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Probabilistic assessment of risks of diethylhexyl phthalate (DEHP) in surface waters of China on reproduction of fish☆

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ABSTRACT

Diethylhexyl phthalate (DEHP) is considered to be an endocrine disruptor, which unlike other chemicals that have either non-specific (e.g., narcotics) or more generalized reactive modes of action, affect the Hypothalamic-pituitary-gonadal (HPG) axis and tend to have specific interactions with particular molecular targets within biochemical pathways. Responding to this challenge, a novel method for deriving predicted no-effect concentration (PNEC) and probabilistic ecological risk assessment (PERAs) for DEHP based on long-term exposure to potentially sensitive species with appropriate apical endpoints was development for protection of Chinese surface waters. PNECs based on potencies to cause lesions in reproductive tissues of fishes, which ranged from 0.04 to 0.20 μg DEHP L^{-1}, were significantly less than those derived based on other endpoints or other taxa, such as invertebrates. An assessment of risks posed by DEHP to aquatic organisms in surface waters of China showed that 88.17% and 78.85% of surface waters in China were predicted to pose risks to reproductive fitness of fishes with thresholds of protection for aquatic organisms based on 5% (HC_5) and 10% (HC_{10}), respectively. Assessment of risks of effects based on effects mediated by the HPG-axis should consider effects on chronic, non-lethal endpoints for specific taxa, especially for reproductive fitness of fishes.

1. Introduction

Diethylhexyl phthalate (DEHP) is one of the most important phthalic acid esters (PAEs). Most of the 1.3 million tons of PAEs produced and consumed in China every year were DEHP and di-n-butyl phthalate (DnBP) (CPCIA, 2009). Because of its versatility, robustness and relatively low cost, DEHP is widely used as an additive to make plastic more flexible and in personal care and medicinal products. However, phthalates are not irreversibly bound to the matrix, so they are easily released once encountered water or organic solvents, and then diffuse into various compartments of the environment. For example, concentrations of DEHP as great as 110 mg L^{-1} have been found in river sediments (Horn et al., 2004). DEHP has been widely detected in Chinese rivers and lakes, including sources of drinking water (He et al., 2013; Li et al., 2015; Shi et al., 2012a, 2012b; Zhang et al., 2015a,b), and therefore concern about DEHP by regulators and the public has been increasingly. DEHP can remain in aquatic ecosystems for relatively long periods of time, and pose risks to aquatic organisms. DEHP causes three primary initiating events in animals (Mathieu-Denoncourt et al., 2015). First, as an endocrine disruptor, DEHP can mimic endogenous estrogen (E2) that can be displaced from carrier proteins and DEHP can bind to the estrogen receptor (ER) where it acts as a weak agonist to activate the ER (Mankidy et al., 2013; Xi et al., 2012). Second, DEHP can affect development of aquatic organisms by disrupting functions of thyroid hormone (TH) and growth hormone (GH), which can increase time to hatching and metamorphosis, histological changes in testes or ovaries (Liu

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et al., 2009). Last, DEHP can alter expression of peroxisome proliferator-activated receptors (PPARs) in mammals, increasing fatty acid oxidation and reducing the animal's ability to cope with the high level of reactive oxygen species (ROS), increasing the occurrences of malformation (Mathieu-Denoncourt et al., 2015). Due to potential for toxicity of DEHP to aquatic organisms, assessing risk of DEHP is crucial to protection of aquatic organisms in surface waters of China.

Despite data on effects of DEHP available for fish, *Daphnia* and algae, no predicted no-effect concentration (PNEC) had been derived for DEHP and its risks to aquatic organisms was thus still unclear, especially in surface waters of China. An important step in assessment of risks of chemicals is determination of PNEC or the maximum concentration at which structure and function of ecosystems are protected. Risks of DEHP to aquatic organisms have been conducted by use of deterministic methods, such as the Hazard Quotient (HQ) (Hu et al., 2012; Luo et al., 2011; Zhang et al., 2015a). However, these assessments have primarily focused on acute lethality of aquatic organisms and derived a toxicity threshold based on assessment factors to correct for uncertainties. DEHP, unlike other chemicals that have either non-specific (narcotics) or more generalized reactive modes of action, is a Hypothalamic-pituitary-gonadal (HPG)-active compound that tends to have more specific interactions with particular molecular targets (Jana et al., 2014). While steroid hormones including estrogen and androgens are core components of the HPG-axis, this system also includes a larger group of tissues and biochemical pathways, which in vertebrates, govern sexual development, maturation, and reproduction (Magdouli et al., 2013). There are a number of consequences arising from this specificity. One important consequence of specificity of xenobiotics, is how criteria are derived when chemicals exceed minimal acute toxicity (Zhang et al., 2015b), but mostly cause longer-term, sub-lethal effects (Ye et al., 2014). In comparison to assessment of risks due to lethality, for those chemicals causing adverse effects on reproduction due to modulation of endocrine function, to be protective of ecosystem structure and function, lesser PNECs, based on sub-lethal effects of reproduction are appropriate (Jin et al., 2014). Second, specificity of molecular targets can also affect specific taxa that are especially sensitive to the chemical MOA of concern. While some biological pathways, such as energy metabolism, tend to be conserved among taxa, others can be quite specific to certain phylogenetic groups. Although control of reproduction through the HPG axis is conserved among classes of vertebrates, taxonomic groups such as invertebrates have different endocrine systems that function differently from those of vertebrates (Caldwell et al., 2008, 2012). As a result, it is likely that data on chronic toxicity for fishes would be the most appropriate for deriving the PNEC for DEHP. Also, it would be unlikely that toxicological data for invertebrate species would drive criteria for these chemicals. Third, assessment factors (AFs) are recognized as a conservative approach for dealing with uncertainty in assessing risks posed by chemicals (Chapman et al., 1998). However, current uses of AFs are based on policy rather than on empirical results, and thus result in values that are protective, but not predictive of effects. These methods are also limited because AFs are somewhat arbitrary and uncertainty of the PNEC is generally not quantified (Chapman et al., 1998). Last, although it is simple, the HQ approach is only appropriate for conservative screening-level risk assessment and for the early stages or tiers of risk assessment. Because risk represents a likelihood or probability of occurrence, it cannot be established from point estimates such as the HQ (Mebane, 2010).

Responding to this challenge, a new method for deriving PNECs and probabilistic ecological risk assessment (PERAs) for DEHP was developed based on long-term exposure to potentially sensitive species with appropriate endpoints. This assessment applied a multi-parametric approach including fitness traits such as survival, growth/development, reproduction, biochemical and molecular biology known to be relevant for health of ecosystems and taxa of aquatic organisms. Probabilistic assessments are considered an improvement on the HQ approach and, thus, recommended for higher tiers of ecological risk assessments (ERA) (Solomon et al., 1996, 2000). Because PERAs can better describe the likelihood of exceeding thresholds for effects and describe risks of adverse effects, this approach has been adopted by a number of researchers (Giesy et al., 1999; Jin et al., 2012a, 2014; Qiao et al., 2007; Zeng et al., 2013). As a higher-tier assessment for a more accurate estimation of ecological risk, the Joint Probability Curve (JPC) was used to describe the likelihood of exceeding the effect thresholds based on different endpoints and the risk of adverse effects for Chinese surface waters.

2. Materials and methods

2.1. Collection of data and generation of SSD

Data for toxic potencies of DEHP were collected from existing toxicity databases (e.g. ECOTOX Database, http://cfpub.epa.gov/ecotox/), published in the literature, and government documents following principles of accuracy, relevance and reliability (Caldwell et al., 2008; Klimisch et al., 1997). For acute toxicity data, selected measurement endpoints were median lethal concentration (LC50) or median effect concentration (EC50) based on immobility for animals and biomass or growth for plants. For chronic toxicity data, no observed effect concentrations (NOECs) were calculated based on values available in the literature. When a NOEC was not available, maximum acceptable toxicant concentration (MATC) or lowest observed effect concentration (LOEC) or ECx values were used. Toxicity data for effects of DEHP on aquatic organisms were divided into four categories of measurement endpoints as follows: survival, growth/development, reproduction and other nontraditional endpoints, e.g. biochemistry and molecular biology (Jin et al., 2014). Effects on fecundity, rate of fertilization, hatchability, expression of vitellogenin in blood plasma (VTG), gonad somatic index, gonadal histology and multiple generation effect to aquatic organisms were classified as effects on reproduction. When the range of values for a taxon was 10-fold or more, or toxicity data of a taxon exhibited greater variability than that for other species, it was eliminated as a outlier (Feng et al., 2015). In the case of multiple values for the same end point and species, the geometric mean was calculated. Toxicity data used for the Species Sensitivity Distributions (SSDs) are reported in the Supporting Information (Table S2).

The SSDs approach is based on the assumption that the toxicity data obtained is a sub-sample of a much larger dataset, and single-species data for many species are fit to a distribution such as the log-normal or log-logistic. In this study, a log-normal distribution model was fitted to different endpoint data points for DEHP, and the fit of the model was evaluated using the Anderson-Darling test. HC_5 (hazardous concentration for 5% species affected) values with 50% confidence were then derived by the ETX 2.0, RIVM software packages. The final PNECs were calculated as the derived HC_5 divided by a factor 1–5, which was a qualitatively chosen factor depending on the amount of supporting evidence, such as non-native species data, multispecies data present, and field data etc (Jin et al., 2014).

2.2. Concentrations of DEHP in surface waters of China

To assess the overall status of DEHP research in aquatic environments of China, data on exposure to DEHP, expressed as concentrations in surface waters including rivers, lakes, reservoirs and urban rivers were collected from literature published in China and abroad during the last 10 years, with master's theses and doctoral dissertations included. Given the large number of studies in the literature, the mean concentration for a location was calculated. For statistical analyses, values that were less than the method detection limits (MDL) were replaced with a surrogate value equal to half the MDL. All investigated sites were classified into the seven main watersheds of China (Yangtze River, Yellow River, Hai River, Huai River, Songhua River, Liao River and Pearl River) those flowing through 31 provinces, autonomous regions and municipalities, and studies that failed to report details of occurrence data and/or geographical information were excluded. Distributions of concentrations of DEHP in surface waters of China determined in this study were tested for normality by use of the Kolmogorov–Smirnov test in SPSS Version 22 software (SPSS Inc., Chicago, Illinois). The statistical summary of the exposure distributions are reported in the Supporting Information (Table S1).

2.3. Assessment of risk of DEHP to aquatic organisms in surface waters of China

The probabilistic ecological risk assessment (PERA) was performed by using the Probabilistic Risk Assessment Tool (PRAT) (Solomon et al., 1996, 2000). In PERA, estimation of risk is described as being proportional to the degree of overlap of the distributions, and one method of displaying risk is through the use of joint probability distributions (JPCs), which describes the probability of a particular set of exposure conditions occurring relative to the number of taxa that would be affected (Giesy et al., 1999; Jin et al., 2012a,b; Jin et al., 2014). The x-axis of the JPC represents the intensity of effects, while the y-axis represents their probability. Each point on the curve represents both the probability that the chosen proportion of species will be affected and the frequency with which that magnitude of effect would be exceeded. The closer the JPC is to the axes, the less the probability of adverse effects (Solomon et al., 2000). Concentrations of DEHP in Chinese surface waters and data on sensitivities of aquatic species to DEHP were compiled and transformed to *probits* respectively by fitting appropriate distributions (Shi et al., 2014; Wang et al., 2014). The *probits* were then plotted as a function of concentration. The linear regression of the two data sets can then be used to calculate the probabilities of concentrations causing adverse effects in a specified percentage of species. For comparison, the HQ approach was also used to assess ecological risk of each site of water. HQs were calculated as the quotient of the measured environmental concentration (MEC) from reports and the PNECs based on fish reproductive toxicity effects as well as all test species reproductive toxicity data.

3. Results and discussion

3.1. PNEC of DEHP based on various measurement endpoints

Toxicity data used to generate SSDs are reported in the Supporting Information (Table S2). A total of 18 toxicity values based on lethality and behavioral effects for aquatic species were collected for DEHP, including 8 fishes and 10 invertebrates. Concentrations for mortality ranged from 60 to 303,974 μg L^{-1}, with a median of 39,126 μg DEHP L^{-1}. Nineteen chronic NOEC values, based on the effect on development and growth, with 9 data points for fishes, 5 for invertebrates, and 5 for planktonic alga, respectively. The range of potencies for effects on growth was from 21 to 2000 μg L^{-1}, with a median of 468.43 μg DEHP L^{-1}. Nine results based on *reproduction* including fecundity, rate of fertilization, hatchability and effects on multiple generations, included 5 fishes, 4 invertebrates. The NOECs (or EC_x) ranged from 1 to 560 μgL^{-1}, with a median of 129.63 μg DEHP L^{-1}. In addition, a total of 11 toxicity data were collected based on *biochemical & molecular biology* endpoints, with concentrations ranging from 10.0 to 300 μg L^{-1}, with a median of 207.40 μg DEHP L^{-1}. Parameters describing species sensitivity distributions for DEHP based on various endpoints are shown in Table 1.

Toxicity data for DEHP based on various endpoints were investigated by use of the Anderson-Darling test ($p < 0.05$) to determine if they met the assumption log-normally for application of parametric statistics. Derived HC_5s for each measurement endpoint ranged from 0.20 to 58.51 μg L^{-1} (Table 1). The final PNECs were calculated as the derived HC_5 divided by an application (uncertainty) factor 1–5, which was a qualitatively chosen factor depending on the amount of supporting evidence. PNECs derived based on survival and growth/development ranged from 11.70 to 58.51 and 7.35 to 36.76 μg L^{-1} respectively, which were greater than the current standards of most countries. Guidelines for drinking-water quality (WHO, 2004) given by the World Health Organization (WHO) and Chinese Sanitary standard for drinking water (CNSMC, 2006) dictate the WQS of DEHP was 8 μg L^{-1}, while US EPA stipulates that concentrations of DEHP must be less than 6 μg L^{-1} in surface waters (EPA, 2009). However, the PNEC derived based on reproductive toxicity using a factor of 1–5 was less by a factor of 10–50 than the US EPA standard of 6 μg/L, which ranged from 0.14 to 0.68 μg L^{-1} (Fig. 1). The SSDs for DEHP based on various endpoints demonstrated that effects on reproduction occurred at lesser concentrations than those based on lethality, growth or biochemical & molecular biology (Fig. 1), and were significantly different

Table 1
Parameters of species sensitivity distributions (SSDs) for DEHP based on different endpoints[a].

Endpoint	N	Mean	SD	A-D test for normality	HC_5 with 50% CI (μg L^{-1})	PNEC (μg L^{-1})
Survival	18	39,126	76,811	0.3030	58.51 (8.00–221.64)	11.70–58.51
Growth/Development	19	468.43	486.74	0.5719	36.76 (15.01–67.45)	7.35–36.76
Biochemical & Molecular biology	11	207.40	302.01	0.4731	1.52 (0.15–5.91)	0.30–1.52
Reproduction (All)	9	129.63	192.26	0.2560	0.68 (0.04–3.25)	0.14–0.68
Fish Reproduction	5	68.34	140.74	0.4324	0.20 (0.001–1.59)	0.04–0.20
Invertebrate Reproduction	4	206.25	240.98	0.3086	9.33 (0.10–41.13)	1.87–9.33

Note: [a] N refers to number. Mean refers to arithmetic mean. SD refers to standard deviation. HC_5 refers to hazardous concentration for 5% of species affected. PNEC refers to predicted no-effect concentration.

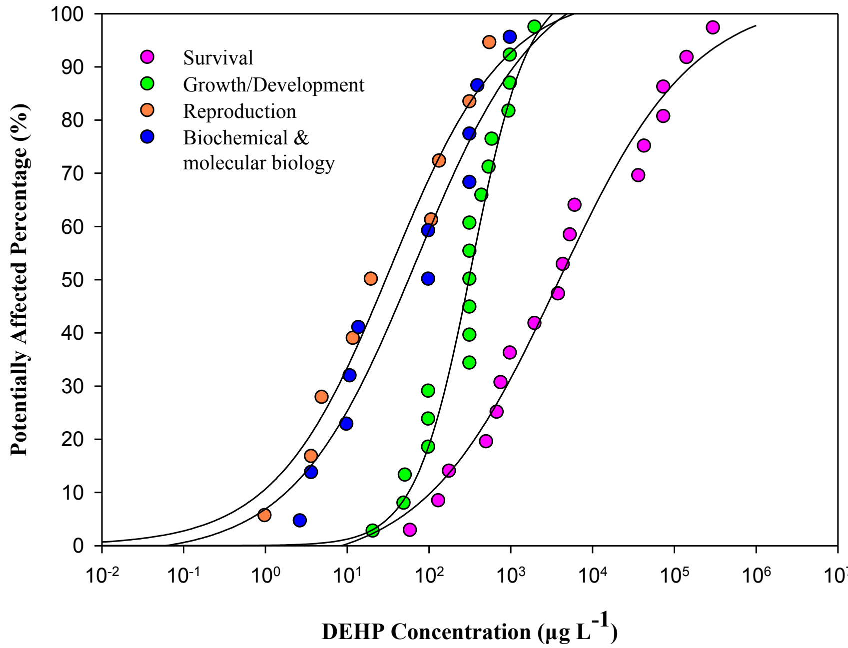

Fig. 1. Species sensitivity distributions (SSDs) for DEHP based on different measured endpoints.

from data survival and growth ($p < 0.05$). PNECs based on reproductive fitness occurred at lesser concentrations than other test endpoints.

SSDs for DEHP, based on various endpoints, demonstrated that reproductive fitness was a more sensitive endpoint than *growth/development* and *survival*. Measurement endpoints of *biochemical & molecular biology* had a similar sensitivity to that of reproduction, but this endpoint was characterized by some "false positives" where the apical endpoint was not affected even though there was an effect on the biomarker. In this case the effects on biomarkers cannot be used to estimate water ecological risk (Hartung, 2009). This conclusion was consistent with the results of the previous investigations, which had shown that DEHP was estrogenic and affected reproduction of vertebrates (Mathieu-Denoncourt et al., 2015). For the effect intensity of the peroxisome proliferator-activated receptors (PPARs) what was believed to be the main mechanism of action behind reproductive organ toxicity (Hurst and Waxman, 2003; Latini et al., 2007), oxidative stress (Lee et al., 2007) and mortality (Abbott et al., 2007) depends on length of alkyl chain, DEHP exhibited lesser carcinogenic and mutagenic effects, but a greater sensitivity for effects on reproduction compared with other PAEs (Mathieu-Denoncourt et al., 2015). The European Commission (EC) Cosmetics Directive banns chemicals in cosmetics classified as carcinogenic, mutagenic, or repro-toxic, and DEHP was classified as a category 2 reproductive toxicant (Commission, 2003). Combined with studies of ecological criteria deriving for NP (Jin et al., 2014) and PNEC for EE2 based on reproductive toxicity (Caldwell et al., 2008), the PNEC (0.14–0.68 µg L^{-1}) derived based on reproductive toxicity in the assessment, results of which are presented here could better protect aquatic life from the exposure of DEHP.

A potential concern about protectiveness of using PNEC based on reproduction of all species was consistent with the steep dose-response for fishes (Caldwell et al., 2012). In the present assessment, SSDs were derived based on NOECs for reproduction of fishes and invertebrates, respectively. This approach further reduced uncertainty by considering data only from fish which are most sensitive to DEHP. In the present assessment, NOECs from four invertebrate species and five fishes were well-described by a log-normal distribution (Table 1). NOECs based on reproduction of invertebrates ranged from 20 to 560 µg L^{-1}, while NOECs for five fishes ranging from 1 to 320 µg L^{-1} (Table S2). Although there is some overlap of NOECs for DEHP derived from SSDs for invertebrates and fishes, in general fish were more sensitive (Fig. 2). HC_5 derived based on reproduction of fishes was 0.2 µg L^{-1}, while the HC_5 based on toxicological data for reproduction of invertebrates was 9.3 µg L^{-1}. That is because reproduction of fishes is regulated by the Hypothalamic-pituitary-gonadal (HPG) axis that was more sensitive to DEHP due to specificity of the molecular target. But invertebrates have different endocrine systems that function differently (Ankley et al., 2005) and are less sensitive than the HPG axis to effects of DEHP. As a result, it is likely that the PNEC derived based on reproduction of fishes would also protect less sensitive aquatic organisms.

3.2. Probabilistic assessment of risks posed by DEHP

Concentrations of DEHP for 31 surface waters from the seven main watershed of China were collected from 20 reports published between 2006 and 2015. Mean concentrations for the various sites ranged from 0.01 to 2634.41 µg L^{-1} (Table S1), which was determined by the Kolmogorov-Smirnov test to follow a log-normal distribution with a median of 1.1 µg L^{-1}. Concentrations varied among uses of surface waters. Concentrations of DEHP in sources of drinking water ranged from 0.09 to 0.35 µg L^{-1}, which were lesser than the Chinese national standard of 8 µg L^{-1} (CNSMC, 2006). However, urban waters in some industrial cities were more contaminated. For example, concentrations of DEHP in Xuanwu lake and urban rivers of Anshan were as great as 1.3×10^3 (Shen et al., 2010) and 1.4×10^3 µg L^{-1} (Yao et al., 2011), respectively. For the different watersheds and drainage basins, DEHP was the most frequently detected in the Yangtze River with values reported for 23 sites in 9 reports for the MDL ranged from 0.0005 to 0.01 µg L^{-1}, and 17.4% of concentrations more than 2 µg L^{-1}.

Assessment of risk of DEHP to affect aquatic organisms was achieved by applying a tiered approach consisting of simple deterministic methods to probabilistic methods for

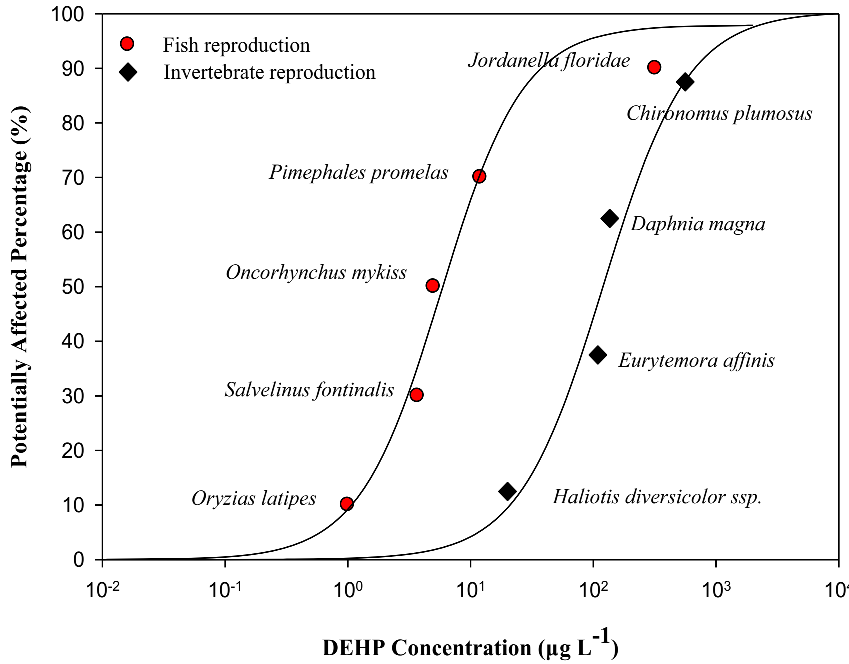

Fig. 2. Species sensitivity distributions (SSDs) for DEHP based on fish reproduction and invertebrate reproduction.

characterization of risk, including concentrations of DEHP from nationwide surface water investigate and toxicity data for different endpoints from previously published reports. HQs of DEHP in surface waters of China were assessed by comparing PNECs based on three reproductive effects to mean concentrations in each water body (Table S1). When PNECs were calculated as HC_5, 69.2% of locations sampled in Chinese surface waters had HQs for DEHP that exceeded 1.0 based on reproductive toxicity to all aquatic organisms, while the proportion of 87.2% and 28.2% greater than 1.0, based on reproduction of fishes and invertebrates, respectively. The result indicated that DEHP posed a risk of reproductive system damage to fish in most surface waters in China, but the risk based on reproduction of invertebrates was less except for two more contaminated urban waters.

JPCs resulting from direct comparison of exceedance probability function (EXF, calculated as 100−cumulative distribution function (CDF)) and SSD offer a representation of overall risk (Fig. 3). The x-axis of the JPC represents intensities of effects, while the y-axis represents their probability of occurring at the observed concentrations. Each point on the curve represents both the probability that the chosen proportion of species will be affected and the frequency with which that magnitude of effect would be exceeded. The closer the JPC is to the axes, the less the probability of adverse effects (Solomon et al., 2000).

Reproduction was the most sensitive endpoint to DEHP, especially for reproductive toxicity of fishes (Fig. 3). Probabilities of exceeding NOEC based on reproductive toxicity for 5% and 10% of the species were 72.22% and 60.05%, respectively, while probabilities of exceeding the NOEC based effects on reproduction of 5% and 10% of fishes were 88.17% and 78.85%, respectively. Because of the specific mechanism of action (MOA) of DEHP, reproduction of invertebrates was less affected than were effects on biochemical and molecular biomarkers. Sensitivities of aquatic species to DEHP, based on survival and growth, were less and probabilities of exceeding thresholds for effects on 5% of species were 16.08% and 17.91%, respectively. In most surface waters of China, including some sources of drinking water, DEHP posed a risk on aquatic ecosystems, especially on reproduction of fishes. Accordingly, use of DEHP might affect the reproductive health of aquatic organisms, and corresponding measures should be taken to minimize ecological risk posed by DEHP.

3.3. Uncertainty analysis

Due to the limited information available for effects of DEHP among species and endpoints some uncertainty in conclusions reached was unavoidable. Another major limitation in the application of a fully probabilistic approach was the fact that concentrations of DEHP in surface waters for China were frequently less than MDL with a detection frequency of 82%. In fact, it was not advisable to use bootstrapping techniques to fill in the censured portion of the frequency distributions when more than 20% of values were non-detected. Additional sources of uncertainty include: the spatial and temporal variability of concentrations of DEHP in a single river or lake water, the ecological relevance of the toxicity data, and model used to assess risk. Some studies indicated that concentrations of DEHP in a single river would change annually as a function of season and runoff. In general greatest concentrations of DEHP occurred during the rainy season, with lesser concentrations during the dry season and least concentrations during the transition seasons (Lu, 2013). Since surveys were conducted from 2006 to 2015 during different seasons, uncertainties were inevitable. Concentrations of DEHP in surface waters varied among regions depending on economic development, urbanization and industrialization. Therefore, nationwide investigations of concentrations of DEHP would be needed to more accurately describe exposes of aquatic organisms to DEHP across China.

Due to durations required for studies to determine effects on reproduction they are limited. Only nine studies of effects of DEHP on reproduction were available for use in this assessment, including five for fishes and four for invertebrates. Considering that OECD (OECD, 1995) requires a minimum of five NOECs for different species for accurate assessment of effects, results for fishes and invertebrates represented only a preliminary, quantitative

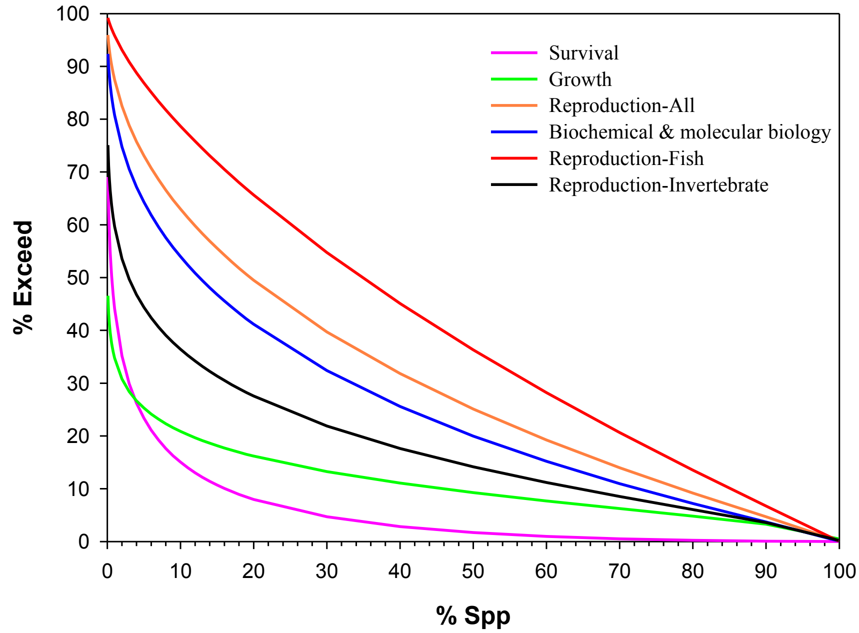

Fig. 3. Joint probability curves (JPCs) for ecological risk of DEHP in surface water from China.

assessment of risks and more data on effects on reproduction among species would be required to allow for conduct of a more accurate ERA. Because of differences among geographical distribution and biological diversity, the potential use of nonnative species in deriving PNEC is controversial (Hose and Van Brink, 2004). When differences in sensitivity between native and nonnative species have been investigated for a few chemicals (Jin et al., 2011, 2012b), there were no statistically significant ($P > 0.05$) differences in criteria and SSDs based on aquatic species endemic to China and non-native species, there is still a desire by regulators to have more data on species native to China. For example, Caldwell et al. (Caldwell et al., 2008) derived a HC_5 of 0.35 ng/L based on effects of EE2 on reproduction of 26 species and recommend as a PNEC in surface water, but Zha et al. (Zha et al., 2008) found the LOEC of EE2 based on reproduction of the Chinese rare minnow (*Gobiocypris rarus*), to be 0.2 ng/L. Therefore, in order to adequately protect native species, an AF of 10 would offer adequate protection to native Chinese species for a 10% uncertainty (Jin et al., 2015).

There are several approaches for deriving SSDs for use in ERAs (Wang et al., 2015). Differences between these approaches lie in the choice of underlying distribution such as the log-normal, log-logistic or Burr Type III (Jin et al., 2011, 2012b). The linearized, log-normal method has become the approach tentatively adopted by most researchers. Besides theses parametric methods, standard non-parametric bootstrap method and bootstrap regression based on a log-logistic regression model were also adopted by some researchers. The results of risk assessments have some uncertainty due to stochasticity of the data as well as systematic errors introduced by processes and models chosen. However, there is no consensus on which methods are most appropriate for assessing risks. Results of the present assessment showed that the choice of toxicity data affects HC_5 more than the statistical method used to extract threshold values from the data (Wheeler et al., 2002).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.03.005.

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