DEVELOPMENT OF SCREEINING METHOD WITH FRACTIONATION OF PCDD/Fs AND PCBs IN ENVIRONMENTAL SAMPLES

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Introduction

Polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) and biphenyls (PCBs) are three related compounds of toxic organochlorinated pollutants that are often found together in environmental and biological samples. However, these compounds cannot not be analyzed in simple purified extraction, even though the analysis is performed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). At present, chemical analysis of the 17 toxic 2,3,7,8-substituded PCDD/F congeners, that use various chromatographic techniques along with mass spectrometry, is the standard method for determining TEQs of there samples.

Here, we described a development of screening method with combination system based on tri-lab levels of unique analysis techniques for PCDD/Fs/PCBs. First, prescreening of these compounds using bioassay that can provide a rapid "Yes or No" answer [1,2]. The next step of monitoring should involve chemical analysis methods that use various chromatographic techniques along with mass spectrometry. These methods provide information on quantitation of dioxin. Bioassay based on cells cannot provide its effect on animals [3]. So, a long-term animal study is needed. To characterize the direct effects of EDCs on organisms, this step must be carried out as an animal assay. Information from these methods will be instrumental in identifying specific compounds and development of biosensors. As a result of combination system of tri – lab monitoring, the goal of this study will be to determine which organic pollutants can definitely be characterized as environmental endocrine disrupting chemicals.

Methods and Materials

Sample preparation: The fly ash (10 g) and soil (20 g) samples were extracted by using soxhlet extraction and Accelerated Solvent Extractor (ASE) (Dionex ASE 2000) extraction, following a EPA method. These samples also required clean-ups using multi silica gel and acid alumina columns [4,5].

Fractionation methods: If complex mixtures cause a significant response in a bioassay, in order to determine the cause and identify the possible source the compounds causing the observed response need to be identified. Instrumental analysis should be applied to the entire mixture or different fractions.

These general steps are (Fig. 1.):
1) Screening of the whole extract: S1 and A1
2) After sulfuric acid treatment: S2 and A2
3) After cleanup with multi silica gel column chromatography: S3 and A3
4) After cleanup with acid alumina column chromatography: S4 and A4

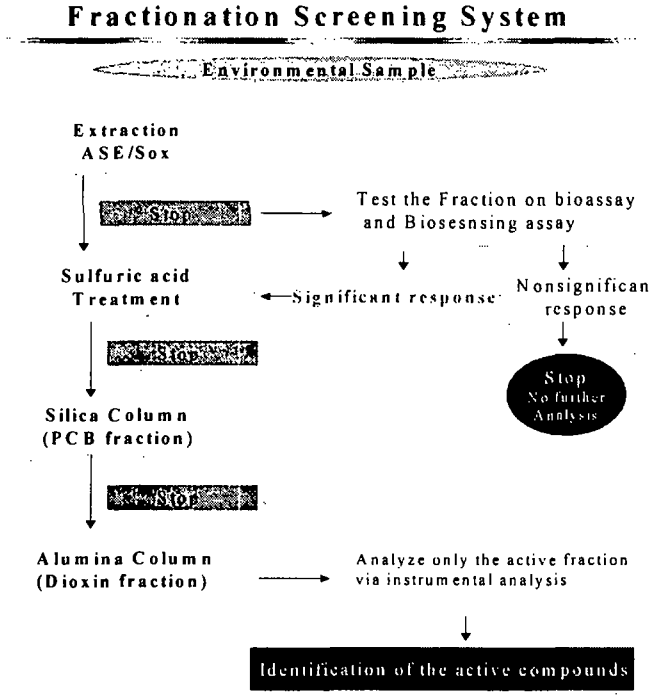

Figure 1. Flow chart of fractionation screening system

Sample analysis: HRGC/HRMS analysis was performed on a JMS-700T (JEOL, Japan) coupled to a HP 6890 gas chromatograph (Hewlett Packard, USA).

Bioassay (Luciferase activity assay): After treatment of cells with appropriate chemicals or extracts for 24 hr, cells were lysed with lysis buffer (20 mM Tris-HCl pH7.8, 1% Triton X-100, 150 mM NaCl, 2 mM DTT). The cell lysate 1 μg/5 μl was mixed with luciferase activity assay reagent 25 μl (Promega) and relative light unit (RLU) was measured using a luminoskan (Labsystems).

Biosesnsing test: The recombinant bacterial strains DPD2794, containing a *recA::luxCDABE* fusion, DPD2511, containing a *katG::luxCDABE* fusion, TV1061, containing a *grpE::luxCDABE* fusion, and DPD2540, containing a *fabA::luxCDABE* fusion, were used in this study. These 0.1 mL cultures in the test tubes were exposed with different concentrations of dioxin, and the bioluminescence was measured at set time intervals using a Model 20e luminometer (Turner Designs, CA). Dioxins stock solutions were prepared to 1 μg/ml concentrations using 0.8%

acetone. The maximum BL ratio is defined in this study as the ratio of the maximum BL of the induced cells to the maximum BL of the positive control cells that were exposed to same concentration of the solvent alone.

Results and Discussion

Fractionation of the samples that were active in bioassays and chemical analysis can be used to determine the most probable contributors to the total activity. The difference between bioassay and chemical assay value is relatively consistent for a specific matrix. For example, the bioassay of soil was about two or three higher than the chemical TEQ level. Similar values were obtained for fly ash. For example, the PCDD/Fs concentration was 0.095 ng-TEQ/g in fly ash, while biological TEQ concentration was 0.3 ng-TEQ/g in the same sample (Fig. 2). In addition to the fraction test, several soil and fly ash samples were analyzed for PCDD/Fs and PCBs extracted with ASE and Soxhlet. The extraction efficiencies were compared with bioassay and biosensing assay. The ASE method showed equal or better extraction efficiency than soxhlet extraction in biological assays. Comparison of toxicity levels of biosensing assay for each fraction in fly ash samples was Dioxin fraction levels higher than the others levels (Fig. 3). Based on the experiences during the bioassay and chemical assay, it is concluded that the biological TEQ values of environmental samples are higher than values derived from the chemical assay.

Bioassay, in combination with simple clean-up procedure, seems to be a very suitable screening method for dioxins and planar PCBs. Since, in most cases, rapid investigation of suspected samples is required, the test functions best when operated in close combination with the GC/MS.

In conclusion, the present study was conducted to establish a rapid and simple combination method for screening of PCDD/F and PCBs in various environmental samples.

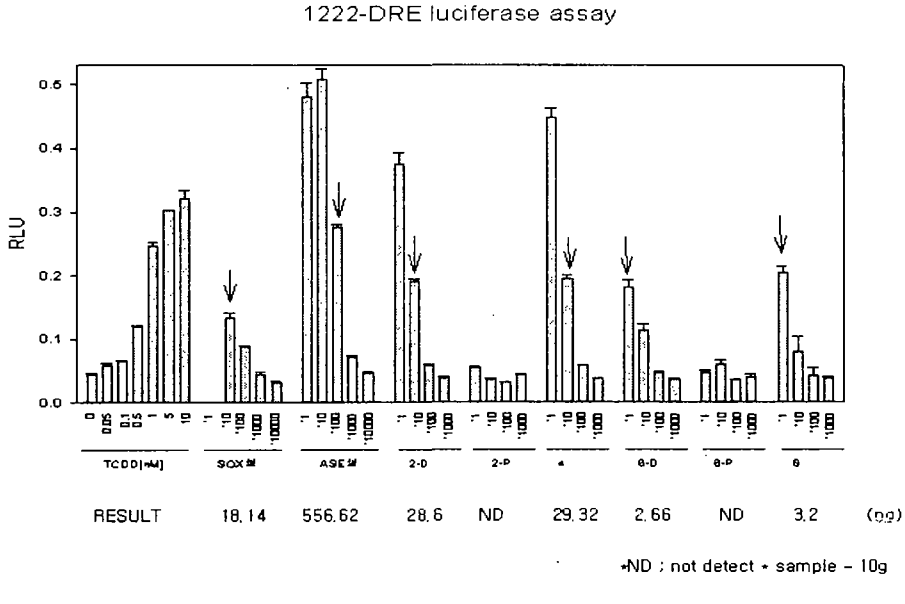

Figure 2. DRE luciferase bioassay for each fraction of fly ash sample

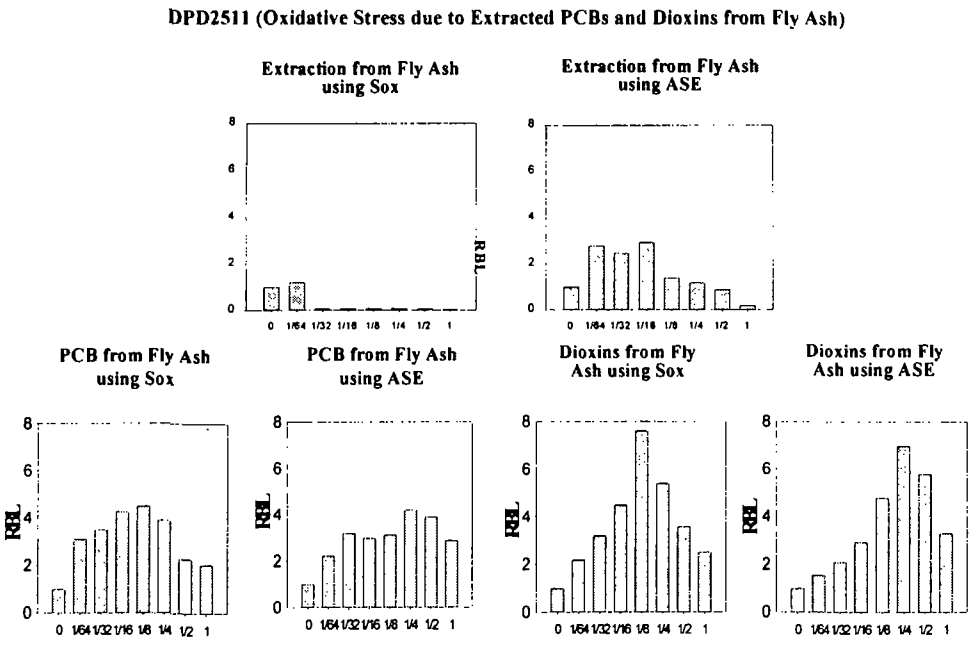

Figure 3. Comparison of toxicity levels between each fraction of fly ash

Acknowledgements

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