Bull Environ Contam Toxicol (2012) 89:161–165
DOI 10.1007/s00128-012-0632-9

Occurrence of (Anti)estrogenic Effects in Surface Sediment from an E-Waste Disassembly Region in East China

Lei Chen · Chunna Yu · Chaofeng Shen · Jinglan Cui · Chen Chen · Yingxu Chen

Received: 6 December 2011 / Accepted: 29 March 2012 / Published online: 10 April 2012
© Springer Science+Business Media, LLC 2012

Abstract Because of the report on the abnormal local fertility rate at Taizhou area, which is a famous e-waste disassembly center in China, the hormone-like effects in the surface sediment from the local river was investigated. Compared to the control site DG, significant estrogenic effects ($p < 0.01$) were observed at e-waste recycling sites ranging from 6.01 to 29.31 nmol/kg dw E2 equivalents by water extraction while ranging from 20.47 to 135.02 nmol/kg dw by organic extraction. When coincubated with E2, the water and the organic extractions displayed significant ($p < 0.01$) synergistic and anti-estrogenic effects respectively.

Keywords E-waste recycling · Yeast estrogen screen assay (YES)

The worldwide rapid increase of obsolete electrical and electronic equipments, such as computers, printers, copying machines, television sets, and mobile phones, results in the emerging environmental issue of electronic waste (e-waste) (Halluite et al. 2005). Both domestic and imported e-waste was concentrated and disassembled in developing countries like China for the potential economical benefits. Recycling of e-waste seems to be a promising way to deal with the increasing e-waste. Nevertheless the techniques used in recycling operations in developing countries are often primitive, such as recycling metals in open-pit acid baths, burning plastic materials in open air, disassembling the electric power capacitor and transformer rudely, and disposing unwanted materials in the farmland and riverbanks (Wu et al. 2009; Xing et al. 2009). Those processes will induce a large quantity of toxic chemicals, including heavy metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, dioxins, and so on, released into the environment (Chen et al. 2009; Ha et al. 2009) and may give rise to high ecological and health risk.

Taizhou area in Zhejiang Province, East China is one of the two largest disassembly centers of China. It has a history of e-waste disassembly for nearly 30 years. Each year, mountains of e-waste from both domestic generation and illegal imports ends up in Taizhou and is disassembled rudely in the simple household e-waste recycling workshops (Shen et al. 2009). Most of the residents, even many children, have been involved in the recycling process. E-waste derived PCBs to ground and surface waters, agricultural soils, rice, eggs, fish and ultimately humans has been demonstrated (Zhao et al. 2009b). Acute toxicity and genetic toxicity in the surface sediment near e-waste recycling sites have been reported (Chen et al. 2010). PBBs, PBDEs and PCBs with high concentrations were found in hair samples from local residents (Zhao et al. 2009a). High incidence of cancer in local residents has been reported. Recently it is pointed out that the local fertility rate has become dramatically low and the born infants are mainly female. The first report of e-waste import and disassembly process in China by Basel Action

L. Chen · C. Shen · J. Cui · C. Chen · Y. Chen (✉)
College of Environmental & Resource Sciences of Zhejiang University, Hangzhou 310058, People's Republic of China
e-mail: yxchen@zju.edu.cn; clymcl@zju.edu.cn

C. Yu
Center for Biomedicine and Health, Hangzhou Normal University, Hangzhou 311121, People's Republic of China

C. Shen · Y. Chen
The Academy of Water Science and Environmental Engineering, Zhejiang University, Hangzhou 310058, People's Republic of China

Network in public (BAN 2002) has driven the international attention to the e-waste recycling industry of China. Also the Chinese government has been doing something to solve this problem. Take Taizhou area for example, about 10 years ago, some of the original simple household workshops were forced to close by the local government, and large recycling plants were constructed in the form of the industrial park. Technologies with higher efficiency and more environmental protection facilities have been adopted in the industrial parks. Incinerators followed by waste gas treatment have been used instead of the open burning. Special treatment is required for waste gas and waters. Unsalvageable materials are collected and afterwards landfilled.

In the present study we focused on the Nanguan River of Taizhou area, which is an extended water source for local and downstream daily use. The objective of this study was to investigate the possible influence of e-waste recycling process on the local reproduction based on the detection of the (anti)estrogenic effects in surface sediments by using the yeast estrogen screen assay (YES), a widely used estrogenic activity screening system for environmental samples (Ma et al. 2007; Thomas et al. 2009).

Materials and Methods

The study area and sample location are shown in Fig. 1. There was no other pollution source except the e-waste recycling industry in this area. The Nanguan River runs through a dense e-waste recycling area containing numbers of simple household workshops and two industrial parks, and is about 7 km long with large areas of crops on each side. Surface sediments (0–10 cm) were collected from one control site (Dongguan, DG)—an upstream site without any known e-waste recycling pollution and six recycling sites—four near the simple household e-waste recycling workshops (A, B, C and D) and two near the industrial parks (E and F) in 2007. Because of the frequent storms and strong soil erosion in this area, the sedimentary rate is relatively high, about 1 cm/year. Thus the samples could represent the contamination in the last several years. All the samples were then air-dried in the dark at room temperature, sieved to <0.250 mm after removing stones and residual roots, and stored at 4°C.

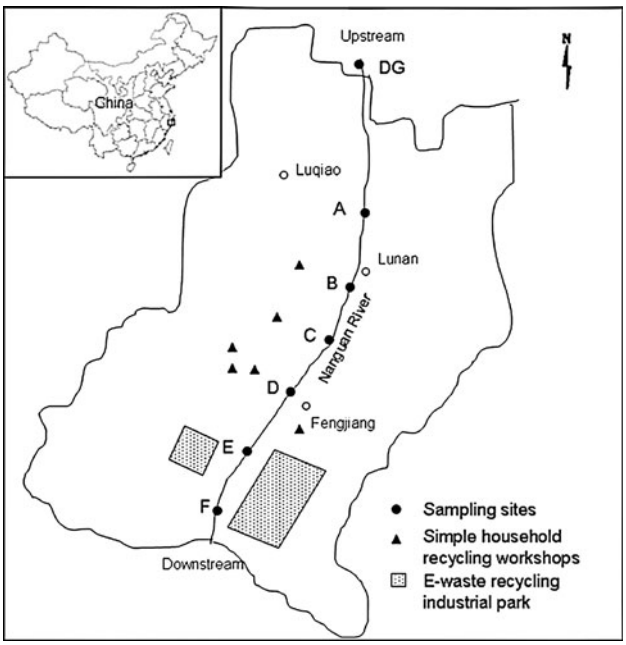

Fig. 1 Map of study area and sampling locations. DG control site; A–D: primitive disassembly sites; E–F: industrial park sites

A total of 10 g of samples were Soxhlet-extracted with 200 mL high purity acetone/hexane (1:1, v/v) for 48 h at four to six cycles per hour. Acid-washed copper was added to remove sulfur. The extracts were concentrated to approximately 2 mL by rotary evaporator (Büchi R-200, Switzerland) and were solvent-exchanged to hexane. The concentrated extracts were then evaporated to near dryness under a gentle stream of nitrogen gas, redissolved in 0.5 mL of dimethyl sulfoxide (DMSO; ACS grade, Sigma Chemical Co.) and stored at −20°C. The original extracts were finally diluted by 100 times with DMSO for the bioassay. Besides the organic extracts, the water extracts were prepared as well. Sediment samples were extracted with double-distilled water (sediment/water, 1/2.5) at room temperature for 24 h at 125 rpm in the mechanical shaker. 1 ml of the upper supernatant of each sample was collected after centrifugation and stored at −4°C. Meanwhile, the dry weight of the sediment was determined by drying sediment samples (about 5 g) at 105°C overnight. 17β-estradiol (E2) was purchased from Sigma-Aldrich, Germany and the test solutions were prepared in DMSO, with concentrations ranging from 0.05 to 500 nmol/L.

The yeast estrogen screen assay which accounts for both unanalyzed chemicals and potential non-additive interactions among compounds can provide a comprehensive characterization of a sample's potential to modulate the estrogen receptor alpha (ER-α) resulting in estrogenic responses. The recombinant yeast (Saccharomyces cerevisiae) cells were kindly provided by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The assay was performed according to the description by Routledge and Sumpter (1996) and Wu et al. (2002). OD420 nm of the reaction solutions were measured on the microplate reader. β-galactosidase activity U. was calculated from the OD420 nm values of test solutions and negative controls (DMSO and deionized water) and the reaction time, as described by Wang et al. (2003). The final estrogenic effects were expressed as E2 equivalents (EEQ)

compared to E2 induction rates. All statistical analyses were performed with Statistical Package for the Social Sciences 16.0 for windows. One-way analysis of variance was applied to detect significant differences.

Results and Discussion

Each assay contained a standard curve for E2, which was used to calculate the EEQ values for the environmental samples. Estrogenic effects in the surface sediment were finally expressed as EEQ, that is nmol E2 per kilogram of dry sediment (nmol/kg dw). Results are shown in Fig. 2. Compared to the control site DG, the estrogenic effects in the sediment were significantly ($p < 0.01$) higher at e-waste disassembly sites (B, C, D, E, and F, except A) ranging from 6.01 to 29.31 nmol/kg dw E2 equivalents by water extraction and from 20.47 to 135.02 nmol/kg dw E2 equivalents by organic extraction. Much higher estrogenic effects were observed at site B, C and D. It is suggested that the e-waste disassembly derived pollution had caused the estrogenic effects in the sediment. For both two different extracting methods, the sediment samples from the sites with numbers of simple household e-waste recycling workshops nearby (B, C, and D) showed higher estrogenic effects than the sites near the e-waste recycling industrial parks (E and F). It is evident that the intensive management and new technologies adopted in the industrial parks worked and have mitigated the contamination and ecological risk derived from e-waste recycling.

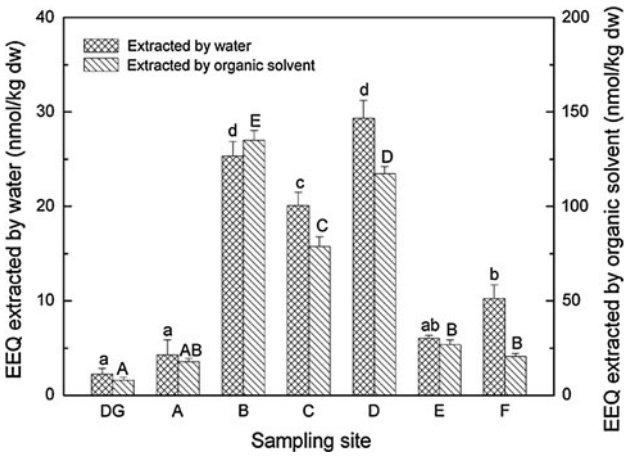

Fig. 2 Estrogenic effects (expressed as EEQ) in the surface sediment. Error bars represent the standard deviation of four samples. Columns denoted by different lowercase letters (*a*, *b*, *c*, and *d*) indicate significant ($p < 0.01$) difference of estrogenic effects extracted by water among different sites, and those by different capital letters (*A*, *B*, *C*, *D*, and *E*) indicate significant ($p < 0.01$) differences of estrogenic effects extracted by organic solvent. DG control site; A–D: primitive disassembly sites; E–F: industrial park sites

Levels of pollutants such as PCBs, PAHs, and heavy metals/metalloid at the same sites were analyzed in our previous study (Chen et al. 2010). Correlation test between the chemical concentrations and the estrogenic effects was performed and results are shown in Table 1. The estrogenic effects in water extractions were significantly correlated with concentrations of total PCBs ($p < 0.01$) and total heavy metals/metalloid ($p < 0.05$), while the estrogenic effects in organic extractions were only significantly correlated with concentrations of total PCBs ($p < 0.01$). The contribution of PAHs to estrogenic effects seems to be little.

The potential estrogenic activity of PCBs has been demonstrated both in vitro and in vivo (Shekhar et al. 1997; Arcaro et al. 1999) and the repression of the Wnt7a signaling pathway was proposed as one of the mechanisms (Ma and Sassoon 2006). It is also believed that metabolites of PCBs, some hydroxylated PCBs for example, may exert estrogenic effects (Nomiyama et al. 2010). Besides, metals are also suspected of exerting estrogenic activity in human and wildlife. Martin et al. (2003) demonstrated estrogen-like activity of several divalent metals including copper, lead and mercury in MCF7 breast cancer cells. Cadmium is currently the most documented metal regarding direct ability to induce estrogen-dependant responses by binding to the hormone binding domain of the receptor (Nesatyy et al. 2006). PCBs and their metabolites, and heavy metals particulary cadmium from e-waste disassembly were the main cause of the estrogenic effects in the sediment samples. Further more, according to Fig. 2, it is suggested that the estrogenic activity from the organic fraction was much higher than the inorganic fraction, nearly 10 times higher.

Impact of the contaminated sediment on the activity of natural estrogen E2 was investigated afterwards. The test solutions of the sediment samples were coincubated with E2 solution and then tested for estrogenic activity. As shown in Table 2, the calculated EEQ was the sum of the EEQ values of the test solution and E2 singlely, while the actual EEQ was the EEQ value of each coincubated solution in the bioassay. According to Table 2, as for water extractions coincubated with E2, there was a significant

Table 1 Correlations between estrogenic activity and pollutant concentrations in the surface sediment

	Estrogenic effects in water extraction	Estrogenic effects in organic extraction
Total PCBs	0.957[b]	0.986[b]
Total PAHs	0.676	0.607
Total heavy metals/metalloid	0.872[a]	0.754

[a] Correlation is significant at the 0.05 level (two-tailed)

[b] Correlation is significant at the 0.01 level (two-tailed)

Table 2 Calculated and actual estrogenic effects (expressed as 17β-estradiol equivalents, EEQ) of sediment extractions coincubated with 17β-estradiol (E2, 10 nmol/L)

Sampling site	Water extraction + E2		Organic extraction + E2	
	Calculated EEQ (nmol/L)	Actual EEQ (nmol/L)	Calculated EEQ (nmol/L)	Actual EEQ (nmol/L)
DG	10.89 ± 0.26a	11.65 ± 0.72a	11.59 ± 0.33A	12.03 ± 0.35A
A	11.70 ± 0.65a	10.83 ± 0.48a	13.55 ± 0.34A	11.04 ± 0.54B
B	20.12 ± 0.62a	25.64 ± 0.93b	37.01 ± 1.02A	31.23 ± 0.78B
C	18.03 ± 0.56a	22.25 ± 1.23b	25.76 ± 1.03A	18.38 ± 0.37B
D	21.73 ± 0.76a	24.32 ± 0.67b	33.45 ± 0.74A	29.56 ± 0.23B
E	12.40 ± 0.14a	14.23 ± 0.85a	15.34 ± 0.53A	17.26 ± 1.06A
F	12.10 ± 0.58a	11.31 ± 0.28a	14.09 ± 0.32A	11.78 ± 0.87B

Different lowercase letters (a and b) indicate significant ($p < 0.01$) difference between calculated and actual EEQ of the mixture of water extractions and 17β-estradiol. Different capital letters (A and B) indicate significant ($p < 0.01$) difference between calculated and actual EEQ of the mixture of organic extractions and 17β-estradiol. DG control site; A–D: primitive disassembly sites; E–F: industrial park sites

($p < 0.01$) synergistic estrogenic effect at site B, C, and D, which were sites with numbers of simple household e-waste recycling workshops nearby. However, the organic extracted test solutions revealed significant ($p < 0.01$) anti-estrogenic effects at all e-waste recycling sites, except site E. The reason might be the combined pollution of both heavy metals and organic compounds. Accordingly to the previous results in this study, the influence was much higher at the sites with simple household e-waste recycling workshops around.The potentiation of E2 activity by metals has been reported by Denier et al. (2009), also using the yeast estrogen screen assay. It was observed that cadmium, copper and zinc increaed the galactosidase activity of E2. This could explain the over-additive estrogenic effect in water extractions coincubated with E2. Meanwhile, the anti-estrogenic effects could be mainly due to the antiestrogentic activity of some PCB congeners. The anti-estrogenic activity of PCBs, which depends on the pattern of chlorine substitutions on the parent PCB molecule, was evident and appeared to be mediated through Ah receptor, as a consequence of a hypothetic defence mechanism to endogenous or exogenous ligands (Calò et al. 2010).

It is believed that the industry of e-waste recycling is a promising way, obtaining large profit at the meantime of dealing with the fast-growing e-waste. Nevertheless, the primitive disassembly processes without good management and technologies often cause serious environmental pollution and may threat ecological safety and human health. The hormone-like effects in the surface sediment from the local river was investigated and significant (anti)estrogenic effects were observed at e-waste recycling sites, especially those with simple household e-waste recycling workshops nearby. However, further studies are necessary to confirm the direct relation between the abnormal fertility rate and the contamination by e-waste recycling. Moreover, the results also provide the evidence that good management and advanced technologies are necessary to reduce the contamination and risk during the development of e-waste recycling industry.

Acknowledgments This work was supported by This work was supported by the National High Technology Research and Development Program of China (2009AA063104), National Natural Science Foundation of China (40901153) and Science and Technology Foundation of Zhejiang Province, China (2009C13003).

References

Arcaro KF, Yi L, Seegal RF, Vakharia DD, Yang Y, Spink DC, Brosch K, Gierthy JF (1999) 2,2′,6,6′-Tetrachlorobiphenyl is estrogenic in vitro and in vivo. J Cell Biochem 72:94–102

Calò M, Alberghina D, Bitto A, Lauriano ER, Cascio PL (2010) Estrogenic followed by anti-estrogenic effects of PCBs exposure in juvenil fish (*Spaurus aurata*). Food Chem Toxicol 48:2458–2463

Chen L, Yu CN, Shen CF, Zhang CK, Liu L, Shen KL, Tang XJ, Chen YX (2010) Study on adverse impact of e-waste disassembly on surface sediment in East China by chemical analysis and bioassays. J Soil Sediment 10:359–367

Denier X, Hill EM, Rotchell J, Minier C (2009) Estrogenic activity of cadmium, copper and zinc in the yeast estrogen screen. Toxicol In Vitro 23:569–573

Ha NN, Agusa T, Ramu K, Tu NPC, Murata S, Bulbule KA, Parthasaraty P, Takahashi S, Subramanian A, Tanabe S (2009) Contamination by trace elements at e-waste recycling sites in Bangalore, India. Chemosphere 76:9–15

Halluite J, Linton JD, Yeomans JS, Yoogalingam R (2005) The challenge of hazardous waste management in a sustainable environment: insights from electronic recovery laws. Corp Soc Responsib Environ Manage 12:31–37

Ma R, Sassoon DA (2006) PCBs Exert an estrogenic effect through repression of the Wnt7a signaling pathway in the female reproductive tract. Nature 114:899–904

Ma M, Rao KF, Wang ZJ (2007) Occurrence of estrogenic effects in sewage and industrial wastewaters in Beijing, China. Environ Pollut 147:331–336

Martin MB, Reiter R, Pham T, Avellanet YR, Camara J, Lahm M, Pentecost E, Pratap K, Gilmore BA, Divekar S, Dagata RS, Bull JL, Stoica A (2003) Estrogen-like activity of metals in MCF-7 breast cancer cells. Endocrinology 144:2425–2436

Nesatyy VJ, Ammann AA, Rutishauser BV, Suter MJ (2006) Effect of cadmium on the interaction of 17beta-estradiol with the rainbow trout estrogen receptor. Environ Sci Technol 40:1358–1363

Nomiyama K, Nomura Y, Takahashi T, Uchiyama Y, Arizono K, Shinohara R (2010) Hydroxylated polychlorinated biphenyls (OH-PCBs) induce vitellogenin through estrogenic activity in primary-cultured hepatocytes of the *Xenopus laevis*. Chemosphere 78:800–806

Routledge EJ, Sumpter JP (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ Toxicol Chem 15:241–248

Shekhar PV, Werdell J, Basrur VS (1997) Environmental estrogen stimulation of growth and estrogen receptor function in preneoplastic and cancerous human breast cell lines. J Natl Cancer Inst 89:1774–1782

Shen CF, Chen YX, Huang SB, Wang ZJ, Yu CN, Qiao M, Xu YP, Setty K, Zhang JY, Zhu YF, Lin Q (2009) Dioxin-like compounds in agricultural soils near e-waste recycling sites from Taizhou area, China: chemical and bioanalytical characterization. Environ Int 35:50–55

BAN and SVTC (The Basel Action Network and Silicon Valley Toxics Coalition) (2002) Exporting harm: the high-tech trashing of Asia. Seattle, WA. http://www.ban.org

Thomas KV, Langford K, Petersen K, Smith AJ, Tollefsen KE (2009) Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in northsea offshore produced water discharges. Environ Sci Technol 43:8066–8071

Wang JX, Wu WZ, Henkelmann B, You L, Kettrup A, Schramm KW (2003) Presence of estrogenic activity from emission of fossil fuel combustion as detected by a recombinant yeast bioassay. Atmos Environ 37:3225–3235

Wu WZ, Chen J, Rehmann K, Schramm KW, Kettrup A (2002) Estrogenic effects from household stoves. Ecotox Environ Safe 53:65–69

Wu JP, Luo XJ, Zhang Y, Chen SJ, Mai BX, Guan YT, Yang ZY (2009) Residues of polybrominated diphenyl ethers in frogs (*Rana limnocharis*) from a contaminated site, South China: tissue distribution, biomagnification, and maternal transfer. Environ Sci Technol 43:5212–5217

Xing GH, Chan JKY, Leung AOW, Wu SC, Wong MH (2009) Environmental impact and human exposure to PCBs in Guiyu, an electronic waste recycling site in China. Environ Int 35:76–82

Zhao GF, Wang ZJ, Zhou HD, Zhao Q (2009a) Burdens of PBBs, PBDEs, and PCBs in tissues of the cancer patients in the e-waste disassembly sites in Zhejiang, China. Sci Total Environ 407:4831–4837

Zhao GF, Zhou HD, Wang DH, Zha JM, Xu YP, Rao KF, Ma M, Huang SB, Wang ZJ (2009b) PBBs, PBDEs, and PCBs in foods collected from e-waste disassembly sites and daily intake by local residents. Sci Total Environ 407:2565–2575