Evaluation of the *in vitro* estrogenicity of emerging bisphenol analogs and their respective estrogenic contributions in municipal sewage sludge in China

Ting Ruan, Dong Liang, Shanjun Song, Maoyong Song*, Hailin Wang, Guibin Jiang

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, PR China

HIGHLIGHTS

- Eight BPs were identified in sewage sludge of waste water treatment plants.
- Eight BPs exhibited estrogenic activity in BLYES assay.
- All sewage sludge samples elicited considerable estrogenic activity.
- BPs made minor contributions to the total estrogenic activity of sewage sludge.

ARTICLE INFO

Article history:

Received 17 July 2014

Received in revised form 1 December 2014

Accepted 6 December 2014

Available online 26 December 2014

Handling Editor: A. Gies

Keywords:

Bisphenols

Estrogenic activity

BLYES assay

Sewage sludge

ABSTRACT

There is a potential risk to the environment from persistent estrogenic compounds in sewage sludge. In this study, eight bisphenols (BPs) were identified in sewage sludge collected from wastewater treatment plants in 15 cities in China. The estrogenic potencies of the eight BPs and the estrogenic activities of sludge samples were evaluated using a bioluminescence yeast estrogen screen (BLYES) assay. All sludge samples elicited considerable estrogenic activity at a range of 2.8–4.7 ng E2 g⁻¹ dry weight (dw). All BPs exhibited estrogenic activity in the BLYES assay, but there were significant differences between the potency of individual chemicals. Bisphenol AF had the highest activity, followed by tetrachlorobisphenol A, bisphenol F, bisphenol A, bisphenol E, bisphenol S and 2,4-dihydroxybenzophenone. Tetrabromobisphenol A showed weak estrogenic activity at 1 × 10⁴ nM, but significant cytotoxicity above this concentration. The total estradiol equivalency quantities (EEQs) of BPs were in the range of 2.16–49.13 pg E2 g⁻¹ dw, accounting for 0.05–1.47% of the total EEQs in sewage sludge samples. The results indicate that BPs made a minor contribution to the estrogenic activity of the investigated sewage sludge. Nevertheless, our results suggest that considerable attention should be directed to the estrogenic potentials of emerging organic pollutants because of their widespread use and their potential to persist in the environment.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Bisphenols (BPs) form a large family of chemicals that are commonly used to produce polycarbonates and epoxy resins. Of these compounds, bisphenol A (BPA) is the most widely used; more than 3 million tons year⁻¹ of BPA is used all over the world in the manufacture of numerous consumer products (Delfosse et al., 2012). BPs, which consist of two phenolic rings joined together by a bridging carbon or other chemical structure, are structurally similar to BPA. Bisphenol B (BPB), bisphenol F (BPF), bisphenol AF (BPAF), bisphenol S (BPS), teramethylbisphenol A (TMBPA), and other

bisphenols are also used as materials for polycarbonate resin. Halogenated derivatives of BPA, which have bromine or chlorine substitutes on the phenolic rings, are used as flame retardants. Tetrabromobisphenol A (TBBPA) is the most-produced brominated flame retardant (global production >150,000 tons year⁻¹) (de Wit et al., 2010). Tetrachlorobisphenol A (TCBPA), closely related to TBBPA, is also used as a flame retardant, but is manufactured in lower quantities (global production <10,000 tons year⁻¹) (Chu et al., 2005).

Extensive industrial production and household applications facilitate the release of BPs into the environment. Several studies have shown that BPA is released from consumer products, and that it is detectable at trace levels in food, drinking water, wastewater, air, and dusts (Vandenberg et al., 2007). TBBPA has been found in

* Corresponding author. Tel./fax: +86 10 62849178.

E-mail address: smsong@rcees.ac.cn (M. Song).

river sediment in Japan and China (Watanabe et al., 1983; Zhang et al., 2009), sewage sludge in Sweden and Canada (Sellström and Jansson, 1995; Alaee et al., 2003), and air samples in Sweden (Sjödén et al., 2001). TCBPA has been detected in waste paper recycling plants in Japan (Fukazawa et al., 2002), drinking water in China (Fan et al., 2013), sediment and sludge in USA (Voordeckers et al., 2002; Chu et al., 2005). BPF has been detected in surface water, sewage and sediments in Germany (Fromme et al., 2002). BPF and BPAF have recently been found in sediments and indoor dust samples obtained from North America and several Asian countries (Song et al. 2012; Liao et al., 2012a,b). Of greater significance is the fact that some studies have identified BPs, such as BPA and TBBPA, in human serum, urine, and placental tissues in Germany, Sweden and Norway (Jakobsson et al., 2002; Thomsen et al., 2002; Dekant and Völkel, 2008; Vandenberg et al., 2010). The major source of consumer exposure is likely to be through food and drinks that have been in contact with BPs-containing materials.

BPA has been identified as a weak estrogenic chemical that modifies natural endocrine functions through binding to the estrogen receptor. It has been shown to cause a range of adverse effects in laboratory animals (O'Connor and Chapin, 2003; Vandenberg et al., 2009; Chung et al., 2011). The estrogenic activity of BPA was reported to be four to six orders of magnitude weaker than that of natural estrogen, 17 β -estradiol (VomSaal et al., 1998; Song et al., 2006). However, until now, little attention has been paid to the toxicity of other BPs. Because of their similar molecular structures and physicochemical properties, other BPs may also exhibit similar toxicological effects. Our recent work demonstrated that exposure to TCBPA, TBBPA, and BPAF resulted in developmental toxicity in zebrafish embryos/larvae, but only BPAF specifically showed estrogenic activity (Song et al., 2014a). Kitamura et al. (2005) compared the endocrine-disrupting activities of BPA and 19 related compounds by means of different *in vitro* and *in vivo* reporter assays. Some BPs exhibited estrogenic activity in the human breast cancer cell line MCF-7, but there were notable differences in activity (Kitamura et al., 2005). Recent studies have shown that the estrogenic activity of BPAF was about one order of magnitude stronger than does BPA (Okada et al., 2008; Matsushima et al., 2010). However, information on the toxicological consequences, environmental presence, and environmental fate of these compounds is still limited.

BPA is a significant contaminant in wastewater and biosolids from sewage treatment plants (STPs), and may affect wildlife at environmental relevant concentrations (Crain et al., 2007). In a recent study, we identified BPs, such as TCBPA, BPAF, and BPE, in sewage sludge for the first time in China (Song et al., 2014b). Because all the compounds in sewage sludge carry an intrinsic risk of off-site transport once applied to land, it is essential to provide information on their exposure risk potentials in the surrounding environment. In this study, the estrogenic potencies of BPA and other BPs found in sewage sludge were examined using a bioluminescence yeast estrogen screen (BLYES). Additionally, the contributions of BPs to the total estrogenic activities of sewage sludge were investigated by calculating estrogenic activities of BPs relative to those of sewage sludge samples. To the best of our knowledge, this is the first attempt to assess the risk of BPs in municipal sewage sludge. The results will not only show the contamination status of BPs, but also may be useful for assessing their risk in sewage sludge.

2. Materials and methods

2.1. Materials

TCBPA and BPAF were purchased from TCI (Portland, OR). 17 β -estradiol (E2), BPA, and TBBPA were purchased from Sigma–Aldrich

(USA). BPE, BPF, BPS, and 2,4-dihydroxybenzophenone (DHBP) were obtained from TCI (Tokyo, Japan). All chemicals had a purity of 98% or greater unless otherwise mentioned. BPs were dissolved in methanol to form stock solutions of 1000 $\mu\text{g mL}^{-1}$ and were stored away from light. Ultrapure water (18.3 MU) was produced by a Milli-Q system (Millipore, Billerica, MA, USA). ENVI-Carb (0.5 g, 6 mL) and Sep-Pak C18 (1 g, 6 mL) solid-phase extraction cartridges were obtained from Supelco (St. Louis, MO, USA) and Waters (Milford, MA, USA), respectively. Other chemicals used in this study were analytical grade.

2.2. Estrogenic potency of BP chemicals

The relative estrogenic activity was determined by a bioluminescence estrogen assay. The re-engineered *Saccharomyces cerevisiae* BLYES was kindly provided by Gary S. Saylor (University of Tennessee, USA). The BLYES assay has been described in detail in Sanseverino et al. (2005) and Eldridge et al. (2007). Briefly, a strain of BLYES was grown overnight in a modified minimal medium without leucine and uracil (YMM leu[−], ura[−]) at 30 °C, and by shaking at 200 revolution(s) per minute (rpm) until an approximate optical density at 600 nm (OD600) of 1.0 was achieved. Cells were centrifuged and re-suspended in fresh YMM (leu[−], ura[−]) until the OD600 was 1.0. Two hundred microliters were transferred to each well of a black 96-well microplate (Thermo Scientific, USA). Appropriate dilutions of test chemicals were added to each well. Bioluminescence was measured every hour for 12 h in a microplate reader (Varioskan Flash, Thermo Scientific). The positive control was E2, and negative controls were either medium only, or medium containing methanol.

The response of each chemical, expressed in mean relative bioluminescence units, was converted to relative response units, expressed as a percentage of the maximum response observed for E2 (% E2max). For each chemical, the response versus the log of the chemical concentration was plotted. A linear regression was determined using the points that fell on the linear portion of the curve. Each 50% effective concentration (EC50) was calculated using a linear regression formula. To allow comparison with the E2 standard, the relative potency, called the estradiol equivalency factor (EEF) was calculated as follows:

$$\text{EEF}_{\text{chemical}} = (\text{EC50}_{\text{E2}}) / (\text{EC50}_{\text{chemical}}) \quad (1)$$

2.3. Estrogenic activity in sludge extracts

Sewage sludge samples were collected from municipal waste water treatment plants in 15 cities. Freshly digested sludge samples were collected at the dewatering stage, packed in aluminum foil, and sealed in polypropylene bags. They were then immediately delivered to the laboratory, where they were stored at −20 °C until analysis. All sludge samples were freeze dried, homogenized, and sieved through a stainless steel 100-mesh sieve. The sample pretreatment procedure was similar to that used in our previous study (Song et al., 2014b). Briefly, 0.3 g of each sample was placed in a 15-mL, polyethylene terephthalate centrifuge tube (Corning Inc., Corning, NY, USA) and extracted with 5 mL of methanol triply by shaking at 350 rpm for 60 min. The supernatant was collected after centrifugation at 3500 $\times$ g for 10 min, and then passed through a 6-mL, methanol preconditioned ENVI-Carb cartridge to eliminate matrix interferences (Pang et al., 2013). Analyte residues on the ENVI-Carb cartridge were then eluted with a mixture of methanol and tetrahydrofuran/methanol (4:6, v/v). All eluates were combined and loaded onto a Sep-Pak C18 cartridge for further purification. The analytes were eluted with methanol and finally concentrated to 1 mL under a nitrogen stream.

The estrogenic activities of sewage sludge samples were determined by the method used earlier for the chemicals (see Section 2.2). The sample extracts were mixed with methanol of six different concentrations, and then were added to wells in the same way as described earlier. Bioluminescence was measured and the EC20 of each sample was then calculated. The estradiol equivalency quantity (EEQ) of each sample was calculated as follows:

$$\text{EEQ}(\text{ng E2 g}^{-1} \text{dw}) = (\text{EC20}_{\text{E2}}) / (\text{EC20}_{\text{sample}}) \times (\text{sample dilution factor}) \quad (2)$$

2.4. Estrogenic activity of BPs in sludge

In this study, the concentrations of BPs in samples were determined by a Quattro Premier XE triple-equadrupole mass spectrometer coupled with an Alliance 2695 high-performance liquid chromatography (HPLC-MS/MS; Waters Inc., Milford, MA, USA). The analytical methods and HPLC-MS/MS conditions have been described in our previous study (Song et al., 2014b). The calculated EEQ (cEEQ) of BPs in sewage sludge sample can be estimated by multiplying the individual EEF by the observed BPs concentrations (C_{chemical}):

$$\text{cEEQ} = \sum ((\text{EEF}_{\text{chemical}}) \times (C_{\text{chemical}})) \quad (3)$$

3. Results and discussion

In our previous study, we investigated the presence and profiles of 13 BPs in 52 municipal sewage sludge samples collected from 30 cities in China. Eight BPs were positively identified in most of the 52 sludge samples, indicating that BPs are widespread in sludge from various regions of China (Song et al., 2014b). In this current study, we selected municipal sewage sludge samples from 15 of these cities for further study (Fig. 1).

Eight chemicals identified in the sewage sludge samples were tested in the BLYES assay. The chemical structure of the test BPs is shown in Fig. 2a. In these experiments, a series of E2 dilutions

(from 1×10^{-3} to 20 nM) were used to generate the standard curves. The reproducibility of the bioluminescence induction was determined by calculating the EC50 of E2 from seven independent experiments. The calculated EC50 value from the bioluminescence assays was 4.4 nM, which agrees with other published data (Sanseverino et al., 2005). The bioluminescence induction of each chemical was measured at a series of concentrations in the BLYES assay and their dose–response curves are shown in Fig. 2b. All BPs exhibited bioluminescence induction activity in the BLYES assay, but the activities varied markedly. BPAF showed significant bioluminescence induction when the concentration was between 1×10^2 and 5×10^3 nM. BPA, TCBPA, BPE, and BPF showed significant bioluminescence induction at concentrations between 0.5×10^2 and 5×10^4 nM. DHBP and BPS showed significant bioluminescence induction when the concentrations were between 1×10^4 and 5×10^5 , and 5×10^4 and 1×10^6 nM, respectively. The bioluminescence maximal induction of TBBPA was 12.7%, and cytotoxicity was observed when concentrations exceeded 1×10^4 nM.

We then used ICI182780, a strong estrogen receptor antagonist, to block the ER in the BLYES assay. There was a marked reduction in bioluminescence when ICI182780 was exposed to E2 and the tested BPs, indicating that BPs induced bioluminescence by activating ER (data not shown). The relative estrogenic potencies of BPs were determined by the EC50 values from the dose–response curve. The EC50 values and the EEF of all BPs (except TBBPA, which was calculated from its maximum induction of 12.7%) relative to E2 are summarized in Table 1. BPAF showed the highest estrogenic activity, followed by TCBPA, BPF, BPA, BPE, TBBPA, DHBP, and BPS. Kitamura et al. (2005) used an ERE-luciferase reporter assay in MCF-7 cells (Kitamura et al., 2005) for BP chemicals, and reported similar values for the relative estrogenic potencies of some BPs.

The detailed EEQ of BPs in each sewage sludge sample was calculated and is shown in Table 2. The $\sum \text{cEEQ}$ of BPs had a range of 2.16–49.13 pg E2 g⁻¹ dw, and the squared correction coefficient (R^2) between the $\sum \text{cEEQ}$ and total concentration of BPs was 0.75. The contribution of each BP to the $\sum \text{cEEQ}$ was related to both its estrogenic activity and concentration in the sludge. TBBPA, detected in all samples, was the dominant compound in the total

Fig. 1. Sample sites of waste water treatment plants in China.

Fig. 2. (a) Chemical structures of BP analogs; (b) dose–response curves for E2 and BPs in BLYES assay. Results are expressed as a percentage of bioluminescence measured per well (mean $\pm$ SD, $n = 4$) relative to the value obtained with 10 nM E2 (E2max).

Table 1

The EC50 values and estradiol equivalency factors (EEFs) of BP analogs.

Chemical	EC ₅₀ (nM)	EEFs
E2	0.44	1
BPA	4.1×10^3	1.07×10^{-4}
BPE	7.43×10^3	5.92×10^{-5}
BPS	4.13×10^5	1.06×10^{-6}
BPAF	6.08×10^2	7.23×10^{-4}
DHBP	8.38×10^4	5.25×10^{-6}
TCBPA	2.53×10^3	1.74×10^{-4}
TBBPA	n.d.	8.91×10^{-6a}
BPF	4.01×10^3	1.08×10^{-4}

^a EEF of TBBPA was calculated by its maximal induction of 12.7%.

BP residues. However, because of its low estrogenic activity, it was only the major contributor to the $\sum$ cEEQs of LY (41.3%) and QH (61.2%). BPAF had the highest EEF, and was the major estrogenic contributor to the $\sum$ cEEQ of GS (66.4%). BPA was the second most abundant BP in sewage sludge samples; its estrogenic activity was moderate, and it made a major contribution to the $\sum$ cEEQ of LW (69.2%), NB (72.3%), QD (72.2%), TA (84.0%), and XA (49.1%). The contributions from DHBP and BPS to all samples were negligible

(<2%) because of their weak estrogenic activity and low sample concentrations.

Significant bioluminescence induction was observed in all of the sludge extracts. The maximal induction (% E2max) from BPs in the sample extracts was between 20% and 45% of the maximal induction elicited by E2. Not all samples reached 50% of the maximal efficacy. Cytotoxicity was determined by the LIVE/DEAD[®] Yeast Viability Kit; maximum cytotoxicity concentrations were measured in all the sludge sample extracts tested. Multiple point estimates of EEQ were calculated to account for the low level of induction and nonparallel slopes observed for the samples. The cytotoxicity was significantly observed for most samples at concentrations that the bioluminescence induction higher than 20% E2max (EC20) (data not shown), thus the relative estrogenic activity was expressed as the amount of sample causing the same level of response as EC20. The EEQs of the extracts did not vary significantly among sites, and had a range of 2.8 ± 0.2 – 4.7 ± 0.1 ng E2 g⁻¹ dw (Fig. 3). There were no obvious geographical trends. The results coincidentally agreed with other published levels of E2 at several ng g⁻¹ dw in sludge in China (Liu et al., 2011; Ye et al., 2012), but were about 5–20 times lower than the estrogenic activities reported for the sediments of the Haihe and Dagu Rivers, China (Song et al., 2006).

Table 2

The calculated EEQ (cEEQ) of BPs in sewage sludge samples.

Sites	cEEQ (pg E2 g ⁻¹ dw)								
	TBBPA	BPA	BPS	BPF	TCBPA	BPAF	DHBP	BPE	ΣBPs
BJ	1.10	1.32	n.d.	15.44	2.59	n.d.	0.046	n.d.	20.50
DY	0.19	1.96	0.06	n.d.	0.037	1.71	0.00074	9.89	13.83
GS	2.31	13.80	0.12	n.d.	n.d.	32.61	0.29	n.d.	49.13
HB	0.59	12.63	0.065	1.09	24.80	1.81	0.00074	n.d.	40.99
HF	0.49	1.40	0.0025	0.86	0.32	n.d.	0.0053	5.52	8.60
LW	0.51	8.24	n.d.	1.09	0.36	1.71	0.00074	n.d.	11.91
LY	1.79	0.03	0.00013	0.90	n.d.	0.22	0.046	1.34	4.33
NB	2.12	10.81	0.022	n.d.	0.38	1.59	0.023	n.d.	14.95
QD	1.16	5.89	0.00782	n.d.	1.10	n.d.	n.d.	n.d.	8.16
QH	1.53	0.89	0.0452	n.d.	n.d.	n.d.	0.038	n.d.	2.50
SH	1.18	5.81	0.0017	1.33	n.d.	n.d.	0.18	6.75	15.25
TA	0.18	16.26	0.003	1.78	0.14	0.99	0.0034	n.d.	19.36
XA	0.90	8.90	0.0004	1.56	n.d.	n.d.	n.d.	6.75	18.11
YT	0.06	n.d.	n.d.	2.10	n.d.	n.d.	n.d.	n.d.	2.16
ZGE	0.67	0.03	0.035	3.38	n.d.	3.13	0.16	0.0024	7.41

n.d.: Not detected.

Fig. 3. Estradiol equivalency quantities (EEQs, ng E2 g⁻¹ dw) of sludge extracts determined by BLYES assay.

The BPs accounted for 0.05–1.47% (mean 0.43%) of the EEQs in sewage sludge samples, indicating minor contributions to the total estrogenic activities. As they are widely used as raw materials in manufacturing, BPs may leak into the environment and may accumulate in biological systems. For example, TBBPA has been found in sewage sludge in Canada (1–46.2 ng g⁻¹ dw; Lee and Peart, 2002), Netherlands (2–600 ng g⁻¹ dw; Morris et al., 2004), United Kingdom (15.9–112 ng g⁻¹ dw; Morris et al., 2004), Sweden (<0.3–220 ng g⁻¹ wet weight; Oberg et al., 2002), and Spain (<3–472 ng g⁻¹ dw; Gorga et al., 2013), indicating its widespread use and distribution. Effluents from STPs are the main source of estrogens in the aquatic environment, and many studies around the world have reported the occurrence and removal of estrogens STPs (Xu et al., 2014).

Further, the persistence and prevalence of BPs in the environment highlight the need to have an in-depth understanding of their adverse effects on humans and other organisms. Most measured concentrations in the environment are below levels associated with adverse effects on aquatic organisms. However, it has been reported that BPA could cause various “low-dose effects”, such as decreasing daily sperm production and fertility in male mice (Gupta 2000; Matsushima et al., 2010). And some occasionally environmental levels of BPA exceed the lowest effect concentrations in aquatic organisms and thus could potentially cause some adverse effects on aquatic ecosystems (Crain et al., 2007). These data supports the conclusion that some of the more sensitive species could be affected at upper-end environmental concentrations

of BPA. To date, most research has focused on estrogens and estrogenic chemicals in sewage effluents, while the chemicals that remain in sludge have received very little attention. However, because sewage sludge may be applied to land, it could be important to consider the tendency of emerging organic chemicals, such as BP analogs, to persist at high levels in dry sewage sludge.

Much evidence suggests that BPA causes reproductive and developmental toxicity in aquatic organisms (Crain et al., 2007). These characteristics of BPA have been attributed to the effects on steroid hormone receptors such as ER (Matsushima et al., 2010). Okada et al. (2008) reported that BPAF bound more strongly to ERα than BPA, and that it could cause adverse reproductive and developmental effects in several different *in vivo* and *in vitro* models (Akahori et al., 2008; Bermudez et al., 2010). TBBPA showed a weak estrogenic response in cultured MCF-7 breast cancer cells (Olsen et al., 2003), and inhibited *in vitro* estradiol sulfonation (Hamers et al., 2004), indicating the potential for both direct and indirect estrogenic activity *in vivo*. In this study, all test BPs showed weak estrogenic activities in the BLYES assay, indicating that certain classes of chemicals may function as major environmental endocrine disruptors, and that they may also induce strong estrogenic activity. Moreover, TBBPA and TCBPA have been shown to inhibit sulfation of the thyroid hormone (Rahman and Yamauchi, 2010), and to act as antagonists for the thyroid hormone receptor (Sun et al., 2009).

4. Conclusions

The results of the present study confirm that BP analogs are widely distributed in China. The extensive use of BPs is causing increasing concern because of their potential adverse impacts on health. Even though their contribution to the total EEQs of sludge was low, all test BPs exhibited considerable estrogenic activities. The molecular structures and estrogenic strengths of BPs are similar to those of BPA, which suggests that BPs could contribute to the adverse effects caused by BPA. Further research is needed to identify their environmental presence, environmental fate, and toxicological consequences.

Acknowledgments

This work supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDB14010300) and the National Natural Science Foundation of China (Nos. 21377146 and 21422705).

References

- Akahori, Y., Nakai, M., Yamasaki, K., Takatsuki, M., Shimohigashi, Y., Ohtaki, M., 2008. Relationship between the results of in vitro receptor binding assay to human estrogen receptor alpha and in vivo uterotrophic assay, comparative study with 65 selected chemicals. *Toxicol. In Vitro* 22, 225–231.
- Alaee, M., Arias, P., Sjödin, A., Bergman, A., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* 29, 683–689.
- Bermudez, D., Gray, L., Wilson, V., 2010. Modeling the interaction of binary and ternary mixtures of estradiol with bisphenol A and bisphenol AF in an in vitro estrogen-mediated transcriptional activation assay (T47D-KBluc). *Toxicol. Sci.* 116, 477–487.
- Chu, S., Haffner, G., Letcher, R., 2005. Simultaneous determination of tetrabromobisphenol A, tetrachlorobisphenol A, bisphenol A and other halogenated analogues in sediment and sludge by high performance liquid chromatography – electrospray tandem mass spectrometry. *J. Chromatogr. A* 1097, 25–32.
- Chung, E., Genco, M., Megrelis, L., Ruderman, J., 2011. Effects of bisphenol A and triclocarban on brain-specific expression of aromatase in early zebrafish embryos. *Proc. Natl. Acad. Sci. USA* 108, 17732–17737.
- Crain, D., Eriksen, M., Iguchi, T., Jobling, S., Laufer, H., LeBlanc, G., Guillelte, L., 2007. An ecological assessment of bisphenol-A, evidence from comparative biology. *Reprod. Toxicol.* 24, 225–239.
- de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment—trends and new candidates. *Sci. Total Environ.* 408, 2885–2918.
- Dekant, W., Völkel, W., 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. *Toxicol. Appl. Pharmacol.* 228, 114–134.
- Delfosse, V., Grimaldi, M., Pons, J., Boulahtouf, A., le Maire, A., Cavailles, V., Labesse, G., Bourguet, W., Balaguer, P., 2012. Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes. *Proc. Natl. Acad. Sci. USA* 109, 14930–14935.
- Eldridge, M., Sanseverino, J., Layton, A., Easter, J., Schultz, T., Sayler, G., 2007. *Saccharomyces cerevisiae* BLYAS, a new bioluminescent bioreporter for detection of androgenic compounds. *Appl. Environ. Microbiol.* 73, 6012–6018.
- Fan, Z., Hu, J., An, W., Yang, M., 2013. Detection and occurrence of chlorinated byproducts of bisphenol A, nonylphenol, and estrogens in drinking water of China: comparison to the parent compounds. *Environ. Sci. Technol.* 47, 10841–10850.
- Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J., Wenzel, A., 2002. Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.* 36, 1429–1438.
- Fukazawa, H., Watanabe, M., Shiraishi, F., Shiraishi, H., Shiozawa, T., Matsushita, H., Terao, Y., 2002. Formation of chlorinated derivatives of bisphenol A in waste paper recycling plants and their estrogenic activities. *J. Health Sci.* 48, 242–249.
- Gorga, M., Martínez, E., Ginebreda, A., Eljarrat, E., Barceló, D., 2013. Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and related compounds in sewage sludge from Catalonia (Spain). *Sci. Total Environ.* 444, 51–59.
- Gupta, C., 2000. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc. Soc. Exp. Biol. Med.* 224, 61–68.
- Hamers, T., Kamstra, J., Sonneveld, E., Murk, A., Zegers, B., Boon, J., Brouwer, A., 2004. In vitro screening of the endocrine disrupting potency of brominated flameretardants and their metabolites. *Organohalogen Comp.* 66, 3016–3020.
- Jakobsson, K., Thuresson, K., Rylander, L., Sjödin, A., Hagmar, L., Bergman, Å., 2002. Exposure to polybrominateddiphenyl ethers and tetrabromobisphenol A among computer technicians. *Chemosphere* 46, 709–716.
- Kitamura, S., Suzuki, T., Sanoh, S., Kohta, R., Jinno, N., Sugihara, K., Yoshihara, S., Fujimoto, N., Watanabe, H., Ohta, S., 2005. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. *Toxicol. Sci.* 84, 249–259.
- Lee, H., Peart, T., 2002. Organic contaminants in Canadian municipal sewage sludge. Part I. Toxic or endocrine-disrupting phenolic compounds. *Water Qual. Res. J. Canada* 37, 681–696.
- Liao, C., Liu, F., Guo, Y., Moon, H., Nakata, H., Wu, Q., Kannan, K., 2012a. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. *Environ. Sci. Technol.* 46, 9138–9145.
- Liao, C., Liu, F., Moon, H., Yamashita, N., Yun, S., Kannan, K., 2012b. Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: spatial and temporal distributions. *Environ. Sci. Technol.* 46, 11558–11565.
- Liu, S., Ying, G., Zhao, J., Chen, F., Yang, B., Zhou, L., Lai, H., 2011. Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry. *J. Chromatogr. A* 1218, 1367–1378.
- Matsushima, A., Liu, X., Okada, H., Shimohigashi, M., Shimohigashi, Y., 2010. Bisphenol AF is a full agonist for the estrogen receptor ERalpha but a highly specific antagonist for ERbeta. *Environ. Health Perspect.* 118, 1267–1272.
- Morris, S., Allchin, C., Zegers, B., Haftka, J., Boon, J., Belpaire, C., Leonards, P., Van Leeuwen, S., De Boer, J., 2004. Distribution and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. *Environ. Sci. Technol.* 38, 5497–5504.
- Oberg, K., Warman, K., Oberg, T., 2002. Distribution and levels of brominated flame retardants in sewage sludge. *Chemosphere* 48, 805–809.
- O'Connor, J., Chapin, R., 2003. Critical evaluation of observed adverse effects of endocrine active substances on reproduction and development, the immune system, and the nervous system. *Pure Appl. Chem.* 75, 2099–2123.
- Okada, H., Tokunaga, T., Liu, X., Takayanagi, S., Matsushima, A., Shimohigashi, Y., 2008. Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-γ. *Environ. Health Perspect.* 116, 32–38.
- Olsen, C., Meussen-Elholm, E., Samuelsen, M., Holme, J., Hongslo, J., 2003. Effects of the environmental oestrogens bisphenol A, tetrachlorobisphenol A, tetrabromobisphenol A, 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl on oestrogen receptor binding, cell proliferation and regulation of oestrogen sensitive proteins in the human breast cancer cell line MCF-7. *Pharmacol. Toxicol.* 92, 180–188.
- Pang, G., Fan, C., Chang, Q., Li, Y., Kang, J., Wang, W., Cao, J., Zhao, Y., Li, N., Li, Z., Chen, Z., Luo, F., Lou, Z., 2013. High-throughput analytical techniques for multiresidue, multiclass determination of 653 pesticides and chemical pollutants in tea – Part III: evaluation of the cleanup efficiency of an SPE cartridge newly developed for multiresidues in tea. *J. AOAC Int.* 96, 887–896.
- Rahman, F., Yamauchi, K., 2010. Characterization of iodothyronine sulfotransferase activity in the cytosol of *Rana catesbeiana* tadpole tissues. *Gen. Comp. Endocrinol.* 166, 396–403.
- Sanseverino, J., Gupta, R., Layton, A., Patterson, S., Ripp, S., Saidak, L., Simpson, M., Schultz, T., Sayler, G., 2005. Use of *Saccharomyces cerevisiae* BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds. *Appl. Environ. Microbiol.* 71, 4455–4460.
- Sellström, U., Jansson, B., 1995. Analysis of tetrabromobisphenol A in a product and environmental samples. *Chemosphere* 31, 3085–3092.
- Sjödin, A., Carlsson, H., Thuresson, K., Sjölin, S., Bergman, A., Östman, C., 2001. Flame retardants in indoor air at an electronics recycling plant and at other work environments. *Environ. Sci. Technol.* 35, 448–454.
- Song, M., Xu, Y., Jiang, Q., Lam, P., O'Toole, D., Giesy, J., Jiang, G., 2006. Measurement of estrogenic activity in sediments from Haihe and Dagou River, China. *Environ. Int.* 32, 676–681.
- Song, M., Liang, D., Liang, Y., Chen, M., Wang, F., Wang, H., Jiang, G., 2014a. Assessing developmental toxicity and estrogenic activity of halogenated bisphenol A on zebrafish (*Danio rerio*). *Chemosphere* 112, 275–281.
- Song, S., Song, M., Zeng, L., Wang, T., Liu, R., Ruan, T., Jiang, G., 2014b. Occurrence and profiles of bisphenol analogues in municipal sewage sludge in China. *Environ. Pollut.* 186, 14–19.
- Song, S., Ruan, T., Wang, T., Liu, R., Jiang, G., 2012. Distribution and preliminary exposure assessment of bisphenol AF(BPAF) in various environmental matrices around a manufacturing plant in China. *Environ. Sci. Technol.* 46, 13136–13143.
- Sun, H., Shen, O., Wang, X., Zhou, L., Zhen, S., Chen, X., 2009. Anti-thyroid hormone activity of bisphenol A, tetrabromobisphenol A and tetrachlorobisphenol A in an improved reporter gene assay. *Toxicol. In Vitro* 23, 950–954.
- Thomsen, C., Lundanes, E., Becher, G., 2002. Brominated flame retardants in archived serum samples from Norway: a study on temporal trends and the role of age. *Environ. Sci. Technol.* 36, 1414–1418.
- Vandenberg, L., Chahoud, I., Heindel, J., Padmanabhan, V., Paumgarten, F., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ. Health Perspect.* 118, 1055–1070.
- Vandenberg, L., Maffini, M., Wadia, P., Sonnenschein, C., Rubin, B., Soto, A., 2007. Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland. *Endocrinology* 148, 116–127.
- Vandenberg, L., Maffini, M., Sonnenschein, C., Rubin, B., Soto, A., 2009. Bisphenol-A and the great divide, a review of controversies in the field of endocrine disruption. *Endocr. Rev.* 30, 75–95.
- VomSaal, F., Cooke, P., Buchanan, D., Palanza, P., Thayer, K., Nagel, S., Parmigiani, S., Welshons, W., 1998. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol. Ind. Health* 14, 239–260.
- Voordeckers, J., Fennell, D., Jones, K., Häggblom, M., 2002. Anaerobic biotransformation of tetrabromobisphenol A, tetrachlorobisphenol A, and bisphenol A in estuarine sediments. *Environ. Sci. Technol.* 36, 696–701.
- Watanabe, I., Kashimoto, T., Tatsukawa, R., 1983. Identification of the flame retardant tetrabromobisphenol-A in the river sediment and the mussel collected in Osaka. *Bull. Environ. Contam. Toxicol.* 31, 48–52.
- Xu, Y., Xu, N., Llewellyn, N., Tao, H., 2014. Occurrence and removal of free and conjugated estrogens in wastewater and sludge in five sewage treatment plants. *Environ. Sci. Process Impacts* 16, 262–270.
- Ye, X., Guo, X., Cui, X., Zhang, X., Zhang, H., Wang, M., Qiu, L., Chen, S., 2012. Occurrence and removal of endocrine-disrupting chemicals in wastewater treatment plants in the Three Gorges Reservoir area, Chongqing, China. *J. Environ. Monit.* 14, 2204–2211.
- Zhang, X., Luo, X., Chen, S., Wu, J., Mai, B., 2009. Spatial distribution and vertical profile of polybrominateddiphenyl ethers, tetrabromobisphenol A, and decabromodiphenylethane in river sediment from an industrialized region of South China. *Environ. Pollut.* 157, 1917–1923.