CALUX BIOASSAY AND ECOLOGICAL RISK ASSESSMENT OF PCDD/F_S AND DL-PCB_S IN SEDIMENT OF HAIHE RIVER, CHINA

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Introduction

Recently, the dioxins and dioxin-like compounds, such as PCDD/Fs (polychlorinated dibenzo-p-dioxin and dibenzofurans) and DL-PCBs (dioxin-like polychlorinated biphenyls), are arousing more and more attention around the world. But, due to a general deficiency of monitoring capacity, until now this class of compounds has been poorly characterized in China [1]. The HRGC/HRMS (High Resolution Gas Chromatography/High Resolution Mass Spectrometry) analysis is the international golden standard method. However, it is a time-consuming, expensive and complex method. In addition, for hazard and risk assessment, HRGC/HRMS analysis only provides information about concentration of several target dioxin compounds. And limited information on the total biological effects of the samples is available, particularly when there are complicated interactions among various dioxins and other compounds. As an alternative, the CALUX (Chemically Activated LUciferase gene eXpression) bioassay, which is a more rapid and cheaper method, can provide a comprehensive measure of total AhR (aryl hydrocarbon receptor) activity and its associated risk [2].

Haihe River, which flows through the Tianjin area, is the most badly polluted one in the seven largest rivers in China. In this area, there's a main producer of PCP (pentachlorophenol) and its sodium salt PCP-Na. Such production has lasted for more than two decades [3,4], to meet the need of schistosomiasis control in endemic provinces of southern China. PCP and PCP-Na production has been identified as a very important source of PCDD/Fs release [5], which may endanger the aquatic organisms in the Haihe River. The goal of this study was to use CALUX bioassay to determine TCDD-TEQs (toxic equivalency) of surface sediments in Haihe River and perform probabilistic aquatic ecological risk assessment of dioxins in Haihe River based on CALUX TEQs in sediments and EqP (equilibrium partitioning) theory.

Materials and Methods

Sampling

The sampling map is shown in Figure 1. It spreads from 117°11.00'E to 117°48.50'E, and from 38°58.10'N to 39°07.83'N. The surface sediment (<10 cm depth) sampling were conducted twice, once in March, 2005 and the other in November, 2006. The samples No.1~10 (denoted as H01~H10) were taken from Haihe River and No.11 (denoted as BHW) was taken from Bohai Bay for comparison.

CALUX Bioassay

The samples were freeze dried, milled and passed through a 60-mesh sieve. 3.50g sediment samples were weighted. The samples were exacted with methanol/toluene (1:4, v/v), cleaned up by a 25ml celite column and a 25 ml sulfuric acid silica gel column, and then separated on a 5 ml disposable X-CARB (Xenobiotic Detection Systems, USA) column into PCDD/Fs fraction and DL-PCBs fraction.

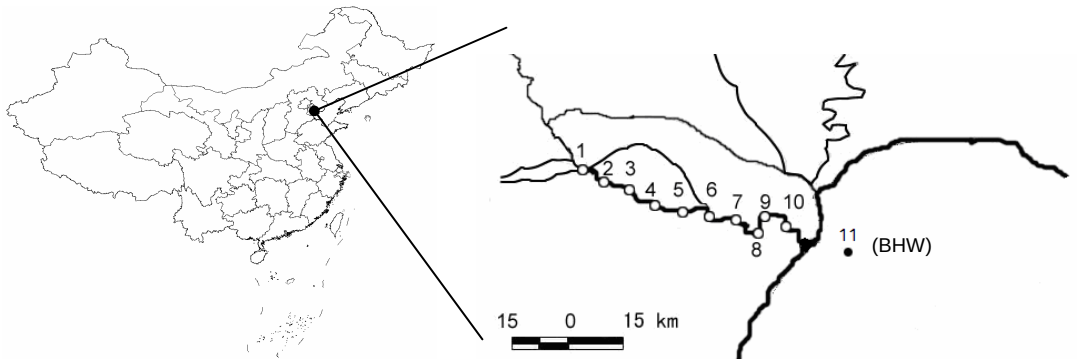


Figure 1 The map of sampling sites

H1L6.1c2 cells (Xenobiotic Detection Systems, USA) were exposed to the samples in 96-well plates. Light output was measured by the microplate luminometer (Berthold Centro LB 960, Germany) and expressed in RLU (relative light unit). The best equation fitting the calibration curve was calculated using a four-variable Hill equation. This equation was used to convert the measured RLU value into a CALUX TEQ value expressed in pg 2,3,7,8-TCDD-TEQ/g sediment.

Ecological risk assessment

The water concentrations (C_{water}) were estimated from CALUX TEQs in sediments (C_{sed}) using the EqP method[6]:

$$C_{water}\ (\text{pg TEQ/L}) = C_{sed}\ (\text{pg TEQ/kg})/K_p = C_{solid}\ (\text{pg TEQ/kg}) \cdot f_{oc}/K_{oc} \qquad (1)$$

Here, Kp is the partition coefficient between the solid and the aqueous phase (2,3,7,8-TCDD), L/Kg; foc is mass fraction of organic carbon. Koc is the partition coefficient normalized by organic carbon content.

Acute toxicity data (LC_{50}, half lethal concentration) of 2,3,7,8-TCDD to various aquatic species were extracted from AQUIRE database (USEPA) and some other sources, and then were used to develop SSDs (Species Sensitivity Distributions) in the following probabilistic ecological risk assessment.

A joint probability curve (JPC) was generated from aquatic ECD (Exposure Concentration Distributions) and SSD. For JPC method, the overall risk was usually illustrated qualitatively by the adjacence degree of the curve to axes[7]. In this research, the Overall Risk Probability (ORP) was introduced to quantitatively characterize the overall ecological risk:

$$ORP = \int_0^1 EPr(x)\, dx \qquad (2)$$

Here, EPr(x) was the exceedance probability for x proportion of species affected ($0 \le x \le 1$). ORP could be just calculated as the area under the JPC. Because sufficient chronic toxicity data, such as NOECs ((No Observed Effect Concentrations), were unavailable, SSDs were developed based on a set of "surrogate NOECs" generated by dividing acute toxicity values by a set of ACRs (Acute to Chronic Ratio) (1, 5, 25, 125 and 1000). A series of JPCs and corresponding ORPs were achieved.

The distribution-based HQs (Hazard Quotients) were generated from ECD and SSD after 20,000-times Monte Carlo (MC) simulation. Accordingly, risk was expressed as the probability of exceeding certain HQ criteria (1, 1/5, 1/25, 1/125, 1/1000) corresponding to a series of ACRs mentioned above.

Results and discussion

Pollution level and character

From Figure 2 and Figure 3, it is showed that PCDD/Fs are the main contributor to dioxin TEQ. DL-PCBs levels are much lower than PCDD/Fs levels. PCDD/Fs in March, 2005 were very close to those in November, 2006. The same case can be seen from DL-PCBs in March, 2005 and November, 2006. The sites near the Bohai Bay were most badly polluted. For the site BHW in Bohai Bay, the dioxin pollution is neglectable because of its distance from potential source and dilution by sea water. Good linear correlations were found between the CALUX results in 2005 and those in 2006 for PCDD/Fs (R^2=0.9706) and DL-PCBs (R^2=0.6227). Also, PCDD/Fs levels are significantly correlated with DL-PCBs levels in Haihe River (Figure 4).

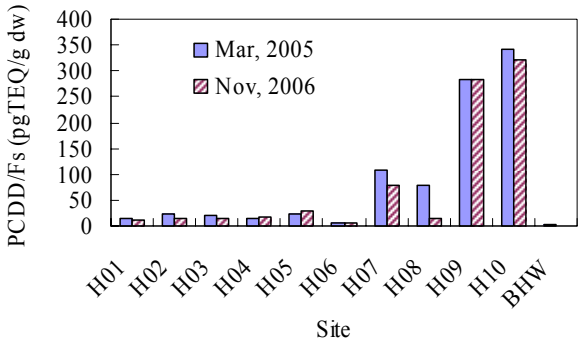


Figure 2 PCDD/Fs in sediment of Haihe River

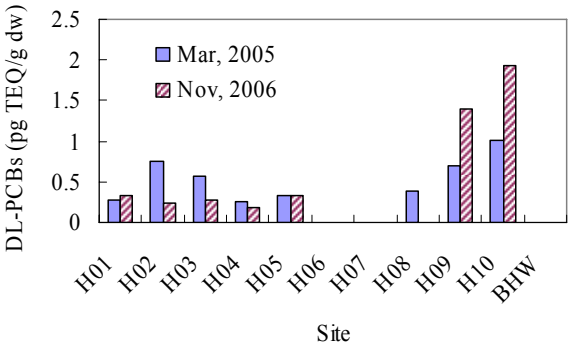


Figure 3 DL-PCBs in sediment of Haihe River

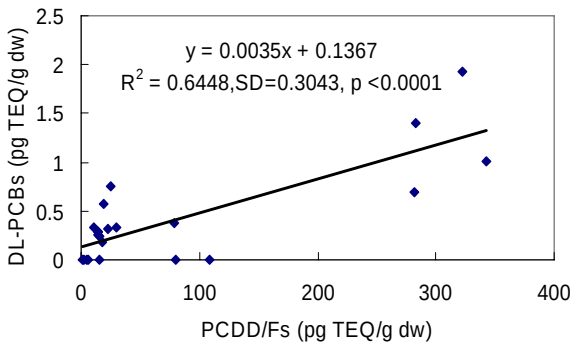


Figure 4 Linear correlation between PCDD/F and DL-PCBs in Haihe River

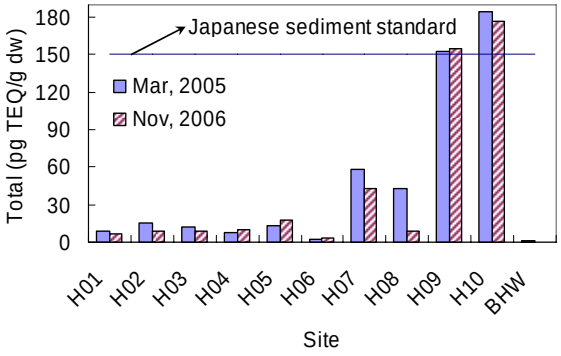


Figure 5 Estimated HRGC/HRMS result

The total HRGC/HRMS TEQ was estimated form CALUX TEQ based on the transformation coefficients that were determined by our previous research of sediment samples in Haihe River:

$$\text{Total TEQ}_{\text{HRGC/HRMS}} = 0.5325\ \text{PCDD/Fs TEQ}_{\text{CALUX}} + 2.7486\ \text{DL-PCBs TEQ}_{\text{CALUX}} \quad (3)$$

It is shown in Figure 5 that the estimated total HRGC/MS TEQs in sediment in Haihe River are generally not very high. But for H09 and H10, the total TEQ exceeds the Japanese sediment standard (150 pg TEQ/g). These sampling sites are both at the downstream of the Haihe River flowing though the chemical industrial area where the PCP and PCP-Na have been manufactured for a long period. Much attention should be paid to their potential hazard and risk.

Ecological Risk

ORPs differ largely for different ACRs. The ORPs calculated at an ACR of 1, 5, 25, 125 and 1000 were respectively 0.002586, 0.008537, 0.02418, 0.05902 and 0.1510. ORPs calculated at the ACR of 5 and 125 could be respectively considered as the lower limit and higher limit of ORPs calculated at the ACR of 25. The ORPs would be underestimated if LC_{50} were directly used to develop JPC (ACR=1), and significantly overestimated if ACR was set as 1,000.

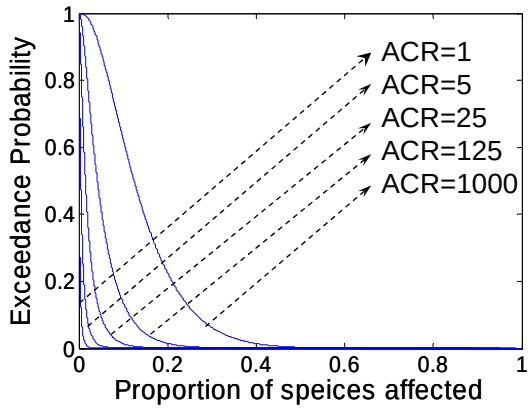
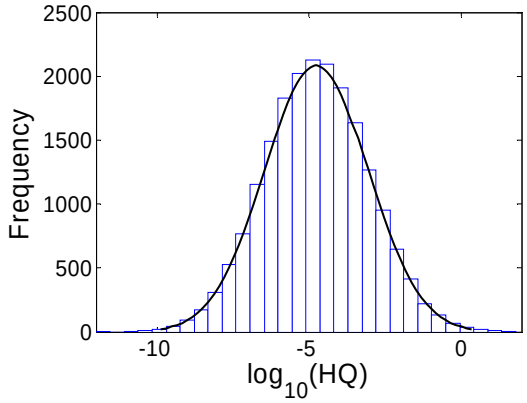


Figure 6 JPC of dioxins at different ACRs



Fiure 7 HQ distribution of dioxins after MC simulation

The geometric mean of HQ is 1.7682×10^{-5}. The probability of exceeding preselected HQ criteria of 1, 1/5, 1/25, 1/125 and 1/1000 were respectively 0.002647, 0.008693, 0.02452, 0.05959 and 0.1519. The probabilities of exceeding the preselected HQ criteria were very close to ORPs at the corresponding ACRs in JPC method. Thus, the same conclusion could be drawn from Monte Carlo simulation and from the JPC method. Monte Carlo simulation and JPC method are just the different risk characterization mode with the same essence. Monte Carlo simulation can calculate the distribution of HQs, which did not vary with ACR. And probability of exceeding preselected HQ criteria could be calculated after Monte Carlo simulation. JPC differ largely for different ACRs, but it can be used to estimate probabilities for different proportions of species affected at various ACRs.

Acknowledgement

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