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RESEARCH ARTICLE

The acute toxicity of bisphenol A and lignin-derived bisphenol in algae, daphnids, and Japanese medaka

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Abstract Risk assessing newly synthesized chemicals prior to their applications is extremely important, if we want to ensure substitution of risky chemicals with more benign ones. During the past two decades, many analogs of bisphenol A (BPA) have been manufactured, while their toxicity remains less studied. The aim of this study was to compare the acute toxicity of a synthesized lignin-derived BPA (LD-BP) with that of BPA in representative aquatic organisms including two algal species (*Chlorella pyrenoidosa* and *Scenedesmus obliquus*), a cladoceran species (*Daphnia magna*), and the Japanese medaka (*Oryzias latipes*). The results revealed that the two algal species showed different responses to the two chemicals. For *C. pyrenoidosa*, both BPA and LD-BP stimulated growth within 48 h of exposure, except for the 50 mg L^{-1} of LD-BP treatment. After 96 and 144 h of exposures, BPA stimulated the growth of *C. pyrenoidosa* at low-exposure concentrations but inhibited its growth at high concentrations, while LD-BP caused a concentration-dependent response in *C. pyrenoidosa*. *S. obliquus* exhibited a monotonic concentration-response curve for both BPA and LD-BP exposures. For both *D. magna* and *O. latipes*, concentration-responses were monotonic with 96 h–LC_{50} of BPA and LD-BP of 11.7 and 5.0 mg L^{-1} and 9.4 and 4.1 mg L^{-1}, respectively. Our results demonstrate that LD-BP is more toxic than BPA in the representative aquatic organisms, and it can pose higher ecological risk to the aquatic ecosystem than BPA.

Keywords Bisphenol A · Lignin-derived bisphenol · Freshwater algae · *Daphnia magna* · *Oryzias latipes* · Acute effects

Dan Li and Ran Bi contributed equally to this study.

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Introduction

Industrial activities have released a large number of pollutants into the aquatic ecosystems over the past several decades. Among them, bisphenol A (BPA, 2,2-bis-(4-hydroxyphenyl) propane) has been receiving increasing attention due to its adverse effects on aquatic organisms and potential deleterious effects on human beings (Im and Loffler 2016; Rosenmai et al. 2014). Bisphenol A is widely used in the synthesis of hard plastic and epoxy resins as the coating on food cans, water bottles, water pipes, thermal paper receipts, digital media (CDs and DVDs), and electronic equipment lines (Im and Loffler 2016). Its wide industrial applications have driven a continuously rising demand for BPA. The global consumption of BPA is estimated to reach approximately 7.7 billion kg in

2015, and it is predicted that 10.6 billion kg will be manufactured in 2022 (Research and Markets 2016). Bisphenol A from different sources including manufacturers, effluents from wastewater treatment plants (WWTPs), landfill leachates, and improperly disposed BPA-containing products will inevitably enter into the aquatic ecosystems. In fact, BPA has been detected in various waters including the effluents of WWTPs (0.097–370.0 μg L^{-1}), soil leachates (128.0–200.5 μg L^{-1}), surface water (ND–3.9 μg L^{-1}), and drinking water (ND–882.5 μg L^{-1}) around the world (Huang et al. 2012; Makinwa and Uadia 2015; Yamamoto et al. 2001).

Over the past two decades, more than 20 new structural analogs of BPA have been manufactured for different purposes (Kitamura et al. 2005; Chen et al. 2015). Some BPA analogs have been widely used for synthesis of specialized epoxy and polycarbonate resins (Suzuki et al. 2010; Vandenberg et al. 2014). For example, bisphenol B (BPB, 2,2-bis(4-hydroxyphenyl)butane) and bisphenol P (BPP, 2,2-bis(4-hydroxy3-methyl) phenyl propane) have one extra methyl group on the methylene bridge and phenyl rings. They can be used as substitutes for BPA in the production of phenolic resin, rubber additives, and raw materials for pesticides and dyes (Chen et al. 2002; Liao et al. 2012). Bisphenol F (BPF, 4,4′-methylenediphenol) has been used mainly in lacquers, varnishes, adhesives plastics, water pipes, dental sealants, and coatings for food packaging (Cabaton et al. 2009). Bisphenol S (BPS, 4-hydroxyphenyl sulfone) is mainly used in can coatings and thermal receipt papers (Naderi et al. 2014). Bisphenol AF (BPAF, 4,4′-hexafluoroisopropylidene)diphenol), a cross-linker in fluoroelastomers, is mainly used in specialty polymer applications (Matsushima et al. 2010). Increasing efforts are expected to be put forth for the synthesis of more BPA analogs to serve new and special needs from industry.

It is well established that BPA can cause a variety of adverse effects in aquatic organisms including endocrine disruption, cytotoxicity, genotoxicity, reproductive toxicity, and neurotoxicity (Bonefeld-Jorgensen et al. 2007; Crain et al. 2007; vom Saal et al. 1998). A few studies have reported the adverse effects of BPA analogs and compared their toxicities with those of BPA in aquatic organisms (Chen et al. 2002; Im and Loffler 2016; Li et al. 2016). For example, it is reported that the EC_{50}-48 h values of BPB, BPP, and bis(4-hydroxyphenyl)sulfide (TDP) in *Daphnia magna* were 5.5, 1.6, and 3.5 mg L^{-1}, respectively, which were lower than the 10 mg L^{-1} reported for BPA (Chen et al. 2002). In terms of the endocrine disruption potential of BPA and BPA analogs, one study finds that the levels of vitellogenin (VTG) in the male zebrafish were elevated after 75 days of exposure to 100 μg L^{-1} of BPS (Naderi et al. 2014). However, the levels of VTG in the male zebrafish were not altered after 90 days of exposure to BPA at concentrations less than 400 μg L^{-1} (Keiter et al. 2012). Furthermore, the estrogenicity of bisphenol B was 10 times higher than that of BPA by virtue of the VTG levels in the liver of male Japanese medaka (Yamaguchi et al. 2015). A recent study showed that the exposure to 1.6 μg L^{-1} of BPA and BPS for 24 h in the zebrafish embryos caused 80 and 140% increase in the newly born neurons in the hypothalamus of the embryos, respectively (Kinch et al. 2015). The above mentioned studies indicate that many BPA analogs might be more toxic than BPA to aquatic organisms. The differences in the toxicity among BPA analogs can be partially attributed to their chemical structures, such as the number of methyl groups on the phenyl rings and the number of methyl groups on the methylene bridge, which can change their hydrophobicity and octanol/water partition coefficient (log K_{ow}) and therefore alter their uptake into aquatic organisms (Kitamura et al. 2005).

Unlike most analogs of BPA which were synthesized from petrol-based chemicals, lignin-derived bisphenol (LD-BP, 5,5′-methylenebis(2-methoxy-4-methylphenol) is synthesized with a biorenewable and recyclable catalyzer from feedstock (Chen et al. 2015). The synthesized product had high purity (> 99%) as evidenced from the analyses of the ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra (Chen et al. 2015). The differences in chemical structures between BPA and LD-BP are seen in Supplementary File 1. In our previous study, it is shown that the biomarkers for effects on the nerve, digestive, antioxidant, and reproductive systems were altered in Japanese medaka exposed to 1.5 mg L^{-1} of BPA and LD-BP for 60 days. In addition, it seemed that LD-BP was more toxic than BPA in medaka (Li et al. 2016). In this study, the acute effects of BPA and LD-BP in some representative aquatic organisms were evaluated and compared. These organisms include two freshwater algal species, i.e., *Chlorella pyrenoidosa* and *Scenedesmus obliquus*, a cladoceran species (*D. magna*) and the Japanese medaka (*Oryzias latipes*). The results of this study will provide basic toxicity data for LD-BP in aquatic organisms and help the ecological risk assessment of LD-BP.

Materials and methods

Chemicals and glassware

Bisphenol A (> 97%) (CAS no. 80-05-7; $C_{15}H_{16}O_2$) was obtained from Aladdin Industrial Corporation (Shanghai, China), and lignin-derived bisphenol ($C_{17}H_{20}O_4$) was synthesized (> 99%) (Chen et al. 2015). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were commercially available and of analytical grade. All glassware and other containers were acid washed, rinsed with deionized water, air-dried, and autoclaved before use.

Test species

The freshwater algae *C. pyrenoidosa* and *S. obliquus* strains were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. The two algal strains have been cultured in a reconstituted medium according to the OECD guideline (no. 201) in the lab for years (OECD 2011). The culture medium was sterilized under pressure of 1.05 kg cm^{-2} at 121 °C for 30 min prior to inoculation of the algae. The culture of two algal species was maintained in the lab by inoculating new flasks with freshly prepared culturing medium at 1:20 (*v*/*v*) once a week. The algae were propagated steadily in the 1-L flasks under illumination of 1500 lx (16 /8 h, light/dark) at 24 ± 1 °C in an incubator. To avoid contamination of the algal species in the lab, the purity of the algae was frequently examined under a microscope with software aiding in algal species counting and identification (Shineso Algacount-Sx, Hangzhou, China).

Daphnids (*D. magna*) and Japanese medaka (*O. latipes*) have been maintained in the laboratory for many generations. They were cultured in moderately hard reconstituted (MHR) water (pH 7.0–7.6; total hardness 69.8 mg L^{-1} as $CaCO_3$) with 16/8 h (light/dark) at 25 ± 1 °C. Daphnids were fed *C. pyrenoidosa* once a day, and fish were fed newly hatched *Artemia* nauplii and fish flakes.

Growth inhibition of BPA or LD-BP in two algal species

The growth inhibition assay was performed according to the OECD guidelines for the testing chemicals—testing no. 201 (OECD 2011) with algae. Algal cells were inoculated in 250 mL flasks with 100 mL medium at 25 ± 1 °C with a light intensity of 28 μmol m^{-2} s^{-1} (16/8 h, light/dark). Six nominal concentrations of BPA or LD-BP were used in the study for each algal species: 1.6, 3.1, 6.3, 12.5, 25.0, and 50.0 mg L^{-1}. Algae exposed to the same volume of DMSO (carrier solvent for BPA or LD-BP) as the abovementioned six concentrations were treated as the control. Each treatment had four replicates. The algae were acclimated to the exposure conditions for 3 days prior to exposure. One hundred microliters of stock solutions of BPA or LD-BP (dissolved in DMSO and 1000 times of their respective nominal exposure concentrations) was added to the flasks containing 100 mL culture medium to achieve the nominal exposure concentrations. Then, 1 mL of *C. pyrenoidosa* or *S. obliquus* from the algal stock was transferred to the flasks with culture medium containing BPA or LD-BP. The solvent control contained DMSO at 0.1% (*v*/*v*). After exposure for 48, 96, and 144 h, the absorbance of algal culture was monitored at 750 nm (OD_{750}) on a Multiskan FC (Thermo Scientific, China) spectrophotometer. The growth inhibition rate (GIR) was calculated using the equation: $GIR = (A_C - A_T)/A_C \times 100\%$, where A_C is the absorbance of the algal culture of the control; A_T is the absorbance of the algal culture of the treatments. EC_{50} values at each assay time were estimated using the nonlinear regression to a log-logistic model (Hill equation) using GraphPad Prism.

Acute toxicity of BPA or LD-BP in *D. magna*

Acute exposure of BPA and LD-BP in *D. magna* was conducted according to the modified guidelines of OECD no. 202 for testing chemicals with *D. magna* (OECD 2004). Approximately 300 adults were divided into 8 containers (each with 1000 mL capacity) to produce neonates for the experiment. The nominal concentrations for BPA or LD-BP were 6.0, 8.0, 10.7, 14.2, 18.9, and 25.3 mg L^{-1}. The solvent control contained 80 μL of DMSO only in 80 mL exposure medium (0.1%, *v*/*v*). Each concentration was replicated 4 times. The nominal concentrations for BPA or LD-BP were prepared similarly as above by adding 80 μL of the stock solution (1000× of the nominal concentration) to a 100 mL beaker with 80 mL of MHR water. Then, ten neonates (< 24 h) were transferred into a beaker with the newly prepared exposure medium. The exposure was conducted using a static regime without renewal of the exposure medium. The concentrations of BPA were not quantified. A previous study has demonstrated that after 48 h of exposure, the measured concentrations of BPA were 93.6–95.7% of their nominal concentrations (range from 0.47–7.5 mg/L) in an exposure medium (total hardness 84 mg/L as $CaCO_3$) (Mihaich et al. 2009), which was similar to the exposure medium (MHR water) using in this study. It is therefore reasonable to believe that BPA concentrations will remain over 90% of the nominal concentrations for the exposure in this study. In another study on the effects of BPA in Japanese medaka, it has shown that measured BPA levels in the exposure chambers using a semi-static system were almost identical to their nominal exposure concentrations after 24 h (Yokota et al. 2000). Therefore, in the present study, nominal concentrations of BPA and LD-BP were used. During the exposure for 4 days, food was withheld for the daphnia. Mortality was checked every 12 h, and dead animals were removed from the container at each checking interval.

Acute toxicity of BPA or LD-BP in Japanese medaka

Juvenile medaka (n = 600, approximately 45 days old; weight 67.3 ± 2.2 mg; standard length 1.01 ± 0.13 cm, n = 20) were randomly selected from more than 1500 individuals. The acute exposure was conducted according to the modified guidelines of OECD no. 203. for testing chemicals with fish (OECD 1992). The fish were exposed to 8.5, 9.0, 9.5, 10.0, 12.0, and 16.0 mg L^{-1} of BPA or 2.0, 4.0, 4.5, 5.0, 5.5, and 6.0 mg L^{-1} of LD-BP, respectively. The concentrations for the two chemicals were based on results of a preliminary experiment on their acute toxicity to Japanese medaka. Each

concentration had four replicates. For the exposure, ten juveniles (45 days) were transferred into a glass beaker (250 mL) with an exposure media of 200 mL MHR water and 200 μL of freshly-made BPA or LD-BP stocks (1000×). The solvent control contained 0.1% DMSO (*v*/*v*). Exposure media were not renewed during the exposure according to a previous study on the acute toxicity of BPA in Japanese medaka, in which both larvae and adult medaka were used (Ishibashi et al. 2005). Mortality was recorded every 12 h for 4 days, and dead animals were removed from the beakers at each checking interval.

Statistical analysis

All data were expressed as mean ± standard error (S.E.M). Analysis of variance (ANOVA) was used to detect the difference in the growth inhibition rates of algae, mortality of daphnids, and mortality of Japanese medaka. Tukey's test was used for the multiple comparisons among treatments. Data were analyzed using the GraphPad Prism 5.0 and OriginPro 7.5 (OriginLab, Northampton, MA, USA).

Results

The inhibitory effects of BPA and LD-BP on the growth of *C. pyrenoidosa* and *S. obliquus*

For *C. pyrenoidosa*, after 48 h of exposure, BPA stimulated its growth in a reversed concentration-dependent manner, i.e., the lowest concentration of BPA had the highest stimulation (Fig. 1a). After 96 and 144 h of exposures, the inhibitory effect on growth at concentrations above 10.0 mg L^{-1} was noticed in *C. pyrenoidosa*, while a stimulator effects were observed at concentrations lower than 10.0 mg L^{-1} (Fig. 1a). For LD-BP, after 48 h of exposure, the growth of *C. pyrenoidosa* was stimulated less relative to BPA, while the inhibition of the growth was observed at the highest concentration (i.e., 50.0 mg L^{-1}) (Fig. 1b). After 96 and 144 h of exposures, *C. pyrenoidosa* showed a concentration-dependent response to LD-BP. The EC_{50}-96 h of LD-BP was significantly lower than ($p < 0.001$) that of BPA (Table 1).

For *S. obliquus*, a clear concentration-dependent response to BPA and LD-BP after 48, 96, and 144 h of exposures was shown (Fig. 2a, b). Its growth was inhibited by approximately 80% compared to the control at the highest test concentrations (50.0 mg L^{-1}) after 96 h. The EC_{50}-96 h of LD-BP was 59% lower than that of BPA ($p < 0.01$) (Table 1).

The acute toxicity of BPA and LD-BP in *D. magna*

For the control, mortality rate was lower than 2.5% after 4 days of exposure. The mortality of *D. magna* exposed to BPA or

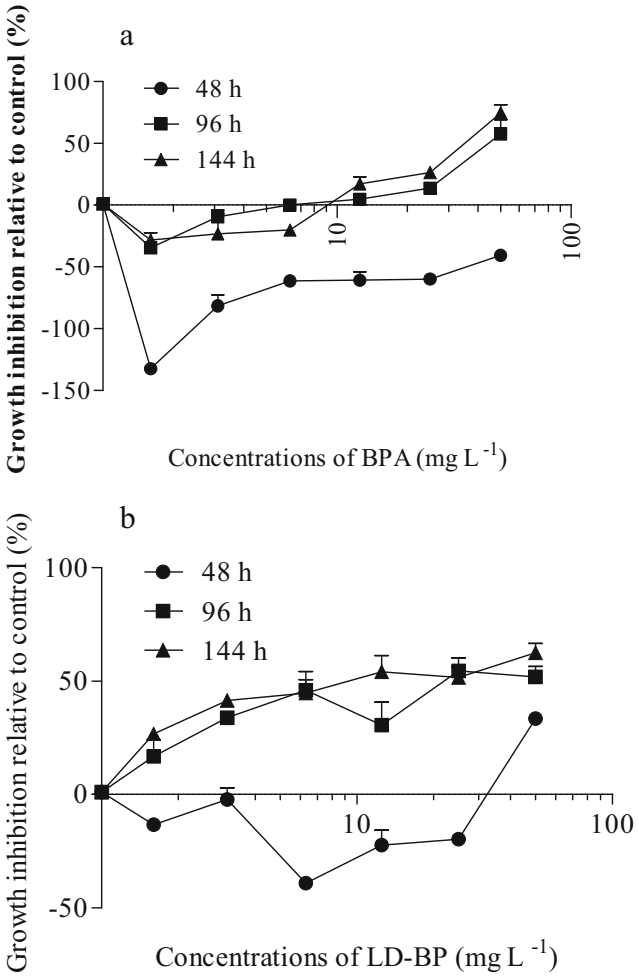

Fig. 1 Growth inhibition rate of BPA (**a**) and LD-BP (**b**) (each chemical with seven concentrations) in *Chlorella pyrenoidosa* at 48, 96, and 144 h. The exposure concentrations were from 0 to 50.0 mg L^{-1}. Data were expressed as the mean ± standard error ($n = 4$)

LD-BP showed a time- and concentration-dependant pattern (Fig. 3a, b). This coincides with the trends of LC_{50} values (24–96 h) of BPA and LD-BP for *D. magna* (Table 1). In general, mortality started earlier in the LD-BP exposure than in the BPA exposure. After 24 h of exposure, mortality started to occur at concentrations higher than 6.0 mg L^{-1} for LD-BP and 10.7 mg L^{-1} for BPA. The LC_{50} values of BPA in the daphnids were approximately 24, 50, 75, and 134% ($p < 0.01$ in all cases) higher than that of LD-BP after 24, 48, 72, and 96 h of exposure, respectively.

The acute toxicity of BPA and LD-BP in the Japanese medaka

The Japanese medaka showed a concentration-dependent response in mortality to the exposure to both BPA and LD-BP, respectively. Mortality started to show at LD-BP concentrations higher than 4.5 mg L^{-1} and BPA concentrations above 9.5 mg L^{-1} after 24 and 48 h of exposures which had a similar

Table 1 The EC_{50} of bisphenol (BPA) and lignin-derived bisphenol (LD-BP) in the freshwater algae (*Chlorella pyrenoidosa* and *Scenedesmus obliquus*) and the LC_{50} in a cladoceran species (*Daphnia magna*) and the Japanese medaka (*Oryzias latipes*)

Exposure duration (h)	BPA (mg L^{-1})	LD-BP (mg L^{-1})	Difference
C. pyrenoidosa			
96 h	44.9 ± 4.8	9.2 ± 1.8	388%***
S. obliquus			
96 h	33.9 ± 1.9	21.3 ± 2.3	59%**
D. magna			
24 h	16.2 ± 2.8	13.1 ± 1.8	24%*
48 h	14.7 ± 1.2	9.8 ± 1.3	50%**
72 h	13.3 ± 1.6	7.6 ± 0.8	75%**
96 h	11.7 ± 0.9	5.0 ± 0.6	134%**
O. latipes			
24 h	10.2 ± 1.3	5.4 ± 0.6	89%**
48 h	9.9 ± 1.5	5.3 ± 0.8	87%**
72 h	9.8 ± 0.8	4.9 ± 0.7	100%**
96 h	9.4 ± 0.9	4.1 ± 0.5	129%**

Data were expressed as the mean ± standard error ($n = 4$). Asterisk (*) indicates significant differences in EC_{50} or LC_{50} between LD-BP and BPA in these organisms. Difference refers to the percentage of change in EC_{50} or LC_{50} values of BPA relative to those of LD-BP

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

response curves for either BPA or LD-BP (Fig. 4). The LC_{50} values of BPA and LD-BP at 24, 48, 72, and 96 h were estimated to be 10.2, 9.9, 9.8, 9.4 mg L^{-1} and 5.4, 5.3, 4.9, 4.1 mg L^{-1}, respectively (Table 1). The differences in LC_{50} values between BPA and LD-BP in medaka after 24, 48, 72, and 96 h of exposures varied from 89 to 129% ($p < 0.01$ in all cases).

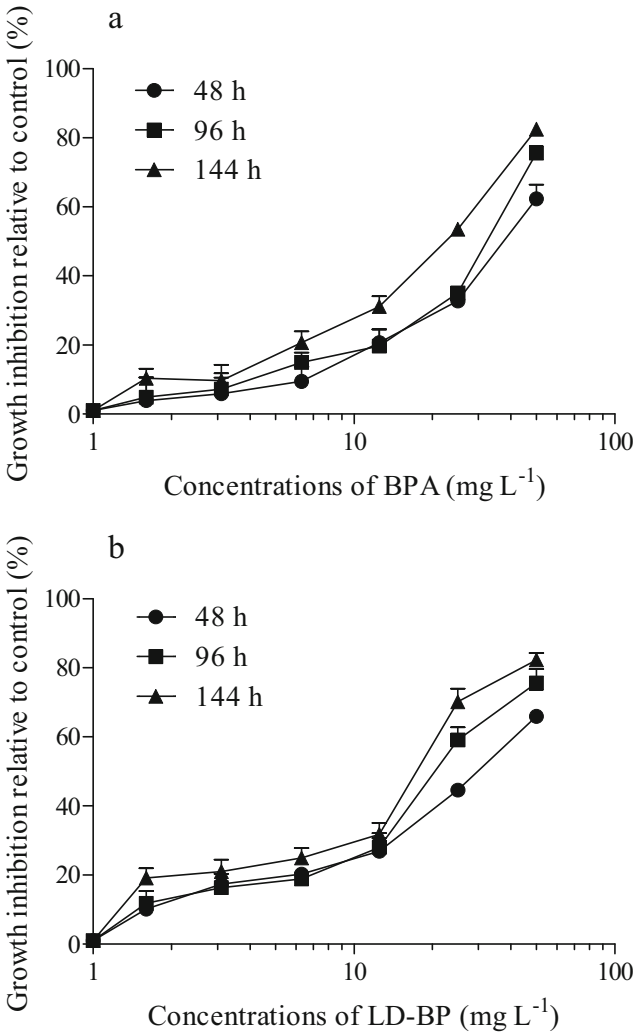

Fig. 2 Growth inhibition rate of BPA (**a**) and LD-BP (**b**) (each chemical with seven concentrations) in *Scenedesmus obliquus* at 48, 96, and 144 h. The exposure concentrations were from 0 to 50.0 mg L^{-1}. Data were expressed as the mean ± standard error ($n = 4$)

Discussion

The comparisons of the effects of BPA and LD-BP

In this study, overall, the toxic effects of LD-BP to the representative aquatic organisms were larger than those of BPA. The EC_{50}-96 h or LC_{50}-96 h values of LD-BP in the two freshwater algae, daphnids and medaka, were 37–80% lower than that of BPA. In our early study in which Japanese medaka were exposed to 1.5 mg L^{-1} of BPA and LD-BP for 60 days, it was found that, relative to the BPA-exposed medaka, the levels of malondialdehyde (a biomarker for lipid peroxidation) were significantly higher in all the tissues (liver, gill, intestine, brain, gonads) of LD-BP exposed medaka (Li et al. 2016). In addition, LD-BP-exposed male medaka had approximately 55% higher concentrations of vitellogenin (a biomarker for exposure to endocrine disruption chemicals in fish) than BPA-exposed male fish (Li et al. 2016). Bisphenol A and LD-BP differ in their chemical structures in that LD-BP has hydroxyl groups at the three positions of the phenyl rings and extra methyl and methoxyl groups at the phenyl rings of BPA, but no methyl and methoxyl groups between the two phenyl rings (Supplementary File 1). It is hypothesized that the toxicity of BPA analogs is probably positively related to the number of methyl/methoxyl group in the phenyl ring and the methylene bridge between two benzene rings, especially in the former case (Chen et al. 2002). The results from our early study and this study support this hypothesis. In addition, the extra methyl and methoxyl groups in LD-BP might alter the hydrophobicity and octanol/water partition coefficient (log K_{ow}) (with an extra $\cdot CH_3O$ and $\cdot CH3$ group in each phenyl ring of BPA). The estimated log K_{ow} by the software (EPI suite v4.11) of LD-BP (3.80) was slightly higher than that of BPA (3.64) (US EPA 2012).

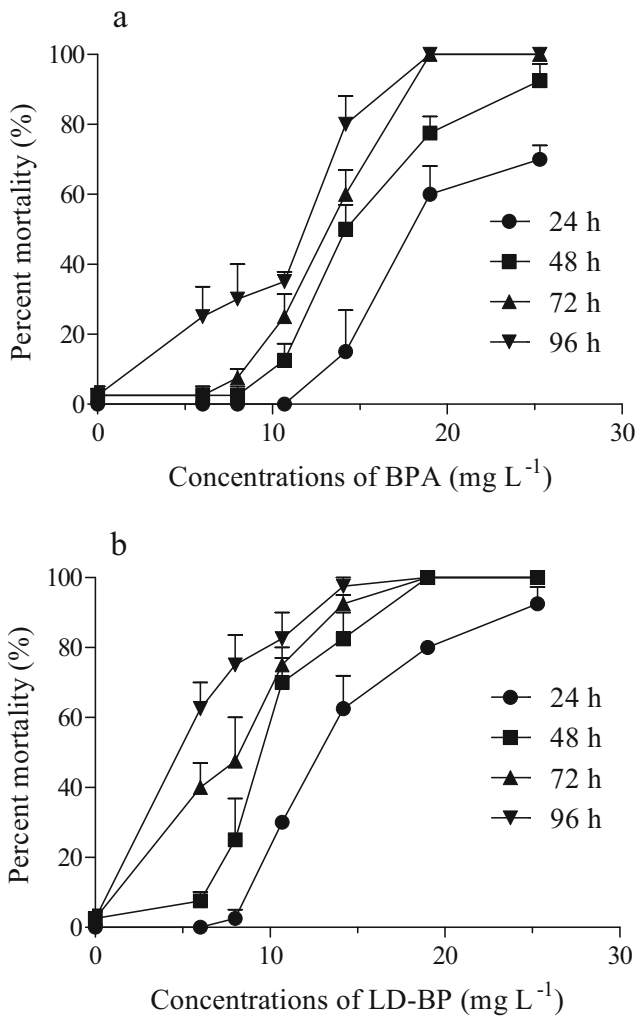

Fig. 3 Lethal effect of BPA (**a**) and LD-BP (**b**) (each chemical with seven concentrations) in *Daphnia magna* at 24, 48, 72, and 96 h. The exposure concentrations were from 0 to 25.3 mg L^{-1}. Data were expressed as the means ± standard errors. No error bar on the pillars means that the standard errors equal zero, because all of the organisms were dead (n = 10)

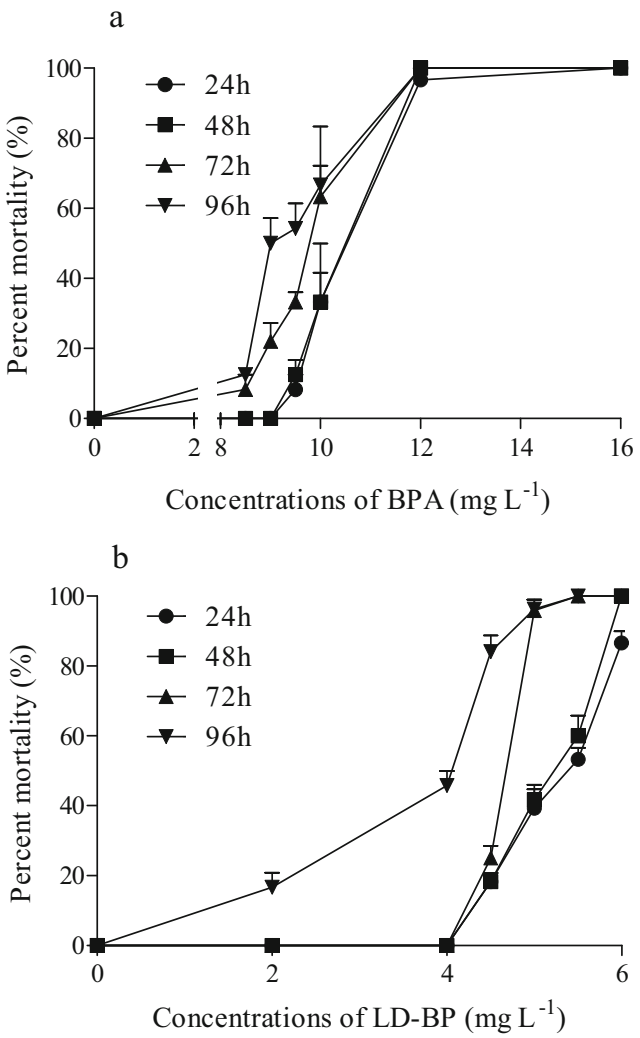

Fig. 4 Lethal effect of BPA (**a**) and LD-BP (**b**) (each chemical with seven concentrations) in Japanese medaka (*O. latipes*) at 24, 48, 72, and 96 h. The exposure concentrations of BPA and LD-BP were from 0 to 16.0 mg L^{-1} and from 0 to 6.0 mg L^{-1}, respectively. The control with no dead is not shown in this figure. Data were expressed as the means ± standard errors. No error bar on the pillars means that the standard errors equal zero, because all of the organisms were dead (n = 10)

The inhibitory effects of BPA and LD-BP in *C. pyrenoidosa* and *S. obliquus*

The toxic effects of pollutants in algae are generally evaluated by phytotoxicity tests based on the growth inhibition. In this study, the EC_{50}-96 h values of BPA on *C. pyrenoidosa* and *S. obliquus* were 44.9 ± 4.8 and 33.9 ± 1.9 mg L^{-1}, respectively, which were comparable to 63.5 and 26.7 mg L^{-1} for the two same species reported in an early study (Zhang et al. 2012). In addition, the two freshwater algal species showed different responses to the exposures to BPA or LD-BP. The growth of *S. obliquus* was inhibited at all the concentrations of these two chemicals, while for *C. pyrenoidosa*, during the first 48 h of exposure, stimulation of growth was observed for both chemicals. The differences in the responses between the two algal species might be due to the differences in the compositions of their cell walls. *C. pyrenoidosa* is a unicellular algal species, and its cell wall is composed primarily of α-cellulose and glycoprotein (Northcote et al. 1958). *S. obliquus* is frequently found in the form of four- or eight-celled coenobia, and its cell wall is mainly constituted of mannose (Takeda 1996). It is known that the cell wall of algae plays a critical role in the transport of materials including contaminants in and out of the cell (Latala et al. 2005). Therefore, differences in the composition of the cell walls were likely to affect the uptake of BPA and LD-BP and therefore resulted in the observed difference in their growth inhibition. Furthermore, the differences in growth inhibition by BPA and LD-BP between the two species might be related to their ability to degrade BPA. One study showed that *C. fusca* can

degrade BPA to monohydroxybisphenol A, a low toxic intermediate (Hirooka et al. 2005). Though we have no direct evidence, it is possible that *C. pyrenoidosa* (a close relative of *C. fusca*) could also degrade BPA to low toxic intermediates and thus was less sensitive to BPA and LD-BP than *S. obliquus*.

Furthermore, BPA and LD-BP affected *C. pyrenoidosa* differently. After 48 h of exposure, LD-BP showed less stimulatory effects in this alga than BPA. After 96 and 144 h of exposure, the stimulatory effects were still obvious for BPA at low concentrations. On the contrary, LD-BP inhibited the growth of *C. pyrenoidosa* at all the tested concentrations. The exact mechanisms underlying the stimulatory and inhibitory effects of BPA remain unknown and deserve future research.

The acute toxicity of BPA and LD-BP in *D. magna*

In this study, the LC_{50}-48 h of BPA in *D. magna* was estimated to be 14.7 mg L^{-1}, which was comparable and within the range of the LC_{50}-48 h values for the same species reported in earlier studies (3.9–20.0 mg L^{-1}) (Alexander et al. 1988; Hendriks et al. 1994; Mu et al. 2005; Stephenson 1983). Again, the higher toxicity of LD-BP in this species than that of BPA might be explained by the differences in their chemical structures. In the evaluation of the toxicity of several BPA analogs in *D. magna*, it was found that their toxicity was positively related to the number of methyl groups (Chen et al. 2002). Therefore, it is likely that substituting methyl groups in the phenyl ring might increase its toxicity to *D. magna*.

The acute toxicity of BPA and LD-BP in Japanese medaka

In this study, the LC_{50}-72 h and LC_{50}-96 h value of BPA in medaka was 9.8 and 9.4 mg L^{-1}, respectively. These values were comparable to the results from earlier studies on the acute toxicity of BPA in Japanese medaka. For example, the LC_{50}-96 h value of BPA in Japanese medaka was 13.9 mg L^{-1} (Ishibashi et al. 2005). The LC_{50}-72 h value of BPA in Japanese medaka was determined to be 6.8 mg L^{-1} for male fish and 8.3 mg L^{-1} for female fish (Kashiwada et al. 2002).

The LC_{50}-96 h of LD-BP was significantly lower than that of BPA in Japanese medaka, indicating that LD-BP was more toxic than BPA in acute toxicity testing. In our previous study, it was shown that LD-BP was more estrogenic than BPA and LD-BP. In addition, LD-BP caused higher level of lipid peroxidation than that of BPA when the juvenile Japanese medaka were exposed to the same concentration of these two chemicals for 2 months (Li et al. 2016). Similarly, early studies on the toxicities of BPA analogs have demonstrated that many BPA analogs are more toxic than BPA in fish at various aspects. For instance, the LC_{50}-96 h of BPB was estimated to be 6.1 mg L^{-1} in larval medaka, which was significantly lower than that of BPA (13.9 mg L^{-1}) (Yokota et al. 2008). In a study on the evaluation of the estrogenicity of BPA and BPB, the lowest observed effect concentration (LOEC) of BPB and BPA to stimulate *Vtg* gene production in the male medaka was 5 and 50 μmol L^{-1} (Yokota et al. 2008; Yamaguchi et al. 2015). Again, the increase in the toxicity of BPA analogs relative to BPA might be closely related to the substitutes at the phenyl rings and at the methylene bridge (Chen et al. 2002).

In summary, this study revealed the acute toxic effects of BPA and LD-BP in *C. pyrenoidosa*, *S. obliquus*, *D. magna*, and *O. latipes*. The two algal species showed different responses to the exposures to both chemicals. The two chemicals affected *C. pyrenoidosa* differently in that the stimulatory effect was more pronounced in BPA exposure than LD-BP exposure. The acute toxicity of BPA and LD-BP to these three species representative of three trophic levels (primary producer, primary consumer and secondary consumer) in the aquatic food webs was in order of *O. latipes* > *D. magna* > *C. pyrenoidosa* and *S. obliquus*. In addition, the results of this study and those from our early study (Li et al. 2016) have demonstrated that LD-BP is more toxic than BPA. The results of our studies and those of others on the toxicities of BPA analogs in aquatic organisms suggest that more data on the toxicity of LD-BP and probably other BPA analogs in aquatic organisms are needed prior to their large scale industrial applications.

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