The derivation of water quality criteria for bisphenol A for the protection of marine species in China
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ABSTRACT
Bisphenol A (BPA) is an environmental estrogen that occurs widely in the aquatic environment and causes feminization to various species, including fishes and gastropods. This study aims to develop the water quality criteria for BPA in the marine environment using the species sensitivity distribution (SSD) methodology from a scientific basis. Both acute and chronic toxicity data tested with saltwater species resident to China were collected. Additional tests were conducted to supplement toxicity data with local saltwater biota, including mollusk (*Ruditapes philippinarum*) and fish species (*Scophthalmus maximus* and *Pagrosomus major*). Based on SSD modelling, the criterion maximum concentration of BPA was estimated to be 273 μg/L. The criterion continuous concentration (CCC) for reproductive and non-reproductive effects was calculated to be 0.46 μg/L and 4.90 μg/L, respectively. Based on the derived criteria, the acute risk of BPA in coastal waters of China was determined to be negligible with RQs (risk quotients) of <0.01. The chronic risk was however much higher with RQs of up to 0.4 and 4.3 based on non-reproductive and reproductive CCC, respectively. The ecological risk assessment for BPA based on reproductive CCC can, therefore, better protect the safety of marine species.

Key words | acute-to-chronic ratio, bisphenol A, endocrine disruptor, risk quotient, species sensitivity distribution, water quality criteria

INTRODUCTION
Bisphenol A (BPA) is a synthetic chemical mainly used to produce polycarbonate plastics and epoxy resins for various products such as reusable bottles, compact discs and protective coatings for cans (*Staples et al. 1998; European Commission 2008*). The commercial production of BPA initiated in the 1950s and since then production has grown rapidly (http://www.bisphenol-a.org). The annual production of BPA reached 6.4 million tonnes in China in 2012, which accounted for about 10% of world production, according to statistics of the China Petroleum and Chemical Industry Federation (CPCIF) (http://www.cpcia.org.cn). Consequently, BPA is widely detected in the aquatic environment at trace level concentrations (ng/L to μg/L) throughout the world (*Kang et al. 2007*). As an endocrine disruptor, BPA interferes with the reproductive system of various species such as fish and gastropods at environmentally relevant concentrations (*Crain et al. 2007*). Due to its ubiquitous occurrence and endocrine-disrupting features, BPA raised widespread environmental concern. Over the past decade, ecological risk assessment in freshwater systems was carried out for this pollutant in several countries and regions such as Japan (*Nakanishi et al. 2007*), Canada (*Environment Canada 2008*) and Europe (*European Commission 2008*). The ecological risks in the marine environment, however, have not been appropriately assessed due to the lack of saltwater quality criteria for this emerging pollutant.

Water quality criteria (WQC) are the maximum concentrations of a pollutant acceptable in the aquatic environment without obvious effects on aquatic organisms and their

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functions (Swietlik et al. 2003; Yang et al. 2014). WQC are crucial in various processes including environmental emergency response, water quality evaluation and ecological risk assessment (Wu et al. 2010). The United States Environmental Protection Agency (US EPA) proposed two-number numerical criteria: criterion maximum concentration (CMC) and criterion continuous concentration (CCC) (Stephen et al. 1985). CMC is derived from acute toxicity data and is used for short term environmental management, whereas CCC is preferentially developed from chronic toxicity data and used for routine environmental surveillance. WQC can be derived through the assessment factor (AF) method and the species sensitivity distribution (SSD) modeling (European Chemicals Bureau 2005). The AF approach is applicable with limited toxicity data, but it shows great uncertainty. The SSD methodology requires a full set of toxicity data and therefore provides more reliable estimates of PNEC (predicted no effect concentration) (Hickey et al. 2013; Lei et al. 2015).

A major problem during the use of SSD for emerging pollutants is the shortage of toxicity data, particularly of chronic data. To overcome this problem, the US EPA proposed the acute-to-chronic ratio (ACR) approach to fill in data gaps (Stephen et al. 1985). ACR is defined as the ratio of acute and chronic toxicity values obtained for the same pollutant and to the same species, which can be used to estimate chronic toxicity to a different species with known acute toxicity (Raimondo et al. 2007). Acute tests usually adopt LC50 (50% lethal concentrations) or EC50 (50% effect concentrations) as customary endpoints, whereas chronic tests usually use long-term sublethal NOECs (no observed effect concentrations) on survival, development and growth as endpoints. As an endocrine disruptor, BPA affects the reproductive system at lower concentrations than non-reproductive endpoints (Guo et al. 2015). Chronic toxicity values may be under-estimated through the ACR method without considering a different category of endpoints. Previous study also showed that WQCs derived with reproductive endpoints were lower than those with traditional endpoints (Guo et al. 2015). Furthermore, difference in mode of action among various taxonomic groups also causes variation in ACRs (Klapow & Lewis 1979).

This study aims to derive marine WQC for BPA using data from the published literature supplemented with acute toxicity data for three saltwater species. The ACR approach was used and both reproductive and non-reproductive ACR for each taxon (i.e., alga, Mollusca, Arthropoda and Chordata) were calculated. With the endpoint and taxon-specific ACR, chronic toxicity values could be calculated from acute values. The SSD models for both acute and chronic (reproductive and non-reproductive) data were constructed, from which the CMC and CCC were derived.

**MATERIALS AND METHODS**

**Screening of toxicity data**

The toxicity data of BPA tested with Chinese resident species were thoroughly reviewed from published literature and the Chinese National Knowledge Infrastructure database (http://epub.cnki.net) up to December 2017. The quality of data was evaluated on reliability, adequacy and relevance (Klimisch et al. 1997) and only the data obtained through standard toxicity tests were collected. For acute toxicity data, short-term (48 h or 96 h) LC50 or EC50 were adopted. For chronic toxicity data, long-term NOECs (≥14 day, ≥7 days and ≥3 days for vertebrates, invertebrates and algae, respectively) for growth, development, survival and reproduction were used. When NOEC was not available, EC10 (10% effect concentration) or half of LOEC (lowest observed effect concentration) was used as an alternative. The geometric mean was adopted if multiple data were available for the same species and endpoint. The chronic toxicity data were separated into two categories: reproductive and non-reproductive data.

**Calculation of ACR**

Acute and chronic toxicity data of BPA generated from the same test were used to calculate ACR. The pairs of acute and chronic toxicity data were divided into four taxa (alga, mollusca, arthropoda and chordata). Both the reproductive ACR and non-reproductive ACR were calculated separately for each taxon. Nevertheless, matching pairs of data for acute and reproductive effects are unavailable. There are however matching pairs of data for non-reproductive and
reproductive effects. It is assumed that the ratio between acute and reproductive effects can be deduced based on the ratio of acute versus non-reproductive effects and the ratio of non-reproductive versus reproductive effects. The reproductive ACR for each taxon was therefore calculated as the ratio between the geometric mean of acute data and reproductive chronic data within each taxon. Similarly, the non-reproductive ACR was calculated as the ratio between the geometric mean of acute data and non-reproductive chronic data. These taxon ACRs were consequently used to estimate chronic values.

Acute toxicity tests

According to the US EPA guidelines, a set of toxicity data covering at least eight families and three phyla are required for the derivation of WQC (Stephen et al. 1985). However, neither acute nor chronic toxicity data were sufficient to derive saltwater WQC for BPA. Additional toxicity tests were therefore undertaken to supplement toxicity data with three saltwater species resident to China, one clam (Ruditapes philippinarum) and two fish (Scophthalmus maximus and Pagrosomus major). All tests were conducted according to relevant international guidelines (OECD 1992; ASTM 1993; US EPA 2002). The test procedures are briefly described as follows.

Specimens of fish Scophthalmus maximus (mean body weight: 4.0 ± 0.5 g), Pagrosomus major (mean body weight: 5.2 ± 0.6 g) and clam Ruditapes philippinarum (mean body weight: 7.5 ± 0.8 g) were obtained from local maricultural sites in Qingdao, China and acclimated to lab conditions (temperature 20 ± 1°C, pH 7.8 ± 1, dissolved oxygen (DO) >5.5 mg/L, salinity 30–32, light dark period 12 h:12 h) for at least one week before toxicity tests. Filtered natural seawater collected from a clean area of Qingdao was sterilized with autoclave sterilizer and used for dilution medium. BPA (from Sigma-Aldrich with a purity of ≥99%) was dissolved in acetone to make 500 mg/L stock solution. Static-renewal tests were performed and the test solutions were totally replenished at 24 h intervals. Ten specimen per group were randomly selected. Both solvent (acetone) and blank control were set up. Three replicates were set up for each exposure concentration. For R. philippinarum, tests were performed in 3-L glass beakers containing 1.5 L test solution with BPA concentrations of 750, 1,060, 1,500, 2,121 and 3,000 μg/L, and the 48-h LC50 was the observed endpoint. For S. maximus and P. major, tests were performed in 9-L glass containers containing 4 L test solution with BPA concentrations of 1,335, 2,000, 3,000, 4,500, 6,750 μg/L and 2,500, 4,242, 6,000, 8,484, 12,000 μg/L, respectively, and the 96-h LC50 was the observed endpoint.

During the toxicity tests, the specimens were not fed and dead individuals were removed immediately. The mortality rate was monitored each day. No mortality was observed in either control or solvent control groups. BPA concentrations in each group were checked at the beginning and at the end of the experiment by gas chromatography-mass spectrometry according to the method of Li et al. (2013) and the detection limit for BPA was 1.0 ng l⁻¹. A total of 126 samples were detected and BPA was not detected in either blank or solvent controls. All the measured concentrations deviated from the nominal ones by <20%. Consequently the nominal concentrations were not calibrated in the following regression and calculation. The 96-h LC50 and its 95% confidence intervals were calculated with probit methodology through SPSS software.

Data integration and criteria derivation

To minimize the effect of geographic difference, only the toxicity data tested with saltwater species commonly resident in China were collected to derive WQC. The selected acute toxicity values, together with three values obtained in this study were compiled to derive CMC. Chronic toxicity values were estimated by dividing these acute values with the corresponding taxon ACRs. The estimated chronic toxicity values together with raw values screened from literature were used to derive CCC.

For the derivation of CMC and CCC, the SSD methodology is now routinely used (ANZECC 2006). This methodology assumes that the acceptable effect level of different species in an ecosystem follows a probability function (Dowse et al. 2013). Based on SSD, the HC5 (hazardous concentration for 5% of species) can be calculated (Kooijman 1987; Wheeler et al. 2002). Consequently, the PNEC can be achieved by dividing the HC5 with an assessment factor.
(AF, usually 1–5). The choice of AF depends on further uncertainties identified, including amount of toxicity data and goodness of model fit. A value of 2 is used for AF in most studies to ensure consistency of results, which is also adopted in this study.

In this study, log-normal distribution recommended by the TGD (Technical Guidance Document on Risk Assessment) of the European Union (EU) was adopted for fitting the toxicity data, including acute toxicity data, chronic reproductive toxicity data and chronic non-reproductive toxicity data. The data were fitted with a scientific software ETX 2.0 (developed by RIVM, the National Institute for Public Health and the Environment) (Van Vlaardingen et al. 2005) and the model goodness of fit was evaluated through the Anderson-Darling test.

### Ecological risk assessment

The ecological risk of BPA in coastal waters of China was assessed with the risk quotient method ($RQ = \frac{MEC}{PNEC}$, where MEC is measured environmental concentration). The risk level is ranked as low, medium and high when the $RQ$ is $<0.1$, $0.1 – 1.0$ and $\geq 1.0$, respectively (Guo et al. 2018).

### RESULTS AND DISCUSSION

#### Acute-to-chronic ratios

The toxicity data used to calculate ACRs in this study are presented in Table 1. A total of 14 pairs of toxicity data (including acute data, chronic reproductive data and chronic non-reproductive data) were used to calculate ACRs. Non-reproductive ACRs were calculated as 2.1, 89.6, 39.2 and 36.6 for the taxa of alga, Mollusca, Arthropoda and Chordata, respectively. Reproductive ACRs were calculated as 1,649, 117.6 and 164.7 for Mollusca, Arthropoda and Chordata, respectively. The ACRs for reproductive endpoints were therefore much higher than those for non-reproductive ones. Especially for the most sensitive taxon, mollusca, the difference between reproductive and non-reproductive ACRs was as high as 18 times.

The ACR approach is commonly used to supplement chronic data for deriving WQC for aquatic species (US EPA 2005; Yang et al. 2012). The US EPA employs final acute-to-chronic ratio (FACR), which is expressed as the geometric mean of all available ACRs for a chemical to estimate chronic toxicity values. The use of FACR assumes that the ratio between acute and chronic toxicity is the same.
among different endpoints and taxa. This assumption is generally satisfied for those chemicals with similar mode of action between acute and chronic toxicities, such as anesthetics (Mayer et al. 1994).

However, it was observed for endocrine disruptors, such as BPA, that chronic effects (e.g. growth inhibition and reproductive impairment) were triggered by different modes of action (Fol et al. 2017). This would cause variation in ACRs among different toxicity endpoints (Hutchinson 2002; Raimondo et al. 2007). Furthermore, difference in mode of action among taxonomic groups also causes ACR variation (Klapow & Lewis 2013). To overcome this problem, the ACR was calculated separately for both reproductive and non-reproductive endpoints for each taxon in this study. The ACR for mollusks, for example, was obtained as 89.6 and 1,649 for non-reproductive and reproductive endpoints, respectively, which was much higher than that for arthropoda (39.2 and 117.6, respectively).

Based on the taxon ACRs, the chronic toxicity of BPA on marine planktonic diatom Skeletonema costatum and harpacticoid copepod Tigriopus japonicas, for example, was estimated as 476 μg/L and 110 μg/L, respectively (Table 2). These values were in agreement with previous laboratory tested chronic values of 400 μg/L and 110 μg/L (Alexander et al. 1988; Lee et al. 2007). With the use of a single FACR, however, the estimated chronic toxicity data deviated greatly from laboratory tested values (data not shown). ACR calculation considering endpoint and taxon differences, therefore, provides a better estimate of chronic toxicity data.

During the calculation of ACR, matching pairs of data for acute versus reproductive effects of BPA were unavailable in the literature (Table 1). The reproductive ACR was estimated in this paper as the ratio between the geometric mean of acute data and reproductive chronic data. The reproductive ACR could be better estimated with more detailed matching pairs of data. The toxicity tests matching acute versus reproductive effects of BPA therefore warrants further study.

### The toxicity data for SSD modeling

The toxicity data for SSD model construction are shown in Table 2. Based on the toxicity tests conducted in this study,
the 48-h \( LC_{50} \) for *Ruditapes philippinarum* was measured as 1,270 μg/L (95% CI: 1,091–1,460 μg/L). The 96-h \( LC_{50} \) for *Scophthalmus maximus* and *Pagrosomus major* was measured as 2,645 μg/L (95% CI: 2,296–3,039 μg/L) and 5,650 μg/L (95% CI: 4,827–65,57 μg/L), respectively. A total of 11 acute values (three obtained in this study and eight screened from literature) tested with saltwater species in China were pooled for SSD modeling. The \( LC_{50} \) ranged from 1,000 μg/L to 56,100 μg/L with a median of 4,200 μg/L. In total, 13 chronic non-reproductive values (11 estimated with ACR and 2 screened from literature) were pooled with NOECs ranging from 14 μg/L to 11,352 μg/L with a median of 110 μg/L. A total of nine chronic reproductive values were estimated by ACR with NOECs ranging from 0.8 μg/L to 477 μg/L with a median of 22 μg/L. The reproductive endpoints were therefore generally more sensitive than non-reproductive ones.

**WQC for BPA**

Based on the toxicity data in Table 2, the SSD models were constructed and the fitted curves shown in Figures 1 and 2. The HC5 for acute toxicity endpoints was calculated as 545 μg/L. The HC5 for chronic non-reproductive and reproductive endpoints was calculated as 9.80 μg/L and 0.92 μg/L, respectively. Based on the HC5, the CMC, non-reproductive CCC and reproductive CCC were derived as 273 μg/L, 4.90 μg/L and 0.46 μg/L, respectively.

The difference in SSD models between reproductive and non-reproductive endpoints is shown in Figure 2. As expected, the reproductive SSD curve shifted to the left of the non-reproductive curve. The resulting HC5 for reproductive endpoints was only about one-tenth of that for non-reproductive endpoints, indicating much higher sensitivity of reproductive effects. A similar phenomenon was reported in the derivation of WQC for nonylphenol (Gao *et al.* 2015).

To date, toxicity data tested with saltwater species have generally been insufficient for the derivation of WQC for emerging pollutants. Freshwater data were therefore used as surrogates or supplements to derive saltwater quality criteria in the literature (Leung *et al.* 2003), based on the untested assumption that freshwater species respond similarly to saltwater species. Compared with our previous study (Guo *et al.* 2015), saltwater WQC (CMC: 275 μg/L, CCC: 0.46 μg/L) in this study were 2–6 times lower than freshwater WQC (CMC: 1,518 μg/L, CCC: 0.86 μg/L). It can therefore be assumed that saltwater species were more sensitive under both acute and chronic exposure for BPA. Without saltwater criteria, the surrogate use of freshwater criteria would not adequately protect saltwater species. The reason for higher toxicity of BPA to saltwater species is probably related to its Kow and lipophilicity. It was demonstrated that the log Kow of BPA was influenced by salinity with a value of 3.44 and 3.53 in freshwater and saltwater, respectively (Borrirukwitsak *et al.* 2012). Higher log Kow also represents stronger lipophilicity and toxicity, which is consistent with the lower criteria derived in saltwater in this study.
BPA was widely reported in coastal waters of China (Table 3). The concentrations ranged from 1 to 1,964 ng L\(^{-1}\), which was among the highest in the world (Huang et al. 2014).

Based on the concentrations in Table 3, the acute RQ ranged from \(10^{-6}\) to \(10^{-3}\) in coastal waters of China, indicating negligible acute risk to marine species under short-term exposure. For chronic risk, the RQ on non-reproductive
endpoints (RQ_in) ranged from 0.0002 to 0.40 with a median of 0.012 and the RQ on reproductive endpoints (RQr) ranged from 0.002 to 4.3 with a median of 0.13 (Figure 3). The chronic RQ on reproductive endpoints were therefore about ten times higher than those on non-reproductive endpoints, indicating that ecological risk assessment based on traditional effects may not guarantee the safety of aquatic biota.

CONCLUSION

Due to its widespread occurrence and endocrine disrupting effect, BPA has aroused worldwide concern. The saltwater quality criteria, however, have not been established, which hinders the ecological risk assessment and environmental management for the pollutant. This study adopted taxon ACR and SSD methodology to estimate chronic toxicity values and derive saltwater quality criteria for BPA. Both acute and chronic toxicity data tested with saltwater species resident in China were screened from published literature, which were however not sufficient for the establishment of SSD models. Additional acute toxicity tests were conducted with three marine species to supplement toxicity data. Chronic toxicity values were further estimated with taxon ACR based on acute toxicity values. Through model simulation, the CMC, non-reproductive and reproductive CCC were derived as 273 \( \mu \text{g/L} \), 4.90 \( \mu \text{g/L} \) and 0.46 \( \mu \text{g/L} \), respectively. The risk quotients based on reproductive CCC were about ten times higher than those based on non-reproductive criteria. The adoption of reproductive CCC can, therefore, better protect the safety of wildlife from environmental estrogens such as BPA.

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