# A novel reporter gene-based bioassay system for dioxins determination in solid waste

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Introduction:

Dioxin and dioxin-like compounds are ubiquitous and persistent environmental contaminants. These compounds produce a variety toxicological effects and most of which are mediated by the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor (Denison and Nagy 2003). Exhaust gas and fly ash from incineration and incomplete combustion of solid waste are a major source of dioxins released into the environment (Rathna et al. 2018). Both in the environmental regulation and in the health assessment, dioxin emissions monitoring is an essential task. The standard method for dioxin measurement relied on high-resolution gas chromatography/high resolution mass spectrometry (HRGC-HRMS) is time consuming and expensive. Therefor we have developed a novel AhR-mediated reporter gene cell sensor which is more sensitive and specific than other similar bioassays cell line (minimal detection limit was 0.1pM). This AhR-dependent sensor has been successfully applied in the dioxin determination in ambient air (Zhang et al. 2018). In this study, we employed this sensor for dioxin determination in solid waste and established a bioassay system including sample extraction to detection dioxin in fly ash.

Material and methods

35 fly ash samples were collected from incinerators. Briefly, for extraction and cleanup, 5g fly ash was extracted by accelerated solvent extraction (ASE) with toluene and hexane: dichloromethane (1:1), respectively. We use a 33% acidic silica gel and a tandem multilayered silica gel-activated carbon column for dioxin like polychlorinated biphenyl (DL-PCBs), Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/Fs) and Polybrominated Dibenzo-p-dioxins and Dibenzofurans (PBDD/Fs).the extraction was eluted with an aliquot of 15 mL ethyl acetate/toluene/hexane (1:1:8 v/v/v) to get the fraction containing DL-PCBs and eluted with 20 mL toluene to get the fraction containing PCDD/Fs. For chemical analysis, using appropriate elution for HRGC-HRMS analysis. For cell-based analysis, the fractionated extracts were evaporated and dissolved in 200 μL DMSO.

Cells were seeded with a density of 40,000 cells/well in white clear bottom 96-well cell culture plates in α-MEM medium supplemented with 10% FBS at a volume of 100 μL per well. After cultivation for 24h, 100 μL growth medium containing the test sample was added to the cells in triplicate (1% DMSO final concentration). In parallel, a calibration standard curve for TCDD was generated with 9 concentrations between $1.5 \times 10^{-13}$ to $10^{-9}$ M. Each plate contained a control solution of 11.93 pg-TEQ per well (called verification solution). After 24h of exposure, the medium was removed, the cells were washed gently with 100 μL PBS, then 100 μL of cell-lysis buffer was added into each test-well. The plate was then shaken at room temperature for 10min and luciferase activity was measured with a microplate luminometer using the Luciferase Reporter Assay System kit

Results and discussion

CBG2.8D cell sensor is an AhR-mediated reporter gene mouse hepatoma cell line which has been stably transfected with a highly sensitive luciferase reporter plasmid, pCL-CR2, containing 8 copies of DREs (dioxin response elements) and the mouse *CYP1A1* core promoter (Figure 1). This new optimization ensures the specificity of response compared with other vectors commonly used for bioassays which contain the mouse mammary tumor virus (MMTV) promoter or *Drosophila* heat-shock promoter (Denison et al. 2004; Takeuchi et al. 2008).

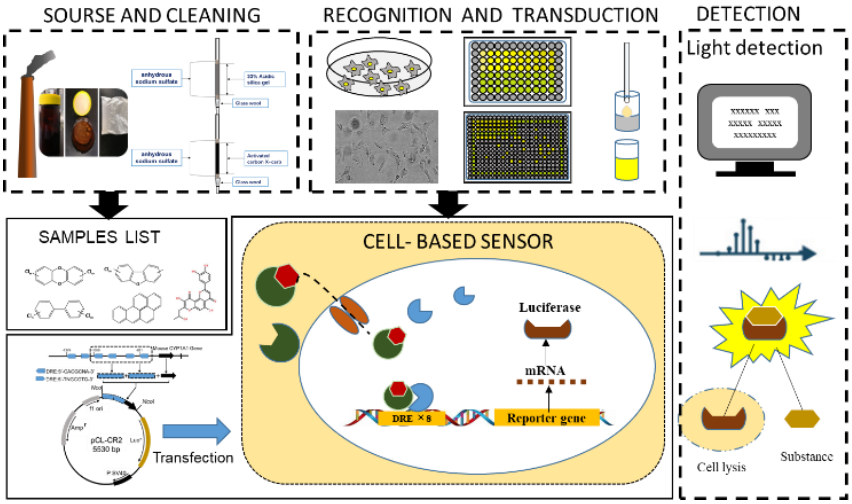


Figure 1: Construction of CBG2.8D cell sensor and diagram of bioassay system process

We determined the concentrations of dioxins in 35 fly ash samples collected in different incinerators around China, using both the CBG2.8D bioassay system and HRGC-HRMS analysis, then compared the respective TEQ values obtained. There was a good correlation between the two methods indicating this bioassay system could be used as a rapid screening method for dioxins determination in solid waste.

## Conclusion

In this study, we successfully applied the novel cell sensor CBG2.8D in detection dioxin in fly ash sample and established a detection system including sample extraction and dilution, cell treatment, data analysis and quality control (data about this part were not shown). The preliminary validation study shows this bioassay system could be used as an alternative screening method for a quick dioxin determination in solid waste.

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## References

Denison MS, Nagy SR. 2003. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annual review of pharmacology and toxicology 43:309-334.
Denison MS, Zhao B, Baston DS, Clark GC, Murata H, Han D. 2004. Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals. Talanta 63:1123-1133.
Rathna R, Varjani S, Nakkeeran E. 2018. Recent developments and prospects of dioxins and furans remediation. J Environ Manage 223:797-806.
Takeuchi S, Iida M, Yabushita H, Matsuda T, Kojima H. 2008. In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, dr-ecoscreen cells, and in vivo mouse liver cytochrome p450-1a induction by propanil, diuron and linuron. Chemosphere 74:155-165.
Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, et al. 2006. The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences 93:223-241.
Zhang S, Li S, Zhou Z, Fu H, Xu L, Xie HQ, et al. 2018. Development and application of a novel bioassay system for dioxin determination and aryl hydrocarbon receptor activation evaluation in ambient-air samples. Environmental science & technology 52:2926-2933.
Zhou ZG, Ren Y, Chu JZ, Li N, Zhen S, Zhao H, et al. 2016. Occurrence and impact of polychlorinated dibenzo-p-dioxins/dibenzofurans in the air and soil around a municipal solid waste incinerator. J Environ Sci-China 44:244-251.