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DRE-CALUX bioassay in comparison with HRGC/MS for measurement of toxic equivalence in environmental samples

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Abstract

CALUX, Chemically Activated LUciferase gene eXpression bioassay, has proven valuable for screening for and assessing toxic equivalents of dioxin-like compounds, because it detects all AhR (arylhydrocarbon receptor) ligands in a variety of sample matrices. In this study, we tried to validate DRE (dioxin-response elements)-CALUX bioassay, which has been developed by cloning mouse *cyp1a1* gene in front of luciferase reporter gene. We compared DRE-CALUX bioassay with high resolution gas chromatography/mass spectrometry (HRGC/MS) for assessing environmental samples from Korea. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dose response study showed high correlation ($r^2=0.89$) between DRE-CALUX bioassay and EROD (ethoxyresorufin *O*-deethylase) bioassay, a commonly used bioassay method. The mean TEQ value of water samples was 0.57 pg-$TEQ_{HRGC/MS}$/L and 4.97 pg-TEQ_{CALUX}/L. For soil samples, HRGC/MS-TEQ values ranged from 0 to 47.18 pg-TEQ/g (dry) and correlated well ($r^2=0.98$) with values obtained by CALUX-TEQ which ranged from 0.92 to 649.97 pg-TEQ/g (dry). The difference between the absolute TEQ values might be due to the presence of dioxin-like compounds without WHO-TEQ values rather than the difference between CALUX-REP and WHO-TEQ. Based on this study, we suggest that DRE-CALUX bioassay can serve as an alternative bioassay method for high-throughput analysis of large number of environmental samples.

Keywords: CALUX; HRGC/MS; Dioxin; EROD; TEQ

1. Introduction

Persistent Organic Pollutants (POPs) represent a group of pollutants that are semi-volatile, bioaccumulative, persistent and toxic (Vallack et al., 1998; Jones and de Voogt, 1999). Due to their long-range transport potential and harmful effects on humans and wildlife, international agreements are now coming into effect for reducing future environmental burdens. Stockholm Convention on POPs, designed to eliminate or at least control 12 POPs, was signed by 151 governments including Korea from 23 May 2001 until May 2002, and ratified by 98 governments (as of September 2006) (UNEP, 2006). Under this convention, the establishment of emission inventories for POPs is mandatory and this should eventually provide the basis for further emission reductions (UNEP, 1999, 2001; Vestreng and Klein, 2002). Thus, monitoring of POPs in environment is an important first step. (WHO, 1999).

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Among the POPs, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), so-called "Dioxin-like" compounds, are now the object of public, regulatory, and scientific attention, because some of these compounds exhibit a wide spectrum of toxic responses including immunotoxicity, developmental and reproductive toxicity, neurotoxicity and carcinogenicity (Kerkvliet, 1995; Brouwer, 1991; Nebert et al., 1993). Until 10 years ago, chemical analysis by high-resolution gas chromatography/mass spectrometry (HRGC/MS) was the only option and the golden standard for detecting dioxin-like compounds. Based on chemical analysis of each congener of the selected dioxin-like compounds, TEQ (Toxic EQuivalence) values can be calculated as a sum of multiples of concentration of each congener with TEF (Toxic Equivalency Factor) values. Although chemical analysis can measure various dioxin-like compounds quantitatively with selectivity and sensitivity, this method has limitations such as high costs, lack of rapidity, and the need for large volumes of samples. Moreover, because chemicl analysis is limited to 17 PCDDs/PCDFs, risk assessment by this methods will not include the potential toxic effects of other toxic polyhalogenated aromatic hydrocarbons (PHAHs), such as fluorinated, brominated, and mixed halogenated PCDDs/PCDFs as well as other polyhalogenated compounds, such as azo and azoxycompounds, biphenyl ethers, naphthalenes, sulfur-analogue dioxins, and alkylated dioxins. In addition, this method will miss potential synergistic or antagonistic effects of dioxin-like compounds. In the environment, dioxin-like compounds coexist as complex mixtures of various congeners and multiple levels of interactions between dioxin-like compounds can modulate their toxic potential. A number of studies demonstrated the non-additive interactions between dioxin-like compounds (Bannister et al., 1987; Haake et al., 1987; Biegel et al., 1989; Davis and Safe, 1989, 1990; Harper et al., 1995). Thus, chemical analysis is sufficient for neither the high-throughput monitoring of dioxin-like compounds in environment nor prediction of combinational toxicity in the biological organisms.

To overcome these limitations of chemical analysis, various biological assays, such as arylhydrocarbon hydroxylase (AHH) assay and ethoxyresorufin-*O*-dealkylase (EROD) assay (Bradlaw and Casterline, 1979; Zacharewski et al., 1989; Tillitt et al., 1991; Hanberg et al., 1991; Safe, 1993), have been developed as alternative methods. These bioassay approaches are based on a number of studies (Nebert et al., 1993; Lucier et al., 1993; Poland and Knutson, 1982; Safe, 1990; Schmidt and Bradfield, 1996) demonstrating that the toxicity of dioxin-like compounds is mediated through arylhydrocarbon receptor (AhR), a ligand dependent transcription factor (Nie et al., 2001). After dioxin-like compounds bind to the AhR, the AhR complex undergoes transformation and interacts with its dimerization partner, AhR nuclear translocator (Arnt). This heteromeric ligand·AhR·Arnt complex binds to Dioxin-Response Elements (DREs) in the 5′ flanking region of certain genes, so-called "AhR gene battery" (Burbach et al., 1992; Hankinson, 1995; Whitlock, 1993). The binding to the DRE sequences leads to DNA bending, chromatin and nucleosome disruption, increased promoter accessibility and increased transcriptional activation of responsive genes.

In this study, we cloned 5′ mouse *cyp1a1* flanking DNA (−1642 ~ +53) containing multiple DREs in front of luciferase reporter gene. Using this plasmid, we established genetically modified cell line by transfecting them for the DRE-CALUX bioassay. This study was aiming at establishing the DRE-CALUX bioassay as a cost-efficient high-throughput screening method for dioxin-like compounds in environmental matrices. In order to test DRE-CALUX bioassay system if it is suitable for the high throughput screening method, we have applied DRE-CALUX, EROD, and HRGC/MS methods for the same sample and compared the results from these three assay systems. Using this DRE-CALUX bioassay, we assessed the dioxin-like compounds in Korean water and soil samples. We also calculated CALUX-REP values for 17 kinds of dioxin-like compounds and compared them with WHO-TEFs of corresponding compounds. Furthermore, we examined dioxin-like activity of compounds without assigned WHO-TEF values in order to elucidate the reason for difference between absolute TEQ values obtained from DRE-CALUX bioassay and HRGC/MS analysis.

2. Materials and methods

2.1. Materials

PCDD/PCDF congeners were purchased from Wellington Laboratory (Ontario, USA) and the purities of these chemicals were greater than 95%. Polyhalogenated aromatic hydrocarbon (PAHs) standards were kindly provided by EPA (USA). DMSO, sodium chloride, sodium acetate, sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), *d*-glucose, ethoxyresorufin, resorufin, β-nicotinamide adenine dinucleotide phosphate reduced form (β-NADPH), sodium bicarbonate, glycerol, tris base,