



## Research article

# Comprehensive evaluation of dioxins and dioxin-like compounds in surface soils and river sediments from e-waste-processing sites in a village in northern Vietnam: Heading towards the environmentally sound management of e-waste



Go Suzuki <sup>a,\*</sup>, Masayuki Someya <sup>b</sup>, Hidenori Matsukami <sup>a,c</sup>, Nguyen Minh Tue <sup>d,e</sup>, Natsuyo Uchida <sup>a</sup>, Le Huu Tuyen <sup>d,e</sup>, Pham Hung Viet <sup>e</sup>, Shin Takahashi <sup>f</sup>, Shinsuke Tanabe <sup>d</sup>, Abraham Brouwer <sup>g</sup>, Hidetaka Takigami <sup>a</sup>

<sup>a</sup> Center for Material Cycles and Waste Management Research, National Institute for Environmental Studies (NIES), Tsukuba 305-8506, Japan

<sup>b</sup> Tokyo Metropolitan Research Institute for Environmental Protection, 1-7-5 Shinsuna Koto, Tokyo 136-0075, Japan

<sup>c</sup> Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa 277-8563, Japan

<sup>d</sup> Center for Marine Environmental Studies, Ehime University, Matsuyama 790-8577, Japan

<sup>e</sup> Research Centre for Environmental Technology and Sustainable Development (CETASD), VNU Hanoi University of Science, 334 Nguyen Trai, Hanoi, Viet Nam

<sup>f</sup> Center of Advanced Technology for the Environment, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan

<sup>g</sup> BioDetection Systems, 1098 XH Amsterdam, The Netherlands

## ARTICLE INFO

## Article history:

Received 15 January 2016

Received in revised form

4 March 2016

Accepted 4 March 2016

Available online 12 April 2016

## Keywords:

Dioxin

Brominated dioxin

E-waste

Open burning

CALUX

## ABSTRACT

The management of electronic waste (e-waste), which can be a source of both useful materials and toxic substances, depending on the processing method, is important for promoting material cycling. In this study, we used the dioxin-responsive chemical-activated luciferase gene expression (DR-CALUX) assay combined with gas chromatography–high-resolution mass spectrometry to evaluate the levels of dioxin-like compounds in surface soils and river sediments collected in and around an e-waste-processing village in northern Vietnam. The WHO-TEQs (Toxic equivalents) of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), coplanar polychlorinated biphenyls (Co-PCBs), and polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) in soils collected in January 2012 ranged from 0.29 to 310 pg/g (median 2.9 pg/g,  $n = 32$ ), and the WHO-TEQs in sediments ranged from 0.96 to 58 pg/g (median 4.4 pg/g,  $n = 8$ ). Dioxin-like activities (CALUX-TEQs [2,3,7,8-TCDD equivalent]) in soils collected in January 2012, 2013, and 2014 ranged from <30 to 4300 pg/g (median <30 pg/g,  $n = 96$ ), and the activities in sediments ranged from <30 to 4000 pg/g (median 33 pg/g,  $n = 24$ ). Dioxin-like compounds accumulated in samples collected around e-waste-processing areas such as open-burning sites and e-waste-processing workshops, and the compounds may be transported from their sources to surrounding areas over the course of several years. Some of the CALUX-TEQs, but not WHO-TEQs, values were higher than the maximum acceptable WHO-TEQs promulgated by various authorities, indicating that all dioxin-like compounds should be evaluated in samples collected from e-waste-processing areas. Our findings suggest that open burning and open storage of e-waste should be prohibited and that wastewater treatment should be implemented at each workshop to reduce contamination by dioxin-like compounds from e-waste.

Copyright © 2016, The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author. Tel.: +81 29 850 2205; fax: +81 29 850 2269.

E-mail address: [g-suzuki@nies.go.jp](mailto:g-suzuki@nies.go.jp) (G. Suzuki).

Peer review under responsibility of KeAi Communications Co., Ltd.

## 1. Introduction

Waste from obsolete electronic devices and appliances such as personal computers, television sets, mobile phones, printers, and refrigerators is generated at a global rate of approximately 41.8 million tons per year, according to a press release by United



Production and Hosting by Elsevier on behalf of KeAi

Nations University [1]. Currently, large quantities of this waste, called e-waste, are recycled in both developed and developing countries because it contains considerable quantities of valuable and reusable metals, plastics, glass, and other materials. However, the use of certain e-waste-processing methods, such as open burning, especially in the developing world, has become an important issue in the last decade because of the adverse human health impacts of these methods [2–5]. Although several developing countries, including China, have enacted legislation focusing on the environmental effects of e-waste disposal and processing, the legislation enacted to date does not effectively regulate e-waste processing [6]. Furthermore, a recent study estimated that by 2018, developing countries will be disposing of more old computers than developed countries [7]. Up to now, the main approach to mitigating the impacts of undesirable e-waste processing has focused on reducing the amount of e-waste that developed countries export to developing ones, but in response to anticipated changes in the flow and generation of e-waste, new approaches will have to be explored.

Numerous studies have targeted contamination hot spots resulting from intensive e-waste-processing activities such as open burning and smelting and acid leaching to retrieve metals (reviewed by Man et al. [4] and Chan and Wong [5]). Many researchers have concluded that intensive e-waste processing is harmful to the environment and to human health. Therefore, our motivation in this study was to provide data for environmental samples collected not only from areas where open burning, an intensive processing activity, is conducted but also from areas where nonintensive processing activities such as collection, storage, and manual dismantling of e-waste are conducted. Since January 2012, our research group has been investigating e-waste-processing activities in Bui Dau, a village in My Hao district, Hung Yen Province, northern Vietnam, to elucidate the current levels of contaminants associated with various types of e-waste processing and to monitor the 3-year temporal trends not only of chemicals that may be initially present in e-waste, such as brominated and chlorinated flame retardants, phosphorus-containing flame retardants, and heavy metals but also of hazardous chemicals that may be generated during e-waste processing, such as chlorinated and brominated dioxins and dioxin-like compounds.

Specifically, we evaluated persistent dioxin-like compounds in surface soils and river sediments collected from the village. The levels of flame retardants such as polybrominated diphenylethers (PBDEs) and possible alternatives have already been reported by Matsukami et al. [8] and Someya et al. [9], and data for heavy metals will be reported elsewhere in the near future. In this study, we used gas chromatography-high resolution mass spectrometry (GC-HRMS) for measurement of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), coplanar polychlorinated biphenyls (Co-PCBs), and polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), and the dioxin-responsive chemical-activated luciferase gene expression (DR-CALUX) assay for all the dioxin-like compounds. First, we evaluated the levels of PCDD/Fs, Co-PCBs, PBDD/Fs, and emerging dioxin-like compounds in samples collected in January 2012, in order to elucidate the source of these contaminants and possible relationships among them. By using DR-CALUX assay, we also evaluated the levels of all the dioxin-like compounds in samples collected in January 2012, 2013, and 2014 to investigate the time-course of the levels of contaminants related to e-waste-processing activities. Finally, we tried to interpret the obtained results for identification of critical processes of contaminant formation and possible countermeasures for environmentally sound management of dioxin-like compounds derived from e-waste processing and to investigate the validity of using the DR-CALUX

assay in combination with GC-HRMS to assess the risks associated with e-waste processing activities.

## 2. Materials and methods

### 2.1. Sampling location and sample collection

The study location was an e-waste-processing site in the village of Bui Dau, My Hao district, Hung Yen Province, northern Vietnam. Detailed information about this area has been reported elsewhere [8,10]. The sampling area, which includes living areas and rice fields, covered a surface area of about 4.2 km<sup>2</sup>. The total population of the village reached approximately 3000 persons by 2015, according to a survey conducted for this study. The population and area of the village are much smaller than those of Guiyu (population, 150,000; land area, 52.4 km<sup>2</sup>) and Taizhou (population, 400,000; land area, 274 km<sup>2</sup>), which are well-known e-waste-processing sites in China [4]. E-waste processing in Bui Dau started in the 2000s [10], whereas Guiyu and Taizhou started processing e-waste in the late 1980s and 1970s, respectively. Hence, Bui Dau is a small-scale processing location and has a short history of e-waste processing; therefore, we expected that the degree of chemical contamination derived from processing activities would be low. A map of the specific sites in Bui Dau from which surface soils and river sediments were collected is shown in Fig. 1 (reproduced from Ref. [8]).

During each of the three sampling surveys in January 2012, 2013 and 2014, surface soils (depth 0–5 cm) were collected from 32 fixed locations including footpaths in rice paddies around the village ( $n = 19$ : SS-1 to SS-19), wires and cables open-burning area ( $n = 3$ : SS-20 to SS-22), and areas adjacent to e-waste-processing workshops ( $n = 10$ : SS-23 to SS-32), as shown in Fig. S1. River sediments were collected from eight locations including upstream of the e-waste-processing area (RS-1), the e-waste-processing area ( $n = 3$ : RS-2 to RS-4), and downstream of the e-waste-processing area ( $n = 4$ : RS-5 to RS-8). The above indicated surface soil and river sediment samples were collected in three sampling campaigns, respectively January 2012, 2013, and 2014; therefore, a total of 96 soil samples and 24 sediment samples were collected. Each sample was composed of five subsamples, which were collected with a stainless steel shovel from an area of approximately 10 m<sup>2</sup> and were placed in re-sealable polyethylene zipper storage bags. All samples were kept in a freezer in the Research Centre for Environmental Technology and Sustainable Development (CETASD), Hanoi University of Science, until they were transported from Vietnam to Japan with permission from the Ministry of Agriculture, Forestry, and Fisheries. All the imported samples were stored at –20 °C until analysis.

### 2.2. Sample pretreatment and extraction

After removal of pebbles, weeds, and twigs, the samples were air-dried and manually homogenized with a wooden hammer. Each air-dried sample was transferred to a stainless steel sieve (<2.0 mm), which was then covered with a steel lid and shaken manually. The sieved material was collected and stored in amber glass bottles at –20 °C until extraction.

Approximately 15 g of each sieved sample was extracted by means of a rapid thermal solvent extractor (SE100, Mitsubishi Chemical Analytech Co., Kanagawa, Japan) first at 35 °C for 40 min with 1:1 (v/v) acetone:*n*-hexane at a flow rate of 2 mL/min and then at 80 °C for 40 min with toluene at 2 mL/min. The combined extracts were subjected to solvent evaporation to a volume of 10 mL, and the resulting crude extracts were stored at 4 °C until WHO-TEQ and CALUX-TEQ determinations.

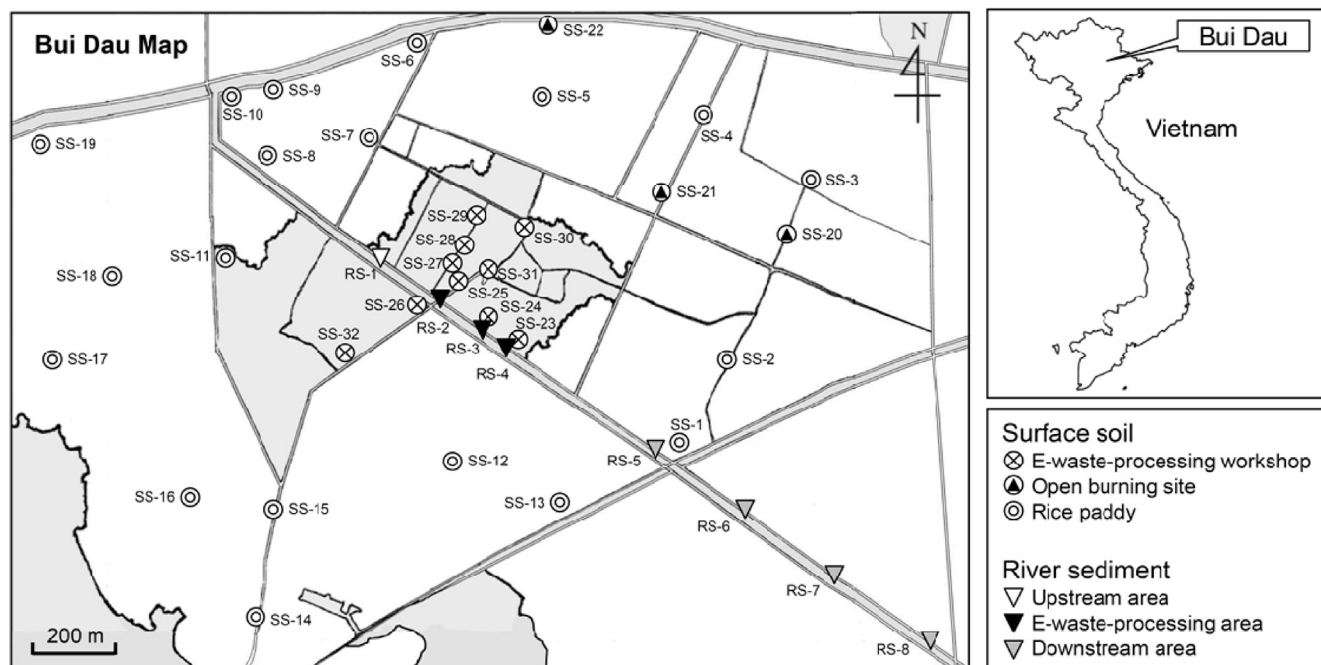


Fig. 1. Sampling locations of surface soils and river sediments in Bui Dau, Hung Yen province, Vietnam, January 2012, 2013, and 2014 (reproduced from Ref. [8]). SS-1 to SS-32: surface soil samples. RS-1 to RS-8: river sediment samples.

### 2.3. WHO-TEQ determination

Surface soils ( $n = 32$ ) and river sediments ( $n = 8$ ) collected in January 2012 were used to measure PCDD/F, Co-PCB, and PBDD/F concentrations for subsequent WHO-TEQ determinations. An aliquot of each crude extract (equal to 3 g of sample) spiked with  $^{13}\text{C}$ -labeled standards was exchanged from toluene to *n*-hexane, purified with sulfuric-acid treatment, a sulfoxide column, a multi-layer column, and then fractionated with an activated-carbon-dispersed silica gel column. Several extracts were treated with activated copper for removal of elemental sulfur. Each eluate was evaporated under a gentle stream of nitrogen gas, and the residue was redissolved in nonane containing  $^{13}\text{C}$ -labeled standards as a syringe spike for the subsequent GC-HRMS analysis. The conditions for measuring PBDD/F concentrations have been described previously [11], and the conditions for measurement of PCDD/F and Co-PCB concentrations using GC-HRMS are described in the supporting information for this paper. The WHO-TEQs for PCDD/Fs and Co-PCBs were calculated by multiplying their measured concentrations by the corresponding WHO-TEFs [12]. In this study, the analogue approach to TEQ estimation was used, as described previously [12], and the WHO-TEQs for the identified and quantified PBDD/Fs congeners were also calculated from the WHO-TEFs of their chlorinated counterparts.

### 2.4. CALUX-TEQ determination

For measurement of total activities of dioxin-like compounds, a portion of the crude extract (equal to 0.1 g of sample) of each sample was evaporated and the solvent was exchanged for *n*-hexane. After removal of elemental sulfur with activated copper, the *n*-hexane fraction was applied to a cleanup column composed of 55% (w/w) sulfuric acid silica gel. After elution with 20 mL of *n*-hexane, the eluate was evaporated, and the residue was dissolved in 50  $\mu\text{L}$  of dimethyl sulfoxide. Dioxin-like activities were measured by means of the DR-CALUX assay using the rat hepatoma H4IIE cell line with

an aryl hydrocarbon receptor (AhR)-regulated luciferase gene construct [13]. The conditions for cell culture and the procedure for using DR-CALUX assay to calculate 2,3,7,8-TCDD equivalent values (CALUX-TEQs) have been described in detail elsewhere [14,15].

### 2.5. Quality assurance/quality control

In this study, all analytical procedures used for determination of the WHO-TEQs and CALUX-TEQs were conducted under UV-cutoff conditions.

#### 2.5.1. WHO-TEQ determination

For  $^{13}\text{C}$ -labeled PCDD/F congeners ( $^{13}\text{C}$ -2,3,7,8-tetraCDD [TCDD],  $^{13}\text{C}$ -1,2,3,7,8-pentaCDD [PeCDD],  $^{13}\text{C}$ -1,2,3,4,6,7,8-heptaCDD [HpCDD],  $^{13}\text{C}$ -1,2,3,4,7,8-hexaCDD [HxCDD],  $^{13}\text{C}$ -1,2,3,6,7,8-HxCDD,  $^{13}\text{C}$ -1,2,3,7,8,9-HxCDD,  $^{13}\text{C}$ -octaCDD [OCDD],  $^{13}\text{C}$ -2,3,7,8-TCDF,  $^{13}\text{C}$ -1,2,3,7,8-PeCDF,  $^{13}\text{C}$ -2,3,4,7,8-PeCDF,  $^{13}\text{C}$ -1,2,3,4,7,8-HxCDF,  $^{13}\text{C}$ -1,2,3,6,7,8-HxCDF,  $^{13}\text{C}$ -1,2,3,7,8,9-HxCDF,  $^{13}\text{C}$ -2,3,4,6,7,8-HxCDF,  $^{13}\text{C}$ -1,2,3,4,6,7,8-HpCDF,  $^{13}\text{C}$ -1,2,3,4,7,8,9-HpCDF, and  $^{13}\text{C}$ -OCDF) and Co-PCBs congeners ( $^{13}\text{C}$ -PCB IUPAC #77, #81, #126, #169, #105, #114, #118, #123, #156, #157, #167, and #180), the average recoveries for surface soils ( $n = 32$ ) and river sediments ( $n = 8$ ) ranged from 74% to 96% and from 76% to 100%, respectively. For  $^{13}\text{C}$ -labeled PBDD/F congeners ( $^{13}\text{C}$ -2,8-DiBDF,  $^{13}\text{C}$ -2,4,8-TriBDF,  $^{13}\text{C}$ -2,3,7-TriBDF,  $^{13}\text{C}$ -2,3,7,8-TBDF,  $^{13}\text{C}$ -2,3,7,8-TBDD,  $^{13}\text{C}$ -2,3,4,7,8-PeBDF,  $^{13}\text{C}$ -1,2,3,7,8-PeBDD,  $^{13}\text{C}$ -1,2,3,4,7,8-HxBDF,  $^{13}\text{C}$ -1,2,3,4,7,8-HxBDD,  $^{13}\text{C}$ -1,2,3,4,6,7,8-HpBDF,  $^{13}\text{C}$ -OBDF, and  $^{13}\text{C}$ -OBDD), the average recoveries were 64%–92% and 60%–89% for surface soils ( $n = 32$ ) and river sediments ( $n = 8$ ), respectively; the average recoveries for  $^{13}\text{C}$ -2-MBDF were outside these ranges: 32.3% and 37.5% for surface soils and river sediments, respectively. Analyses of procedural blanks ( $n = 4$ ) revealed no detectable concentrations of any of the test substances except for PCB #118. For PCB #118, the mean procedural blank value was calculated and subtracted from values obtained for the samples.

### 2.5.2. CALUX-TEQ determination

Half-maximal expected concentration ( $EC_{50}$ ) values were calculated from a dose–response calibration curve for 2,3,7,8-TCDD standards (blank sample, 0.3, 1.0, 3, 10, 30, 100, and 300 pM/well in dimethyl sulfoxide; Fig. 2a). The calculated  $EC_{50}$  of the 2,3,7,8-TCDD standard for the DR-CALUX assay was  $8.2 \pm 2.3$  pM in the microplate well (average  $\pm$  SD,  $n = 98$ ). The induction value (i.e., the luciferase activity of 300 pM 2,3,7,8-TCDD in the DR-CALUX cells divided by the luciferase activity of dimethyl sulfoxide) was  $9.6 \pm 2.8$  (average  $\pm$  SD,  $n = 98$ ). The obtained  $EC_{50}$  and induction values met the quality levels specified in the standard operating procedure provided by the supplier of the DR-CALUX cells (BioDetection Systems B.V.), indicating inter-assay consistency for 2,3,7,8-TCDD activity against the DR-CALUX cells. Luciferase activities that exhibited a response between 1 and 4 pM 2,3,7,8-TCDD were interpolated from the fitted 2,3,7,8-TCDD calibration curve to calculate CALUX-TEQ per gram of sample. The relative standard deviations of the CALUX-TEQs obtained by repeated experiments ( $n = 3$ ) were less than 30% for surface soils and river sediments. As a quality assurance/quality control measure, the same extract of surface soil was repeatedly applied to a sulfuric acid column cleanup for the subsequent measurement of the CALUX-TEQs in this study. The obtained CALUX-TEQ was  $98 \pm 20$  (average  $\pm$  SD,  $n = 11$ ), indicating that the conducted experiment was highly reproducible. No significant DR-CALUX response was detected in any of the procedural blank samples ( $n = 10$ ).

### 2.6. Statistical analysis

In this study, we investigated the correlation between PBDF concentrations and PBDE concentrations (reported by Matsukami et al. [8]) in soils and sediments by using SigmaPlot for Windows (ver. 13.0, Systat Software, Chicago, IL, USA). Spearman rank order correlation coefficients were calculated because the PBDF and PBDE concentrations were not normally distributed, as determined by the Shapiro–Wilk test for normality. The correlation between the WHO-TEQs and the CALUX-TEQs and the correlation between the CALUX-TEQs and the concentration of each homologue in the soils and sediments collected in January 2012 were also investigated by means of the above-mentioned statistical analysis method.

## 3. Results

### 3.1. WHO-TEQs

We measured PCDD/F, Co-PCB, and PBDD/F concentrations in surface soil and river sediments collected in January 2012 by using GC-HRMS, and then determined the WHO-TEQs and homologue profiles. The analytical results are summarized in Table 1 and presented in detail in Tables S1–S4. Target dioxin-like compounds were detected in all samples. Total WHO-TEQ ranges for PCDD/Fs, Co-PCBs, and PBDD/Fs in surface soil samples collected from footpaths in rice paddies ( $n = 19$ ), open-burning sites ( $n = 3$ ), and around e-waste-processing workshops ( $n = 10$ ) were 0.29–20 pg/g (median 1.5 pg/g), 4.5–230 pg/g (median 100 pg/g), and 1.7–310 pg/g (median 28 pg/g), respectively. The median total WHO-TEQs in soils collected from open-burning sites and from around e-waste-processing workshops tended to be 1 to 2 orders of magnitude higher than the median values for soils collected from footpaths in rice paddies. Total WHO-TEQ ranges for PCDD/Fs, Co-PCBs, and PBDD/Fs in river sediments collected from the upstream area ( $n = 1$ ), the e-waste-processing area ( $n = 3$ ) and the downstream area ( $n = 4$ ) were 1.0 pg/g, 13–58 pg/g (median 13 pg/g), and 1.1–7.1 pg/g (median 1.6 pg/g), respectively. The median total WHO-TEQs in river sediments collected around e-waste-processing workshops were higher than the medians for samples collected from other sites (Table 1). As shown in Table 1, PCDFs were the most important contributors to WHO-TEQs in surface soils collected from open-burning sites, whereas PBDFs strongly contributed to WHO-TEQs in soils collected around e-waste-processing workshops. In the river sediments, PCDFs tended to be the most important contributors to total WHO-TEQs, followed by PBDFs.

### 3.2. CALUX-TEQs

CALUX-TEQs derived from all the dioxin-like compounds in surface soils and river sediments collected in January 2012, 2013, and 2014, which were measured by means of the DR-CALUX assay, are summarized in Table 2, and detailed data are listed in Tables S5 and S6. Dose-dependent dioxin-like activity was detected in 25%, 78%, and 83% of the surface soils collected from footpaths in rice paddies, open-burning sites, and e-waste-processing workshops, respectively, and in 0%, 89%, and 33% of the river sediments collected from the upstream area, the e-waste-processing area, and the downstream

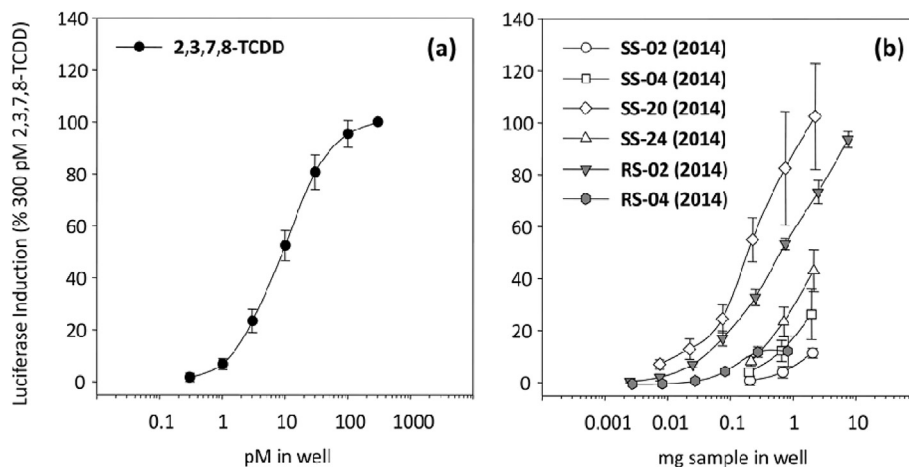


Fig. 2. Dose-response curves for (a) 2,3,7,8-TCDD standard ( $n = 98$ ) and for (b) four surface soils and two river sediments ( $n = 3$  each) in the DR-CALUX assay. Values are means  $\pm$  SDs.



**Table 1**  
WHO-TEQs (pg/g dry weight) of PCDD/Fs, Co-PCBs, and PBDD/Fs in surface soils and river sediments collected in Bui Dau, Hung Yen province, Vietnam, January 2012.<sup>a</sup>

| Surface soils           | Footpaths in rice paddies (n = 19) |         |     | Open-burning sites (n = 3)      |      |        | E-Waste-processing workshop (n = 10) |        |      |
|-------------------------|------------------------------------|---------|-----|---------------------------------|------|--------|--------------------------------------|--------|------|
|                         | Median                             | Min     | Max | Median                          | Min  | Max    | Median                               | Min    | Max  |
| PCDDs                   | 0.72                               | 0.076   | 1.1 | 13                              | 1.2  | 13     | 0.84                                 | 0.070  | 4.6  |
| PCDFs                   | 0.46                               | ND      | 13  | 64                              | 2.6  | 120    | 3.7                                  | 0.21   | 13   |
| Co-PCBs                 | 0.32                               | 0.00078 | 1.7 | 4.8                             | 0.55 | 6.6    | 1.3                                  | 0.29   | 5.8  |
| PBDDs                   | ND                                 | ND      | ND  | ND                              | ND   | 3.2    | 0.013                                | ND     | 15   |
| PBDFs                   | 0.025                              | ND      | 5.4 | 14                              | 0.19 | 83     | 20                                   | 0.83   | 270  |
| Sum of target compounds | 1.5                                | 0.29    | 20  | 100                             | 4.5  | 230    | 28                                   | 1.7    | 310  |
| River sediments         | Upstream area (n = 1)              |         |     | E-Waste-processing area (n = 3) |      |        | Downstream area (n = 4)              |        |      |
|                         | Median                             | Min     | Max | Median                          | Min  | Max    | Median                               | Min    | Max  |
| PCDDs                   |                                    | 0.92    |     | 1.0                             | 0.79 | 9.2    | 1.2                                  | 0.38   | 1.6  |
| PCDFs                   |                                    | 0.011   |     | 6.3                             | 2.2  | 42     | 0.043                                | 0.025  | 4.6  |
| Co-PCBs                 |                                    | 0.0050  |     | 0.89                            | 0.67 | 4.9    | 0.0055                               | 0.0027 | 0.54 |
| PBDDs                   |                                    | ND      |     | 0.0050                          | ND   | 0.0063 | ND                                   | ND     | ND   |
| PBDFs                   |                                    | 0.028   |     | 4.3                             | 2.2  | 8.9    | 0.33                                 | ND     | 1.0  |
| Sum of target compounds |                                    | 1.0     |     | 13                              | 12   | 58     | 1.6                                  | 1.1    | 7.1  |

<sup>a</sup> ND, not detected.

area, respectively. The dose–response curves of 2,3,7,8-TCDD and four surface soils and two river sediments collected in 2014 are shown in Fig. 2 as examples; the samples from SS-20 and RS-02 showed marked dioxin-like activity. The dose–response curves for SS-20 and RS-02, which were similar to that of 2,3,7,8-TCDD, indicate that the major compounds responsible for the activity in these samples were full AhR agonists like 2,3,7,8-TCDD.

CALUX-TEQs in soils from footpaths in rice paddies (n = 57), open-burning sites (n = 9), and e-waste-processing workshops (n = 30) ranged from <30 to 180 pg/g (median <30 pg/g), from <30 to 4300 pg/g (median 1200 pg/g), and from <30 to 580 pg/g (median 62 pg/g), respectively. The ranges for river sediments from the e-waste-processing area (n = 9) and the downstream area (n = 12) were from <30 to 4000 pg/g (median 200 pg/g) and from <30 to 64 pg/g (median <30 pg/g), respectively. In contrast, none of the sediments from the upstream area during 2012, 2013, or 2014 showed any dioxin-like activity. The median CALUX-TEQs of surface soils and river sediments collected around e-waste-processing locations such as workshops and open-burning sites were also higher than the medians for samples from other sites (Table 2). Especially, the levels in surface soils collected around open-burning sites were in the range of thousands of picogram CALUX-TEQs per gram.

## 4. Discussion

### 4.1. WHO-TEQ monitoring

To evaluate the PCDD/F, Co-PCB, and PBDD/F contamination levels at the study location, we compared the obtained WHO-TEQs

for soils and sediments with guideline values by regulatory authorities. There are various international TEQs mainly for PCDD/Fs in residential soil, as summarized by Paustenbach et al. [16]. Paustenbach et al. have stated that the current toxicological, epidemiological, and exposure assessment data indicate that 1000 pg-TEQ/g soil is a reasonable screening value for most residential sites [16]. We found that the WHO-TEQs obtained in this study for PCDD/Fs, Co-PCBs, and PBDD/Fs were lower than this screening value.

Several countries have issued guidelines for PCDD/Fs and/or Co-PCBs in sediments: Canada, 21.5 pg-TEQ/g (action level for ecotoxicological assessment) [17]; Japan, 150 pg-TEQ/g (remedial action level based on risks to humans) [18]; the Netherlands, 1000 pg-TEQ/g (remedial action level based on risks to humans) [19]; and the United States, 2.5–25 pg-TEQ/g (low- and high-risk levels for mammalian wildlife) [19]. The WHO-TEQs obtained for the sediments collected in this study were below the reference values for human health issued by Japan and the Netherlands.

WHO-TEQs for the samples from our study varied with the sampling location. The values for soils and sediments collected around open-burning sites and e-waste-processing workshops tended to be higher than the values for control soils (Table 1). There have been several studies reporting WHO-TEQs derived from PCDD/F, Co-PCB, or/and PBDD/F concentrations in soils and sediments collected from e-waste-processing sites (Table 3) [20–27]. The results of these previous studies indicate that soils collected from e-waste open-burning sites tend to contain the highest concentrations of dioxin-like compounds, followed by soils collected from e-waste-processing areas without open-

**Table 2**  
CALUX-TEQs (pg/g dry weight) of all dioxin-like compounds in surface soils and river sediments collected in Bui Dau, Hung Yen province, Vietnam, January 2012, 2013, and 2014.

| Surface soils   | Footpaths in rice paddies (n = 19) |     |     | Open-burning sites (n = 3)      |     |      | E-Waste-processing workshops (n = 10) |     |     |
|-----------------|------------------------------------|-----|-----|---------------------------------|-----|------|---------------------------------------|-----|-----|
|                 | Median                             | Min | Max | Median                          | Min | Max  | Median                                | Min | Max |
| January 2012    | <30                                | <30 | 180 | 390                             | 73  | 2300 | 81                                    | <30 | 580 |
| January 2013    | <30                                | <30 | 130 | 1900                            | <30 | 4300 | 51                                    | <30 | 400 |
| January 2014    | <30                                | <30 | 110 | 1200                            | <30 | 3900 | 55                                    | <30 | 300 |
| River sediments | Upstream area (n = 1)              |     |     | E-Waste-processing area (n = 3) |     |      | Downstream area (n = 4)               |     |     |
|                 | Median                             | Min | Max | Median                          | Min | Max  | Median                                | Min | Max |
| January 2012    |                                    | <30 |     | 200                             | <30 | 200  | <30                                   | <30 | 44  |
| January 2013    |                                    | <30 |     | 1100                            | 200 | 4000 | 33                                    | <30 | 64  |
| January 2014    |                                    | <30 |     | 250                             | 65  | 1000 | <30                                   | <30 | 45  |

burning. As shown in Table 3, the median TEQs for control soils were at least 4 to 5 orders of magnitude lower than TEQs in samples collected around e-waste-processing areas, including open-burning sites and e-waste-processing workshops. Our results together with previous studies clearly indicate that e-waste-processing activities generate dioxin-like compounds. The WHO-TEQs that we obtained also indicate that PBDD/Fs were the primary dioxin-like compounds of concern associated with the e-waste-processing activities at our study location. However, there have been only a few studies of PBDD/F concentrations in environmental samples collected from sites associated with e-waste processing [22,26]. Based on recommendation by WHO and United Nations Environmental Programme [12], WHO-TEQs should be determined not only for PCDD/Fs and Co-PCBs but also for PBDD/Fs for environmental samples collected from areas associated with e-waste processing.

#### 4.2. Sources of the major contributors to WHO-TEQs

In this study, we found that PCDFs were important contributors to WHO-TEQs in surface soils collected from open-burning sites, and PBDFs strongly contributed to WHO-TEQs in surface soils collected around e-waste-processing workshops. That is, PCDFs and PBDFs in soils and sediments were the primary contaminants derived from e-waste-processing activities based on obtained WHO-TEQs.

The PCDD/F homologue profiles clearly indicated that soils collected around open-burning sites tended to contain TCDFs, PeCDFs, and HxCDFs (Fig. 3), which indicates that combustion-

related activities were the source of the contamination [28]. PCDD/Fs are known to be byproducts of combustion processes such as open burning at low temperatures. In fact, in January 2013, we observed open burning of PVC-coated wires and cables to retrieve copper in this village (Fig. S2). PVC-coated wires from a workshop were taken to a footpath in the rice paddy, and burned (Fig. S2(A) and (B)), and then the copper with burnt residues were back to the workshop for copper collection (Fig. S2(C) and (D)). Therefore, the PCDD/F profiles in soils from e-waste-processing workshops and sediments collected around the e-waste-processing area might fall somewhere between the profile for open-burning sites and that for control sites such as footpaths in rice paddies, and the areas upstream from the e-waste-processing area (Fig. 3). The OCDD-dominated profiles observed at the control sites are thought to be derived mainly from atmospheric deposition [28].

We investigated the correlation between the concentrations of PBDD/Fs and PBDEs [8] in soils and sediments collected in January 2012 (Fig. 4). We found that the PBDF and PBDE concentrations were positively correlated not only in the surface soils (correlation coefficient = 0.84,  $P < 0.001$ ,  $n = 31$ ) but also in the river sediments (correlation coefficient = 0.82,  $P = 0.015$ ,  $n = 7$ ). Furthermore, when the data for the soils collected around the e-waste-processing workshops were analyzed separately, the correlation coefficient increased to 0.94 ( $P < 0.001$ ,  $n = 10$ ) (Fig. S3), suggesting that the PBDFs were derived from PBDEs contained in the e-waste. This is consistent with the fact that PBDFs have been detected in several commercial PBDE mixtures by Hanari et al. [29]. Among commercial PBDE mixtures analyzed, DE-83 (a commercial flame retardant containing >97% BDE-209), which contains a high proportion of

**Table 3**

Comparison of TEQs (pg/g dry weight) of dioxin-like compounds in soils and sediments collected from various e-waste-processing areas.<sup>a</sup>

| Sample   | Location       | Note   | Year | n  | Target dioxin-like compounds | pg TEQ/g |         |       |        | Ref                            |
|----------|----------------|--|------|----|------------------------------|----------|---------|-------|--------|--------------------------------|
|          |                |  |      |    |                              | Median   | Average | Min   | Max    |                                |
| Soil     | Accra, Ghana   | Control site                                       | 2010 | 4  | PCDD/Fs, Co-PCBs, PBDD/Fs    | 0.16     | NA      | 0.002 | 2.4    | Tue et al. 2016 <sup>b</sup>   |
| Soil     | Eastern China  | Control site                                       | NA   | 17 | PCDD/Fs, Co-PCBs             | 0.35     | NA      | 0.017 | 5.04   | Liu and Liu 2009 <sup>c</sup>  |
| Soil     | Guiyu, China   | Control site                                       | 2004 | 5  | PCDD/Fs                      | NA       | NA      | 0.565 | 28.4   | Wong et al. 2007 <sup>c</sup>  |
| Soil     | Guiyu, China   | Control site                                       | 2004 | 3  | PCDD/Fs                      | NA       | NA      | 0.39  | 1.5    | Leung et al. 2007 <sup>e</sup> |
| Soil     | Taizhou, China | Control site                                       | 2006 | 10 | PCDD/Fs                      | 5.3      | NA      | 2.3   | 45     | Shen et al. 2009 <sup>b</sup>  |
| Soil     | Taizhou, China | Control site                                       | 2005 | 3  | PCDD/Fs                      | 0.5      | NA      | 0.5   | 0.5    | Shen et al. 2008 <sup>b</sup>  |
| Soil     | Taizhou, China | Control site                                       | 2007 | 3  | PCDD/Fs                      | NA       | 0.34    | NA    | NA     | Ma et al. 2008 <sup>e</sup>    |
| Soil     | Taizhou, China | Control site                                       | 2007 | 3  | PBDD/Fs                      | NA       | ND      | ND    | ND     | Ma et al. 2009 <sup>b,f</sup>  |
| Soil     | Guiyu, China   | Control site                                       | 2004 | 36 | Co-PCBs                      | NA       | NA      | ND    | 9.21   | Wong et al. 2007 <sup>d</sup>  |
| Soil     | Taizhou, China | Control site                                       | 2006 | 10 | Co-PCBs                      | 8.1      | NA      | 0.42  | 11     | Shen et al. 2009 <sup>b</sup>  |
| Soil     | Taizhou, China | Control site                                       | 2005 | 3  | Co-PCBs                      | 1.0      | NA      | 0.3   | 1.1    | Shen et al. 2008 <sup>b</sup>  |
| Soil     | Accra, Ghana   | E-waste-processing site                            | 2010 | 5  | PCDD/Fs, Co-PCBs, PBDD/Fs    | 120      | NA      | 24    | 290    | Tue et al. 2016 <sup>b</sup>   |
| Soil     | Eastern China  | E-waste-processing site                            | NA   | 4  | PCDD/Fs, Co-PCBs             | 7.6      | NA      | 6.52  | 16.7   | Liu and Liu 2009 <sup>c</sup>  |
| Soil     | Southern China | E-waste-processing site                            | 2009 | 24 | PCDD/Fs                      | NA       | 43      | 7.7   | 191.3  | Hu et al. 2013 <sup>c</sup>    |
| Soil     | Taizhou, China | E-waste-processing site                            | 2005 | 8  | PCDD/Fs                      | 9.5      | NA      | 6.1   | 20.1   | Shen et al. 2008 <sup>b</sup>  |
| Soil     | Taizhou, China | E-waste-processing site                            | 2007 | 5  | PCDD/Fs                      | NA       | 92      | NA    | NA     | Ma et al. 2008 <sup>e</sup>    |
| Soil     | Taizhou, China | E-waste-processing site                            | 2007 | 5  | PCDD/Fs                      | NA       | 49.3    | NA    | NA     | Ma et al. 2008 <sup>e</sup>    |
| Soil     | Taizhou, China | E-waste-processing site                            | 2007 | 10 | PBDD/Fs                      | NA       | 799     | 46.3  | 3680   | Ma et al. 2009 <sup>b,f</sup>  |
| Soil     | Taizhou, China | E-waste-processing site                            | 2005 | 8  | Co-PCBs                      | 179.4    | NA      | 118.5 | 377.5  | Shen et al. 2008 <sup>b</sup>  |
| Soil     | Guiyu, China   | E-waste-processing site (Acid leaching)            | 2004 | 3  | PCDD/Fs                      | NA       | NA      | 203   | 1100   | Leung et al. 2007 <sup>e</sup> |
| Soil     | Guiyu, China   | E-waste-processing site (Printer roller dump site) | 2004 | 3  | PCDD/Fs                      | NA       | NA      | 2.1   | 8.2    | Leung et al. 2007 <sup>e</sup> |
| Soil     | Guiyu, China   | Near open-burning site                             | 2004 | 2  | PCDD/Fs                      | NA       | NA      | 129   | 213    | Wong et al. 2007 <sup>c</sup>  |
| Soil     | Guiyu, China   | Near open-burning site                             | 2004 | 6  | PCDD/Fs                      | NA       | NA      | 10    | 48     | Leung et al. 2007 <sup>e</sup> |
| Soil     | Guiyu, China   | Near open-burning site                             | 2004 | 8  | Co-PCBs                      | NA       | NA      | ND    | 64.4   | Wong et al. 2007 <sup>d</sup>  |
| Soil     | Accra, Ghana   | Open-burning                                       | 2010 | 5  | PCDD/Fs, Co-PCBs, PBDD/Fs    | 7100     | NA      | 510   | 24,000 | Tue et al. 2016 <sup>b</sup>   |
| Soil     | Guiyu, China   | Open-burning                                       | 2004 | 3  | PCDD/Fs                      | NA       | NA      | 627   | 13,900 | Wong et al. 2007 <sup>c</sup>  |
| Soil     | Guiyu, China   | Open-burning                                       | 2004 | 5  | Co-PCBs                      | NA       | NA      | 6.99  | 878    | Wong et al. 2007 <sup>d</sup>  |
| Sediment | Eastern China  | E-waste-processing site                            | NA   | 6  | PCDD/Fs, Co-PCBs             | 5.2      | NA      | 2.25  | 34.6   | Liu and Liu 2009 <sup>c</sup>  |
| Sediment | Southern China | E-waste-processing site                            | 2009 | 1  | PCDD/Fs                      | NA       | 128     | NA    | NA     | Hu et al. 2013 <sup>e</sup>    |

<sup>a</sup> ND, not detected; NA, not available.

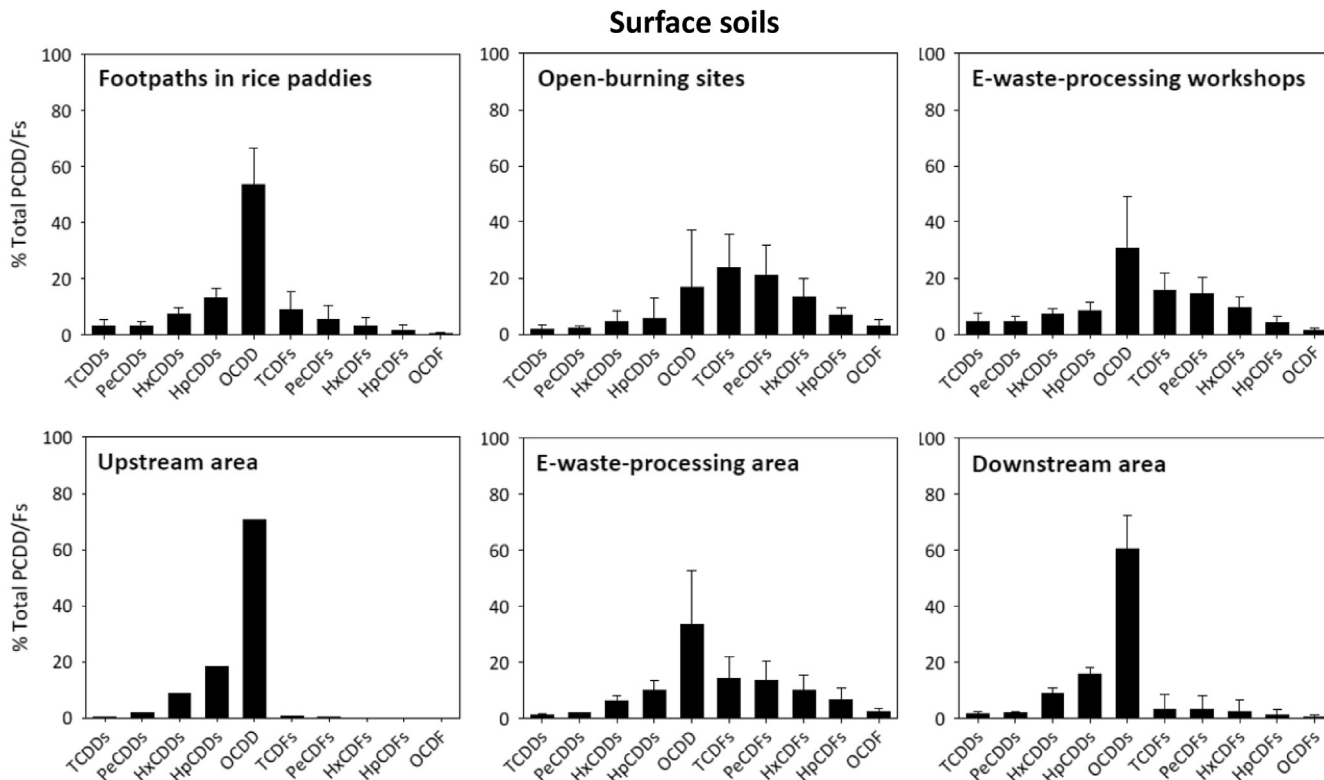
<sup>b</sup> TEQ values were calculated using WHO<sub>2005</sub>-TEFs.

<sup>c</sup> TEQ values were calculated using WHO-TEFs.

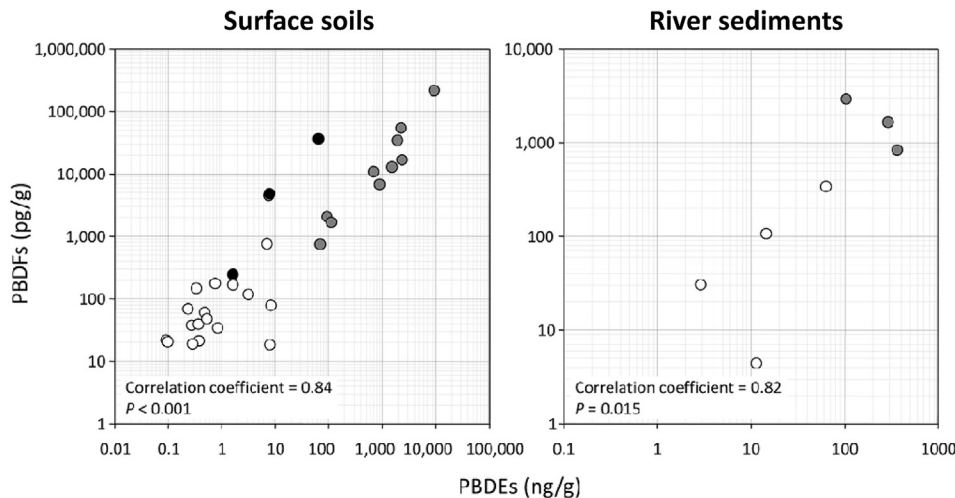
<sup>d</sup> TEQ values were calculated using I-TEFs.

<sup>e</sup> TEQ values were calculated using WHO<sub>1998</sub>-TEFs.

<sup>f</sup> TEQ values were calculated using CALUX-TEFs.



**Fig. 3.** PCDD/F homologue profiles in surface soils and river sediments collected in January 2012. Surface soils were collected from footpaths in rice paddies ( $n = 19$ ), open-burning sites ( $n = 3$ ), and areas adjacent to e-waste-processing workshops ( $n = 10$ ). River sediments were collected from the upstream area ( $n = 1$ ), the e-waste-processing area ( $n = 3$ ), and the downstream area ( $n = 4$ ). Values are means  $\pm$  SDs.



**Fig. 4.** Relationship between PBDF and PBDE concentrations in surface soils ( $n = 31$ ) and river sediments ( $n = 7$ ) collected in January 2012: surface soils from footpaths in rice paddies ( $\circ$ ), from open-burning sites ( $\bullet$ ), and from areas adjacent to e-waste-processing workshops ( $\bullet$ ) and river sediments from the upstream and downstream areas ( $\circ$ ) and the e-waste-processing area ( $\bullet$ ). SS19 and RS08 were excluded from the statistical analysis because PBDFs were not detected at those sites. The Spearman rank order coefficients and  $P$  values are indicated in the figure. PBDE data are from Ref. [8].

OBDF, have a PBDF homologue profile similar to the profiles in the soils and sediments collected from e-waste-processing areas in this study. In fact, BDE-209 made up about 86% of the total PBDEs detected in surface soils ( $n = 10$ ) from areas adjacent to e-waste-processing workshops in January 2012 [8]. However, more careful comparison of the data indicated that there were big differences both in the PBDF/PBDE ratio and in the PBDF profile. The average PBDF/PBDE ratios for DE-83 ( $n = 2$ ) [29] and soils collected around

the workshops in January 2012 ( $n = 10$ ) were 0.000040 and 0.016, respectively; that is, the ratio for the soils was about 3 orders of magnitude higher than that for DE-83. OBDF is a major homologue in DE-83, accounting for about 96% of total PBDFs; and in this study, the proportions of HpBDFs and OBDF, as well as PeBDFs and HxBDFs, were high in soils from the e-waste-processing workshops (Fig. 5). That differs to the PBDF profiles in DE-83. These results suggest that the potential environmental emissions of lower-

brominated compounds may be higher than those of higher-brominated compounds. In fact, their vapor pressures indicate that the potential emissions may be higher for lower-brominated compounds than higher-brominated compounds (2,3,7,8-TBDF,  $4.7 \times 10^{-6}$  Pa; HxBDF,  $6.4 \times 10^{-8}$  Pa; HpBDF,  $7.2 \times 10^{-9}$  Pa; OBDF,  $7.9 \times 10^{-10}$  Pa; calculated with EPI Suite ver. 4.11; <http://www.epa.gov/tsc-screening-tools/download-epi-suite-estimation-program-interface-v411>). Furthermore, debromination and formation of PBDFs during BDE-209 photolysis by sunlight exposure in soils [30] and/or TBDFs, PeBDFs and HxBDFs derived from other commercial PBDE mixtures such as DE-71 and DE-79 [29] also may affect the PBDF profiles in soils from the e-waste-processing workshops.

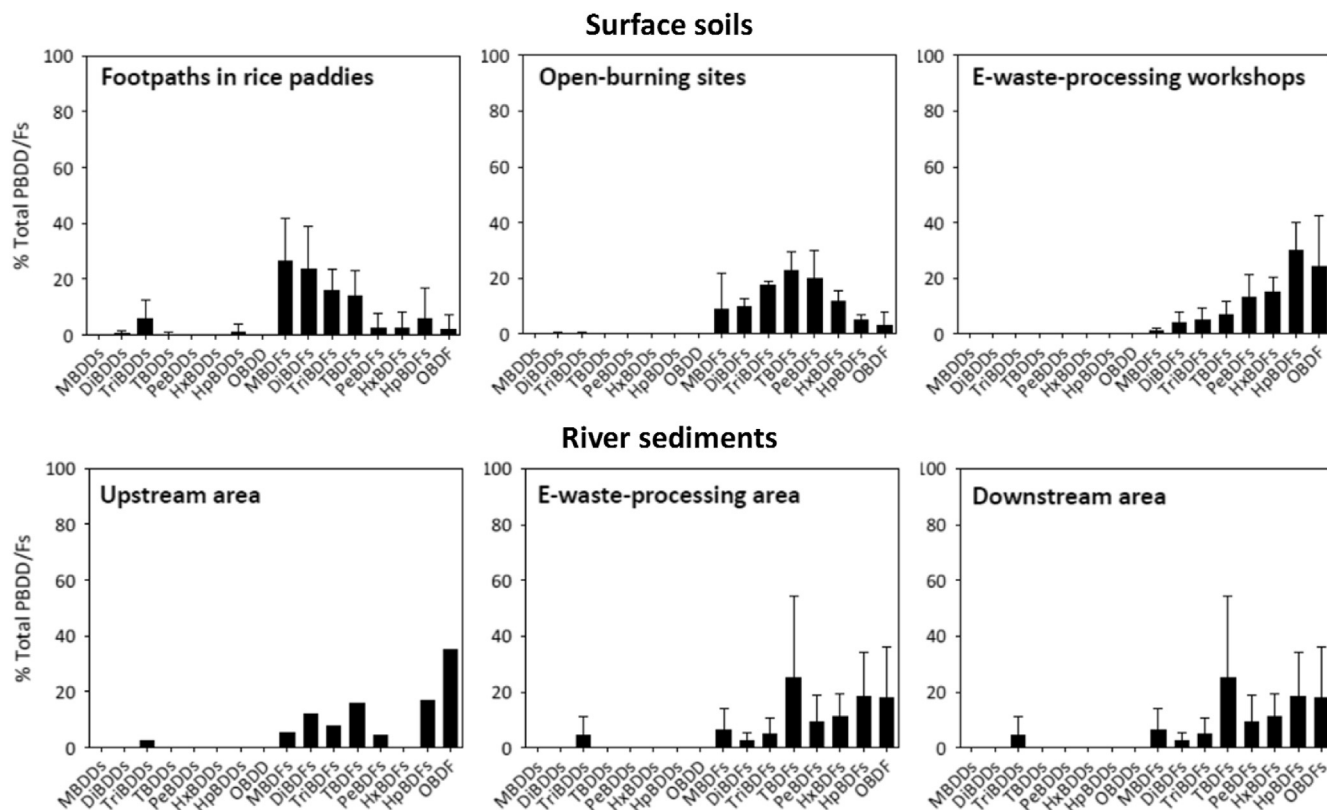
In addition, note that PBDF homologue profiles characterized by high proportions of TriBDFs, TBDFs, and PeBDFs were observed in soils from open-burning sites (Fig. 5). Although PBDE concentrations in soils from open-burning sites tended to be lower than those in soils from areas adjacent to e-waste-processing workshops, the average PBDF/PBDE ratio was 0.46 for soils from open-burning sites. These results suggest that the PBDFs were derived from pyrolysis of BDE-209 and subsequent debromination/hydrogenation reactions as well as HBr/Br<sub>2</sub> elimination catalyzed by metal oxides, such as antimony trioxide (Sb<sub>2</sub>O<sub>3</sub>), which are used with BDE-209, and zinc borate (B<sub>6</sub>O<sub>11</sub>Zn<sub>2</sub>), which is used mainly in PVC [31–33].

#### 4.3. Quantitative comparison between WHO-TEQs and CALUX-TEQs

By using the DR-CALUX assay to detect AhR agonists, we aimed to evaluate various dioxin-like compounds, including not only PCDD/Fs, Co-PCBs and PBDD/Fs but also emerging compounds

contained in soils and sediments collected from the study village in northern Vietnam. On the basis of the data obtained from the DR-CALUX assay and instrumental analysis, we also tried to elucidate the levels of dioxin-like compounds that were not measured by GC-HRMS in this study. Although Shen et al. [24,25] used a 7-ethoxyresorufin-*O*-deethylase-based assay (EROD assay) with rat hepatoma H4IIE cells for detection of AhR agonists contained in soils from crop lands closed to e-waste-processing sites, there were marked differences in main target compounds between their studies and our study. In this study, the sample extracts were cleaned up with a sulfuric acid-treated silica gel column and then subjected to the DR-CALUX assay, whereas Shen et al. used crude extracts in the EROD assay. That is, our study focused on detection of persistent compounds such as polyhalogenated compounds, including PCDD/Fs, Co-PCBs, and PBDD/Fs, after compounds labile to sulfuric acid, such as polyaromatic hydrocarbons, were removed. We focused on persistent compounds because persistence is one of the criteria for classification of a compound as dioxin-like [34].

To evaluate the validity of the DR-CALUX assay as a tool for monitoring various types of dioxin-like compounds in samples collected from areas associated with e-waste processing, we investigated the correlation between the WHO-TEQs derived from PCDD/F, Co-PCB, and PBDD/F concentrations, and the CALUX-TEQs in the soils and sediments collected in January 2012 (Fig. 6). The WHO-TEQs and CALUX-TEQs datasets were positively correlated for the surface soils and river sediments (correlation coefficient = 0.86,  $P < 0.001$ ,  $n = 16$ ). On the basis of our GC-HRMS results and previous results on the chemical impact of e-waste-processing activities [23,26], we suggest that not only PCDD/Fs and Co-PCBs but also PBDD/Fs must be major

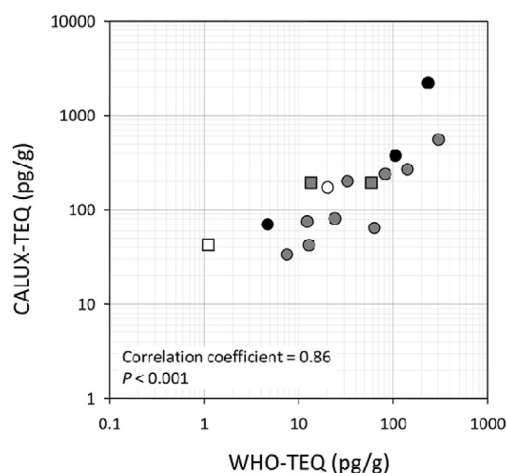


**Fig. 5.** PBDD/F homologue profiles in surface soils and river sediments collected in January 2012. Surface soils were collected from footpaths in rice paddies ( $n = 19$ ), from open-burning sites ( $n = 3$ ), and from areas adjacent to e-waste-processing workshops ( $n = 10$ ). River sediments were collected from the upstream area ( $n = 1$ ), the e-waste-processing area ( $n = 3$ ), and the downstream area ( $n = 4$ ). Values are means  $\pm$  SDs.



contributors to CALUX-TEQs. Note, however, that there were differences between the obtained WHO-TEQs and CALUX-TEQs, even though the correlation between them was statistically significant. On the basis of our experiences with combining the DR-CALUX assay with instrumental analysis [35–37], we expected that there would be gaps among TEQs due to differences between WHO-TEFs and relative effect potency (REP) values for DR-CALUX cells [38]. As shown in Table S7, DR-CALUX REPs were higher than WHO-TEFs [39] for PCDFs, whereas DR-CALUX REPs were lower than WHO-TEFs for PBDFs. Therefore, to assess the CALUX-TEQs obtained in our study location by using GC-HRMS data for PCDD/Fs, Co-PCBs, and PBDD/Fs, we calculated theoretical CALUX-TEQs using the concentrations of PCDD/Fs, Co-PCBs, and PBDD/Fs (Tables S1–S4) and their DR-CALUX REPs (Table S7) for soils and sediments. CALUX-TEQs and theoretical CALUX-TEQs are shown in Fig. S4, indicating that CALUX-TEQs/theoretical CALUX-TEQ ratios were 1.3–15 (median 3.8). That is, unidentified dioxin-like compounds also contributed strongly to the total dioxin-like activity of the soils and the sediments that were collected around e-waste-processing activities.

As also mentioned in previous studies [35–37], our results (Tables S1–S4) and those of previous studies of samples collected from areas associated with e-waste-processing activities [20–27] indicate that non-2,3,7,8-substituted PCDD/Fs (mainly PCDFs) and PBDFs, which have never been evaluated for AhR activity because of the lack of standards, might be important contributors. In fact, the concentrations of TCDDs (correlation coefficient, 0.79), PeCDDs (0.73), HxCDDs (0.60), TCDFs (0.70), PeCDFs (0.70), HxCDFs (0.71), HpCDFs (0.71), and PeBDFs (0.63) were significantly correlated with corresponding CALUX-TEQs ( $P < 0.01$ ), indicating that lower-halogenated PCDD/Fs and PBDFs might be primary homologues. Mixed halogenated dibenzofurans (PXDFs) (X = Cl and Br), which have been reported to be AhR agonists [38], could also become important contributors to samples collected from areas associated with open burning of e-waste [26,40]. Furthermore, halogenated polyaromatic hydrocarbons (with more than four rings) showing AhR-mediated activity are thought to have dioxin-like characteristics [41,42].



**Fig. 6.** Relationship between WHO-TEQs and CALUX-TEQs of surface soils ( $n = 13$ ) and river sediments ( $n = 3$ ) collected in January 2012: surface soils from footpaths in rice paddies (○), from open-burning sites (●), and from areas adjacent to e-waste-processing workshops (●) and river sediments from the downstream area (■) and the e-waste-processing area (■). Soils and sediments in which target compounds were not detected in the DR-CALUX assay were excluded from the statistical analysis. The Spearman rank order coefficient and  $P$  value are indicated in the figure. WHO-TEQs and CALUX-TEQs of soils and sediments are listed in Tables S1–S5.

#### 4.4. Time-course of levels of dioxin-like compound by CALUX-TEQ

In addition to determining CALUX-TEQs and WHO-TEQs in soils and sediments collected in January 2012, we also evaluated samples collected in January 2013 and 2014 by means of the DR-CALUX assay, to evaluate the levels of all the dioxin-like compounds derived from e-waste-processing activities. As shown in Tables 2 and S5 (which include data for all three years), the CALUX-TEQs in soil and sediments collected from areas associated with e-waste-processing activities such as open-burning sites and e-waste-processing workshops tended to be higher than those of samples collected from control sites. These results also suggest that e-waste processing is an important source of dioxin-like compounds, in agreement with our findings based on the samples collected in January 2012. With regard to the soils, the CALUX-TEQs varied with sampling location. In particular, with regard to soils collected during January 2012 and 2014, it is notable that the CALUX-TEQs of SS-20 and SS-21 (open-burning sites) were often higher than the CALUX-TEQs of samples from other locations, and transport of the contaminants at these sites may have led to high CALUX-TEQs at SS-02 to SS-05, which are nearby, on footpaths in the rice paddies (Fig. 1). That is, the dioxin-like compounds derived from open burning of e-waste may be transported from the source to the surrounding areas over the course of several years.

The CALUX-TEQs in sediments collected in January 2013 and 2014 tended to be higher than those of sediments collected in January 2012. For example, the CALUX-TEQs of RS-03 in 2013 were more than 100 times those in 2012. As shown in Fig. S2(D), to collect copper, the villagers used large quantities of water to flush combustibles from the open-burning area around the workshop near the RS-03 and RS-04 sampling points in January 2013, leading to heavy contamination of the river and sediment with various types of dioxins. In addition, we observed several drain ports leading from workshops located near the RS-02, RS-03, and RS-04 sampling points. Therefore, another possible reason that the CALUX-TEQs of samples collected at RS-02, RS-03, and RS-04 were much higher than those of samples collected upstream and downstream is the accumulation of dioxin-like compounds contained in the effluent that was released from the workshops via the drain ports. These DR-CALUX assay-monitoring results improved our understanding of the temporal and spatial changes in the levels of all dioxin-like compounds during the period from January 2012 to January 2014 at our study location.

The joint WHO/United Nations Environmental Programme consultation that took place in 2011 [12] focused not only on PBDD/Fs but also on PXDD/Fs (which we anticipated would be important contributors to CALUX-TEQs as unidentified, emerging, dioxin-like compounds) for inclusion in the TEF scheme, just as PCDD/Fs, Co-PCBs, and PBDD/Fs are. Therefore, to assess contamination levels of all dioxin-like compounds in surface soils, we evaluated the CALUX-TEQs that we obtained for soils by comparing them to the above-mentioned recommended value of 1000 pg-TEQ/g as the maximum acceptable WHO-TEQ level for residential soil [16]. The CALUX-TEQs in soils from areas adjacent to open-burning sites (SS20–SS22) exceeded this value; these soils were collected from footpaths in paddy fields around the residential area of this village (Fig. 1). The CALUX-TEQs in all the sediments collected around the e-waste-processing area also exceeded the reference values determined on the basis of the risks to humans by Japan [18] and the Netherlands [19]. For sediments, the Japanese government set a value of 150 pg-TEQ/g as the remedial action level based on consumption of fish that live in the vicinity of contaminated sediments. If residents eat fish from the river near the village, sediments containing several dioxin-like compounds should be remediated. However, our interviews with residents suggested that they do not

regularly eat fish from the river. Therefore, although the CALUX-TEQs in some of the soils and sediments in this study exceeded the criterion for human health, this situation may not be a state of emergency requiring immediate countermeasures such as remediation. However, mitigation measures are necessary to prevent further increase in contamination levels in the river.

#### 4.5. Implication of our results for environmental management practices

Waste processing strategies based on appropriate management of useful resources and toxic substances generated during a product's life cycle should be promoted. E-waste is an important target waste because of its environmental and human health impacts. In this study, we identified critical e-waste-processing processes and evaluated possible countermeasures for environmentally sound management of dioxin-like compounds derived from e-waste processing. In the study village, metals and plastics are retrieved from e-waste such as computers, TVs, video and DVD players, mobile phones, and printers. Printed circuit boards from mobile phones, computers, and TVs are among the most important parts of e-waste. In the village, processing operations such as manual dismantling of wires and circuit boards, separation of metal and plastic, sorting of electrical parts, and removal of parts from printed circuit boards tend to be conducted in workshops located within or near living area. Open storage of large amounts of e-wastes including cathode ray tubes, TV casings, and printed circuit boards along the roadsides near workshops was regularly observed during the study period (Fig. S1). In the paddy fields around the village, PVC-coated cables were burned to retrieve copper, and then combustibles were taken back to the workshop (Fig. S2). Among these processing operations, open burning was the biggest source of PCDD/Fs and other emerging dioxin-like compounds such as PXDD/Fs; and open storage of e-waste-containing PBDEs can lead to PBDF contamination. Furthermore, wastewater from e-waste-processing workshops may also be a source of dioxin-like compounds derived from e-waste, as indicated by our results for river sediments. Therefore, our findings suggest that open burning and open storage of e-waste should be prohibited and that wastewater from the workshops should be treated to reduce pollution by e-waste-derived chemicals such as dioxin-like compounds.

Our results, along with previously reported results [20–27,33], indicate that PCDD/Fs tended to form at open-burning sites, that PBDFs were present at relatively high concentrations around e-waste-processing areas if the e-waste contained brominated flame retardants such as PBDEs, and that open burning of e-wastes such as PVC-coated cables generated not only PCDFs and PBDFs but also other emerging dioxin-like compounds. On the basis of these findings, we concluded that cell-based AhR-binding reporter gene assays are an ideal tool for screening and monitoring of all types of dioxin-like compounds derived from e-waste-processing activities, because these assays measure not only PCDD/Fs, Co-PCBs, and PBDD/Fs but also PXDD/Fs. As mentioned above, PXDD/Fs are thought to be comparable to PCDD/Fs in their persistence and toxicity [12,43]. However, there are 4600 possible PXDD/Fs (3050 PXDFs and 1550 PXDDs) [44], and the use of GC-HRMS to analyze PXDD/Fs, PCDD/Fs, Co-PCBs, and PBDD/Fs in all samples is unrealistic because of the time and expense required. Therefore, a better strategy involves detection of dioxin-like compounds by means of cell-based AhR-binding reporter gene assays, followed by analysis of samples with high concentrations of all dioxin-like compounds by means of GC-HRMS analysis of PCDD/Fs, Co-PCBs, PBDD/Fs, and/or PXDD/Fs for source identification and WHO-TEQ determination. The obtained results can be used to choose and implement appropriate countermeasures.

## 5. Conclusion

The results obtained from this study elucidated the levels, possible sources, and temporal trends in the levels of persistent dioxin-like compounds, including PCDD/Fs, Co-PCBs, and PBDD/Fs, in surface soils and river sediments collected in and around an e-waste-processing site in a village in northern Vietnam. Dioxin-like compounds were found to have accumulated in samples collected in areas where e-waste-processing activities such as open burning and open storage were conducted, as well as in areas near e-waste-processing workshops. Moreover, our results indicate that dioxin-like compounds derived from e-waste-processing activities such as open burning may be transported from their source to surrounding areas over the course of several years. The CALUX-TEQs, but not the WHO-TEQs, obtained for some of the samples were higher than the maximum acceptable WHO-TEQs promulgated by various regulatory authorities, suggesting that the levels of all dioxin-like compounds should be evaluated in samples collected from areas associated with e-waste processing. These findings indicate that open burning and open storage of e-waste should be prohibited and that wastewater treatment measures should be implemented at each e-waste-processing workshop to reduce pollution by e-waste-derived, dioxin-like compounds. Furthermore, our results indicate that cell-based AhR-binding reporter gene assays such as the DR-CALUX assay, in combination with GC-HRMS, should be used to determine the levels of all the dioxin-like compounds derived from e-waste-processing sites.

## Acknowledgments

We gratefully acknowledge analytical support from Ms. Chieko Michinaka, Ms. Kyoko Yoneoka, Mr. Hiroo Takagi, and Mr. Akinori Hashimoto of the NIES in Japan and sampling assistance from members of the CETASD of VNU Hanoi University of Science in Vietnam. This research was supported by the Society for the Promotion of Science and Environment Research and Technology Development Fund (no. 3K133010 and no. 3K153001) of the Ministry of the Environment, Japan. This study was also partly supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) to a project on Center of Excellence for Cooperative and Collaborative Studies – Leading Academia in Marine and Environmental Research (LaMer).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.emcon.2016.03.001>.

## References

- [1] United Nations University, Discarded Kitchen, Laundry, Bathroom Equipment Comprises Over Half of World E-Waste, 2014. Press Releases, <http://unu.edu/media-relations/releases/discarded-kitchen-laundry-bathroom-equipment-comprises-over-half-of-world-e-waste-unu-report.html#info>.
- [2] T. Wang, E-waste creates hot spots for POPs, *Environ. Sci. Technol.* 41 (8) (2007) 2655–2656.
- [3] H.-G. Ni, E.Y. Zeng, Law enforcement and global collaboration are the keys to containing e-waste Tsunami in China, *Environ. Sci. Technol.* 43 (11) (2009) 3991–3994.
- [4] J.K. Chan, M.H. Wong, A review of environmental fate, body burdens, and human health risk assessment of PCDD/Fs at two typical electronic waste processing sites in China, *Sci. Total Environ.* 463–464 (2013) 1111–1123.
- [5] M. Man, R. Naidu, M.H. Wong, Persistent toxic substances released from uncontrolled e-waste processing and actions for the future, *Sci. Total Environ.* 463–464 (2013) 1133–1137.
- [6] K. Zhang, J.L. Schnoor, E.Y. Zeng, E-waste processing: where does it go from here? *Environ. Sci. Technol.* 46 (20) (2012) 10861–10867.
- [7] J. Yu, E. Williams, M. Ju, Y. Yang, Forecasting global generation of obsolete personal computers, *Environ. Sci. Technol.* 44 (9) (2010) 3232–3237.

- [8] H. Matsukami, N.M. Tue, G. Suzuki, M. Someya, L.H. Tuyen, P.H. Viet, S. Takahashi, S. Tanabe, H. Takigami, Flame retardant emission from e-waste recycling operation in northern Vietnam: environmental occurrence of emerging organophosphorus esters used as alternatives for PBDEs, *Sci. Total Environ.* 514 (2015) 492–499.
- [9] M. Someya, G. Suzuki, A.C. Ionas, N.M. Tue, F. Xu, H. Matsukami, A. Covaci, L.H. Tuyen, P.H. Viet, S. Takahashi, S. Tanabe, H. Takigami, Occurrence of emerging flame retardants from e-waste processing activities in the northern part of Vietnam, *Emerg. Contam.* 2 (2016) 58–65.
- [10] N.M. Tue, G. Suzuki, D. Schrenk, S. Safe, C. Tohyama, A. Tritscher, M. Tysklind, R.E. Peterson, Polybrominated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls: inclusion in the toxicity equivalency factor concept for dioxin-like compounds, *Toxicol. Sci.* 133 (2) (2013) 197–208.
- [11] A.J. Murk, J. Legler, M.S. Denison, J.P. Giesy, C. van de Guchte, A. Brouwer, Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water, *Fundam. Appl. Toxicol.* 33 (1996) 149–160.
- [12] G. Suzuki, H. Takigami, Y. Kushi, S. Sakai, Evaluation of mixture effects in a crude extract of compost using the CALUX bioassay and HPLC fractionation, *Environ. Int.* 30 (2004) 1055–1066.
- [13] G. Suzuki, H. Takigami, K. Nose, S. Takahashi, M. Asari, S.-I. Sakai, Dioxin-like and transthyretin-binding compounds in indoor dusts collected from Japan: average daily dose and possible implications for children, *Environ. Sci. Technol.* 41 (2007) 1487–1493.
- [14] D.J. Paustenbach, K. Fehling, P. Scott, M. Harris, B.D. Kerger, Identifying soil cleanup criteria for dioxins in urban residential soils: how have 20 years of research and risk assessment experience affected the analysis? *J. Toxicol. Environ. Health B Crit. Rev.* 9 (2) (2006) 87–145.
- [15] Canadian Council of Ministers of the Environment, Canadian Sediment Quality Guidelines for the Protection of Aquatic Life, 2001.
- [16] Government of Japan, Dioxins, 2012.
- [17] J. Stronkhorst, P. Leonards, A.J. Murk, Using the dioxin receptor-CALUX in vitro bioassay to screen marine harbor sediments for compounds with a dioxin-like mode of action, *Environ. Toxicol. Chem.* 12 (2002) 2552–2561.
- [18] A.O. Leung, W.J. Luksemburg, A.S. Wong, M.H. Wong, Spatial distribution of polybrominated diphenyl ethers and polychlorinated dibenzo-*p*-dioxins and dibenzofurans in soil and combusted residue at Guiyu, an electronic waste processing site in southeast China, *Environ. Sci. Technol.* 41 (8) (2007) 2730–2737.
- [19] J. Liu, W. Liu, Distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/Fs) and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) in the soil in a typical area of eastern China, *J. Hazard. Mater.* 163 (2–3) (2009) 959–966.
- [20] J. Ma, K. Kannan, J. Cheng, Y. Horii, Q. Wu, W. Wang, Concentrations, profiles, and estimated human exposures for polychlorinated dibenzo-*p*-dioxins and dibenzofurans from electronic waste processing facilities and a chemical industrial complex in Eastern China, *Environ. Sci. Technol.* 42 (22) (2008) 8252–8259.
- [21] J. Ma, R. Addink, S. Yun, J. Cheng, W. Wang, K. Kannan, Polybrominated dibenzo-*p*-dioxins/dibenzofurans and polybrominated diphenyl ethers in soil, vegetation, workshop-floor dust, and electronic shredder residue from an electronic waste processing facility and in soils from a chemical industrial complex in eastern China, *Environ. Sci. Technol.* 43 (19) (2009) 7350–7356.
- [22] C. Shen, S. Huang, Z. Wang, M. Qiao, X. Tang, C. Yu, D. Shi, Y. Zhu, J. Shi, X. Chen, K. Setty, Y. Chen, Identification of Ah receptor agonists in soil of e-waste processing sites from Taizhou area in China, *Environ. Sci. Technol.* 42 (1) (2008) 49–55.
- [23] C. Shen, Y. Chen, S. Huang, Z. Wang, C. Yu, M. Qiao, Y. Xu, K. Setty, J. Zhang, Y. Zhu, Q. Lin, Dioxin-like compounds in agricultural soils near e-waste processing sites from Taizhou area, China: chemical and bioanalytical characterization, *Environ. Int.* 35 (1) (2009) 50–55.
- [24] N.M. Tue, A. Goto, S. Takahashi, T. Itai, K.A. Asante, T. Kunisue, S. Tanabe, Release of chlorinated, brominated and mixed halogenated dioxin-related compounds to soils from open burning of e-waste in Agbogbloshie (Accra, Ghana), *Hazard. Mater.* 302 (2016) 151–157.
- [25] M.H. Wong, S.C. Wu, W.J. Deng, X.Z. Yu, Q. Luo, A.O. Leung, C.S. Wong, W.J. Luksemburg, A.S. Wong, Export of toxic chemicals – a review of the case of uncontrolled electronic-waste processing, *Environ. Pollut.* 149 (2) (2007) 131–140.
- [26] J. Baker, R.A. Hites, Is combustion the major source of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to the environment? A mass balance investigation, *Environ. Sci. Technol.* 34 (14) (2000) 2879–2886.
- [27] N. Hanari, K. Kannan, Y. Miyake, T. Okazawa, P.R. Kodavanti, K.M. Aldous, N. Yamashita, Occurrence of polybrominated biphenyls, polybrominated dibenzo-*p*-dioxins, and polybrominated dibenzofurans as impurities in commercial polybrominated diphenyl ether mixtures, *Environ. Sci. Technol.* 40 (14) (2006) 4400–4405.
- [28] N. Kajiwara, Y. Noma, H. Takigami, Photolysis studies of technical decabromodiphenyl ether (DecaBDE) and ethane (DeBDethane) in plastics under natural sunlight, *Environ. Sci. Technol.* 42 (12) (2008) 4404–4409.
- [29] R. Dumler, D. Lenoir, H. Thoma, O. Hutzinger, Thermal formation of polybrominated dibenzofurans and dioxins from decabromodiphenyl ether flame retardant: influence of antimony (III) oxide and the polymer matrix, *Chemosphere* 20 (1990) 10–12.
- [30] D. Lenoir, B. Zier, D. Bieniek, A. Kettrup, The influence of water and metals on PBDD/F concentration in incineration of decabromobiphenyl ether in polymeric matrices, *Chemosphere* 28 (1994) 1921–1928.
- [31] M. Zhang, A. Buekens, X. Li, Brominated flame retardants and the formation of dioxins and furans in fires and combustion, *J. Hazard. Mater.* 304 (2016) 26–39.
- [32] M. Van den Berg, L. Birnbaum, A.T. Bosveld, B. Brunström, P. Cook, M. Feeley, J.P. Giesy, A. Hanberg, R. Hasegawa, S.W. Kennedy, T. Kubiak, J.C. Larsen, F.X. van Leeuwen, A.K. Liem, C. Nolt, R.E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillitt, M. Tysklind, M. Younes, F. Waern, T. Zacharewski, Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife, *Environ. Health Perspect.* 106 (12) (1998) 775–792.
- [33] G. Suzuki, N.M. Tue, S. van der Linden, A. Brouwer, B. van der Burg, M. van Velzen, M. Lamoree, M. Someya, S. Takahashi, T. Isobe, Y. Tajima, T.K. Yamada, H. Takigami, S. Tanabe, Identification of major dioxin-like compounds and androgen receptor antagonist in acid-treated tissue extracts of high trophic-level animals, *Environ. Sci. Technol.* 45 (23) (2011) 10203–10211.
- [34] N.M. Tue, G. Suzuki, S. Takahashi, K. Kannan, H. Takigami, S. Tanabe, Dioxin-related compounds in house dust from New York State: occurrence, in vitro toxic evaluation and implications for indoor exposure, *Environ. Pollut.* 181 (2013) 75–80.
- [35] N.M. Tue, K. Katsura, G. Suzuki, L.H. Tuyen, T. Takasuga, S. Takahashi, P.H. Viet, S. Tanabe, Dioxin-related compounds in breast milk of women from Vietnamese e-waste processing sites: levels, toxic equivalents and relevance of non-dietary exposure, *Ecotoxicol. Environ. Saf.* 106 (2014) 220–225.
- [36] P.A. Behnisch, K. Hosoe, S. Sakai, Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polycyclic aromatic compounds, *Environ. Int.* 29 (6) (2003) 861–877.
- [37] M. Van den Berg, L.S. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk, C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker, R.E. Peterson, The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds, *Toxicol. Sci.* 93 (2006) 223–241.
- [38] M. Zennegg, X. Yu, W.M. Wong, R. Weber, Fingerprints of chlorinated, brominated and mixed halogenated dioxins at two e-waste processing sites in Guiyu/China, *Organohalogen Comp.* 71 (2009) 2248–2252.
- [39] T. Ohura, K. Sawada, T. Amagai, M. Shinomiya, Discovery of novel halogenated polycyclic aromatic hydrocarbons in urban particulate matters: occurrence, photostability, and AhR activity, *Environ. Sci. Technol.* 43 (2009) 2269–2275.
- [40] Y. Horii, J.S. Khim, E.B. Higley, J.P. Giesy, T. Ohura, K. Kannan, Relative potencies of individual chlorinated and brominated polycyclic aromatic hydrocarbons for induction of aryl hydrocarbon receptor-mediated responses, *Environ. Sci. Technol.* 43 (6) (2009) 2159–2265.
- [41] World Health Organization, Polybrominated dibenzo-*p*-dioxins and dibenzofurans, environmental health criteria 205, in: International Programme on Chemical Safety (WHO/IPCS), Geneva, Switzerland, 1998.
- [42] M. Rose, A. Fernandes, Are BFRs responsible for brominated dioxins and furans (PBDD/Fs) in food, in: Proceedings of the 5th International Symposium on Brominated Flame Retardants, 2010.