DMEM) (GIBCO, Germany) supplemented with 10 % (v/v) fetal bovine serum, 100 units/mL penicillin, and 100 mg/mL streptomycin. The cells were then incubated at 37 °C with 5 % CO_2 and 80 % humidity (Sanyo, Japan). The stock cultures of H4IIE cells were detached from the well by trypsin. The cells were quantified and diluted into the growth medium to achieve the desired number of cells (1×10^5/mL). The cells were seeded into 96-well culture plates at 100 μL per well (i.e., about 10,000 cells per well), and the plates were incubated at 37 °C with 5 % CO_2 in an incubator. After 24 h, a monolayer was formed embracing of 70–80 % cell coverage (Luo et al. 2009; Qiao et al. 2006).

EROD assay

The culture medium was removed and replaced by a culture medium containing TeCDD standard solutions or the sample solutions. The standard solutions (0.020, 0.039, 0.078, 0.156, 0.3125, 0.625, 1.25, 2.5, 5, 10 ng/mL DMSO, n=10) were prepared by serial dilution of TeCDD stock solution, producing a dose–response curve of EROD induction. The standard solutions were added on each plate as positive controls for calibration purposes. The extract solutions of each sample were diluted to six concentration levels by two-fold dilution. In each well, the final concentration of DMSO was 0.5 %. A 0.5 % DMSO solution was also used as solvent control. Each test solution was assayed in triplicates, including three solvent controls and three blanks. The wells at the edges of the 96-well culture plates were added with PBS and incubated for 24 h at 37 °C. The culture medium was removed and was replaced with 100 μL fresh medium containing 10 μM 7-ethoxyresorufin (Sigma, E3763, USA) as substrate and 10 μM dicumarol (Sigma, M1390, USA). Cells were incubated for another 60 min at 37 °C in an atmosphere of 95 % air and 5 % CO_2. The reaction was stopped by the addition of 130 μL of methanol. The cell medium was carefully mixed in the titer plate shaker and a 100 μL aliquot from each well was transferred to a new 96-well plate for fluorescence measurement using a microplate reader (TECAN-Genios, Austria) at the excitation and emission wavelengths of 535 and 590 nm, respectively. Resorufin standard curve was analyzed at the same time. Resorufin EROD enzyme activity was expressed as mean picomoles of resorufin produced per minute per milligram of microsomal protein (pmol min^{-1} mg $protein^{-1}$). Data were analyzed by a sigmoid nonlinear curve-fitting module. The function is a four-parameter logistic equation (B) (Luo et al. 2009; Qiao et al. 2006) listed in the supplementary data.

The EROD assay or biologically derived TeCDD equivalent concentrations (expressed as TEQ_{bio}) in soil samples were then obtained by comparing with the dose–response curve of the standard solution on the same plate. Dilution of the extract of soil samples might be necessary so as to fit the TeCDD dose–response curve. The mean solvent control response was subtracted from both sample and standard responses on a plate-by-plate basis. The detection limit for the H4IIE assay was 18.5 fg TeCDD/well, corresponding to an activity equal to three times the standard deviation of the mean solvent control response (Luo et al. 2009; Qiao et al. 2006). Finally, the EROD responses of the samples were corrected with the percentage of cell viabilities with MTT assay (Mosmann 1983).

Statistical analysis

Statistical analyses including variance analysis and Pearson correlation were conducted using SPSS 16.0 for Windows. Variance analysis (alpha=0.05) of EROD assay activity among 12 different types of land use was performed using one-way ANOVA test. Pearson correlation (alpha=0.01 and alpha=0.05) was used to test correlation between TEQ_{cal} and TEQ_{Bio}.

Results and discussion

Table S2 (supplementary data) lists the PCDD/F WHO-TEQ or $TEQ_{PCDD/F}$ of 12 different types of land use, which were all above The New Dutch List (Dutch Indicative Level (1 pg/g)) (VROM 2000) and Canadian Environmental Quality Guidelines for agricultural and industrial use (4 pg/g) (CCME 2010). In addition, soil samples from OBS and EW (OBS) contained PCDD/F WHO-TEQ of 24,922 and 3918 pg/g, respectively, which exceeds the Environmental Quality Standard (1000 pg/g) from Japan (MOE 2003).

When comparing the present results with more stringent soil quality guidelines (SQGs) such as the Dutch Indicative Level (VROM 2000), all of the land use soils in this study may need to be remediated in order to lower their potential risks to humans. However, when comparing with a less stringent one, such as the Environmental Quality Standard (MOE 2003), remediation may not be necessary among all types of land use soils. However, in the case of the OBS and EW (OBS) sites, urgent remediation action is potentially needed since values were approximately 25 and 4 times higher, respectively, than the less stringent Japanese SQG (MOE 2003).

The sample from OBS contained the most elevated concentrations of dioxins (PCDD/F WHO-TEQ=24,922 pg/g) among the 12 sampling sites since the combustion of municipal waste, especially those containing PVC plastic, gives rise to this (Carroll and William 1995). Municipal waste incineration and fossil fuel combustion are common sources of dioxin emission to the atmosphere of industrialized countries and likely to generate a high level of PCDD/Fs to the surrounding environment (Chien 2004). The burning activities of municipal waste observed in OBS and EW (OBS) are thus the possible origin of dioxins. In addition, the frequent transportation of waste to and from the sites would also cause the release of dioxins via engine combustion as a result of energy consumption from fossil fuels.

According to Table 2, ingestion and dermal contact exposure Hazard Quotient (HQ) of adults indicate that soil samples from OBS and EW (OBS) far exceeded the unity with different levels of non-cancer risk (50.9 (high), 8.00 (moderate); and 10.1 (high), 1.58 (low), respectively). In contrast, HQ of children showed soil samples from Ab (1.77), CS (1.47), OBS (407), EW (OBS) (64.0), and CDW (2.23) exceeded the unity via the exposure pathway of ingestion, and OBS (34.2) and EW (OBS) (5.38) exceeded the unity via the exposure pathway of dermal contact. These sites may potentially cause adverse non-cancer effect on adults and children. Alternatively, very low non-cancer risks were noted among all types of land use via the exposure pathway of inhalation of soil particles. Therefore, human exposure to soil of EW (OBS) and OBS exerted relatively higher non-cancer risks via dermal contact and accidental ingestion of soil/dust particles than other soil types.

Ma et al. (2008) also demonstrated that changing agricultural land use to e-waste recycling sites (dismantling workshop and open burning) can exert a greater human health risk for dioxin exposures via dermal contact and accidental ingestion of soil/dust particles in Fengjiang town, Taizhou city, Zhejiang province, eastern China. The estimated daily intakes of TEQs of PCDD/Fs via the above exposure pathways were 0.363 and 2.3 pg TEQ/kg bw day for adults and children, respectively, which were at least 1200 times (0.0003 and 0.0013 pg TEQ/kg bw day for adults and children, respectively) higher than residents living at an urban reference site in Wenling town, Taizhou, Zhejiang province, eastern China.

In addition to the non-cancer risk, based on the ingestion exposure pathway, all of the 12 types of land use soils were classified as either having very low or low cancer risks, except for the soil samples from OBS and EW (OBS) which registered as high (1654×10^{-6}) and moderate (260×10^{-6}) cancer risks, respectively. However, soil samples from OBS and EW (OBS) were registered as moderate (327×10^{-6}) and low (51.5×10^{-6}) cancer risk, respectively, via the dermal contact exposure pathway. In contrast, again, very low cancer risks were noted among all types of land use via inhalation of soil particles (Table 2). Based on the concentrations of PCDD/Fs, soils from OBS and EW (OBS) posed the greatest non-cancer and cancer risk among the 12 different sampling sites, which probably resulted from the combustion activities of these sites. It has been reported that combustion and thermal processes are the major sources of the release of PCDDs (Fiedler 1996).

Chan and Wong (2012) studied the magnitude of exposures for dioxins in e-waste recycling sites in China and noted the following descending order: (1) soil and dust ingestion,(2) air inhalation, and (3) dermal contact. However, the observed results were in the following descending order: (1) soil and dust ingestion,(2) dermal contact, and (3) air inhalation. The observed inhalation non-cancer and cancer risks seemed very low when compared to other exposure routes (ingestion and inhalation). This can be greatly attributed to the lack of detection of PCDD/Fs in air samples of the respirable particles (PM_{10} and/or $PM_{2.5}$). Rovira et al. (2011) indicated that when air samples of the respirable particles (PM_{10}) were applied for detecting PCDD/Fs concentrations, inhalation was found to be the most important exposure route in the vicinity of a cement plant. PCDD/Fs fine particles are likely to produce in the open burning sampling sites (OBS and EW (OBS)) as a consequence of burning activities. One of the limitations of this study was the use of soil particles of less than 2 mm instead of PM_{10} and/or $PM_{2.5}$ for the risk assessment evaluation via the inhalation pathway. Only one sample for each type of land use was employed for the analysis of PCDD/Fs (due to the high costs), and therefore the assessment of cancer risk was restricted to 12 samples only. However, the present study provided a general trend on the release of PCDD/Fs and the potential human health impacts due to changing land use.

The samples of this study were collected once, with only five composite samples per site. In order to have a fuller picture of seasonal (wet and dry seasons) studies of soil

Table 2 Non-cancer and cancer risks via ingestion, dermal contact, and inhalation of 12 different soils based on the total burden of PCDD/F WHO-TEQ or $TEQ_{PCDD/F}$

Sampling sites	Hazard quotient (HQ) of adult			Hazard quotient (HQ) of children			Cancer risk of human		
	Ingestion	Dermal contact	Inhalation	Ingestion	Dermal contact	Inhalation	Ingestion	Dermal contact	Inhalation
OF	0.0285	0.00565	1.26E-09	0.228	0.0192	1.84E-09	0.927	0.184	1.33E-11
A	0.0119	0.00236	5.26E-10	0.0954	0.00802	7.68E-10	0.388	0.0768	5.56E-12
Ab	0.221	0.0437	9.73E-09	**1.77**	0.148	1.42E-08	**7.17**	**1.42**	1.03E-10
EW (S)	0.0197	0.00389	8.67E-10	0.157	0.0132	1.26E-09	0.639	0.127	9.15E-12
CS	0.0102	0.00202	4.50E-10	0.0816	0.00686	6.57E-10	0.332	0.0657	4.75E-12
MRW	0.0293	0.00579	1.29E-09	0.234	0.0197	1.88E-09	0.951	0.188	1.36E-11
CW	0.184	0.0365	8.13E-09	**1.47**	0.124	1.19E-08	**5.99**	**1.19**	8.58E-11
PS	0.00481	0.000953	2.12E-10	0.0385	0.00323	3.10E-10	0.156	0.031	2.24E-12
EW (OBS)	**50.9**	**10.1**	2.24E-06	**407**	**34.2**	3.27E-06	**1654**	**327**	2.37E-08
OBS	0.0937	0.0185	4.13E-09	0.749	0.0629	6.03E-09	**3.04**	0.603	4.36E-11
EW (DW)	**8**	**1.58**	3.53E-07	**64**	**5.38**	5.15E-07	**260**	**51.5**	3.73E-09
CDW	0.278	0.0551	1.23E-08	**2.23**	0.187	1.79E-08	**9.05**	**1.79**	1.30E-10

Bold entries indicate potential non-cancer risk (all cancer risks are presented in units of 10^{-6})

OF organic farm, *A* agricultural, *Ab* abandoned agricultural, *EW* (*S*) e-waste storage, *CS* container storage, *MRW* metal recycling workshop, *CW* constructions waste, *PS* petrol station, *OBS* open burning site, *EW* (*DW*) e-waste dismantling workshop, *EW* (*OBS*) e-waste open burning site, *CDW* car dismantling workshop

PCDD/Fs, concentrations of different types of land use and their subsequent risks exerted on human health should be included as different weather conditions may affect the PCDD/Fs concentrations retained in soil and local population, with different tendencies and behaviors of soil exposure (US EPA 2014).

It is worth noting that there are uncertainties associated with the results of risk assessments. All the exposure factors, such as ingestion rate (*IngR*) and surface area of the skin that contacts the soil (SA) of adults and children via ingestion, and dermal contact for the health risk assessments were fixed by default values (supplementary data) (Calabrese et al. 1987; US EPA 2011, 2014). Default values of exposure factors are commonly used in health risk assessments due to the fact that it is more handy for screening a large number of potentially polluted sites and the resulting risk values can be compared more easily between site to site. Nonetheless, these default values may not sufficiently and accurately describe the reality of all the sites as the exposure factors would change during various stages of life (especially the physiological and behavioral factors of children) and environmental conditions (US EPA 2011, 2014).

Furthermore, oral slope factor and inhalation unit risk (IUR) in the US EPA, (2014) Mid-Atlantic Risk Assessment Regional Screening Level Summary Table, were used to calculate cancer risk from 2,3,7,8-TeCDD exposure. However, the US EPA agency-wide toxicity/dose–response assessment for 2,3,7,8-TeCDD has not yet been finalized. Furthermore, the US EPA risk assessment assumes TeCDD to be a direct-acting non-threshold carcinogen. In contrast, there is a broad-based consensus among international agencies (EC 2000; Health Canada 2009; WHO 1999, 2002) that TeCDD is a threshold carcinogen based on the current scientific understanding on its mode of action. The strongest evidence of cancer-causing effects of TeCDD can be found in individuals whom have been exposed to it at 100 to 1000 times higher than the general population (WHO 2002). The resulting cancer effects are not the most sensitive when compared to other toxic effects in which they only occur at higher body burdens in animals unlike developmental, reproductive, and hormonal effects. The WHO (2002) concluded that the establishment of a tolerable intake based on these effects would also address any cancer risk. As a result, countries (Netherlands, UK, Japan, Australia, European Commission, Germany, Canada) other than the US have either adopted the WHO's evaluation or evaluated TeCDD using a threshold model. Based on a conservative approach, the oral slope factor and IUR in the US EPA (2014) Mid-Atlantic Risk Assessment Regional Screening Level Summary Table were used to conduct the cancer risk assessment of this study.

Figure 2 shows the EROD assay-derived or biologically derived 2,3,7,8- TeCDD concentration (TEQ_{bio}) of the soil samples. It showed that the TEQ_{bio} in the composite samples ranged from $\log_{10}$ 1.21 (OF) to $\log_{10}$ 2.71(OBS)pg/g (16.3 (OF) to 935(OBS)pg/g). Samples from OBS revealed the highest TEQ_{bio} and $TEQ_{PCDD/F}$ $\log_{10}$ 4.40 pg/g (24,922 pg/g). Bioassays are applied to estimate the total biological toxicity of composite pollutants in complex environmental matrices (Giesy et al. 2002). EROD assay is commonly used as an integrated response by AhR agonists (such as PCDD/Fs, dioxin-like PAHs, and PCBs) in soil samples in comparison

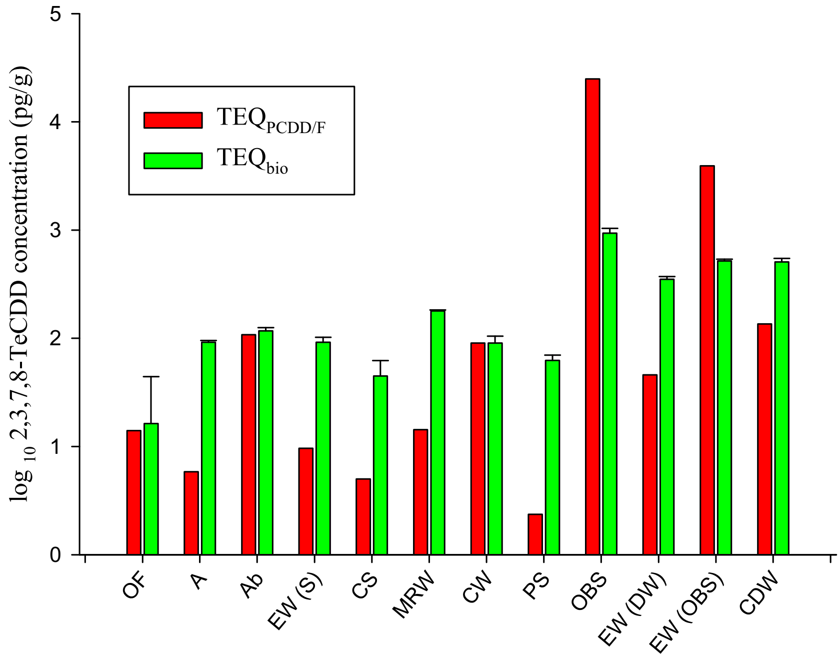

Fig. 2 Comparison of $TEQ_{PCDD/F}$ (pg/g) and TEQ_{bio} (pg/g) of the 12 different types of land use. *OF* organic farm, *A* agricultural, *Ab* abandoned agricultural, *EW* (*S*) e-waste storage, *CS* container storage, *MRW* metal recycling workshop, *CW* constructions waste, *PS* petrol station, *OBS* open burning site, *EW* (*DW*) e-waste dismantling workshop, *EW* (*OBS*) e-waste open burning site, *CDW* car dismantling workshop, $TEQ_{PCDD/F}$ chemically derived 2,3,7,8-TeCDD toxic equivalent concentration, and TEQ_{bio} biologically derived TeCDD equivalent concentrations; and the data was log transformed

with chemical analysis (Shen et al. 2008). Therefore, EROD assay was conducted to anticipate the integrated response induced by AhR agonists in the composite soil samples in the present study. Soils from OBS and EW (OBS) revealed relatively higher TEQ_{bio} and $TEQ_{PCDD/F}$ than other types of land use. TeCDD is a member of PCDD/F (dioxins) that is present as by-products in the manufacture of organochlorides, incineration of chlorine-containing substances such as PVC (polyvinyl chloride), chlorine bleaching process at pulp, and is formed during chlorination by waste and drinking water treatment plants (ATSDR 1999). However, in this study, relatively high concentrations of TeCDDs detected in OBS and EW (OBS) were probably attributed to combustion activities (burning of agricultural waste, municipal waste, furniture, and electronic waste) in these sites.

In the current study, TEQ_{bio} were significantly related to TEQ_{cal} (r=0.770, p <0.05) among the soils of 12 different types of land use. As an AhR agonist, PCDD/Fs (95.4 to 99.9 %) contributed much more to TEQ_{cal} than PAHs did (0.04 to 3.22 %) (Figs. S1 and S2, supplementary data). A previous study showed a prominent positive correlation (r= 0.960, p <0.001) between TEQ_{bio} and TEQ_{cal} (sum of chemically derived dioxin-like PCBs toxicity equivalent concentration TEQ_{PCB}, $TEQ_{PCDD/F,}$ and TEQ_{PAH}) in soils of e-waste site, with the following descending order: (1) PCBs (87.2 to 98.2 %), (2) PCDD/Fs (1.7 to 11.6 %), and (3) PAHs (0 %) (Shen et al. 2008). In addition, Qiao et al. (2006) demonstrated that there was a significant positive correlation between TEQ_{bio} and TEQ_{cal} (sum of TEQ_{PCB}, $TEQ_{PCDD/F,}$ and TEQ_{PAH}) (r=0.85, p <0.01) for sediment samples, with the following descending order: (1) PAHs (70 to 93 %), (2) PCDD/Fs (<30 %), and (3) PCBs (0 %). Our study is in line with the above two studies related to the dose–response relations between $TEQ_{bio\ and}$ TEQ_{cal} (Qiao et al 2006; Shen et al. 2008). However, the present study identified that PCDD/Fs (95.4–99.9 %) was the major stressor contributed to TEQ_{cal} in the soil samples (Fig. S2, supplementary data), whereas Shen et al. (2008) and Qiao et al (2006) showed that PCBs (87.2–98.2 %) and PAHs (70–93 %) were major stressors contributed to TEQ_{cal} in soil and sediment samples, respectively. One of the limitations of this study was that TEQ_{PCB} was not available for comparison with the studies of Shen et al. (2008) and Qiao et al (2006). However, this study has proven that EROD assay is also a reliable and sensitive method to determine the $TEQ_{PCDD/F}$-related toxic potency in H4IIE cells for contaminated soil.

Conclusion

The soil samples from OBS and EW (OBS), which contained the relatively higher PCDD/F WHO-TEQ or TEQ_{TCDD} among the 12 land use types would exert relatively higher potential non-cancer and cancer risks on humans. The observed non-cancer and cancer risks via the exposure pathway of inhalation seem very low when compared to other exposure routes (ingestion and dermal contact) by using soil particles less than 2 mm. Future studies using the respirable particles (PM_{10} and/or $PM_{2.5}$) to assess non-cancer and cancer risk via inhalation in OBS and EW (OBS) are essential. TEQ_{bio} and TEQ_{cal} were also significantly correlated among soils of 12 different types of land use. This suggests that the higher concentration of dioxin may reflect a higher potency in inducing EROD activity.

Acknowledgments The authors would like to thank the Start-up Research Grant for Newly Recruited Professors/(Research) Chair Professors, The Hong Kong Institute of Education (RG24/13-14R) for financial support.

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