

In Vitro Bioassay Determination of Dioxin-Like Activity and Estrogenic Activity in Sediment and Biota from Taihu Lake, China

Yan Xu, Qinting Jiang, Maoyong Song, H.L Wong, Michael H.W Lam, Paul K.S Lam, Hongxia Yu, John P Gisey

1. Introduction

Some persistent halogenated organic contaminants, such as polychlorinated biphenyls (PCB), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and certain polycyclic aromatic hydrocarbons (PAHs), have been shown to elicit a wide variety of adverse effects in organisms, which are mediated by an aryl hydrocarbon receptor (AhR)-dependent mechanism.¹ Recently, effects of estrogenic compounds on endocrine functions of aquatic organisms are of concern.² Comparing to 17- β -estradiol (E2) and estrone, alkylphenols (APs), such as nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA) are a class of weakly estrogenic compounds known to exist in the environment since the 1940s.^{3,4} Instrumental analysis alone provides little information regarding the integrated biological potency of samples. Thus, bioassay is often employed to supplement the deficiency of chemical analysis in characterizing toxicity of environmental samples. In this work, recombinant rat hepatoma cells (H4IIE-luc), with a stable transfected luciferase reporter gene under the control of dioxin-responsive elements (DREs) were used to characterize sediment and biota samples obtained from the Taihu Lake, China, for their capacity and potency to induce dioxin-like responses *in vitro*.⁵ Recombinant MCF-7 human breast cancer cells (MVLN cells) with a luciferase reporter gene under the control of estrogen-responsive elements (EREs) were also used to screen the overall estrogenic potency of sediment samples.⁶

2. Materials and methods

2.1. Sample collection

Taihu basin of the Yantzs River Delta is situated in East China and is a very important region for the economics of the country. It is also one of the regions in China where many major chemical industries are concentrated. Nine surface sediment samples, four species of fish and one species of mud snails were collected from Meiliang Bay, Taihu Lake, China, on 23rd August 2004. All samples were stored at -20°C until analyzed.

2.2. Sample extraction and fractionation

Muscle of individual fish, a combination of 100 individual mud snails' tissue and sediment samples were freeze-dried and homogenized. The biota tissues (4 g) and sediment sample (20 g) were then Soxhlet extracted for 18hr using 400 ml of high purity dichloromethane and hexane (3:1 v/v). Raw extracts of sediment were treated with acid-activated copper powder to remove sulfur. All of the extracts were concentrated to 1.0 ml. Fractionation of sediment extracts was performed in accordance with literature method.⁷ 10% of sediment raw extracts were treated by 98.3% H₂SO₄ to remove PAHs and coloring matter interference.

2.3 Cell culture and bioassay

The cell culturing procedures and bioassay methodologies were in accordance with literature method.⁷ Cells were plated into 96-well culture plates (0.25ml/well) with a concentration of 7.5×10⁴ cells/ml for H4IIE-luc and 12.5×10⁴ cells/ml for MVLN and were incubated for 24hr, prior to dosing. Test and control wells were dosed with 2.5ul of the appropriate extract or solvent. Standard wells received different concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for H4IIE-luc and 17- β -estradiol (E2) for MVLN, while blank wells received no dose. Control wells received solvent only. Cell viability assay and luciferase assays were conducted after 72hr of exposure. Data analysis was carried out in accordance to the literature.⁸

3. Results and Discussion

3.1 Dioxin-like activity

Expressions of the *in vitro* H4IIE-luc bioassay on raw tissue extracts of fish and snails are summarized in Fig.1. All expressions were below detection limit (0.16 pg TCDD /well, 4.2% of the maximum response observed for 30pg TCDD/well), except for snails which showed a TCDD-EQ of 47.4 pg/g DW (EC10). This indicates that extracts of fish may contain some chemicals which may inhibit luciferase activity. Such negative responses were also observed by Jiang *et al.* in fish samples obtained from the East China Sea.⁹ Snails living in sediment may be more exposed to dioxin-like contaminants.

Each of the nine sediment sample was tested as raw extract (RE) and 6 of them were fractionated and acid treated. Based on the initial screening of the RE, all of the sediment samples showed significant dioxin-like activity in H4IIE-luc bioassay. The dose-response curves are shown in Fig. 2. The difference of the slopes was significant. Single point estimate is not suitable. The dose-response curves fit the sigmoidal model. TCDD-EQs derived from the level of response equivalent to EC50 and the range of TCDD-EQs calculated from responses equivalent to EC20 and EC80 are shown in Table 1. Comparing the response of the three fractions, the mid-polar (F2) and most polar (F3) fractions were responsible for the significant reporter gene expression in H4IIE-luc bioassay. F2 and F3 samples were estimated to contain 44.9–256 pg TCDD-EQ/g DW and 26.3–46.3 pg TCDD-EQ/g DW. F1 was found to be unable to induce significant response. After acid treatment, the maximum response of all of the tested RE was greatly decreased. This indicates that PAHs appeared to account for most of the dioxin-like responses observed. Four of the F1 samples were either cytotoxic or caused morphological changes in H4IIE-luc cells. Similar toxicity was also observed in their corresponding raw extracts. No cytotoxicity was seen in F2 and F3, which means the unknown cytotoxic compounds may be unipolar and acid stable.

3.2 Estrogenic activity

Most of the RE of the sediment samples did not exhibit estrogenic response in MVLN bioassay. The highest induction was shown in Sample 2 (2A) with a maximum response of 38.5% according to the maximum activity derived by 30nM E2 standard. Seven samples showed relatively negative estrogenic activity compared with the solvent control samples. As there was no obvious cell death, the negative activity might be an indication of indigenous inhibiting substances in these samples.

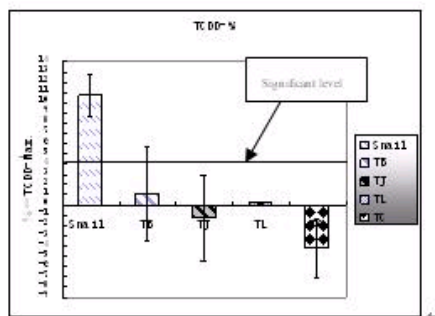
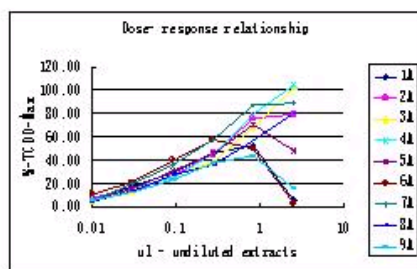


Fig.1. Luciferase induction in the H4IIE cell bioassay elicited by biota samples of Taihu lake. Response magnitude presented as percentage of the maximum response derived by 30pg TCDD/well (%-TCDD-Max). Snail *Bullacta exarata*; TB, white carp; TJ, crucian; TL, common carp; TC, grass carp.

Fig.2. Dose-response relationship curves of dioxin-like activity for raw extracts of Taihu Lake sediment. X-Axis stands for the concentration level of sample in well. Data points represent the mean value of three measurements; Coefficient of variation (CV) of each point are less than 15% except the ones with cytotoxicity.



4. Acknowledgement

The work described in this paper was supported by the Areas of Excellence Scheme established under the University Grants Committee of the Hong Kong Special Administrative Region, China (Project No.

AoE/P-04/2004), and a Central Allocation Grant (Project No. 8730011).

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