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Simple and rapid determination of PCDD/Fs in flue gases from various waste incinerators in China using DR-EcoScreen cells

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HIGHLIGHTS

- We construct an automated sample preparation device (SPD-600) for bioassay.
- We construct a new, sensitive and rapid reporter gene system for PCDD/Fs.
- Utilizing SPD-600 coupled cell to determine four different flue gases in China.
- Utilizing SPD-600 coupled cell can be a useful and prescreening method in China.

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ABSTRACT

In developing countries such as China, there is a strong need for simple and rapid bioassays for the determination of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in environmental samples; i.e., flue gas and fly ash from waste incinerators. In this study, we applied the DR-EcoScreen cell (DR-cell) assay to determination of PCDD/Fs in 78 flue gas samples obtained from various waste incinerators in China between 2009 and 2011. The flue gas samples were obtained from four kinds of incinerators, classified into hazardous, medical and municipal-solid waste, and iron ore sintering, and the flue gas extracts were cleaned up using an SPD-600 automated-sample preparation device for DR-cell assay. The PCDD/Fs values obtained from the DR-cell assay were compared with those obtained from conventional high resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS) analysis. The bioanalytical equivalent (BEQ) values obtained from the DR-cell assay were very closely correlated with the international toxicity equivalent (I-TEQ) values from HRGC–HRMS analysis ($r^2 = 0.98$, $n = 78$), while the BEQ values were 5.52-fold higher than the I-TEQ values, as the PCDFs, which account for 80% of the total I-TEQ value, were overestimated by DR cell-assay. Therefore, we multiplied the BEQ values from the DR-cell assay by a conversion coefficient (0.181, the reciprocal of 5.52), and could approximate the TEQ values from the HRGC–HRMS analysis. These results suggest that the DR-cell assay combined with SPD-600 cleanup provides a promising method for the simple and rapid screening of PCDD/Fs levels in flue gas samples, such as those from various waste incinerators in China.

1. Introduction

China is facing growing environmental pressure due to the rapid economic development and urbanization occurring over the last three decades. Consequently, the quantity of various wastes has increased at a high rate, and their disposal has had a great impact on the environment and on public health. To dispose of

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huge amounts of solid waste at low-cost and in an environmentally friendly manner, many incinerators have been constructed in China. Although incineration offers many advantages, such as significant volume and mass reduction, some secondary pollution with the release of compounds, such as heavy metals (Jung et al., 2004; Yao et al., 2012), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Shibamoto et al., 2007; Ni et al., 2009).

These PCDD/Fs, so-called dioxins, are produced as unintentional by-products, and enter the environment via incineration, thermal processes and chemical manufacture (Zheng et al., 2008). These PCDD/Fs are widely distributed contaminants that are persistent and bio-accumulative, and can induce various toxic responses including immunotoxicity, carcinogenicity, as well as having adverse effects on reproduction, development, and endocrine functions via aryl hydrocarbon receptor (AhR) (Poland and Knutson, 1982; Safe, 1986; Fernandez-Salguero et al., 1996; Mimura et al., 1997). Therefore, the Chinese government has imposed standarzdized limits on PCDD/F emissions from flue gas of 1.0 ng I-TEQ N m^{-3} for municipal solid waste incinerators and 0.5 ng I-TEQ N m^{-3} for medical and hazardous waste incinerators. A better understanding of the levels and distribution of these compounds will allow more appropriate measures to be employed to reduce their emission.

The "gold standard" chromatographic technique based on high resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS) has been extensively used for the conventional determination of 17 PCDD/Fs (Firestone, 1991; Jong et al., 1993; Singh and Kulshrestha, 1997). The measured values of 17 PCDD/Fs are individually multiplied by a toxicity equivalency factor (TEF) and totaled to give I-TEQ values (Van den Berg et al., 2006). This method provides reliable data including the concentration of each of the 17 congeners in the test samples. However, it also requires expensive equipment and highly trained analysts, whilst the sample preparation procedures are time-consuming and costly. In particular, this method might be less than useful when rapid data on PCDD/Fs from a large set of test samples is required. For this reason, the development of a rapid and inexpensive screening method for PCDD/Fs remains a high priority, especially in developing countries with limited resources, such as China. Thus, there is a definite need to develop a faster and lower-cost bioassay methods for the determination of PCDD/Fs.

Reporter gene assays using hepatocarcinoma cells, which express the AhR gene and luciferase reporter gene containing the dioxin-responsive element (DRE), are applicable to the detection of dioxin-like compounds based on their activation of AhR (Garrison et al., 1996; Murk et al., 1996). Recently, Takeuchi have developed a new, sensitive and rapid reporter gene assay (DR-cell assay) using a genetically engineered stable cell line, designated DR-EcoScreen cells. The minimal detection limit (MDL) and 50% effective concentration (EC_{50}) of 2,3,7,8-TetraCDD (TCDD) in this DR-cell assay are 0.1 pM and 2.8 pM, respectively, with little variance observed in the data (within CV 10%), but other reporter gene assays, such as, Hepa1c1c7- and H4IIE-based CALUX assays, the MDL of 2,3,7,8-TCDD were reported to be 1 and 0.3 pM, and the EC_{50} of 2,3,7,8-TCDD were reported to be 10 and 10 pM, respectively (Behnisch et al., 2002; Han et al., 2004). Besides high sensitivity, the DR-cell assay has unique advantages compared to other bioassays. As the DR-EcoScreen cells have very strong luminescence intensity and can be measured using a long-lived luciferase substrate, a bioassay using these cells does not require well-washing or medium changes during the procedure. Thus, the DR-cell assay is compatible with high-throughput automation and can reduce the overall workload in a laboratory. Most recently, based on a comparative study with HRGC–HRMS analysis, it has been reported that the DR-cell assay was helpful in determining low levels of PCDD/Fs and dioxin-like polychlorinated biphenyls (PCBs) in ambient air samples (Anezaki et al., 2009) as well as in fish and seafood samples (Kojima et al., 2011).

In the present study, we investigated the applicability of the DR-cell assay to the determination of PCDD/Fs in 78 flue gas samples from four kinds of incinerators, including medical waste and municipal solid waste incinerators, in China as a prescreening step to the HRGC–HRMS method. In addition, we have now combined the DR-cell assay with a cleanup procedure utilizing an SPD-600 automated-sample preparation device. The bioanalytical equivalent (BEQ) values from the DR-cell assay were compared with the I-TEQ values from the HRGC–HRMS analysis, and we found that the values from both methods showed a very close correlation. Here, we provide evidence that the DR-cell assay coupled with SPD-600 cleanup might afford a promising method for the simple and rapid screening of PCDD/Fs in flue gas, such as that from various waste incinerators in China.

2. Materials and methods

2.1. Chemicals and cell culture materials

Acetone, *n*-hexane, toluene, and dichloromethane were obtained from J.T. Baker, Co., Ltd. (USA). Dimethyl sulfoxide (DMSO) and some kinds of silica gel for multi-layer column chromatography were obtained from Wako Pure Chemicals Inc., Ltd. (Osaka, Japan). The PCDD/F standards were obtained from Wellington Laboratories (Canada).

Fetal bovine serum (FBS), alpha-modified Eagle's minimum essential medium (α-MEM) and hygromycin were obtained from Invitrogen (San Diego, CA, USA). Glutamine and penicillin–streptomycin (antibiotics) solutions were obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). A 0.25% trypsin/0.02% ethylenediamine tetra-acetic acid (EDTA) disodium salt solution was obtained from Life Technologies (Paisley, UK). The luciferase substrate, Steady-Glo™ reagent, was purchased from Promega (Madison, WI, USA).

2.2. Collection, extraction, and cleanup of flue gas samples

We collected 19, 20, 21, and 18 flue gas samples from hazardous waste incinerators (i.e., chemical plants, and pesticide and paint factories), medical waste incinerators, municipal solid waste incinerators, and iron ore sintering furnaces, respectively, in China between 2009 and 2011. PCDD/Fs in flue gas were captured by a quartz filter cylinder, and XAD-2 resin with a vacuum pump (TCR TECORA, Italy) (gas volume approximately 3 m^3, 0 °C and 1 atm). Each sample was then extracted with 300 mL of toluene by soxhlet for 24 h. The solvent was reduced to around 1 mL in a rotary evaporator, then 100 mL *n*-hexane was added. The *n*-hexane solution was treated with 20 mL of concentrated sulfuric acid until the *n*-hexane layer became colorless. After washing the extract twice with 50 mL of 2% NaCl solution, it was evaporated to a 1 mL in a rotary evaporator.

As shown in Fig. 1a, the cleanup of samples was conducted using two different methods: cleanup for the DR-cell assays employed an SPD-600 automated-sample preparation device (Kyoto Electronics Company, Ltd., Kyoto, Japan), whereas the cleanup for the HRGC–HRMS analysis used conventional manual chromatography columns, including a multi-silica gel column, an alumina column and a florisil column (JIS K0311, 1999). The SPD-600 device has a multilayer silica gel column (12.5 × 200 mm) and an alumina column (0.8 g). The multilayer column is composed, from bottom up, of silica gel (0.5 g), 10% $AgNO_3$ silica gel, 44% H_2SO_4 silica gel (10 g), and silica gel (0.5 g). The PCDD/Fs were adsorbed on the