Ecotoxicology (2009) 18:685–692
DOI 10.1007/s10646-009-0340-3

Endocrine-disrupting equivalents in industrial effluents discharged into Yangtze River

Wei Shi · Xiaoyi Wang · Wei Hu · Hong Sun · Ouxi Shen · Hongling Liu · Xinru Wang · John P. Giesy · Shupei Cheng · Hongxia Yu

Accepted: 18 May 2009 / Published online: 9 June 2009
© Springer Science+Business Media, LLC 2009

Abstract The endocrine-disrupting equivalents in effluents from three chemical industry wastewater treatment systems in the vicinity of Yangtze River were determined by several transactivation reporter gene assays. Transient transfections of African green monkey kidney cell line (CV-1) were used to determine the estrogenic, anti-androgenic and anti-thyroid equivalents in the effluents. Organic extracts of the effluents contained compounds that were potent anti-androgens and the activities measured as an equivalent concentration of flutamide were 45.53, 34.65 and 91.61 nM, respectively. The extracts also contained detectable concentrations of thyroid antagonists. Estrogenic activities, measured with the reporter gene assay, were near or below the method detection limit (0.58 pM as E2). Concentrations of some of the major constituents such as di(2-ethylhexyl)phthalate, dibutyl phthalate, 2,6-dinitrotoluene and nitrobenzene were quantified. The data suggest that the reporter gene assay is useful to predication of endocrine disrupting effects in polluted aquatic body.

W. Shi · X. Wang · W. Hu · H. Liu · J. P. Giesy · S. Cheng · H. Yu (✉)
State Key Laboratory of Pollution Control and Resource Reuse & School of the Environment, Nanjing University, 210093 Nanjing, People's Republic of China
e-mail: yuhx@nju.edu.cn

H. Sun · O. Shen · X. Wang
Key Laboratory of Reproductive Medicine & Institute of Toxicology, Nanjing Medical University, 210029 Nanjing, People's Republic of China

J. P. Giesy
Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada

Keywords Waste · Treatment · Chemical industry complexes · Reporter gene assay

Introduction

Some of pollutants existing in surface waters and drinking water have been classified as endocrine disrupting chemicals (EDCs; Takako and Satoshi 2002). Chemicals in effluents from wastewater treatment plants (WWTPs) can interfere with the normal endocrine homeostasis and development, which can lead to reproductive disorders and population declines (Gracia et al. 2008; Hecker and Giesy 2008; Marina et al. 2007). Both androgen receptor (AR) and estrogen reporter (ER)-mediated processes are important in reproduction. Chemicals can mimic ER and AR agonists and cause morphologic abnormalities. Research about chemicals that can interact with the AR as agonists or antagonists showed that industrial chemicals were AR antagonists (Sun et al. 2008a). More recently, compounds that can interact with the thyroid hormone receptor (TR) have aroused more concern (Gaido et al. 1997; Devito et al. 1999). Reporter gene assays have been proposed by the United States Environmental Protection Agency (US EPA) for detecting EDCs, especially in mixtures. A transient reporter gene assay based on CV-1 African green monkey kidney cells, was used to determine the total estrogen (E2), androgen (DHT) and thyroid hormone (T3) equivalents in effluents. This in vitro assay is effective in characterizing the potential of individual chemicals, such as alkylphenols and pyrethroid pesticides, acting as receptor agonists and antagonists (Xu et al. 2005; Sun et al. 2006, 2008b).

There are more than 40 chemical industry complexes, including oil refineries, plastic factories and textile mills along the Yangtze River, with three-fourths of them located

in Jiangsu Province. Although the effects of Yangtze River water on reproduction have been previously studied, little information is available on the endocrine disrupting potential of chemical industry parks along the river (Yuan et al. 2005). In this study, reporter gene assays were applied to evaluate the potential effects of three effluents interact with the AR, ER, or TR.

Because reporter gene assays were found to have the greatest sensitivity and least variability in determining the potential endocrine disrupting effects, cell lines were chosen for detailed studies of organic extracts of effluents. Besides inhibitory effect detection, instrumental analysis was used for comparison with analytical results, and four major products in the chemical industry parks were quantified.

Materials and methods

Sampling locations and collection

Water samples were collected in October of 2007 from WWTP effluents of three chemical industry complexes (P1–P3) along the Yangtze River, China (Fig. 1). Location P1 is one of the 10 national industry parks in China founded 10 years ago. P2, larger than P1, is one of 10 provincial parks in Jiangsu province and was founded in 2006. Location P3 is adjacent to YQ village, where people exhibit a relatively great incidence of cancer. Samples of effluent water (about 14 l) were collected from each location in cleaned 7 l glass bottles. Composite samples collected at each site were split into two glass bottles for instrumental analysis and bioassay. Samples were immediately placed on ice and sent to the laboratory where they were stored at 4°C until filtered. To minimize alteration of sample constituents and avoid the need to preserve or stabilize samples, they were extracted within 24 h.

Sample preparation

Samples were extracted by use of methods that have been described in detail elsewhere (Koh et al. 2004). Briefly, before extraction, water samples were separated into dissolved water and particulate fraction by passing through glass fiber filters (GF/F) with a pore size of 0.45 μm. Solid phase extraction cartridges, 1 g Suplco, USA, were washed with methanol, followed by distilled water. About 5 l aliquants of the prepared samples were passed through each washed and conditioned cartridge, by use of a vacuum pump. After the cartridges were dried, retained compounds were eluted with 10 ml hexane, 10 ml hexane and dichloromethane (4:1), followed by 10 ml dichloromethane and methanol (1:1). Extracts were concentrated by rotary evaporation in a thermostatic bath. Extracts for instrumental analysis were concentrated under a gentle nitrogen stream to achieve a final volume of 0.1 ml at 30°C, and extracts for bioassay were solvent-exchanged with dimethylsulfoxide (DMSO) aquatic solution. Blanks extracted from purified water were used to exclude endocrine-disrupting activities during the working procedure because of experimental conditions. The extracts were stored at −20°C until analysis.

Chemicals and quantification

17β-Estradiol (E2), triiodothyronine (T3), 5α-dihydrotestosterone (5α-DHT), flutamide, bisphenol A (BPA), di (2-ethylhexyl)phthalate, dibutyl phthalate, 2,6-dinitrotoluene as well as nitrobenzene with a purity of over 99% were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions of extracts were prepared in absolute dimethyl sulphoxide (DMSO), stored at −20°C, and diluted to desired concentrations in phenol red-free DMEM medium (Sigma–Aldrich) immediately before use. The final DMSO concentrations in the culture medium were not

Fig. 1 Map of sampling points (P1, P2 and P3) for endocrine disruption evaluation along the lower reaches of the Yangtze River in China

higher than 0.1% (v/v) to avoid the potential effect to cell yields. Di(2-ethylhexyl)phthalate, dibutyl phthalate, 2,6-dinitrotoluene and nitrobenzene were quantified using gas chromatography/mass spectrometry (GC/MS), following the steps of EPA Method 8270.

Plasmids

Recombinant plasmids pERE-aug-Luc, pMMTV-luc, and pUAS-tk-luc, with luciferase under control of the estrogen response (ERE), androgen response (ARE) and thyroid response (TRE) elements, respectively, were developed as previously described (Takeyoshi et al. 2002, 2003; Sun et al. 2008b).

Cell culture and transfection

The CV-1 cell line was obtained from Institute of Biochemistry and Cell Biology in Shanghai, Chinese Academy of Science and maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C in an atmosphere containing 5% CO_2. Cell culture and exposure were conducted according to Sun et al. (2008a) and Xu et al. (2006). For different endocrine-disrupting activity testing systems, cells were transfected with different plasmids and transfection reagents (Table 1). After an incubation of 12 h, the transfection medium was removed and various concentrations of chemicals and sample extracts dissolved in medium were added for measurement of ER agonist, anti-androgenic and anti-thyroid activities. Solvent control wells contained medium with 0.1% DMSO.

Reporter gene assays

Cells were harvested after treated with test chemicals and extractions for 24 h. Dosing solutions of the extracts were diluted with DMSO to a maximum DMSO concentration of 0.1%. After rinsing three times with phosphate-buffered saline (PBS, pH 7.4), cells were lysed with 1× lysis buffer (Promega, Madison, WI, USA, 50 µl/well). Cell lysates were analyzed immediately using a 48-well plate luminometer (Berthold Detection System, Pforzheim, Germany) to detect the luminescence and determine the amount of expressed luciferase. Following the manufacturer's instructions, the amount of luciferase was measured with the luciferase reporter assay system kit (Promega). The relative transcriptional activity was converted to fold induction relative to the solvent control (*n*-fold).

Measurement of estrogen equivalency

Equivalent hormone concentrations (EE2) were derived using the methods previously reported (Urbatzka et al. 2007; Conroy et al. 2007; Villeneuve et al. 2000). EC20 values for the hormones were divided by sample concentration factors, which produce the same response to convert estrogenic activities in samples. The method detection limit (MDL) was the E2 concentration, which produced a test response equal to blank, divided by the highest concentration factor tested.

Measurement of antagonism equivalency

Anti-androgenic response in the bioassay was derived by a known AR antagonist flutamide concentration necessary to produce an equivalent reduction in androgenic activity (Urbatzka et al. 2007; Conroy et al. 2007). A 20% reduction in the normal androgenic response to 1 nM DHT was selected for calculation of equivalent antagonist activities (AC20). Anti-androgenic activity was reported as an equivalent flutamide concentration, defined as the flutamide divided by the sample concentration factor that produced an equivalent (20%) depression in the bioassay response to 1 nM DHT.

Quantification of TR antagonist equivalents was accomplished by comparison to BPA as a reference TR antagonist. A similar method was utilized trying to measure the equivalency for TR antagonist activities.

Statistical analysis

Values are reported as the mean ± SD ($n = 3$). In the bioassays tripplicate wells were dosed. Calculations were made with SPSS 12.0. Before parametric analysis, the normality of each sample set was assessed with the Kolomogrov–Smirnov one-sample test, followed by Duncan's multiple comparisons when appropriate. The level of significance was set at $*p < 0.05$ and $**p < 0.01$. For agonists, treatments were compared to the solvent control

Table 1 Concentrations of plasmids and transfection reagent for three reporter gene assay systems

	Estrogenic activities		Androgenic activities		Thyroid activities	
Plasmid	pERE-aug-Luc	0.25 µg/well	pMMTV-Luc	0.25 µg/well	pUAS-tk-Luc	0.2 µg/well
	rERa/pCI	0.1 µg/well	AR/pcDNA3.1	0.05 µg/well	pGal4-L-TRβ	0.075 µg/well
Transfection reagent	Sofast^{TM}	0.5 µl/well	Sofast^{TM}	0.5 µl/well	Sofast^{TM}	0.5 µl/well

group; while for androgen antagonists/hypothyroid treatments were compared to the DHT/T3 positive control groups.

Results

Cell viability and system creditability

Before reporter gene assay, the extract dilutions in the range of 1, 0.5, 0.1 and 0.05 were tested to determine their effects on cell viability by use of the MTT assay and microscopic examination. None of the extracts were deemed to be overtly toxic and they did not affect growth, viability or proliferation of CV-1 cells, alone or with 1 nM DHT/5 nM T3 at any dilution. The CV-1 cell reporter assay systems exhibited appropriate responses to the natural estrogen E2, AR agonist DHT and TR ligand T3. All of the model test chemicals caused transcription of luciferase in a concentration-dependent manner (Fig. 2). From the dose response relationship, E2 induced luciferase activity in the range of 10^{-10} M–10^{-7} M (Fig. 2a), with maximal induction of 17.4-fold relative to that of the vehicle control achieved at a concentration of 5×10^{-8} M E2. The median effective concentration (EC50) value was 0.15 nM E2. DHT induced luciferase activity in the range of 10^{-11} M–10^{-6} M DHT (Fig. 2) with the maximal induction of 11.2-fold, relative to that of the solvent control achieved at a concentration of 10^{-8} M DHT. The standard curves of flutamide with 1 nM DHT showed the general suitability of this assay to detect anti-androgenic activities. The AC20 for flutamide was 0.51 μM (Fig. 3). For T3, the maximal induction of 27.3-fold relative to that of solvent control was achieved at a concentration of 10^{-6} M T3, and the EC50 was 9.4 nM T3 (Fig. 2). BPA with 5 nM T3 also

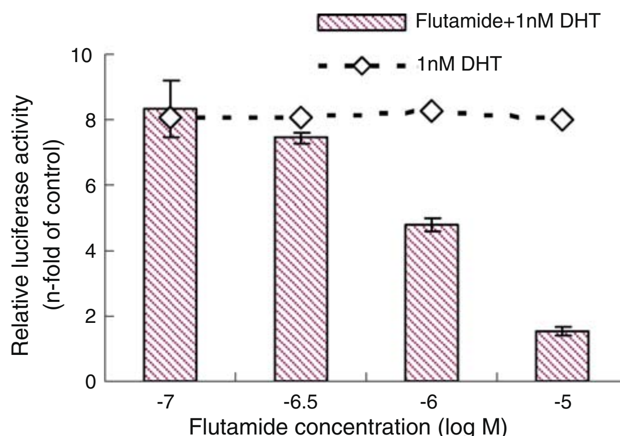

Fig. 3 Anti-androgenic activity as measured by reporter gene assay. Flutamide was diluted as indicated. Anti-androgenic equivalents in environmental samples were calculated using the sample concentration factor necessary to reduce 1 nM androgenic activity by 20%, which yields a flutamide concentration of 0.51 μM

caused transcription of luciferase in a concentration-dependent manner (Fig. 4). BPA is a weak thyroid hormone antagonist, although the result indicated the general suitability of this assay to detect anti-thyroid activities. No significant induction of luciferase was observed in any of the solvent controls (data not shown). Curve-fitting analyses were carried out with Excel 2003 (Microsoft, USA).

Estrogenic equivalents

When the test effluent extracts were administered to the transfected cells, none of them resulted in statistically significant expression of luciferase relative to the solvent control (Fig. 5). While extracts from P1 induced expression of luciferase, the level of effect was small, 1.2-fold at most, and not statistically different from the solvent controls

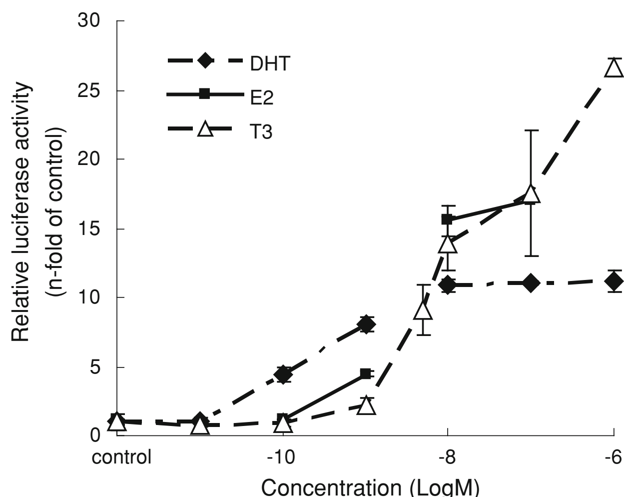

Fig. 2 Effects of E2, DHT and T3 in reporter gene assay

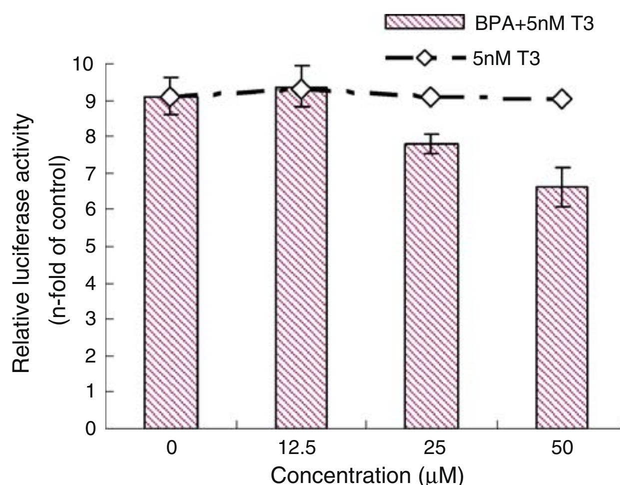

Fig. 4 Anti-thyroid activity as measured by reporter gene assay. BPA was diluted as indicated

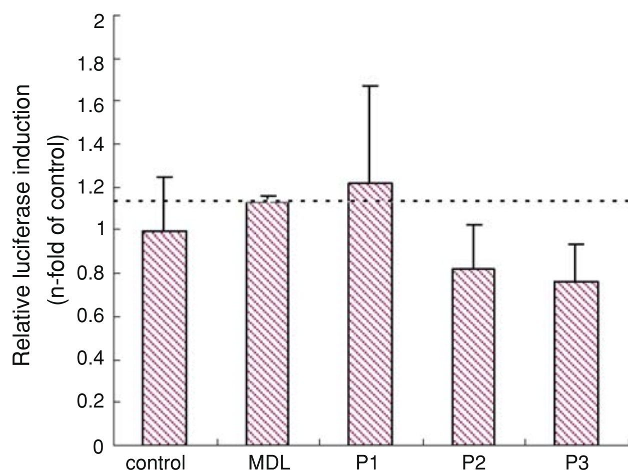

Fig. 5 Estrogenic activities in the effluents measured with reporter gene assay. The results for 3 effluent extracts (P1, P2 and P3), control and MDL (0.1 nM E2, as indicated by the *dashed line*) were shown in the figure. The estrogenic activity is expressed as relative expression versus the untreated cells (control) (mean ± SD). The expression of luciferase in the figure was caused by extracts with the maximal concentration factors (73-fold)

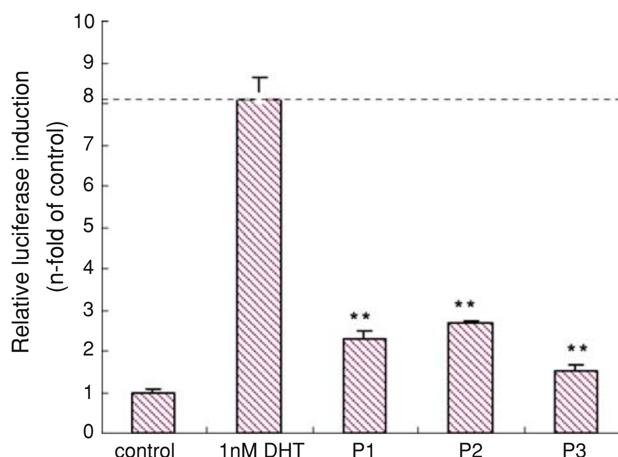

Fig. 6 Anti-androgenic activities in the effluents measured with reporter gene assay. The results for control and 1 nM DHT, 3 effluents extracts (P1, P2 and P3) were shown in the figure. The flutamide and extracts samples were treated in parallel with DHT in the concentration of 1 nM as indicated by the dashed line. The anti-androgenic activity is expressed as relative expression versus the untreated cells (control) (mean ± SD). The expression of luciferase was caused by extracts with the maximal concentration factors. Significant differences between the extracts and the DHT treatment were tested using ANOVA, Dunnett's test. Significant differences are indicated by *asterisks* (** $p < 0.01$; * $p < 0.05$)

($p > 0.05$). Furthermore, there was no significant difference in luciferase expression among the three effluents ($p > 0.05$). All of the concentrates derived from effluents produced estrogenic activity in the bioassay that was less than the MDL (0. 1 nM). Consequently, estrogenic activities in extracts from P1, P2 and P3, measured as an equivalent concentration of E2, were no more than 0.58 pM in effluents.

Androgen equivalents

All three extracts contained potent anti-androgens that significantly ($p < 0.01$) inhibited luciferase activity in the presence of 1 nM of DHT (Fig. 6). Extracts of the P1, P2 and P3 effluents with the maximal concentration factors (73-fold) resulted in decreases in luciferase expressions as 28.6, 33.0 and 18.7% of 1 nM DHT activity, respectively. Because of limited sample volumes, extracts from P1, P2 and P3 could not be tested at higher concentrations. In the three cases they exhibited significant activity but did not reach a maximal response, so significant extrapolation was required to generate equivalents. Because the extracts did not result in equal efficacy with the standard curve used, the results presented here would tend to be overestimates of the antagonist equivalents (Villeneuve et al. 2000). The corresponding flutamide equivalents for P1, P2 and P3 were 1.9, 1.4 and 3. 8 μM in extracts, corresponding to 45.5, 34.7 and 91.6 nM in effluents, respectively. The extract of the P3 effluent caused the most anti-androgenicity while that of the P2 effluent caused the least anti-androgenic effect.

TR antagonist equivalents

Extracts of all three effluents contained anti-thyroid hormone antagonist equivalents. The observed maximum antagonism of extracts of P1, P2 and P3 was greater than that of the maximum concentration of 50 μM BPA studied. BPA was such a weak TR antagonist (Fig. 4) that it was not a reliable reference compound for calculation of TH antagonism equivalents. Consequently, T3 antagonist equivalents were reported as the proportional decrease in luciferace activity relative to the response to 5 nM T3. Greater values of fold change relative to that of the control indicate lesser T3 antagonist equivalency. While they may be suitable for comparative purposes, they should not be used for risk assessment or mass balance analysis. Luciferase expression for extracts from P1, P2 and P3 expressed 7.36, 7.35 and 8.68-fold of control, respectively. Based on the calculation, the rank order of potency among the samples was P1 $\approx$ P2 > P3. The activity at P3 also represented significant difference compared with P1 and P2, demonstrating that there may be more key toxins in P1 and P2.

Water quality parameters and concentrations of the main products

Quality parameters for the effluents were quantified, and concentrations of the four chosen representative chemicals

from the major products of the chemical industry parks are shown in Table 2. The volume of discharges from P1, P2 and P3 were 0.3472, 0.4629 and 0.3688 m^3/s, respectively. Chemical oxygen demand (COD) and volatile phenol of the effluents varied among the locations. In general, the highest COD (74.0 mg/l) was found in the vicinity of the effluents from P3. For P1 and P2, COD concentrations were 58.5 and 55.1 mg/l, respectively. Volatile phenol concentration was more than 0.004 mg/l in treated sewage from P2 and P3, and more than 0.009 mg/l in the effluent from P1.

Discussion

Reporter gene assays have been regarded as a powerful tool in screening endocrinal activity chemicals (Sun et al. 2007). The advantages of in vitro assays over in vivo procedures, are characterization of receptor mediated endocrine activities and screening of endocrine disruptors (Kojima et al. 2004; Freyberger and Schmuck 2005). Although this type of in vitro assay has been widely used as an effective method to characterize the endocrine-disrupting potential of individual chemicals acting as receptor agonists or antagonists (Sun et al. 2006, 2007, 2008b), little work has been carried out for mixtures or environmental samples. Luciferase was used as the reporter gene in a battery of transactivation assays to screen effluents for their ability to interact with the ER, AR and TR as an indicator of the potential to affect endocrine functions of aquatic organisms and human. In previous studies investigating the effects of exposure to industrial sewage effluents, genotoxicity and reproductive toxicity were identified by in vitro bioassays (Houk 1992; Li et al. 2008; Schiliro et al. 2004; White et al. 1996). The in vitro effects were found to be related to health effects, affecting aquatic biota and humans (Ra et al. 2007; Júnior et al. 2007). However, the information for the key toxins identification provided by those in vitro assays was limited. The battery of reporter gene bioassays can provide the total endocrine-disrupting potentials of the effluent extracts, and the detailed targets of the key toxins, which is much more useful for contamination identification. Moreover, the TRs may be the targets of industrial chemicals especially for the petroleum products (Gaido et al. 1997; Devito et al. 1999; Gentes et al. 2007). However, few studies have focused on the potential effects of industrial effluents in China to endocrine functions.

Table 2 Concentrations of industrial pollutants in effluents

Chemical industry complexes	P1	P2	P3	Analysis methods
2,6-Dinitrotoluene (μg/l)	0.8	0.9	0.7	EPA8270
Di(2-ethylhexyl)phthalate (μg/l)	8.3	5.2	4.7	EPA8270
Dibutyl phthalate (μg/l)	3.0	2.4	2.1	EPA8270
Nitrobenzene (μg/l)	0.2	49.3	0.7	EPA8270

In this study, estradiol-equivalents determined in the effluents with the CV-1 cell assay were less than the MDL of 0.58 pM in effluents. Treatment of male trout revealed an increase of the vitellogenin synthesis at the concentration no less than 1 ng/l (3.7 pM) E2-equivalents (Jobling et al. 1995). It has to be suspected in this study that the estrogenic potential of the effluents was insufficient to affect the reproduction of fish. Therefore, the concentrations of the compounds often associated with estrogen equivalents such as propylphenol, butylphenol and bisphenol-A, were insufficient to be detected by the assay (Sun et al. 2008b).

Organic extracts of all effluents could suppress AR-mediated gene transcription induced by DHT. The concentrations of flutamide equivalents for effluents in our study were less than that for surface waters from the river Lambro in North Italy as 1.34 μM (Urbatzka et al. 2007). Flutamide in the nM range (100 μg l^{-1}) was shown to effectively inhibit nest building in male sticklebacks (Sebire et al. 2008). The significant anti-androgenic activity, indicating the presence of AR antagonists, could have been due to some of the chemicals commonly used or produced by these industry parks that are known to be anti-androgenic, such as flutamide, nilutamide, di(2-ethylhexyl)phthalate and dibutyl phthalate (Sun et al. 2007; Kruger et al. 2008). The P3, effluent from which exhibited the greatest androgen antagonist equivalence, is not far from the small village of YQ, where there has been reported to be a cancer node. The effluents from the P3 chemical industry complex WWTP are discharged immediately to the area people living nearby. Therefore, there may be some relation between the incidence of cancer and water pollution (Fenton 2006). Concentrations of the main products of companies in the P3 complex have been measured, but the concentrations were not particularly high. However, these scenarios remain speculative, and further analysis, such as fractionation techniques. Furthermore, until some epidemiological investigations are conducted the potential causes of the cancer node remains unknown.

The TH has been reported to be the target of industrial chemicals (Zoeller 2005). A broad range of chemicals have been reported to bind to the TR and might produce complex effects on thyroid hormone signaling (Zoeller 2005; Cheng 2005; Theodorakis et al. 2006). The mean concentration of nitrobenzene in effluents from P2, which exhibited the greatest T3 antagonist equivalency, was 100-fold greater than the national standard. Nitrobenzene may be one of the TR antagonists. Most of the more recent work

focuses on the genotoxicity and reproductive toxicity of nitrobenzene, and little has been done on its endocrine-disrupting activities. Exposure to oil sands associated chemicals such as polycyclic aromatic hydrocarbons can enhance hormone synthesis by the thyroid glands independently of activation of the pituitary–thyroid axis in birds (Gentes et al. 2007). Di(2-ethylhexyl)phthalate, which has been found to bind to the TRs and perhaps have selective effects on TR functions (Meeker et al. 2007) is the main product of the chemical plants at P3, but further analysis are required to identify source of toxicity at P1 and other toxins at P2.

Regardless of the causes, the results from our study suggest that wastewater treatment processes used in the three chemical industry parks are not effective in removing the chemicals that can interact with the endocrine system. Moreover, the concentration factor in this study is no more than 73-fold for all the samples, which is much less than the Bioconcentration Factors of the main products in these chemical industry parks. This indicates that the effluents from chemical industry complexes discharged directly into Yangtze River have potential endocrine disruption to aquatic animals and residents nearby. Our study firstly showed that the toxicity of the mixed effluents could be identified directly through reporter gene assay. Due to the important role of the Yangtze River, effluents should be identified by both instrumental analysis and reporter gene assays to determine whether it is safe enough for the humans and aquatic animals before going into the river.

In conclusion, reporter gene assays were utilized to identify the toxicity of the effluents from chemical industry park WWTPs along the Yangtze River, which were discharged directly into the river. Observed suppression to the response of reporter gene suggested the presence of pollutants in sewage effluents could cause disruptions to the normal function of TRs and ARs. Both TRs and ARs may be the targets of the present industrial effluents. Although di(2-ethylhexyl)phthalate, dibutyl phthalate and nitrobenzene might be responsible for the observed toxicity, further analyses, such as identification of toxicity source with fractionation techniques, are necessary. Furthermore, the battery of assays can be utilized to identify the source of key toxins, associated with chemical analysis.

Acknowledgments This work was funded by Major State Basic Research Development Program (No. 2008CB418102), the Environmental Monitoring Research Foundation of Jiangsu Province (0710) and Innovation Foundation for Youth Scholars of the State Key Laboratory of Pollution Control and Resource Reuse (PCR-REF07002). Prof. John P. Giesy was supported by the Canada Research Chair program and an at large Chair Professorship at the Department of Biology and Chemistry and Research Centre for Coastal Pollution and Conservation, City University of Hong Kong. The research was also financially supported by a Discovery Grant from the National Science and Engineering Research Council of Canada (Project # 6807) and a grant from the Western Economic Diversification Canada (Project # 6971 and 6807).

References

Cheng SY (2005) Thyroid hormone receptormutations and disease: beyond thyroid hormone resistance. Trends Endocrinol Metab 16:176–182. doi:10.1016/j.tem.2005.03.008

Conroy O, Sáez AE, Quanrud D, Ela W, Arnold RG (2007) Changes in estrogen/anti-estrogen activities in ponded secondary effluent. Sci Total Environ 382:311–323. doi:10.1016/j.scitotenv.2007.04.033

Devito M, Biegel L, Brouwer A, Brown S, Brucker DF, Cheek AO, Christensen R, Colborn T, Cooke P, Crissman J, Crofton K, Doerge D, Gray E, Hauser P, Hurley P, Kohn M, Lazar J, McMaster S, McClain M, McConnell E, Meier C, Miller R, Tietge J, Tyl R (1999) Screening methods for thyroid hormone disruptors. Environ Health Perspect 107:407–415. doi:10.2307/3434545

Fenton SE (2006) Endocrine-disrupting compounds and mammary gland development: early exposure and later life consequences. Endocrinology 147:S18–S24. doi:10.1210/en.2005-1131

Freyberger A, Schmuck G (2005) Screening for estrogenicity and antiestrogenicity: a critical evaluation of an MVLN cell-based transactivation assay. Toxicol Lett 155:1–13. doi:10.1016/j.toxlet.2004.06.014

Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ, McDonnell DP (1997) Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol 143:205–212. doi:10.1006/taap.1996.8069

Gentes M, McNabb A, Waldner C, Smits JEG (2007) Increased thyroid hormone levels in tree swallows (Tachycineta bicolor) on reclaimed wetlands of the Athabasca oil sands. Arch Environ Contam Toxicol 53:287–292. doi:10.1007/s00244-006-0070-y

Gracia T, Jones PD, Higley EB, Hilscherova K, Newsted JL, Murphy MB, Chan AKY, Zhang X, Hecker M, Lam PKS, Wu RSS, Giesy JP (2008) Modulation of steroidogenesis by coastal waters and sewage effluents of Hong Kong, China, using the H295R assay. Environ Sci Pollut R 15:332–343. doi:10.1007/s11356-008-0011-6

Hecker M, Giesy JP (2008) Novel trends in endocrine disruptor testing: the H295R steroidogenesis assay for identification of inducers and inhibitors of hormone production. Anal Bioanal Chem 390:287–291. doi:10.1007/s00216-007-1657-5

Houk VS (1992) The genotoxicity of industrial wastes and effluents. Mutat Res 277:91–138

Jobling S, Reynolds T, White R, Parker MG, Sumpter JP (1995) A variety of environmental persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103(6):582–587. doi:10.2307/3432434

Júnior HM, Silva J, Arenzon A, Portela CS, Ferreira IC, Henriques JAP (2007) Evaluation of genotoxicity and toxicity of water and sediment samples from a Brazilian stream influenced by tannery industries. Chemosphere 67:1211–1217. doi:10.1016/j.chemosphere.2006.10.048

Koh CH, Khim JS, Kannan K, Villeneuve DL, Senthilkumar K, Giesy JP (2004) Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2, 3, 7, 8-TCDD equivalents (TEQs) in sediment from the Hyeongsan River, Korea. Environ Pollut 132:489–501. doi:10.1016/j.envpol.2004.05.001

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004) Screening for estrogen and androgen receptor activities in 200

pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ Health Perspect 112:524–531

Kruger T, Long M, Bonefeld-Jørgensen E (2008) Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. Toxicology 246:112–123. doi:10.1016/j.tox.2007.12.028

Li J, Ma M, Cui Q, Wang Z (2008) Assessing the potential risk of oil-field produced waters using a battery of bioassays/biomarkers. Bull Environ Contam Toxicol 80:492–496. doi:10.1007/s00128-008-9465-y

Marina I, Margherita L, Palumbo M, Piccioli V, Parrella A (2007) Influence of alkylphenols and trace elements in toxic, genotoxic, and endocrine and endocrine disruption activity of wastewater treatment plants. Environ Toxicol Chem 26:1686–1694. doi:10.1897/06-320R2.1

Meeker JD, Calafat AM, Hauser R (2007) Di(2-ethylhexyl)phthalate metabolites may alter thyroid hormone levels in men. Environ Health Perspect 115:1029–1034

Ra JS, Kim SD, Chang NI, An KG (2007) Ecological health assessments based on whole effluent toxicity tests and the index of biological integrity in temperate streams influenced by wastewater treatment plant effluents. Environ Toxicol Chem 26:2010–2018. doi:10.1897/06-542R.1

Schiliro T, Pignata C, Fea E, Gilli G (2004) Toxicity and estrogenic activity of a wastewater treatment plant in Northern Italy. Arch Environ Contam Toxicol 47:456–462. doi:10.1007/s00244-003-0153-y

Sebire M, Allen Y, Bersuder P, Katsiadaki I (2008) The model anti-androgen flutamide suppresses the expression of typical male stickleback reproductive behaviour. Aquat Toxicol 90:37–47. doi:10.1016/j.aquatox.2008.07.016

Sun H, Xu L, Chen J, Song L, Wang X (2006) Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene. Food Chem Toxicol 44:1916–1921. doi:10.1016/j.fct.2006.06.013

Sun H, Xu X, Xu L, Song L, Hong X, Chen J, Cui L, Wang X (2007) Antiandrogenic activity of pyrethroid pesticides and their metabolite in reporter gene assay. Chemosphere 66:474–479. doi:10.1016/j.chemosphere.2006.05.059

Sun H, Shen O, Xu X, Song L, Wang X (2008a) Carbaryl, 1-naphthol and 2-naphthol inhibit the beta-1 thyroid hormone receptor-mediated transcription in vitro. Toxicology 249:238–242. doi:10.1016/j.tox.2008.05.008

Sun H, Xu X, Qu J, Hong X, Wang Y, Xu L, Wang X (2008b) 4-Alkylphenols and related chemicals show similar effect on the function of human and rat estrogen receptor a in reporter gene assay. Chemosphere 71:582–588. doi:10.1016/j.chemosphere.2007.09.031

Takako N, Satoshi S (2002) Contributions of genotoxic precursors from tributary rivers and sewage effluents to the Yodo River in Japan. Water Res 36:989–995. doi:10.1016/S0043-1354(01)00297-4

Takeyoshi M, Yamasaki K, Sawaki M, Nakai M, Noda S, Takatsuki M (2002) The efficacy of endocrine disruptor screening tests in detecting anti-estrogenic effects downstream of receptor–ligand interactions. Toxicol Lett 126:91–98. doi:10.1016/S0378-4274(01)00446-5

Takeyoshi M, Kuga N, Yamasaki K (2003) Development of a highperformance reporter plasmid for detection of chemicals with androgenic activity. Arch Toxicol 77:274–279

Theodorakis CW, Rinchard J, Carr J, Park J, Mcdaniel L, Liu F, Wages M (2006) Thyroid endocrine disruption in stonerollers and cricket frogs from perchlorate-contaminated streams in east-central texas. Ecotoxicology 15:31–50. doi:10.1007/s10646-005-0040-6

Urbatzka R, Cauwenberge A, Maggioni S, Vigano L, Mandich A, Benfenati E, Lutz I, Kloas W (2007) Androgenic and antiandrogenic activities in water and sediment samples from the river Lambro, Italy, detected by yeast androgen screen and chemical analyses. Chemosphere 67:1080–1087. doi:10.1016/j.chemosphere.2006.11.041

Villeneuve DL, Blankenship AL, Giesy JP (2000) Derivation and application of relative potency estimates based on in vitro bioassay results. Environ Toxicol Chem 19:2835–2843. doi:10.1897/1551-5028(2000)019<2835:DAAORP>2.0.CO;2

White PA, Rasmussen JB, Blaise C (1996) Comparing the presence, potency, and potential hazard of genotoxins extracted from a broad range of industrial effluents. Environ Mol Mutagen 27:116–139. doi:10.1002/(SICI)1098-2280(1996)27:2<116::AID-EM7>3.0.CO;2-E

Xu L, Sun H, Chen J, Bian Q, Qian J, Song L, Wang X (2005) Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol in vitro. Toxicology 216:197–203. doi:10.1016/j.tox.2005.08.006

Xu L, Sun H, Chen J, Bian Q, Song L, Wang X (2006) Androgen receptor activities of p, p′-DDE, fenvalerate and phoxim detected by androgen receptor reporter gene assay. Toxicol Lett 160:151–157. doi:10.1016/j.toxlet.2005.06.016

Yuan J, Wu X, Lu W, Cheng X, Chen D, Li X, Liu A, Wu J, Xie H, Stahl T, Sundermann VM (2005) Chlorinated river and lake water extract caused oxidative damage, DNA migration and cytotoxicity in human cells. Int J Hyg Environ Health 208:481–488. doi:10.1016/j.ijheh.2005.09.002

Zoeller RT (2005) Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals? Mol Cell Endocrinol 242:10–15. doi:10.1016/j.mce.2005.07.006