Detection of Ah receptor agonists in sediments from the Three Gorges Reservoir and its feeder rivers using the H4IIE-luc Cell Line

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Introduction

Sediments play a major role as a sink for many kinds of environmental pollutants. Potentially contaminated sediments in the regions of the **Yangtze Three Gorges Reservoir** (TGR) in **China** pose a **risk to aquatic organisms**. Additionally, annual changes in water level cause **flooding events**, which lead to a relocation of particulate matter onto **agricultural areas** along the river bank and thus can pose a certain **risk to humans** as well.
Dioxin-like compounds (DLCs) form an important group of hazardous compounds which can be found almost everywhere in the environment and are known to pose a threat to different organization levels of **mammals**. The initial event of the adverse outcome pathway is the binding of a DLC to the mammal **Aryl hydrocarbon receptor** (AhR). **The H4IIE-luc Assay** is a reliable alternative to the more commonly used **EROD Assay** for detection of DLCs.

Method

Sampling

For the investigation of the sediments using a weight-of-evidence approach [1], a German-Chinese team took samples at **multiple locations** along the TGR and its feeder rivers near the cities of **Chongqing, Fengdu, Yunyang** and **Wushan** in September 2011 (Fig. 1; 2).

H4IIE-luc Assay

To examine **AhR activity** in the sediments, 19 of these samples were tested in the **H4IIE-luc Assay**. H4IIE **rat hepatoma cells** were stably-transfected with an inducible **reporter plasmid** which contains the **firefly luciferase gene** under control of **four dioxin response elements**. Exposure of these H4IIE cells to Ah receptor agonists results in **induction** of measurable **luciferase activation** in a time- and dose-dependent manner.
All sediment extracts were tested in different sediment equivalent concentrations to generate **concentration-response curves**. 2,3,7,8-Tetrachlorodibenzodioxin (**TCDD**) was used as **standard** (Fig 3). All curves were **linearized**. 25% maximal effective concentrations EC_{25} of TCDD were divided by EC_{25} of the samples to calculate **relative potency values (RP_{25})**:

$$RP_{25} = \frac{EC_{25}\ (TCDD)}{EC_{25}\ (sample)}$$

EROD Assay

Results of the H4IIE-luc Assay were compared to results of the more commonly used EROD Assay. The permanent fish liver cell line **RTL-W1** from **rainbow trout** *Oncorhynchus mykiss* was used for identification and quantification of dioxin-like effects. Exposition of RTL-W1 cells to DLCs results in increasing expression of CYP1A. This leads to induction of **7-ethoxyresorufin-O-deethylase** (EROD) which is a measurable biomarker. Relative potency values were calculated in the same manner as in the H4IIE-luc Assay.

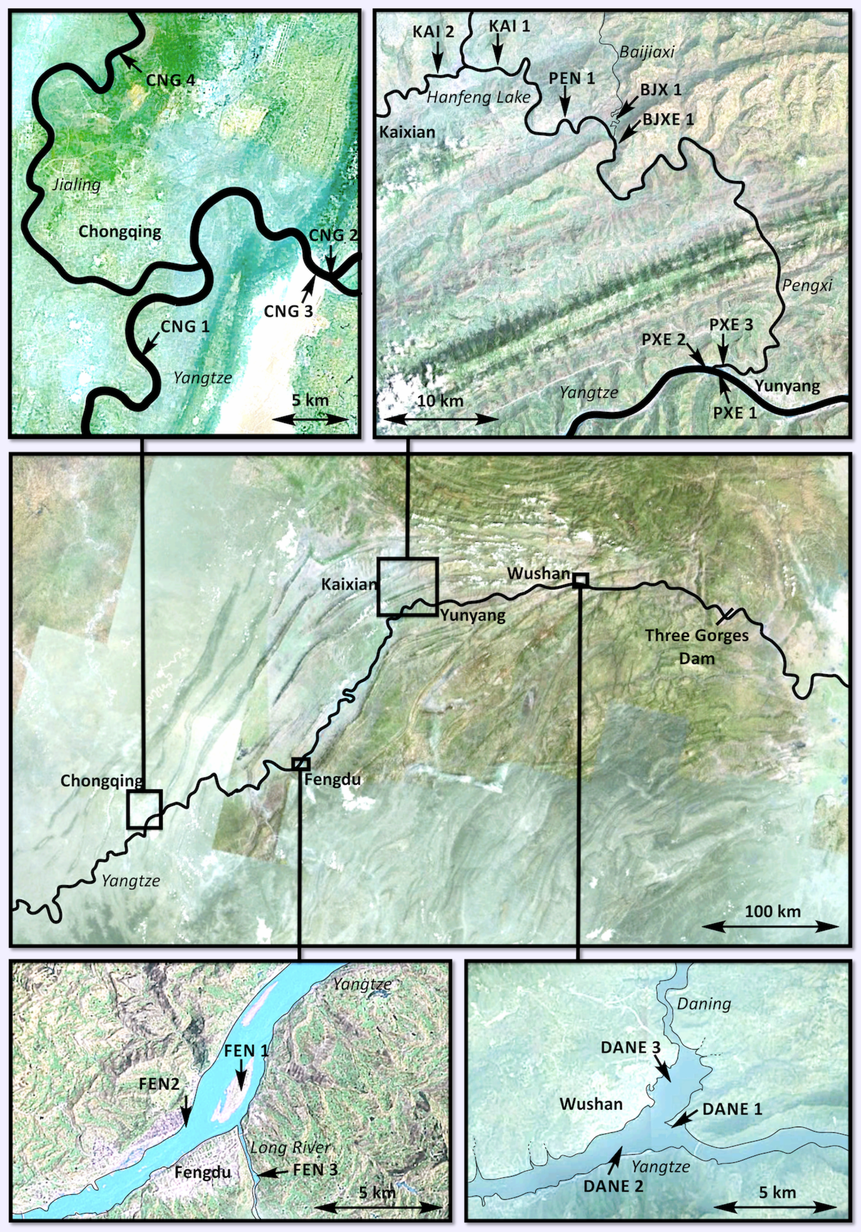

Fig. 2: Map of sampling sites along the Yangtze River (modified satellite imagery from Google Earth)

Fig. 1: Pictures of sampling using a Van Veen Grab Sampler

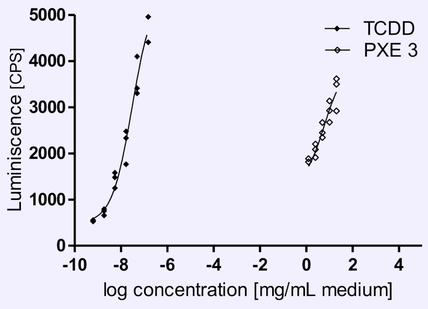

Fig. 3: Representative concentration–response curve measured in the H4IIE-luc Assay for PXE 3 and TCDD for reference

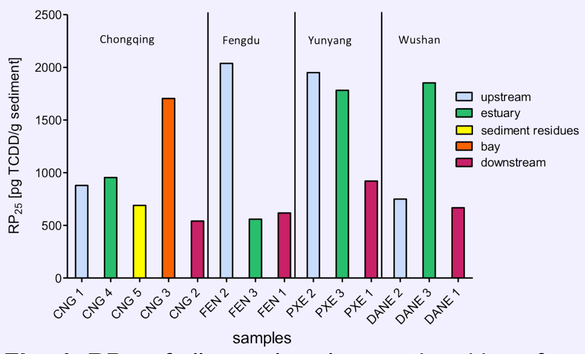

Fig. 4: RP_{25} of all samples close to the cities of Chongqing, Fengdu, Yunyang and Wushan using the H4IIE-luc Assay; n=1

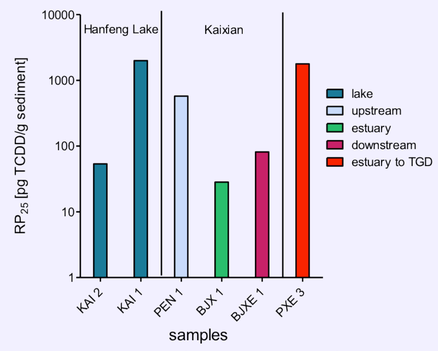

Fig. 5: RP_{25} of all samples from the Pengxi River (upper right box of Fig. 2) using the H4IIE-luc Assay; n=1 (logarithmic scale)

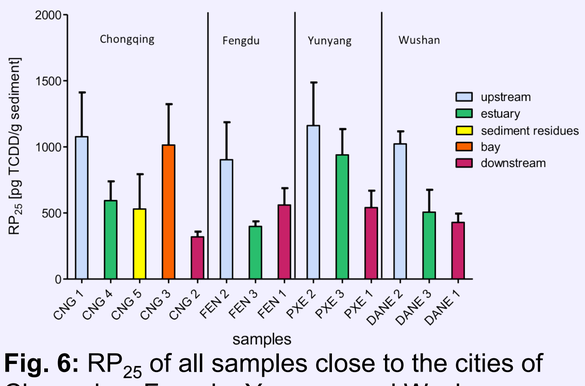

Fig. 6: RP_{25} of all samples close to the cities of Chongqing, Fengdu, Yunyang and Wushan using the EROD Assay; n=3

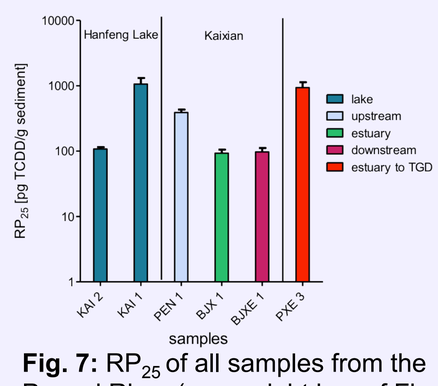

Fig. 7: RP_{25} of all samples from the Pengxi River (upper right box of Fig. 2) using the EROD Assay; n=3 (logarithmic scale)

Results

RP_{25} values **range** from **28 pg/g** (BJX 1) to **2037 pg/g** (FEN 2) using the **H4IIE-luc** Assay (Fig. 4; 5) and from **93 (±13) pg/g** (BJX 1) to **1161 (±326) pg/g** (PXE 2) using the **EROD** Assay (Fig. 6; 7).

RP_{25} values of the **upstream** samples from Chongqing, Fengdu, Yunyang, Wushan and Kaixian were **higher** compared to the **downstream** samples (Fig. 4; 5; 6; 7).

Except for KAI 1, for all samples from the **Pengxi River** area relatively **low** RP_{25} values were calculated (Fig. 5; 7).

Discussion

Sediment pollution by DLCs seems to **decrease from upstream to downstream** regarding all cities in the study areas. Water-processing units in the urban areas might have an influence on the river's water and sediment quality. Additionally, the local **feeder rivers** may cause dilution of the contaminated waters.

According to the comparatively low RP_{25} values, calculated for the **Pengxi River** sediment samples, this feeder river seems to be **less toxic** than the Yangtze River. A **nature reserve**, located in this area, may be one reason for the relatively low pollution by DLCs.

Both Assays for detection of DLCs show very **similar results** when comparing them qualitatively. The H4IIE-luc Assay proves to be a **reliable alternative method** to the EROD Assay. A higher number of replicates in the H4IIE-luc Assay will enhance the comparability in a quantitative way.

In chemical analysis of the sediment extracts, **polycyclic aromatic hydrocarbons** were detected as the only known hazardous group of compounds in the samples. Therefore, these substances are considered as main cause for observed dioxin-like effects.

References: [1] Chapman PM, Hollert H (2006) Should the sediment quality triad become a tetrad, a pentad, or possibly even a hexad? JSS - Journal of Soils and Sediments 6: 4-8

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