Derivation of aquatic life criteria for four phthalate esters and their ecological risk assessment in Liao River

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Abstract:

As a critical family of endocrine disruptors, phthalate esters (PAEs) attracted considerable attentions due to increasingly detected worldwide. Aquatic life criteria (ALC) for PAEs are crucial for their accurate ecological risk assessment (ERA) and have seldom been derived before. Given this concern, the purpose of the present study is to optimize the ALCs of four priority PAEs to estimate their ecological risks in Liao River. Reproductive endpoint was found to be more sensitive than other endpoints. Thus, reproduction related toxicity data were screened to derive ALCs applying species sensitivity distribution (SSD) method. ALCs of DEHP, DBP, BBP and DEP were calculated to be 0.04, 0.62, 4.71 and 41.9 μg·L^{-1}, which indicated decreased toxicity in sequence. Then, the derived ALCs of the four PAEs were applied to estimate their ecological risks in Liao River. A total of 27 sampling sites were selected to detect and analyze the exposure concentrations of PAEs. ERA using the hazard quotient (HQ) method was conducted. The results demonstrated that DEHP exhibited higher risks at 92.6% of sampling sites, and risks posed by DBP were moderate at 63.0% sampling sites. However, risks posed by BBP were low at 70.4% of sampling sites, and there were no risks posed by DEP at 96.3% of sampling sites. The results of probabilistic ecological risk assessment (PERA) indicated that probabilities of exceeding effects thresholds on 5% of species were 60.41%, 0%, 0.12%, 14.28% for DEHP, DEP, BBP and DBP, respectively. The work provides useful information to protect aquatic species in Liao River.

Keywords: Phthalate ester, Reproductive toxicity, Aquatic life criteria, Endocrine disruptor chemical, Ecological risk assessment

1. Introduction

Phthalate esters (PAEs) are used primarily as plasticizers to impart flexibility to polyvinylchloride plastics. They are intensively applied in a variety of products such as food packaging, cosmetics, various kinds of toys and medical equipment(Weir et al., 2014). As PAEs can be easily released into the environment from plastic products, they are ubiquitously detected in various environmental media including water (He et al., 2013; Zhang et al., 2018); Zhang et al., 2018), soil and sediment (Kang et al., 2016; Tan et al., 2016), and air (Deutschle et al., 2008; Wang et al., 2017). PAEs can enter water ecosystems through the discharge of domestic and industrial wastewater, surface runoff input from agricultural and urban areas, and atmospheric wet and dry deposition (Sha et al., 2007; Wang et al., 2008; Chai et al., 2010). PAEs are hardly degraded and the environment fate and bioaccumulation were investigated (Staples et al., 1997; Wofford et al., 1981; Yang et al., 2013). Recently, PAEs raised concerns as suspected endocrine disruptor chemicals (EDCs). They were shown to be carcinogenic, which mimic estrogenic activities in animals and can induce adverse effects in the development of the male reproductive system (Cheung et al., 2007). Also, PAEs posed great threat to aquatic organism (Adams and Gorsuch, 1995; Staples et al., 2000; Qu et al., 2015). Many countries have now limited the usage of PAEs and relevant policies and laws have been established (EC 2003, 2005; EPA, 2014; PRC-NS 2002). Six PAEs including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP) were listed in the 126 priority pollutants filed by the US Environmental Protection Agency (EPA) (EPA, 2014). DBP, DnOP, DEHP and BBP were four PAEs in the list of priority pollutants of the European Communities (EC, 1994; EC, 1995; EC,1997), China also has listed DMP, DBP and dimethyl octyl phthalic (DOP) as environmental priority pollutants (PRC-NS, 2002).

ALC are defined as the maximum water pollutant concentrations that do not pose short-term or long-term adverse or hazardous effects on aquatic life, which are based on scientific experiments and extrapolations (Wu et al., 2010). SSD is an important extrapolation approach to derive ALC based on all available toxicological data of the pollutant and extrapolate the concentration corresponding to the target percentage (Wheeler et al., 2002). Thresholds of protection for aquatic organisms is usually set up as 5% of species (HC_5).

China has launched national-level systematic ALC studies based on the regional characteristics and established comprehensive ALC research frameworks. ALCs of a large number of priority pollutants, including ammonia nitrogen (Wang et al., 2016), heavy metals (Wu et al., 2015; Zheng et al., 2017; Zhang et al., 2017) and organic pollutants(Yan et al., 2012; Wang et al., 2013) have been derived. Most of these researches adopted the lethal endpoint of three phyla and eight families to generate SSD of the pollutants. EDCs are considered to alter hormone levels leading to reproductive effects in aquatic organisms at environmental concentration continuously (Tisler et al., 2016). Previous toxicological studies have demonstrated reproduction was identified as the most sensitive endpoint for EDCs (Caldwell et al., 2008; Jin et al., 2014). Reproductive toxicity includes fecundity, rate of fertilization, hatchability, gonadosomatic index sustained for multiple generations, and the synthesis of vitellogenin (VTG) in fish (Martino-Andrade and Chahoud, 2010). Since the lethal endpoint lacks consideration of the adverse effects from development and function of aquatic organisms at non-lethal concentrations, ALC based on lethal effect was recognized to be unable to provide adequate protection from such adverse effects. PAEs as typical EDCs displayed low-acute toxicity in general, and the reproductive system is particularly susceptible long-term low-dose integrated exposure (Martino-Andrade and Chahoud, 2010). However, comparing with the extensive studies on derivation of ALCs for heavy metals and organic pollutants, there were limited researches available for ALCs of EDCs, especially based on the sublethal effects of reproduction.

Ecological risk assessment (ERA) is a process that evaluates the likelihood of adverse ecological effects occurring as a result of exposure to stressors (US EPA, 1998)， which can provide useful information for risk managers and decision makers. The most rudimentary approach to assessing the potential risk is the calculation of a hazard quotient (HQ), which compare the measured exposure concentration with ALC determined from SSD. HQ is point estimate and is generally considered to be overly conservative, and hence more useful for preliminary ecological risk assessment（Liu et al., 2016）. Probabilistic ecological risk assessment (PERA) is another method of ERA and based on a continuum of potential exposures and effects probability distributions to qualify and quantify ecological risks (Solomon and Giesy, 2000). Risk expresses as a joint probability curve (JPC) in PERA describes that the probability of a particular set of exposure conditions occurring relative to the number of taxa that would be affected. However, the ecological risk assessment of PAEs adopting various methods in water basin were seldom reported before.

Liao River is located in northeast China and belongs to one of the seven main watersheds of China with a water area of approximately 1345 km^2, and is also the critical drinking water source for the residents of the catchment. The rapid economic expansion, along with human activities, has caused enormous environmental pressures on Liao River so that it has suffered from severe contaminations of different pollutants including EDCs. To our knowledge, there has been no systematic investigation of PAEs concentration in Liao River to identifying their potential risk levels to aquatic ecosystem.

This objective of this study was to optimize the ALCs derivation of PAEs applying untraditional endpoints. The concentrations of PAEs in Liao River were monitored. In addition, based on the ALCs and field monitored data of four typical phthalates, their ecological risks in Liao River were comprehensively evaluated with two methods.

2. Materials and methods

2.1 Collection of data

Toxicity data of four PAEs were collected from ECOTOX database (http://cfpub.epa.gov/ecotox), CNKI database, and published literatures. Traditional effects such as lethal endpoints were excluded and only reproductive related endpoints were screened. The reproduction data mainly consisted the effects of fecundity, rate of fertilization, hatchability, expression of VTG, gonad somatic index, gonadal histology and multiple generation effects to aquatic organisms. The principles below were followed. Strictly, NOEC values were limited to calculate the ALC. As there were not enough data of reproduction endpoints for new pollutants, maximum acceptable toxicant concentration (MATC) or lowest observed effect concentration (LOEC) or EC_{10} values were adopted when NOEC is not available. For each chemical, in order to minimize the uncertainty and maximize protective values of the resulting HC_5, flow-through were preference to static/renewal for exposure style and measured chemical analysis were preference to unmeasured chemical analysis. Geometric mean value were calculated when multiple toxicity values were available per species.

2.2 Construction of SSD

The log-logistic distribution was generally a good-fitting model for species sensitivity distribution (SSDs) (Versteeg et al., 1999). In this study, the log-logistic distribution was adopted to construct SSDs and derive the ALCs of four PAEs (Wheeler et al., 2002), The equation is as follows:

$$y = \frac{1}{1+\exp\left(\frac{p_1-x}{p_2}\right)} \qquad (1)$$

where y is the cumulative probability of species, defined as “the order of the data point” divided by one plus the total number of data points; x is the log-transformed NOEC /EC_{10} / MATC/ LOEC; p1 is a parameter representing the location (or intercept); and p2 is a parameter representing the slope of the curve.

2.3 Field monitoring in the Liao River

A total number of 27 representative sampling sites were selected to investigate the variation of concentrations for PAEs in the Liao River (Fig. 2).The sampling sites covered main stream and tributaries along the upstream, middle, downstream of the

Liao River, and industrial cities adjacent to the river. In July 2014, one liter of water samples were collected from every sampling site and stored in brown glass bottles. All samples were refrigerated at 4ºC before extraction and analysis. The samples were filtered through 0.45 m glass-fiber membrane filter and then passed through activating solid phase extraction cartridges at a flow rate of 10 mL·min^{-1}. The cartridges were then eluted with a 5 mL ethyl acetate followed by a 5 mL methylene chloride and 3 mL ethyl acetate/methylene chloride (1:1 v/v) (USEPA, 1995; MWRPRC, 2007). The eluates were dried using anhydrous sodium sulfate and concentrated to 1 mL by the rotary evaporator and gentle stream of nitrogen. The extracts were stored at 4ºC before GC/MS analysis. The sample extracts were analyzed by an Aglient Gas Chromatograph/Mass Selective Detector (GC/MSD) system (Aglient7890-5975C) with an autosampler under full scanning mode. A mixed standard solution of the four target pollutants was used. The pollutants were separated using a DB-5 silica fused capillary column (length: 30 m, id: 250 μm, Am film thickness: 0.25 μm) with a 1.0 μL injection volume at split radio 10:1. The oven temperature was programmed from 45ºC to 300ºC at 8ºC·min^{-1}, and then kept for 5 min. The ion source and Quadrupole temperatures were 230ºC and 150ºC, respectively. The recovery tests of pollutants were performed using an external standard. Before the detection of each sample, the solvent blank was analyzed. The limits of detection for DEHP, BBP, DEP and DBP were 0.13, 0.14, 0.12 and 0.13 μg/L, respectively. The recoveries of DEHP, BBP, DEP and DBP were 93.5%, 88.4%, 102%, 91.2%. The exposure concentrations were summarized in the Table 2.

2.4 Preliminary ecological risk assessment for four PAEs

The exposure concentrations of four PAEs in Liao River were determined measured, and the ERA for these PAEs in Liao River were conducted by the HQ method (Lemly, 1996). HQ is the ratio of measured exposure concentration divided by a statistically derived effect concentration. Deterministic HQ was calculated by the Eq. (2):

$$HQ = EEC/ALC \tag{2}$$

Where EEC is the environmental exposure concentration.

The hypothesis of this method is that potential hazard is likely to occur at any moment if EEC of a pollutant is higher than its ALC. Otherwise the least possible hazard is anticipated. The mathematical explanations of this method were listed below (Lemly, 1996):

$HQ \leq 0.1$, no risk exists;

$HQ = 0.1 \sim 1.0$, risk is low;

$HQ = 1.1 \sim 10$, risk is moderate;

$HQ \geq 10$, risk is high.

2.5 Probabilistic ecological risk assessment (PERA) of PAEs to aquatic organisms in surface water of Liao River.

PERAs were performed by use of MATLAB2017 software. JPCs were used to describe the estimated risks of PAEs in Liao River. The probability of exceedance estimates are derived from both effect and exposure distributions. When the exposure data were plotted on the same axes as the effects data, the extent of overlap between the curves indicated the probability of exceeding an exposure concentration associated with a particular probability of effects of PAEs. For JPCs of PAEs in Liao River, the x-axis of the JPC represented the intensity of toxicity effects, and the y-axis stand for exceeded probability (Solomon et al., 1996, 2000). Each point on the curves represented both the probability that chosen proportion of species would be affected and the frequency with which that magnitude of the effect would be exceeded. The closer the JPCs were to the axes, the less the probability of adverse effects.

3. Results and discussion

3.1 Derivation of the ALCs for four PAEs based on the reproductive endpoint

In the process of data screening according to the principle (Section 2.1), values of reproductive effect were found to be far lower than those of survival effect often by orders of magnitude (data were not shown). In this study, only toxicity data based on reproductive effect were gathered and a number of 11, 6, 7 and 11 data were collected for DEHP, BBP, DEP and DBP, respectively. The details of collected reproductive toxicity data were listed in Table 1. The 35toxicity data in all for four PAEs were from a total of 24 species including fishes, invertebrates, and alga. A wide variation was found in the NOEC/EC_{50}/EC_{10} values for DEHP with values ranged from 1 to 960 $\mu g \cdot L^{-1}$ and a mean value of 196.5 $\mu g \cdot L^{-1}$. Concentrations for BBP ranged from 60 to 1000 $\mu g \cdot L^{-1}$ with a mean value of 347.4 $\mu g \cdot L^{-1}$. Concentrations for DEP ranged from 427.2 to 21000 $\mu g \cdot L^{-1}$ with a mean value of 6699.6$\mu g \cdot L^{-1}$. Concentrations for DBP ranged from 5 to 30200 $\mu g \cdot L^{-1}$ with a mean value of 3678.4 $\mu g \cdot L^{-1}$. Due to the paucity of the available reproductive toxicity data, non-native species were included. Recent study indicated that there were no statistically significant ($p > 0.05$) differences in criteria and SSD values abstained between aquatic species endemic to China and non-native species (Jin et al., 2015). The most sensitive species to DEHP and DEP were two fishes *Oryzias latipe* and *Danio rerio*, individually. Interestingly, the most sensitive species were two aquatic plants for BBP and DBP, *Pseudokirchneriella subcapitata* and *Lemna minor*. The result was in accordance with the previous study, in which aquatic plants showed greater sensitivities to BBP and DBP than others (Yan et al., 2015). All the reproductive toxicity data of aquatic species were used to generate SSD curves and the derived HC_5 values for the four PAEs were shown in Fig.1. SSDs of DEHP, DBP, BBP and DEP were shifted from left to the right, suggesting that DEHP exhibited the maximum toxicity. Although there were some overlaps of SSDs for DBP and BBP, DBP was more toxic according to the lower part of the SSD curve. ALCs of the four PAEs were calculated through dividing HC_5 by an assessment factor (AF) of 2. The derived ALCs were demonstrated in Table 2. The HC_5 value of DEHP was.0.08 $\mu g \cdot L^{-1}$ and ALC was 0.04 $\mu g \cdot L^{-1}$, which was far below the Chinese national standard of 8 $\mu g \cdot L^{-1}$(PRC-NS, 2002). The ALC of DBP was 0.62 $\mu g \cdot L^{-1}$, which was also lower than Chinese national standard of 3 $\mu g \cdot L^{-1}$ (PRC-NS, 2002). DEP was less toxic than the others and its ALC was 41.9 $\mu g \cdot L^{-1}$, which was also lower than Chinese national standard of 300 $\mu g \cdot L^{-1}$ (PRC-NS, 2006). ALC of BBP was not compared due to lack of its standard. In the whole, ALC derivations of DEHP, DBP and DEP in present study were essential since there were some aquatic organisms under protection according to Chinese current standards.

USEPA recommended short-term and long-term thresholds of 940 μg·L^{-1}and 3 μg·L^{-1} for PAEs (USEPA, 1980) and the criteria of individual PAEs such as DEHP, DBP, and DEP were not developed. Obviously, unified criteria for PAEs were not entirely suitable for individual PAE due to their different toxicities. However, Chinese environmental quality standards for surface water dictated the WQSs of DEHP and DBP were 8 μg·L^{-1} and 3 μg·L^{-1}, respectively (PRC-NS, 2002). China also provided DEP content less than 300 μg·L^{-1} in standards for drinking water quality (PRC-NS, 2006). However, Chinese current standards of DEHP, DBP and DEP were only about four hundreds, five, and eight times compared with their ALCs in our study. Among the four PAEs concerned in this study, DEHP have been studied before and its HC_5 was reported to be 0.68 μg·L^{-1} (Liu et al., 2016), which was 8 folds higher than the derived HC_5 in this study. There were several possible reasons for the discrepancy between the two HC_5 values, including selected species, various endpoints for data analysis and adopted statistical models. Firstly, data of aquatic plant was used in the present study while only data on fish and invertebrate were included in the previous study (Liu et al., 2016). Aquatic plant was considered to be an essential part of the aquatic ecosystem, and species used for ALC generation should come from various phyla and families. Then the ecological risks of PAEs can be evaluated comprehensively. Secondly, the previous research adopted toxicity data of one seawater organism to construct the SSD of DEHP, while only the data of freshwater organisms were used in the present study. Furthermore, fitting parameters varied due to different statistical models. As no statistic model always provides the best fit, log-logistic distribution was selected to draw the fitting curve in this study, while log-normal was used in the former study.

Estrogens are sex hormones with a receptor-mediated mode of action（MOA）at lower concentrations. With the researches going on, more investigations indicated that PAEs mimic estrogenic biological activity (Martino and Chahoud, 2010). PAEs were considered to impede the normal reproductive function via an estrogen receptor-mediated MOA (Takeuchi et al., 2005). The MOAs of PAEs and non-EDC pollutants such as heavy metals are different, so that approaches of ALC derivation are obviously distinct. A variety of methods have been proposed for deriving of ALCs (USEPA, 1980; CCME, 1999; RIVM, 2001). As limited toxicity data are available for a pollutant, AF can be used to derive ALC (CCME, 1999). For example, if only acute data are available, the lowest acute toxicity value applying an AF of 10-1000 is allowed by EU guideline (EC, 2003).Since the reproductive toxicity was more sensitive among the chronic data, HC_5 was only divided by 2 to reduce the uncertainty of ALCs in present study. However, It was possible to cause potential reproductive effect at exposure concentrations much lower than ALC (Lyche et al., 2009), because the concentration of PAEs may be sufficient to induce receptor-mediated effects. This possibility explains why ALC extrapolating acute data using an AF may be not adequately protective of reproductive effects. PAEs were able to induce production of the female-specific, egg-yolk precursor VTG in livers of males and decreased fecundity and fertility (Martino and Chahoud, 2010; Maradonna et al., 2013). Correspondingly, traditional measurement endpoints in ecotoxicology survival, development, and growth were also inappropriate for ALC derivation of EDCs. Instead, nonlethal biomarkers were considered better endpoints in the risk assessment of EDCs (Caldwell et al., 2008; Jin, et al., 2013; Liu et al., 2016). In fact, the endpoints adopted in our study mainly included morphological effect, reproductive effect, population effect, growth effect, hormone effect. Aquatic organisms exhibited greater sensitivity when reproduction related effects were used as the measurement endpoint. Therefore, the ALCs derived based on reproductive toxicity in our study may better protect aquatic organisms from exposure to PAEs.

3.2 Preliminary ecological risk assessment of the four PAEs in Liao River

The measured concentrations of the four PAEs for 27 sampling sites in the Liao River were provided in Table 3. Concentrations for DEHP varied in different sites and ranged from 0.54 to 37.33 $\mu g \cdot L^{-1}$ except two sites undetected. Concentrations for DBP varied in different sites and ranges from 1.43 to 16.58 $\mu g \cdot L^{-1}$. However, concentrations for BBP ranged from 0.15 to 6.55 $\mu g \cdot L^{-1}$ except five sites undetected. Concentrations for DEP varied in different sites and ranged from 0.34 to 1.75 $\mu g \cdot L^{-1}$ except one site undetected. On the whole, the exposure concentrations of DEHP and DBP were relatively higher, which were similar to the results of the nationwide survey in China during 2009-2012 (Liu et al., 2014). In fact, exposure concentration level among various sites reflected regional economic development, levels of urbanization and industrialization. For example, sampling sites near provincial capital Shenyang city had higher concentrations of DEHP, DBP, and BBP. The HQs of the four PAEs in different sites of the Liao River were assessed by comparing exposure concentrations to their ALCs. The information of HQs in different sites were presented in Table 3.

Among the 27 sampling sites in the Liao River, PAEs were not detected in 8 sites and it posed no risk at these sites. The HQ indices of DEP were all below 0.1, representing that DEP posed no risks in all sampling sites. BBP had moderate risks at 2 sampling sites located in the estuary of the Liao River and low risks in other sites. DBP posed high risks in 10 sampling sites and moderate risk in 17 sampling sites. DEHP posed high risks in 25 sites. The HQs of DEHP in most sites were greater than 10, which indicated that DEHP might pose nonnegligible harmful effects on aquatic organisms in Liao River. Table 2 and Table 3 indicated the exposure concentrations of BBP and DEP were relatively lower and their ALC values were higher than DEHP and DBP. Compared with Chinese environmental quality standards (8 $\mu g. L^{-1}$), DEHP concentrations for only 37.04% of the sampling sites exceeded. DBP concentrations measured at 16 sites, about 59.26% of all the sampling sites, exceeded 3 $\mu g \cdot L^{-1}$. Thus, the HQ values adopting Chinese current standards instead of their derived ALCs in this study illustrated that DEHP and DBP in Liao River were at relatively lower risk levels. In summary, the ecological risk of PAEs at some regions was underestimated if adopting Chinese current standards.

3.3 Probabilistic ecological risk assessment of the four PAEs in Liao River

JPCs constructed using exceedance probability function and SSD could better describe the overall PAEs risks than HQ method. The x-axis of the JPC represented the intensity of toxicity effects, and the y-axis standed for exceeded probability. Risks in Liao River by PERA analysis were shown for each PAE (Fig.3). However, DEHP posed higher potential ecological risk than others, followed by BBP, The JPCs of DEP and DBP were closer to the axes, indicating the less probability of adverse effects. Therefore, the results of the JPC analysis indicated that probabilities of exceeding the NOEC for 5% of the species were 60.41%, 0%, 0.12%, 14.28% for DEHP, DEP, BBP and DBP, respectively. Overall, DEHP exhibited higher risks adopting both HQ and PERA methods. In fact, HQ method is point estimate approach, which could not provide detailed information on probability or magnitude of ecological risks and cannot be used to establish a level of risk (Solomon et al., 1996, 2000).Thus, HQ is useful as a screening tool that can help to focus risk assessment. In the present study, the results of HQ approach showed that DEHP exhibited higher risks at 92.6% of sampling sites, and risks posed by DBP were moderate at 63.0 % sampling sites. Thus, predicted large HQs indicated that potential risks for the whole area could not be excluded. However, the higher tier approaches allowed the estimation of the proportional risk of measured PAEs concentrations to fresh organisms in Liao River. This will help the risk manager to make the decisions according to the degree of overlap between the exposure and effects function that is acceptable and the level of certainty required in a particular situation. The probabilities of exceeding the NOEC for 5% of the species were 60.41% for DEHP.

Correspondingly, the appropriate measures were applicated to achieve the required degree of certainty that the desired level of protection would be achieved.

3.4 Uncertainty analysis

Uncertainty in ERA adopting both HQ method and probabilistic risk method is inevitable. The uncertainty came from endpoint chosen as available reproductive toxicity data are far less than the acute data. The minimum toxicity data for developing SSD were considered four (Traas and Bruijn, 2001), five (Hose and Brink, 2004), six (Maltby et al., 2005), eight (Wheeler et al., 2002) or more than ten (EC, 2011). Thus, limited toxicity data used to generate SSD model met the minimum acquirement. Also, the toxicity data for non-native species to derive ALC brought the uncertainty due to the paucity of toxicity data applicable for native species. Limited information on temporal and spatial variation in PAEs exposure concentrations especially in Liao River also introduced uncertainty. Further work should be conducted to get more PAEs exposure data in a wide range of temporal and spatial scales. Thus, more accurate ecological risk assessment of risks will be conducted.

4. Conclusions

In the present study, reproductive endpoint was found to be most sensitive and adopted to derive ALCs of four PAEs. Reproduction toxicity data were screened to construct SSD to calculate ALC. ALCs of DEHP, DBP, BBP and DEP were calculated to be 0.04, 0.62, 4.71 and 41.9 $\mu g.L^{-1}$, which indicated decreased toxicity in sequence. Therefore, their ALCs were far less compared with long-term ALCs from USEPA and Chinese current WQS. The exposure concentrations of PAEs of 27 sampling sites in the Liao River were measured and ERA were conducted with two methods. According to the derived ALCs of the four PAEs, risk assessments by HQ approach showed that DEHP exhibited higher risks at 92.6% of sampling sites, and risks posed by DBP were moderate at 63.0 % sampling sites. However, risks posed by BBP were low at 74.1% of sampling sites, and there were no risks posed by DEP at all sampling sites. Furthermore, the results of PERA in Liao River showed that probabilities of exceeding effects thresholds on 5% of species were 60.41%, 0%, 0.12%, 14.28% for DEHP, DEP, BBP and DBP, respectively. These findings demonstrated that PAEs level in the area of the basin may have done harm to aquatic ecosystem structure and function in Liao River.

Acknowledgement

This research was financially supported by National Major S&T Program for Water Pollution Control and Treatment (2017ZX07301002-01).

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Table 1 Reproductive toxicity data used to construct SSDs of DEHP, BBP, DEP, and DBP

PAE	species	Observed Duration (Days)	Endpoint	Effect	Concentration($\mu g \cdot L^{-1}$)	Exposure Type	Reference
DEHP	*Oryzias latipes*	91	NR	Morphology	1	renewal	Kim et al.,2002
	Danio rerio	21	NOEC	Reproduction	2	renewal	Carnevali et al., 2012
	Gobiocypris rarus	21	NOEC	Morphology	3.6	renewal	Wang et al., 2013
	Salvelinus fontinalis	150	NR	Morphology	3.7	flow through	Mayer et al., 2012
	Hydra viridissima	7	NOEC	Population growth rate	10	renewal	Ganeshakumar et al., 2009
	Pimephales promelas	28	NOEC	Morphology	12	renewal	Crago et al., 2012
	Chironomus riparius	30	NOEC	Reproduction	100	renewal	Kim and Lee,2002
	Eurytemora affinis	21	NOEC	Reproduction	109	renewal	Forgetleray et al., 2005
	Stephanodiscus hantzschii	4	EC_{50}	Growth	320	static	Adema et al., 1981
	Daphnia magna	21	NOEC	Reproduction	640	renewal	Adams and Heidolph，1985
	Pseudokirchneriella subcapitata	5	EC_{50}	Population growth rate	960	static	Richter，1982
BBP,	*Pseudokirchneriella subcapitata*	4	NOEC	Population growth rate	60	static	USEPA,1978
	Pimephales promelas	21	NOEC	Reproduction	64.6	flow through	Hick, 2008
	Fundulus heteroclitus	28	NOEC	Reproduction	100	renewal	Kaplan et al., 2013
	Daphnia magna	21	NOEC	Reproduction	260	flow through	Gledhill et al., 1980
	Navicula pelliculosa	4	EC_{50}	Population growth rate	600	static	Gledhill et al., 1980
	Anacystis aeruginosa	4	EC_{50}	Population growth rate	1000	static	Gledhill et al., 1980
DEP,	*Danio rerio*	3.8	NOEC	Reproduction	427.2	renewal	Xu et al., 2013
	Cyprinus carpio	28	NOEC	Morphology	1000	renewal	Barse et al., 2007
	Chlamydomonas reinhardtii	3	EC10	Population growth rate	1020	static	Brack and Rottler,1994
	Pseudokirchneriella subcapitata	4	NOEC	Population growth rate	3650	static	Adams and Gorsuch ,1995

	Daphnia magna	21	NOEC	Reproduction	3800	renewal	Kühn et al., 1989
	Anodonta cygnea	4	EC_{50}	Population growth rate	16000	static	Adams and Gorsuch ,1995
	Scenedesmus subspicatus	4	EC_{50}	Population growth rate	21000	static	Kuhn et al., 1989
DBP	*Lemna minor*	7	LOEC	Morphology	5	static	Huang et al.，2006
	Melanotaenia fluviatilis	7	NOEC	Reproduction	14	renewal	Bhatia et al.，2014
	Gasterosteus aculeatus	22	NOEC	Hormone effect	15.23	flow through	Aoki et al.，2011
	Oncorhynchus mykiss	99	NOEC	Growth	100	flow through	Rhodes et al.，2010
	Pseudokirchneriella subcapitata	4	NOEC	Population growth rate	210	static	Adams and Gorsuch ,1995
	Glandirana rugosa	21	NOEC	Morphology	278.34	renewal	Ohtani et al.，2000
	Danio rerio	95	NOEC	Population growth rate	400	renewal	Chen et al.，2015
	Scenedesmus subspicatus	3	NOEC	Population growth rate	500	static	Scholz，1995
	Daphnia magna	21	NOEC	Reproduction	960	flow through	Rhodes et al.，2010
	Chlorella vulgaris	4	EC_{50}	Population growth rate	7780	static	Chi et al.，2006
	Scenedesmus acutus	3	NOEC	Population growth rate	30200	static	Kuang et al.，2003

Note: NR stands for not report

Table 2 Parameters of SSDs for four PAEs based on reproduction endpoints

PAEs	N	Mean	Adj-R^2	HC_5 (μg·L^{-1})	ALC (μg·L^{-1})
DEHP	11	196.5	0.951	0.08	0.04
DBP	11	3678.4	0.966	1.23	0.62
BBP	6	347.4	0.931	9.42	4.71
DEP	7	6699.6	0.939	83.7	41.9

Table 3 The exposure concentrations of the four PAEs in the Liao River detected in July 2014 and their HQ values

	PAEs	DEHP		BBP		DEP		DBP	
Number	Sampling site	EEC (μg·L^{-1})	HQ	EEC (μg·L^{-1})	HQ	EEC (μg·L^{-1})	HQ	EEC (μg·L^{-1})	HQ
1	Hun he Bridge	0.900	22.5	ND	-	0.59	0.001	1.56	2.52
2	Lu jia Bridge	0.750	18.8	1.11	0.24	0.75	0.002	2.57	4.15
3	Jiang jun Bridge	24.4	609	1.21	0.26	0.61	0.001	10.1	16.2
4	Da huo fang Reservoir	0.800	20.0	4.34	0.92	0.76	0.002	2.00	3.23
5	Bei dao gou Hun he Bridge	1.44	36.0	2.18	0.46	0.75	0.002	2.01	3.24
6	Liao River estuary	12.9	322	1.27	0.27	1.15	0.003	7.19	11.6
7	Tian zhuang tai Bridge	0.750	18.8	6.54	1.39	0.65	0.002	2.11	3.40
8	Pan jin Bridge	17.9	448	2.13	0.45	0.71	0.002	7.87	12.7
9	Tian hu Bridge	23.2	581	1.12	0.24	0.45	0.001	5.98	9.65
10	He ping Bridge	24.9	622	1.21	0.26	0.56	0.002	11.3	18.2
11	Xiao bei bo Bridge	0.650	16.3	1.31	0.28	0.74	0.002	1.43	2.31
12	San cha he Bridge	0.780	19.5	ND	-	0.73	0.002	2.11	3.40

13	East Wang ben Bridge	20.1	501	ND	-	1.13	0.003	8.48	13.7
14	West Wang ben Bridge	32.8	819	0.960	0.20	1.75	0.005	16.6	26.7
15	Sheng li Bridge	37.3	933	1.05	0.22	0.74	0.002	9.98	16.1
16	Sha keng li	ND	-	0.150	0.03	ND	-	4.31	6.95
17	Zhao jia wo peng	5.39	135	ND	-	0.75	0.002	11.2	18.0
18	Bao li Bridge	18.9	472	6.55	1.39	1.55	0.004	8.45	13.6
19	Duan chuan fang zi Bridge	ND	-	1.12	0.24	0.34	0.001	5.53	8.92
20	Tong jiang kou Bridge	4.99	125	2.29	0.49	0.66	0.002	5.61	9.05
21	Gong zhu tun Bridge	0.540	13.5	1.31	0.28	0.71	0.002	4.58	7.39
22	Xin liu Bridge	0.98	24.5	ND	-	0.45	0.001	1.55	2.50
23	Yu bao tai Bridge	14.58	365	1.32	0.28	0.69	0.002	9.81	15.8
24	Hong miao zi Bridge	0.73	18.3	1.11	0.24	0.75	0.002	2.01	3.24
25	Nan za mu Bridge	1.12	28.0	2.12	0.45	0.63	0.002	1.82	2.94
26	Meng jia wo pu	7.58	190	1.15	0.24	0.42	0.001	2.74	4.42
27	Wei ning Bridge	0.75	18.8	2.21	0.47	0.54	0.001	3.25	5.24

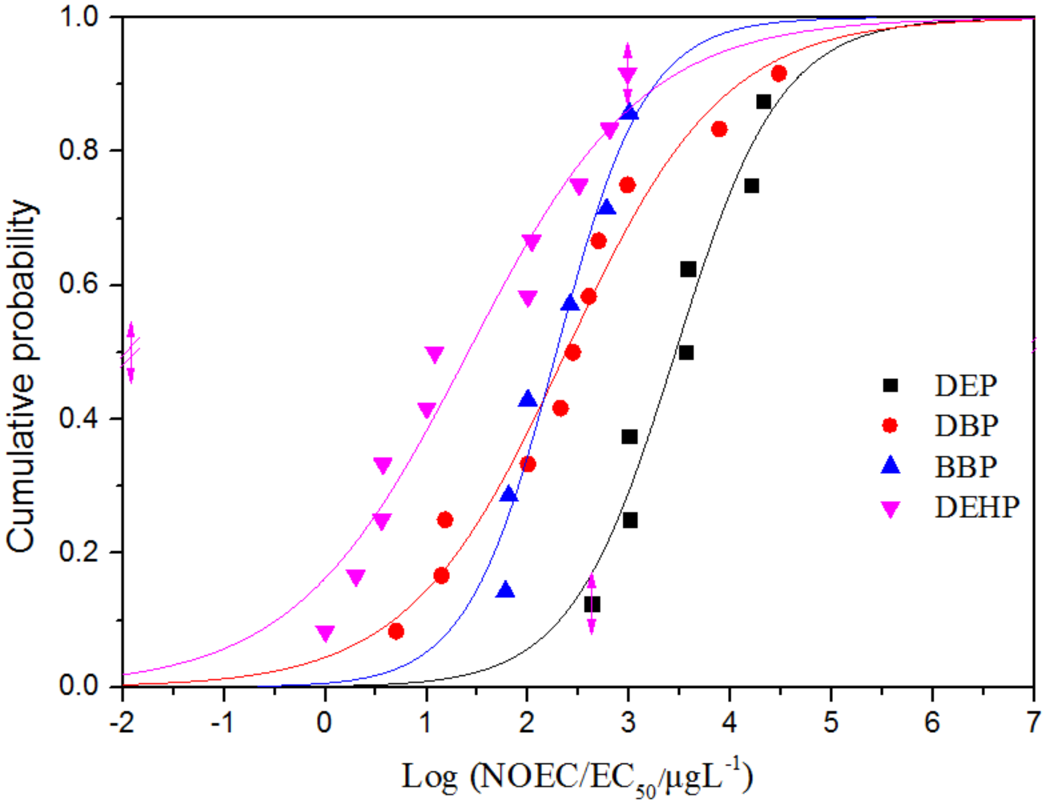

Fig.1 The SSDs for four PAE based on reproductive effects

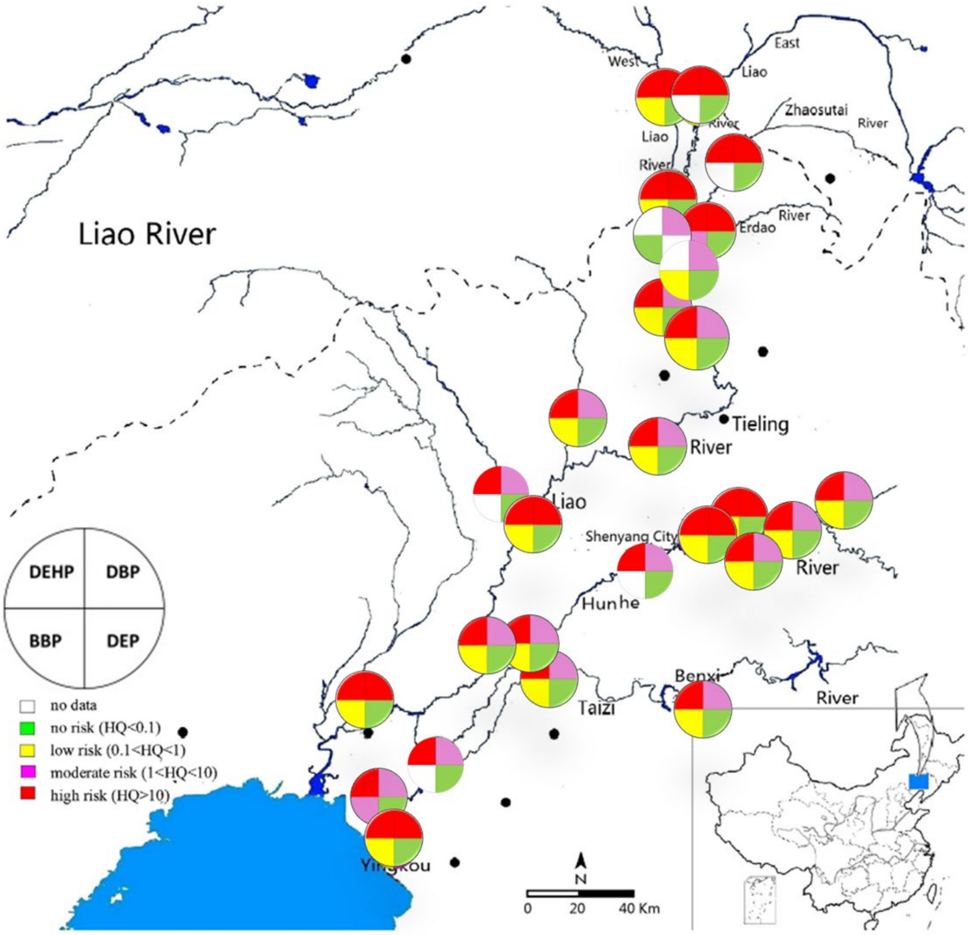

Fig.2 The preliminary ecological risks of four PAEs in Liao River Basin

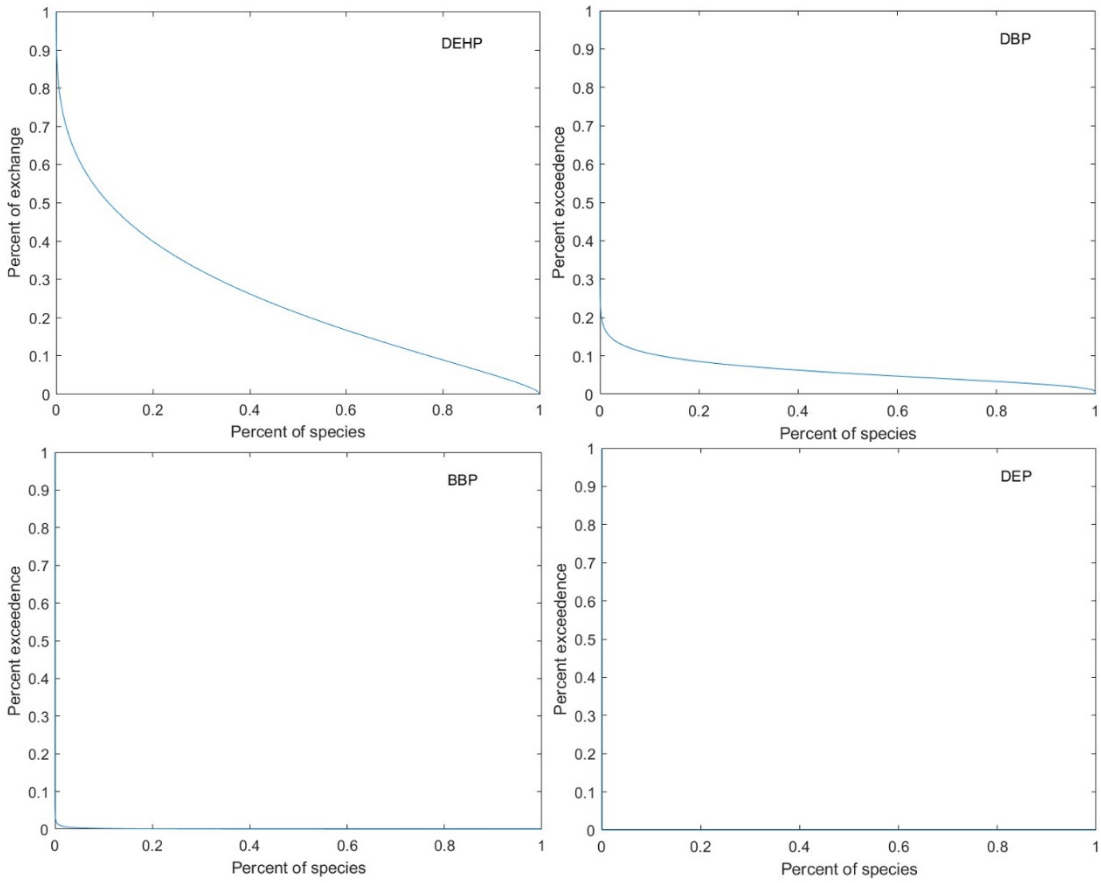

Fig. 3. Joint probability curves for ecological risk of PAEs in Liao River.