Introduction

Various electronic appliances, such as personal computers, television sets, mobile phones, and printers, are being used all over the world. It was estimated that the rate of E-waste generation globally was approximately 40 million tons per year according to the report by United Nation University (2008). Currently, huge quantities of E-waste have been widely recycled in not only developed countries but also developing countries because E-waste contains considerable quantities of valuable and reusable metals and plastics. However, especially in the developing countries, serious health impacts by primitive E-waste recycling operations have become a big issue in the last decade. Although several developing countries such as China have provided legislations mainly focusing on environmental aspects, they do not seem to ensure law enforcement to effectively control primitive E-waste recycling up until now. Furthermore, recent study estimated that the developing countries would be disposing of more old computers than developed countries by 2018. Main approach to mitigating the impacts by informal E-waste recycling has focused on reducing the amount of E-waste that developed countries export to developing ones up to that time. Accordingly, countermeasures responding to changing the flow and generation of E-waste should be properly discussed in the future. Thus there still could be E-waste issues including health effects caused by primitive recycling.

Previously, lots of studies had targeted on hot spots for contaminants derived from “intensive” E-waste recycling activities such as open-burning and smelting process for retrieving metals. Many research concluded that intensive E-waste recycling is harmful to human and the environment. Therefore, our motivation in this study is to provide data in environmental samples collected from “not-intensive” recycling such as collection, storage and manually dismantling of E-waste. Since 2011, our research group has begun such environmental investigations surrounding E-waste recycling workshops. Our study is trying (1) to elucidate the current status of contamination on different categories and conditions of E-waste recycling including not only not-intensive but also intensive recycling such as open-burning, (2) to clarify spatial diffusion of contamination by various primitive recycling, and (3) to monitor the short-term temporal trends (at least three years) in terms of several types of chemicals. Our final goal is to provide useful data for the environmentally sound management of E-waste in the developing countries by identifying critical control process.

In January 2012, we collected soil and sediment in and around the village which had E-waste recycling workshops in northern part of Vietnam. As primitive recycling methods, dismantling and crashing of electronic products, sorting of recyclable materials, and manually removing coated materials from copper wire are conducted in the E-waste workshop. Furthermore, open-burning of E-waste were performed in paddy field footpaths around village. In our study, we analyze not only chemicals potentially contained in E-waste, such as brominated and chlorinated flame retardants (FRs), phosphorus-containing FRs, and heavy metals, but also unintentionally occurred hazardous chemicals associated with E-waste and its recycling activity, such as chlorinated and brominated dioxins and other dioxin-related compounds, to elucidate their contamination status and diffusion from the sources. Preliminary results obtained so far will be introduced and discussed about dioxin-related compounds detected with DR-CALUX assay in this presentation. FRs, chlorinated and brominated dioxins also will be presented elsewhere in this conference.
Materials and methods

Sample collection and pretreatment. In January 2012, sampling was performed in Bui Dau (BD), Hung Yen province, located in northern part of Vietnam. Target area including habitat and rice field was about 3.0 km × 1.2 km. It has 283 households with approximately 1,000 people. The village’s main industry is not only rice agriculture but also recycling of metals and plastics from E-waste such as disposed computers, TVs, video players, phones, and printers since the early 2000s. In recent, most households are closely related to E-waste recycling activities. Recycling operation such as manually dismantling of wires and circuit boards, fractionation of metal and plastic, and sorting of electric parts were family based and conducted in their workplace near their home. Burning of wires and cables for retrieving copper was also performed in the field around the village. A total of 32 soil samples (0-5 cm) were collected from the footpath in rice paddies around village (n=19: BD Soil 01, 02, 04, 05, 07 and 09 to 22), areas adjacent to E-waste recycling workshop (n=10: BD Soil 23 to 32) and to open-burning sites of wires and cables (n=3: BD Soil 03, 06 and 08) as shown in Figure 1. Total eight river sediments were collected from upstream (n=1: BD Sediment 03) to downstream village (n=7: BD Sediment 01, 02, and 04 to 08).

![Figure 1 Soil sample collection from the footpath in rice paddies around village (A), area adjacent to E-waste recycling workshop (B) and open-burning of wires and cables (C)](image)

Each samples was composed of five subsamples and collected with a stainless steel shovel into a zip-locked polyethylene bag from an area of approximately 10 m². All samples were air-dried and manually homogenized with wooden hammer after removal of pebble, weeds and twig. Air-dried sample was transferred to a stainless-steel sieve (<2.0 mm) that was covered with a steel lid and shaken manually. Sieved sample was collected and stored in brown glass bottles at –20 °C until analysis.

Extraction and cleanup. Approximately 10 g of each sample was extracted by using a rapid solvent extractor (SE100, Mitsubishi Chemical Analytech) at 35 °C for 1 h with acetone:n-hexane (1:1, v/v) at a flow rate of 6 mL/min first and then at 80 °C for 1 h with toluene at 10 mL/min. A portion of combined extract (equal to 1.0 g of sample) for each sample was evaporated and transferred to n-hexane by rotary evaporation. After removing elemental sulfur with activated copper for sediment but not soil, then n-hexane fraction was treated with sulfuric acid and applied to a cleanup column composed of sodium sulfate, 22% (w/w) sulfuric acid silica gel, 44% (w/w) sulfuric acid silica gel, and sodium sulfate in that order. After elution with n-hexane, the extract was evaporated and dissolved in 50 µL of dimethyl sulfoxide (DMSO).

DR-CALUX assay. Dioxin-like activity was measured by means of the DR-CALUX assay using the rat hepatoma H4IIIE cell line with an AhR-regulated luciferase gene construct. The conditions for cell culture and the procedure for the DR-CALUX assay have been described in detail elsewhere. In this study, 2,3,7,8-TCDD was used as reference compound for DR-CALUX assay for calculation of TCDD equivalent (CALUX-TEQ). All measurements were conducted in 3 wells. Experiments have been repeated 3 times independently at least.

The calculated EC₅₀ of the 2,3,7,8-TCDD and the average induction (i.e., the luciferase activity of maximum 2,3,7,8-TCDD in the DR-CALUX cells was divided by that of DMSO vehicle control) satisfied the quality levels indicated in the standard operating procedure provided by the supplier of DR-CALUX cells (BioDetection Systems B.V.).
Results and discussion

Dioxin-like activities were detected in the all soil and sediment extracts applied for the DR-CALUX assay in a dose-dependent manner. Among soil samples, BD Soil 03, 08 and 29 had marked dioxin-like activity (Figure 2). The results suggest that the major causative compounds in the extract of BD Soil 03 and 08, but not 29, extract seem to be complete AhR agonist due to dose-response curves almost resembling that of 2,3,7,8-TCDD (data not shown). BD Soil 03 and 08 were collected from areas adjacent to open-burning of wires and cables of E-waste for recovering copper, while BD Soil 29 was sampled around an E-waste recycling workshop.

CALUX-TEQs of soil samples were 6.2 to 2,500 pg/g (average 160 pg/g, median 22 pg/g, n = 32) and those of river sediment samples were 1.7 to 210 pg/g (average 62 pg/g, median 18 pg/g, n = 8). As shown in Figure 3, CALUX-TEQs of soil and sediment extracts collected around E-waste recycling activities tended to be higher than those of other samples. The results suggest that E-waste recycling activity is one of biggest source of dioxin-like compounds. In fact, with the exception of the samples related with E-waste recycling activities, the average values of CALUX-TEQs were 23 pg/g (6.2-180 pg/g, median 13 pg/g, n = 19) and 15 pg/g (1.7-26 pg/g, median 15 pg/g, n = 5) for other soils and sediments, which were comparable to CALUX-TEQ (1.71-44.2 pg/g, mean 11.0 pg/g, n = 24) of soil samples collected in the vicinity of four different kinds of municipal solid or hazardous waste incineration plants in China. The potency of BD Soils collected from the footpath in rice paddies around village (i.e. not closed to “hot spots”) in inducing dioxin-like activity were two to three orders of magnitude lower than those of BD Soil 03, 08 and 29. Among river sediment samples, BD Sediment 04 and 01 taken in the vicinity of E-waste recycling workshops had higher activity than other samples. On the other hand, the potency of BD Sediment 03 obtained in the upstream BD village was the lowest among sediment samples. Our data suggested that dioxin-like compounds from E-waste recycling activity in BD may have low potential for diffusion pollution.

Figure 2  Dose-responses of selected soil extracts on DR-CALUX cells. BD soil 03 (A) and 08 (B) were collected in areas adjacent to E-waste recycling workshop, BD Soil 29 was sampled around open-burning of wires and cables (C). Values represent the mean ± S.D. from triplicate data. BD, Bui Dau.

Figure 3  CALUX-TEQs of BD Soils (A) and Sediment (B). Values represent the mean ± S.D. from three independent assays. ▲, Collected around E-waste recycling workshop; ▲, Collected around open-burning of wires and cables of E-waste. BD, Bui Dau.
Based on previous results for chemical impact of E-waste recycling activities\textsuperscript{8,10}, we expected that not only dioxins (polychlorinated dibenzo-\textit{p}-dioxins and dibenzofurans [PCDD/Fs], and Coplanar-polychlorinated biphenyls [Co-PCBs]) but also polybrominated dibenzo-\textit{p}-dioxins and dibenzofurans (PBDD/Fs) must be major contributors to CALUX-TEQ. Our research group analyzed dioxins and PBDD/Fs and calculated those WHO-TEQs in same BD soil and sediment samples\textsuperscript{11}. As a results, there were good correlations between CALUX-TEQs and WHO-TEQs for soils ($R^2=0.57$, $P<0.001$) and sediments ($R^2=0.52$, $P=0.043$) respectively. Among them, PCDFs and PBDFs analyzed were identified as a major causative compounds. Although PCDFs strongly contributed to CALUX-TEQs in soils collected around open-burning place, PBDFs were causative compounds for dioxin-like activities in soils around not only open-burning site but also E-waste recycling workshops.

Despite of the significant correlation between CALUX-TEQs and WHO-TEQs, CALUX-TEQs were higher than WHO-TEQs including dioxins and PBDD/Fs in almost all the samples. The CALUX-TEQs of BD Soil 29 and 32 collected near the E-waste recycling workshops were about two times higher than those WHO-TEQs. We suspect that the unidentified PBDFs, because of the lack of standards, contributed strongly to the total dioxin-like activity of BD Soil 29 and 32. For instance, there were only 3 isomers of 2,3,7,8-substituted TetraBDFs to HexaBDFs identified with standards in our study\textsuperscript{11}. Total concentrations of TetraBDF to HexaBDF including potential peaks (110,000 pg/g for BD Soil 29; 5,700 pg/g for BD Soil 32) were about 25 to 100 times higher than their concentrations (1,100 pg/g for BD Soil 29; 260 pg/g for BD Soil 32). On the other hands, for soils related with open-burning of wires and cables, CALUX-TEQs for BD Soil 03 and 08 were about 10 and four times higher than respective WHO-TEQs. In this case, we assume that mixed halogenated dibenzofurans (PXDFs) ($X = \text{Cl}, \text{Br}$) were likely to be important in addition to unidentified PBDFs. Markus et al. reported that PXDFs were present in significantly higher concentrations compared to PCDFs and PBDFs in composited soil collected from open E-waste burning place\textsuperscript{12}. They also confirmed extremely high CALUX-TEQs in soils collected from same places, which were five to 14 times higher than PCDD/Fs-derived WHO-TEQs\textsuperscript{13}.

Results indicate that DR-CALUX assay are useful for screening various types of dioxin-like compounds because instrumental analysis is time consuming for the determination of limited number of target compounds. Therefore, we will use DR-CALUX assay as screening tool in our three year investigation at BD in order to clarify spatial diffusion during the period of survey and to monitor their short-term temporal trends.

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References


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