



Reproductive toxicity assessment of surface water of the Tai section of the Yangtze River, China by *in vitro* bioassays coupled with chemical analysis

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ARTICLE INFO

Article history:

Received 20 January 2011

Received in revised form

15 May 2011

Accepted 18 May 2011

Keywords:

Surface water

Reproductive toxicity

Persistent organic pollutants

ABSTRACT

Reproductive toxicity of organic extracts of the surface water from the Tai section of the Yangtze River was assessed by *in vitro* cytotoxicity assays and selected persistent organic pollutants including PCBs, OCPs and PAHs were quantified by instrumental analysis. Eleven of the US EPA priority PAHs were detected. Individual PAHs were found to range from 0.7 to 20 ng/L. Concentrations of BaP did not exceed the national drinking water source quality standard of China. However, a 286-fold concentrated organic extract induced significant reproductive toxicity in adult male rats. The morphology of cells, MTT assay and LDH release assay were all affected by exposure to the organic extracts of water. The results of the reproductive toxicity indicated that PAHs posed the greatest risk of the chemicals studied. The compounds present in the water could be bioconcentrated and result in adverse effects.

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1. Introduction

There is growing global concern about contamination of surface water, especially that was used as drinking source water. The Yangtze River (also called the Changjiang River), the longest river in China, is one of the most important rivers used as sources of drinking water. There are a large number of factories, farms, and cities that are potential sources of contamination to the river. Although the Yangtze River is a large river with significant dilution and self-purification potential, there are still toxicants found in water, sediment and biota, especially persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs) (Liu et al., 2000; Liu et al., 2003), which could be accumulated and bio-magnified in the food chain. There is concern by the public about the safety of drinking water from the lower reaches of the Yangtze River.

The routine monitoring of organic compounds in the surface water focuses on volatile and semi-volatile organic compounds (VOCs & SVOCs), such as benzene congeners and several congeners

of PCBs, OCPs and PAHs. Although concentrations of these compounds in the lower reaches of the Yangtze River meet the national water quality requirements individually, this research attempted to determine the joint effects of mixtures. Specifically, estrogenic activity and genotoxicity of surface water in the Yangtze River have been assessed by use of a variety of bioassays (Shen et al., 2001; Lu et al., 2004; Wu, 2005; Li et al., 2006), but there is little information on the reproductive toxicity of surface water in the lower reaches of the Yangtze River.

The purpose of the present investigation was to evaluate the potency of water in the Tai section of the lower reaches of the Yangtze River, to cause effects in a battery of *in vitro* bioassays coupled with chemical analysis. Specifically, the study (1) determined concentrations of PCBs, OCPs and PAHs; and (2) assessed the reproductive toxicity of organic extracts of surface water with a battery of *in vitro* bioassays.

2. Materials and methods

2.1. Sampling

Surface waters were collected in October, 2008 from the Tai section of the Yangtze River at a routine monitoring point of drinking water source in a highly urbanized drainage region, and located in the middle of the Yangtze River in

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Jiangsu Province. The related waterworks treats about 10,000 m³ water per day and provides water to an average of 650,000 people in this region. The waterworks treatment processes in the related waterworks is representative of the common conditions in this region, including filtration, coagulation, aerobic biodegradation and chlorination. The sites were selected and sampled by government personnel with local knowledge of contaminant sources and hydrologic conditions to get the source waters entering waterworks. According to the state standard of Technical Specifications Requirements for Monitoring of Surface Water and Waste Water (HJ/T 91-2002), experimental samples were composites of four sampling points (two sampling points at each transect were) from the Tai section. Samples were collected in brown glass bottles that had been prewashed with purified water and methanol and shipped to the laboratory within 24 h and stored at -20 °C. The sample was split in two sub-samples for the toxicity and chemical analysis. Procedure blanks prepared with Milli-Q water were used to exclude reproductive chemicals during the working procedure, using the same procedure as for the environmental samples.

2.2. Chemical analysis

After being filtrated through glass fiber membrane (0.45 μm, fisherbrand, USA) filters, two separate 1-L water samples were extracted by use of two solid-phase extraction (SPE) cartridges containing octadecylsilane (Envi-C₁₈, 1 g, 6 ml, Suplco, USA), respectively. One C₁₈ column was eluted with 10 ml of high purity hexane to removed PCBs and OCPs, while the another column was eluted with 10 ml of 20% dichloromethane in hexane for PAHs. Target analytes included 16 US EPA priority PAHs, 20 PCBs congeners, including CB-8, CB-18, CB-28, CB-44, CB-52, CB-66, CB-77, CB-101, CB-105, CB-118, CB-126, CB-128, CB-138, CB-153, CB-180, CB-187, CB-195, CB-206, CB-209, and 16 OCPs, concerning α-, β-, γ-, δ- hexachlorobenzene, aldrin, heptachlor, epoxy heptachlor, endosulfan 1, endosulfan 11, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, dieldrin, endrin, endosulfan sulfate endrin aldehyde. Concentrations of OCPs and PCBs were determined by use of an Agilent 6890 gas chromatograph (GC), equipped with a micro cell electron capture detector (μECD) and DB-5 capillary columns (35 m × 0.32 mm i.d. × 0.25 μm film thickness). Concentrations of PAHs were measured by use of an HP 6890 GC/5973 MSD (Agilent, USA) and HP-5MS capillary columns (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The carrier gases were helium and nitrogen for MSD and μECD respectively. The temperature program for PAHs was 40 °C for 4 min, rising to 320 °C at 8 °C/min and held for 5 min. The oven temperature program for organochlorines was set at 50 °C (held 5 min), rising to 220 °C at 35 °C/min, and held for 20 min and for PCBs was set at 50 °C (held 5 min), raised to 280 °C at 4 °C/min, and held for 10 min. The GC injector and μECD was held at 250 °C and 280 °C respectively. Injector and interface temperatures for GC/MSD were held at 270 °C and 250 °C, respectively. The method blanks contained no detectable amounts of target analytes. The average recoveries in matrix spikes varied from 76.6% to 126% for PCBs, from 59.6% to 87.4% for PAHs, and from 82.8% to 107% for OCPs. The detection limits were 0.1 ng/L for PCBs, 0.1 ng/L for OCs and 0.5 ng/L for PAHs.

2.3. Battery of in vitro bioassays for reproductive toxicity

Two 5 L samples of water were extracted using two solid-phase extraction (SPE) cartridges containing the resin octadecylsilane (Envi-C₁₈, 1 g, 6 ml, Suplco, USA), respectively, after filtrated through a glass fiber membrane. PCBs, OCPs and PAHs were eluted from two C₁₈ columns by the above mentioned solvents and eluted solutions combined in a vial, then rotary-evaporated and then evaporated to dryness under a gentle stream of nitrogen. The residue was solvent-exchanged into 3.5 ml of 1% dimethylsulfoxide (DMSO) aqueous solution and stored at -20 °C. Blanks extracted from purified water were used to exclude artifacts due to experimental conditions.

Sertoli cells, leydig cells and spermatogenic cells were isolated from adult male Sprague–Dawley rats (Nanjing Medical Univ. China), according to the methods of Anway et al. (2003); Kukucka and Misra, 1994; Aravindan et al., 1990 respectively,

with some modification. Detailed isolation conditions and method were described elsewhere (Li et al., 2006; Gong and Han, 2006).

Fluorescein diacetate (FDA)–Propidium iodide (PI) staining was used to distinguish viable (FDA+PI-) and dead cells (FDA-PI+) (Li and Han, 2006). Cell viability was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test and LDH (lactate dehydrogenases) release. Annexin V–PI staining combined with flow cytometry was used to quantitatively differentiate apoptotic and necrotic cells as previously described (Li and Han, 2006).

Cells were exposed to an extract that contained organic residues that had been concentrated 286-fold from water. The final concentration of DMSO was about 0.1%. The 0.1% DMSO solution and blank extract were used as solvent control and procedure control, respectively. Solutions were assayed in triplicate, and three solvent controls and three blank extracts were assayed simultaneously on each 96-well plate.

2.4. Statistical analysis

All calculations and statistical analysis were carried out in SPSS for windows version 11.0 (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. Differences between the exposed samples and the controls were considered to be significantly different when *P* < 0.05. Results are presented as mean ± standard deviation (S.D.).

3. Results and discussion

3.1. Results of chemical analysis

Concentrations of PCBs, OCPs and PAHs were measured in surface waters of the Tai section of the Yangtze River. Concentrations of 16 OCPs and 20 PCBs were less than the limit of quantification (LOQ = 0.1 μg/L). Only 11 PAHs of 16 US EPA priority PAHs were observed at quantifiable concentrations (Table 1). The total concentration of PAHs was 92 ng/L and the total concentration of 6 potentially carcinogenic PAHs was 38 ng/L. Most PAHs were found in the range from 0.7 to 20 ng/L. The PAHs observed in decreasing order of concentration were: FL > NaP > BbFL = Phe > BaA > Flu > IP = BaP > BkFL > Ant. The total concentration of PAHs with 3 and 4 rings, such as NaP, Phe, Flu, FL, were detected at greater concentrations than that of PAHs containing 5 or 6 rings. The concentration of BaP (4.1 ng/L) was less than the national drinking water sources quality standard of China, which is 10 ng/L, but greater than the water quality limit set by the World Health Organization (WHO), which is 0.7 ng/L. Much of the BaP would likely be removed during the treatment of prospective drinking water at Tai such that people would not likely be exposed to a significant amount of BaP via their drinking water.

The results of this study were compared to those of similar studies of concentrations of PAHs in surface waters worldwide. The compiled results including concentrations of BaP (the most toxic PAH), the total concentrations of 6 potentially carcinogenic PAHs and parent PAHs are presented (Table 2). Total concentrations of PAHs in water from the Tai section of the Yangtze River were less than those observed in rivers of Greece, Taiwan and the Jiulong River, Tonghui River, West Lake in China, but similar to those observed in the Esthwaite River (UK). Concentrations of BaP and 6 potentially

Table 1
Concentrations^a of priority polycyclic aromatic hydrocarbons (PAHs) in surface water of the Tai section of the Yangtze River, China.

Compound	Concentration	Compounds	Concentration	Compound	Concentration
NaP	1.5 × 10 ¹	Pyr	n.d. ^b	BaP	4.1
AcP	n.d.	FL	2.0 × 10 ¹	DBA	1.1
AcPy	n.d.	Chry	n.d.	BP	n.d.
Ant	0.7	BaA	1.1 × 10 ¹	IP	4.1
Phe	1.4 × 10 ¹	BbFL	1.4 × 10 ¹	∑16 PAH	9.2 × 10 ¹
Flu	4.8	BkFL	2.7	∑6 PAH _{CarC} ^c	3.8 × 10 ¹

^a Concentration is expressed as ng/L for PAHs.

^b n.d., not detected (<limit of determination).

^c ∑6 PAH_{CarC}, sum of the 6 Carcinogenic PAHs: BaA, BbFL, BkFL, BaP, DBA and IP (IARC probable and possible human carcinogens).

Table 2
Compilation of concentrations of BaP, total parent PAHs, total 6 carcinogenic PAHs in surface waters from various global sites (ng/L).

Location	BaP	$\sum 6 \text{ PAH}_{\text{Carc}}$	$\sum \text{ PAH}$	Ref.
Yangtze River at Tai section, China	4.1	3.8×10^1	9.2×10^1	This study
Surface water in Northern Greece	1.3–4.4	1.1×10^1 – 2.9×10^1	2.3×10^2 – 6.7×10^2	Manoli et al., 2000
Inland water of Central Greece	n.d.	n.d.	1.9×10^2 – 3.3×10^2	Kanaki et al., 2007
Coastal and inland water in Lesbos island, Greece	2.2×10^2 – 3.5×10^2	2.2×10^2 – 3.5×10^2	2.2×10^2 – 4.1×10^2	Kanaki et al., 2007
Esthwaite water lake, UK	–	–	9.1×10^1	Gevao et al., 1998
Gao-ping River, Taiwan	7×10^2 – 1.0×10^2	4.8×10^2	10 – 9.4×10^3	Doong and Lin, 2004
Jiulong River Estuary and Western Xiamen Sea	2.8×10^2 – 1.8×10^3	4.1×10^3 – 1.6×10^4	7.0×10^3 – 2.7×10^4	Maskaoui et al., 2002
Tonghui River in Beijing, China	2.4 – 2.4×10^2	5.1×10^1	1.9×10^2 – 2.7×10^3	Zhang et al., 2004
West Lake in Hangzhou, China	6.4×10^1	2.1×10^2	9.9×10^2 – 2.4×10^3	Chen et al., 2004

–: Cannot obtain the exact information from the Reference; n.d.: not detected.

carcinogenic PAHs were also less than those of the Gao-ping River (Taiwan), Jiulong River, Tonghui River and West Lake (China).

Sources of PAHs can be assessed by use of ratios of concentrations of individual PAHs (Yunker et al., 2002; Budzinski et al., 1997; Gschwend and Hites, 1981). The ratios of Phe/Ant within the two-ring PAH group and FL/Pyr within the four-ring PAH group were used to differentiate among sources. A Phe/Ant ratio of >15 suggests petrogenic sources and Phe/Ant ratios of <10 are

suggestive of pyrogenic sources. The FL/Pyr ratio of >1.0 observed in this study indicates that PAHs originated from pyrogenic sources. The Phe/Ant ratio observed in surface water from the Tai section of the Yangtze River was 19.9, and the FL/Pyr ratio was greater than 1.0, which indicates that PAHs were from both petrogenic and pyrogenic sources. Petrogenic PAHs generally originate from the leakage of crude oil and the refined products such as gasoline (Yunker et al., 2002). Pyrogenic PAHs originate primarily from

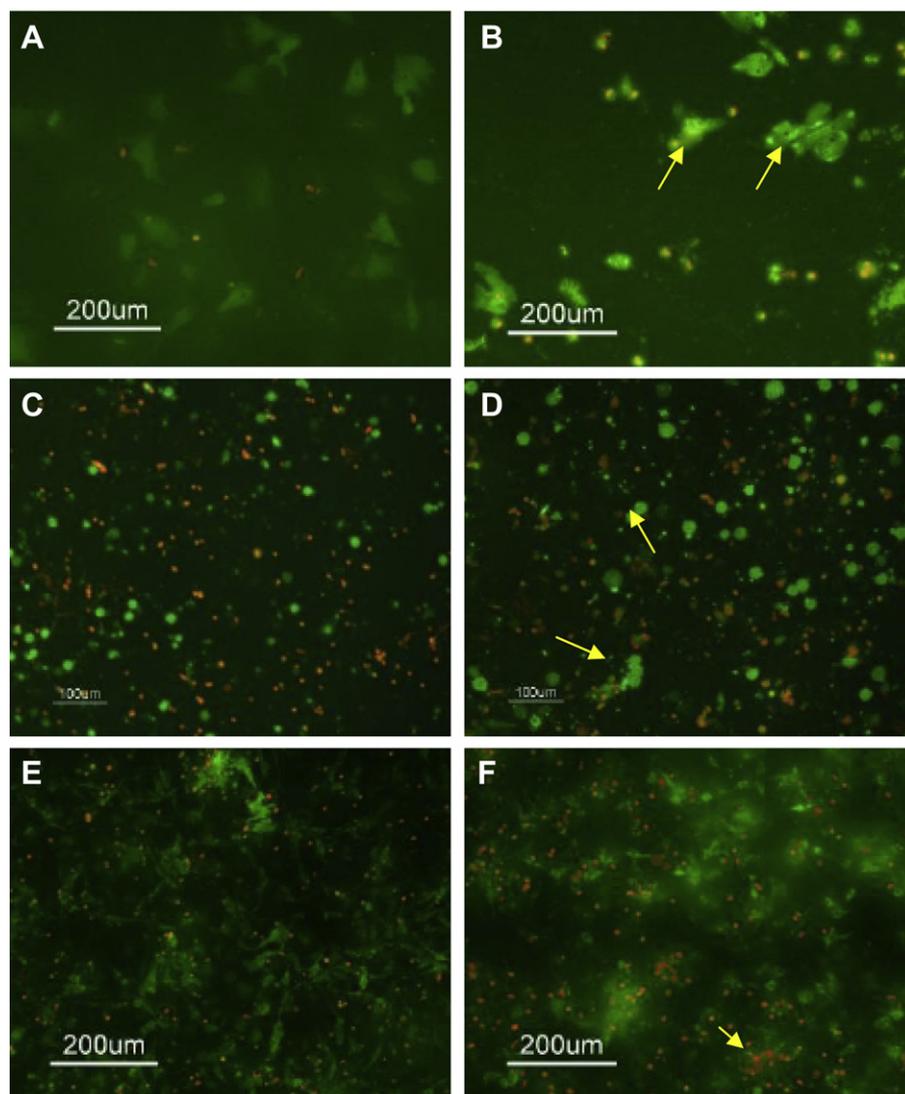


Fig. 1. Fluorescein diacetate (FDA) and propidium iodide (PI) staining for morphologic evaluation for sertoli cells (A, B), spermatogenic cells (C, D) and leydig cells (E, F) treated with organic extracts. Green cells represent the living cells, and the red ones show the dead cells. (blank extracts(A, C, E); Tai water(B, D, F).

combustion, especially of fossil fuels (Yunker et al., 2002). More than 90 petrochemical enterprises are situated along the Tai area of the Yangtze River, and the crude oil seepage and combustion of these enterprises might be the major sources of PAHs. Only 11 of the priority PAHs designated by the US EPA were detected in organic extracts of water from the Tai section of the Yangtze River, and the concentration of BaP was less than the national drinking water sources quality standard of China. This concentration of PAHs would not be expected to have an adverse effect on wildlife or humans. However, long-term consumption of drinking water with this concentration of PAHs, coupled with their bioaccumulation into aquatic organisms in the diets of people, might have adverse effects upon wildlife and human health, by considering only exposure via drinking water.

3.2. Reproductive toxicity

In our study, sertoli cells, spermatogenic cells and leydig cells of adult male rats were chosen to assess the reproductive toxicity of organic extracts. Sertoli cells are one of the most important somatic cells in testis (Karl and Capel, 1998), spermatogenic cells are precursor cell of sperm cells (Reyes et al., 1997) and leydig cells play a role of nutrition, support and mediated transfer (Ewing and Zirkin, 1983).

Earlier studies have suggested that a variety of individual PAHs can have adverse effects on male reproductive systems (Raychoudhury and Kubinski, 2003). Studies of reproductive toxicity have been conducted in animals with BaP and other

individual PAHs. An *in vivo* study found that BaP affected testes (Payne, 1958), causing effects including atrophy of seminiferous tubules, lack of spermatids and spermatozoa, and tumors of the interstitial cells. Sertoli cells cultured in the presence of FL showed morphologic changes, F-actin and α -tubulin distributions and altered cell protein content (Raychoudhury and Kubinski, 2003). Exposure of humans to PAHs resulted in an excess prevalence of low birth weight and premature birth (Raychoudhury and Kubinski, 2003). Raychoudhury and Kubinski (2003) reported that 10 $\mu\text{g/L}$ FL would significantly alter the morphology of sertoli cells. Based on the concentrations of FL observed and concentrations known to cause adverse effects *in vivo* bioassay, in this study the surface water was concentrated 286-fold so that the total concentration of PAHs would be in the $\mu\text{g/L}$ range, and the reproductive toxicity of organic extracts could be detected by *in vitro* bioassays, and screen with sensitive cells and bio-indicators. If effects caused by FL alone were observed, there would be a significant margin of safety. Our study was designed to detect interactions between residues or unidentified residues that would have the potential to affect male reproductive performance.

3.2.1. Cell viability and morphological assessment

Effects of the extract, on morphology of sertoli, spermatogenic, and leydig cells, including necrosis and apoptosis as determined by fluorescein diacetate (FDA) and propidium iodide (PI) staining are presented (Fig. 1). Cells stained green were classified as viable while those stained red were nonviable. Cells incubated without extract were not adversely affected and their morphologies were normal

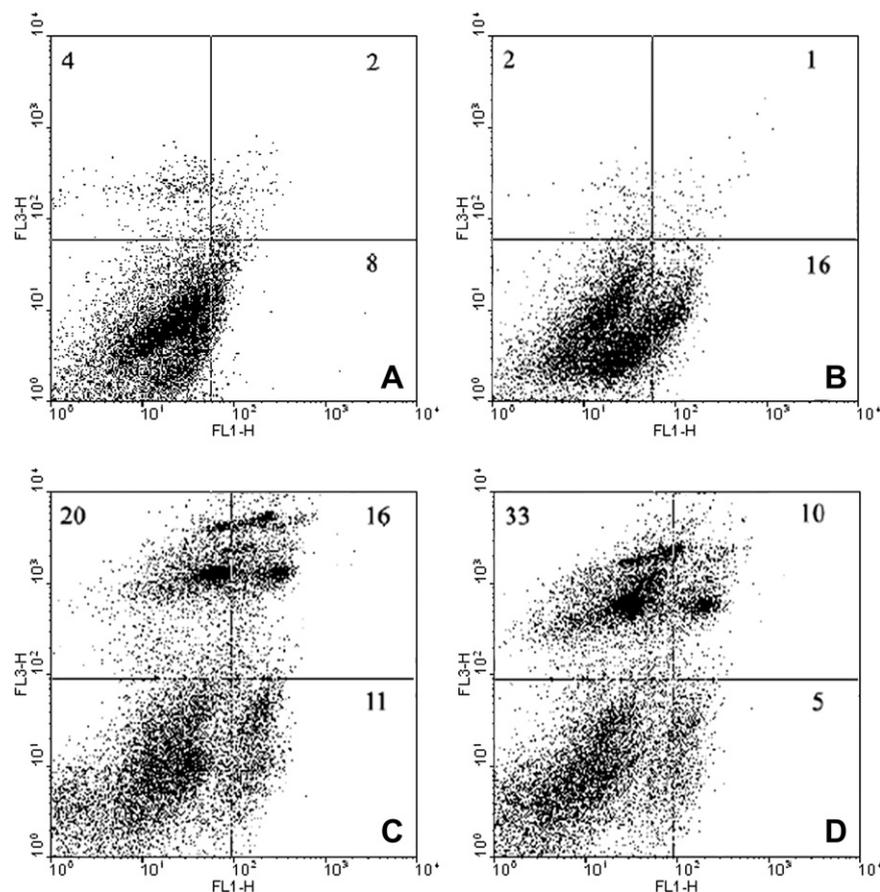


Fig. 2. Flow cytometry dot plots of the simultaneous binding of annexin V FITC and propidium iodide (PI). Sertoli cells (A, B) and spermatogenic cells (C, D) were assessed for annexin V FITC binding (FL1) and PI uptake (FL3) at the 24 h shown (blank extracts, A, C; Tai extracts, B, D). The numbers represent the percentage (%) cells present in the three quadrants. This experiment is representative of three others which gave almost identical results.

Table 3Effects of organic extracts on the ability of cultured testicular cells ($\bar{X} \pm SD$, $n = 3$, treated for 24 h with blank water extracts and Tai water extracts, respectively).

	MTT			LDH activity		
	Sertoli cells	Spermatogenic cells	Leydig cells	Sertoli cells	Spermatogenic cells	Leydig cells
Blank extracts	0.19 ± 0.01	0.68 ± 0.07	0.17 ± 0.01	3.0 ± 1.0	2.6 × 10 ² ± 63	1.7 ± 0.58
Tai extracts	0.17 ± 0.01	0.53 ± 0.06**	0.15 ± 0.01*	10 ± 1.2*	2.7 × 10 ² ± 65	8.3 ± 3.1*

** $p < 0.01$; * $p < 0.05$.

(Fig. 1A, C, E). Incubation of cells with the organic extract of Tai water for 24 h resulted in death or damage to sertoli, spermatogenic, and leydig cells. Cellular integrity was lost, cells became small and attained anomalous shapes and dead cells were more round (Fig. 2B, D, F). Sertoli cells treated with 1.0 mg FL/L or 10 µg FL/L showed morphologic changes (Raychoudhury and Kubinski, 2003). In our study, sertoli cells showed morphologic changes when exposed to extracts containing 4.3 µg FL/L. The lesser concentration of FL required to cause adverse effects could be due to additive or supra-additive interactions among individual PAHs in the mixture extracted from water, or due to contributions of other unquantified PCBs or OCPs.

Results of the MTT assay of cell proliferation and survival and the LDH release assay of membrane damage are given (Table 3). The extract caused few effect on the results of the MTT assay for sertoli cells. In contrast, the optical densities of leydig and spermatogenic cells were significantly less in cells treated with extract than that of the control. Exposure of sertoli cells to 1.0 mg FL/L, 10 µg FL/L or 100 ng FL/L resulted in a statistically significant less mitochondrial activity (Raychoudhury and Kubinski, 2003). In the study on which we report here, the extract which contained 4.3 µg FL/L, resulted in significantly less mitochondrial activity of sertoli cells. This result is consistent with joint toxicity action of the detected PAHs or their metabolites or the presence of undermined compounds.

LDH levels of sertoli and leydig cells were greater when incubated with Tai extracts than that of the controls. LDH concentrations in the medium of spermatogenic cells were significantly different between the Tai extract and controls. The results of both the MTT assay and LDH release assay indicated that exposure to organic Tai extracts resulted in less viability of sertoli, spermatogenic and leydig cells.

3.2.2. Measurement of apoptotic cells, necrotic cells and dead cells

In addition to the qualitative identification of live and dead cells stained by FDA–PI, annexin V–PI staining with flow cytometry was used to quantitatively determine the percentages of the live, apoptotic, necrotic and dead cells. Based on the annexin V and PI staining patterns, cells progressing through apoptosis can be monitored. Normal viable cells in culture will stain negative for annexin V FITC and negative for PI. Cells that stain positive for annexin V FITC and negative for PI are undergoing apoptosis. Both cells in later stages of apoptosis and necrotic cells will stain double positive for annexin V FITC and PI. Representative results of sertoli and spermatogenic cells staining with annexin V FITC/PI for flow cytometric assay at 24 h are given (Fig. 2). The lower left quadrant of the histograms is populated by viable cells, which excluded PI and were negative for annexin V FITC binding. The lower right quadrant represents cells in early apoptosis, which were PI negative and annexin V positive, which includes translocation of phosphatidylserine (PS) to the external cell surface but maintained integrity of the plasma membrane. The upper right quadrant represents nonviable, necrotic cells and late-stage apoptotic cells, which are positive for annexin V binding and PI. The upper left quadrant represents nonviable cells which were positive only for PI. The numbers represent the relative percentage (%) of cells present in the upper left, and upper and lower right quadrants. The relative

percentage of apoptotic sertoli cells incubated in blank extracts and Tai extracts were 8% and 16%, respectively (Fig. 2A, B) and the percentage of necrotic spermatogenic cells was 20% and 33%, respectively (Fig. 2C, D). The percentage of apoptotic sertoli cells and necrotic spermatogenic cells was significantly greater when incubated with Tai extract. Cytotoxic effects induced by PAHs have previously been shown to result in different apoptotic responses (Raychoudhury and Kubinski, 2003). Sertoli cells exposed to 10 ng FL/L for 24 h appeared nonapoptotic. However, exposure to 10 ng BaP/L caused apoptosis and smaller nuclei. Exposure to 10 ng BbFL/L resulted in smaller nuclei and staining of apoptotic DNA fragments (Raychoudhury and Kubinski, 2003). However, in the study upon which we report here, where cells were exposed to 0.94 and 3.3 ng/L of BaP and BbFL, respectively, sertoli cells were apoptotic. These results are consistent with the conclusion that coexposure to PAHs metabolites with undermined compounds might be synergistic.

When exposed to the 286-fold organic extracts of Tai surface water, the morphology and viability of sertoli, spermatogenic and leydig cells were significantly changed. Cell viability was significantly less, and the percentage of apoptotic sertoli cells and necrotic spermatogenic cells was significantly greater. The extract was enriched to determine if there were adverse effects due to exposure to interactions among measured residues or from unquantified residues that might have been in the mixture. To observe the levels of effects that were caused by the concentrated extract *in vitro* in wildlife or humans would require an enrichment of 286-fold. Enrichment factors (EFs) for OCs were 39–68 fold, PCBs were 5.8×10^2 to 4.2×10^4 fold in fish of the Yangtze River in Jiangsu area (Hu et al., 2009). Also, some of the effects observed *in vitro* were fairly severe and thus less enrichment might cause lesser, but still measurable effects. The results presented here indicate that there might be possible direct adverse effects upon reproduction of humans and wildlife during long-term consumption of drinking water in Tai section. While the results of the assays presented here are indicative of potential effects, future studies could be expanded to include epidemiological studies focused on the endpoints found to be affected in these studies and serial dilutions of the extracts performed.

4. Conclusions

Twenty PCBs, 16 OCPs and 16 PAHs were measured in surface water of the Tai section of the Yangtze River, but only 11 PAHs were identified at detectable concentrations. The total concentration of PAHs was 92 ng/L and concentrations of individual PAHs ranged from 0.7 to 20 ng/L. Concentrations of PAHs did not exceed the national drinking water source quality standards of China (GB5749–2006). The total concentration of PAHs in surface water was less than those reported for other locations in China. However, when exposed to the organic extracts of Tai surface water, the morphology and viability of sertoli, spermatogenic and leydig cells were significantly changed. Cell viability was significantly less, and the percentage of apoptotic sertoli cells and necrotic spermatogenic cells was significantly greater. Morphology of cells and the results of the MTT assay and LDH release assay were all effective indicators for organic extracts of surface water. While some of the detectable PAHs

are likely causes of the observed effects, they do not account for all of the observed effects so there might be unidentified compounds or synergisms between and among the PAHs. While it is difficult to relate the potency of the extract to responses to long-term exposure through drinking water, the results presented here indicate that there might be possible direct adverse effects upon reproduction of humans and wildlife. However, since the potential for biomagnifications to fishes is equal to or greater than the enrichment factor of 286 used in these *in vitro* studies, there is the potential for long-term exposure via both water and food to cause adverse effects on male reproductive factors. Therefore, surface water of Yangtze River at Tai section should be purified to eliminate the toxic organic pollutants such as PAHs, before being employed as a drinking water source. Also, additional studies to investigate interactions among residues and the isolation and identification of the potential unidentified active chemicals should be conducted. Finally, studies to determine the potential for accumulation and to be able to calibrate the meaning of the response to concentrated extracts are needed before a definitive interpretation of these results can be made. The results presented here will serve as a baseline to which the results of future studies can be compared to assess the status and trends in potency of male-factor reproductive effects in water from the Lower Yangtze River. Future studies could also include a range of exposure concentrations so that the actual threshold for effects in water volume equivalents can be established.

Acknowledgments

This work was supported by the Scientific Plan Project of Jiangsu Province (BS2007050), the Environmental monitoring Research Foundation of Jiangsu Province (0710), the Scientific Research Foundation for Graduate of Jiangsu Province (CX07B_170z), the Scientific Research Foundation of Graduate School of Nanjing University (2007CL11). The authors wish to thank Mr. Yang Wu for his assistance with collection of samples and extractions. The authors wish to acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure. Prof. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship in the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong. Prof. Giesy was also supported by the Einstein Professors program of the Chinese Academy of Science and the distinguished visiting professor of King Saud University, Riyadh, Saudi Arabia.

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