DIOXIN-LIKE ACTIVITY IN WATER OF THREE GORGES RESERVOIR SAMPLED BY SEMIPERMEABLE MEMBRANE DEVICES

Wang JX[1,2,3 *], Henkelmann B[2], Bernhöft S[2], Pfister G[2], Bi YH[1],Zhu KX[1], Schramm K-W[2,3 *]

[1] The State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China

[2] Helmholtz Zentrum München - German Research Center for Environmental Health (GmbH), Institute of Ecological Chemistry, Ingolstädter Landstr.1, D-85764 Neuherberg, Germany

[3] TUM, Wissenschaftszentrum Weihenstephan für Ernährung und Landnutzung, Department für Biowissenschaftliche Grundlagen, Weihenstephaner Steig 23, 85350 Freising, Germany

Abstract

Bioavailable hydrophobic pollutants in the Three Gorges Reservoir (TGR) of China were sampled by semipermeable membrane devices in May 2008. The chemical analyses and ecotoxicity of the residuals of SPMD dialysate were examined. The comparison of the EROD results with the potential response values calculated by PAH and PCB chemical analysis results indicates that PAH are main contributor to EROD activity of non persistent compounds and other persistent compounds besides PCB not detected in the study exist in the samples and contribute to EROD activity.

Introduction

The Yangtze River is the third largest river in the world and the Three Gorges Dam (TGD) is the largest dam in the world. The closing of the TGD may result in drastic environmental alterations such as changes in the whole ecosystem structure and functioning. These changes will cause great disadvantages on transfer and degradation of water pollutants by reducing intrinsic depuration ability.

Persistent organic pollutants (POPs), such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) or biphenyls (PCBs), are ubiquitous environmental contaminants. POPs cause a multitude of effects such as hepatotoxicity, teratogenesis, immunotoxicity, and tumorigenesis[1]. However, trace POPs in water of TGR were only reported sporadically[2,3], their ecotoxicological effects have never been studied there.

EROD is generally regarded as being an early warning signal for the Ah-receptor-related toxic effects of PCBs, PAHs, and related compounds[4]. EROD with H4IIE rat hepatoma cells bioassay is a bioassay established to measure CYP1A1 induction caused mostly by dioxin-like chemicals in environmental samples.

In this study, triolein SPMD technology was applied to sample and concentrate the priority organic pollutants in water of TGR. Chemical analysis was performed for PCBs congeners, PAHs, and organochlorine pesticides (OCP). EROD-bioassay was used to assess the potential toxicological effects of water in TGR. The aim of this study was to investigate the levels and distribution patterns of Ah-agonists effects in surface water from TGR by combining SPMD-based biomimetic sampling technology with relevant bioassay and link the chemical analyses components to their ecotoxicity.

Materials and Methods

Semipermeable membrane devices were prepared using lay-flat polyethylene tubing (from VWR Ismaning, Germany; 2.5 cm wide and 65 μm thick). The tubing was cut into 29 cm pieces and 700 μL of triolein (Sigma, Munich, Germany, 99%) were pipetted into each piece of tubing before sealing the ends[3].

The SPMD were deployed for a minimum of 5 d to a maximum of 24 d in six sites across 600 km in the TGR of China during May 2008. The sampling places are S1 Maoping, S2 Zigui, S3 BadongI, S4 BadongII, S5 Wanzhou, S6 Changshou. The SPMD were deployed in stainless steel cages and immerged into water at about 1 m depth and at a distance of 10-20 m from the river bank. After exposure, the SPMD were retrieved from each site and transported immediately to the laboratory and were kept in a freezer at -28 °C until processing.

The SPMD were rinsed with on site water to remove surface-defouled residues. After the inspection for cuts and leakages, the membranes were quickly rinsed with acetone and air-dried. Each SPMD was dialyzed in 95:5 (v/v) cyclopentane: dichloromethane (two batches, each was 150 mL for 24 hrs). The volume of the extracts was reduced to about 2 mL using rotary evaporation and then blown to dryness with a gentle nitrogen flow. The residues were dissolved in 4:1 (v/v) DMSO: isopropanol.

The EROD bioassay was performed according to the method described by Donato et al.[5] and Schwirzer et al.[6]. H4IIE rat hepatoma cells were grown in Dulbecco's minimum essential medium supplemented with 10% fetal calf serum. The cells were incubated at 37 °C, in a humidified 5% CO_2 incubator. For the EROD assay, cells were seeded at a density of 2×10^4/ well in a 96 – well plate. The cells were exposed to 2,3,7,8 TCDD standard concentrations and to the sample extracts for 24 and 72 hours. Appropriate blanks were also carried out. After incubation, the medium was substituted by a 7-Ethoxyresorufin solution 8 μM and the Resorufin produced was measured by fluorescence (excitation 535 nm, emission 590 nm) after 30 minutes incubation. The protein determination was performed according to Pierce (Micro BCA Protein Assay Kit). The EROD induction of the sample after incubation was compared to the 2,3,7,8-TCDD response. The obtained values were interpolated in a dose-response function regarding TCDD induction. Chemical analysis was performed according to the method described by our recent published paper[3].

Results and Discussion

The samples concentrated with SPMD were analyzed for target chemicals, including 16 PAHs, 18 PCB congeners, and 28 OCPs on a gas chromatograph coupled to a mass spectrometer (GC-MS)[3]. The total concentrations of PAH, PCB and OCP per sample are illustrated in Fig. 1. The highest total PAH concentration was S6 of 7 d and 24 d exposure. The second highest one was S5 of 24 d exposure. There was no much difference of total PCB concentration both of 7 d and 24 d exposure in the six sampling sites. The highest concentration of total OCP occurred in S5 and S6 of 24 d exposure.

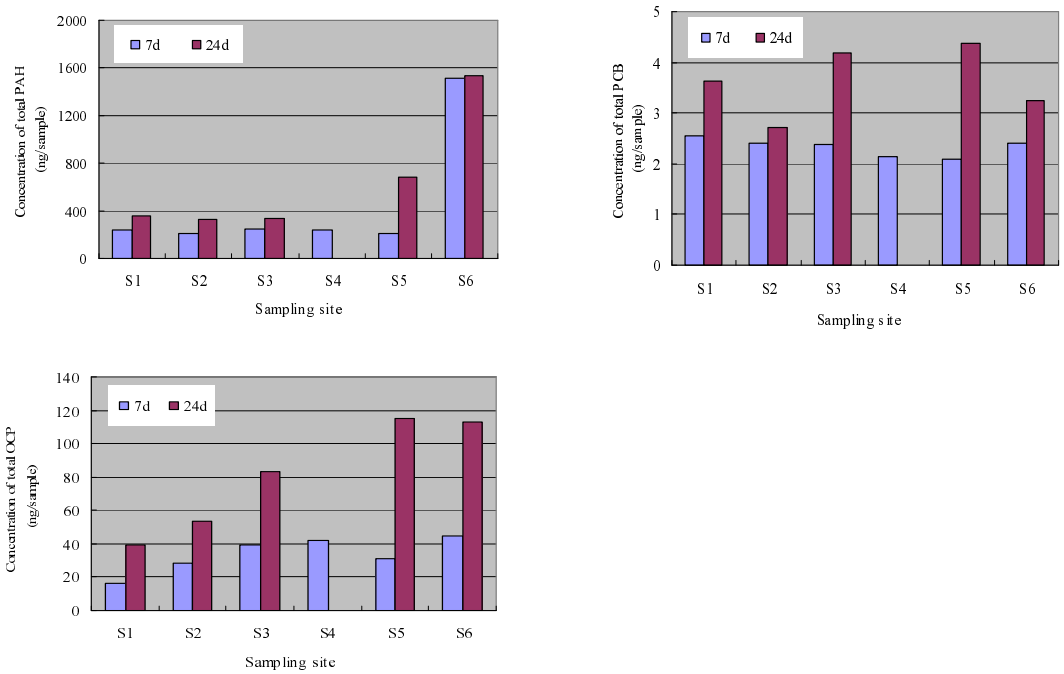

Fig.1. Concentrations of total PAH, PCB, and OCP in six sampling sites in TGR

The EROD results calculated as toxicity equivalent values (TEQ values) are illustrated in Fig.2. After 24 h incubation the TEQ value is related to all the compounds able to elicit a response (persistent and non-persistent compounds). Compounds that still elicit a response after 72 h incubation in the EROD bioassay are defined as persistent. The chemical analytical results are given as Toxic Equivalent values (TEQ) according to WHO (1998)[7] in Fig.3 in order to compare them with the EROD results. EROD of 7 and 24 d exposure ranged from 27-990 pg TCDD/sample. However, the TEQ of PCB only ranged from 0.04-2.5 pg TCDD/sample. Therefore, PCB contributes very little to the EROD activity in the samples from water of TGR. The TEQ of PAH ranged from 6.4-42.3 ng TCDD/sample. Therefore, PAH in SPMD may be main contributor to EROD activity of 24 h incubation. The reason of the EROD activities of 24 h incubation lower than TEQ value of PAH may be due to some compounds coexisting in the samples which inhibited the EROD activity. In case of 72 h incubation, other persistent compounds not detected in the study may exist to cause EROD induction. Further study warranted to clarify the differences.

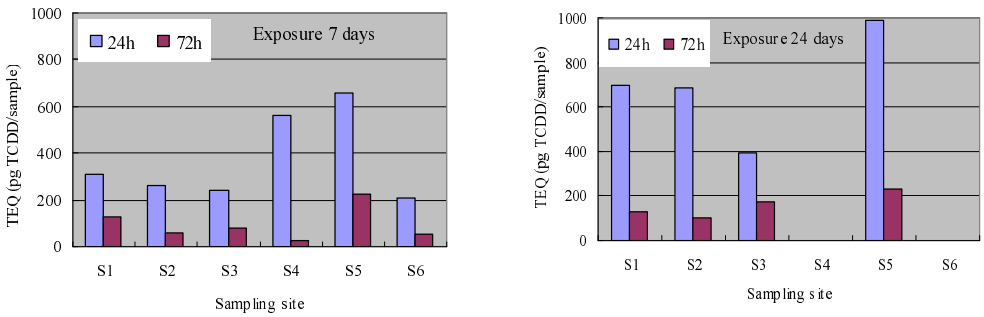

Fig.2. TEQ-EROD values after 24 h and 72 h incubation of samples from TGR water

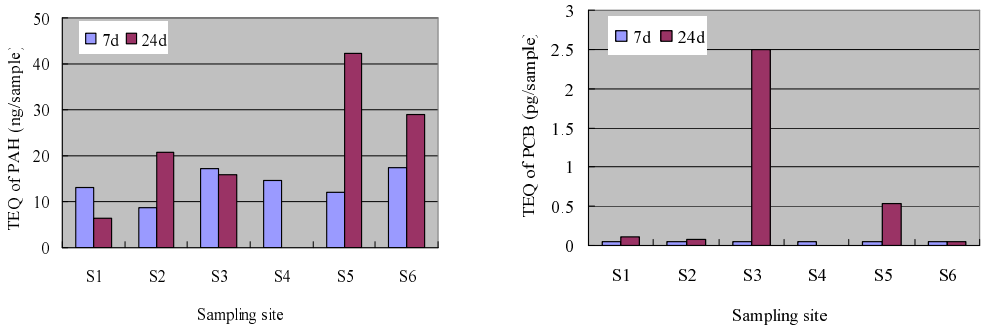

Fig.3. TEQ values of PAH and PCB in samples from TGR water

Acknowledgements

This research was supported by Intellectual Innovation Project of the Chinese Academy of Sciences (Grant No. KZCX1-YW-08-01) and DAAD (Deutscher Akademischer Austausch Dienst) special program (A/07/92706).

References

1 DeVito M. and Birnbaum L.S. In Dioxins and Health (A. Schechter, Ed), pp. 139-162. Plenum, New York.

2 Chen J., Luo J., Qiu Z., Xu C., Huang Y., Jin Y., Saito N., Yoshida T., Ozawa K., Cao J., Shu W. Chemosphere 2008; 70, 1545-1551.

3 Wang J., Bi Y., Pfister G., Henkelmann B., Zhu K., Schramm K.-W. Chemosphere 2009, 75, 1119-1127

4 Safe S. Crit Rev Toxicol 1990; 21, 51-88.

5 Donato M.T., Gómez-Lechón M.J., and Castell J.V. Anal Biochem 1993; 213, 29-33.

6 Schwirzer S.M.G., Hofmaier A.M., Kettrup A., Nerdinger P.E., Schramm K.-W., Thoma H., Wegenke M., Wiebel F.J. Ecotoxicol Environ Safety 1998; 41, 77-82.

7 Schramm K.-W. Biomonitoring ausgewählter organischer Chemikalien mit Haaren. Herbert Utz Verlag Wissenschaft 1999.