Predicted No-Effect Concentration and Risk Assessment for 17-[Beta]-Estradiol in Waters of China

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Contents

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D.M. Whitacre (ed.), *Reviews of Environmental Contamination and Toxicology Volume 228*, Reviews of Environmental Contamination and Toxicology 228,
DOI 10.1007/978-3-319-01619-1_2,

1 Introduction

Endocrine-disrupting chemicals (EDCs) comprise a group of environmental contaminants that can disturb the normal functioning of the hormonal system, can cause adverse effects in wildlife and humans (Jobling et al. 2003; Arcand-Hoy and Benson 1998), and have garnered increasing concern in recent decades (Jobling and Sumpter 1993; Desbrow et al. 1998; Heberer 2002; Silva et al. 2002; Ying et al. 2008, 2009, 2002a, b). EDCs can act as mimics of natural hormones, agonists or antagonists of hormone receptors, and may cause indirect effects by modulating certain processes (e.g., synthesis, transport, metabolism) that disrupt endocrine function (Klaassen and Admur 2001).

One class of EDC chemicals that has received a great deal of attention is those that bind to estrogen receptors (ERs), stimulating the ER-dependent response, or influencing expression of estrogen-responsive genes (Byford et al. 2002; Okubo et al. 2001). These substances are classified as estrogenic chemicals. They include some synthetic estrogens, such as 17-[alpha]-ethinylestradiol (EE2), diethylstilbestrol (DES), natural estrogens, such as estrone (E1), 17-[beta]-estradiol (E2), and some xenoestrogens, such as 4-tert-octylphenol (4-t-OP), 4-nonylphenol (4-NP), bisphenol-A (BPA), and phthalic acid esters (PAEs) (Zhao et al. 2011a).

E2, which plays an essential role in the reproductive physiology of females (Choudhry and Chaudry 2008), is released into the aquatic environment of several countries mainly from livestock, sewage treatment plant (STP), and industrial effluents (Kang et al. 2002). E2 also occasionally exists in some rivers (Furuichi et al. 2004; Ping 2011; Zhao et al. 2009), streams (Kolpin et al. 2002), and coastal water ecosystems (Hashimoto et al. 2005; Pojana et al. 2007) at ng/L levels, and has been detected in some sediments at ng/g levels (Hashimoto et al. 2005; Labadie and Hill 2007; Schlenk et al. 2005; Wang et al. 2011). E2 as a potent endocrine-disrupting compound, binds to organs, activates a hormone response at relatively small concentrations (Melnick 1999), and disrupts normal sex differentiation and gametogenesis (Thompson et al. 2009). Multiple potential endocrine-disrupting effects of E2 have been observed in aquatic species; such effects include reduced egg production, altered sexual characteristics, and aberrant expression of mRNA for vitellogenin (Vtg), estrogen (ER), and androgen (AR) receptor isoforms (Jobling et al. 1998).

Derivation of a predicted no-effect concentration (PNEC) is a key step in assessing the ecological risk of chemicals. In recent decades, the approach for deriving PNECs for a limited number of chemicals has been well defined. However, deriving PNECs for EDCs in the aquatic environment is a more recent challenge that is not as well developed. In 2008, a draft document entitled “aquatic life criteria for contaminants of emerging concern” was submitted by the American OW/ORD Emerging Contaminants Workgroup; this group proposed a PNEC for EE2 in this report (Caldwell et al. 2008). In 2012, Caldwell and his colleagues, after reviewing the reproductive effect of E2 on fishes, derived a PNEC for E2 by using the species sensitivity distribution (SSD) methodology. The Caldwell group constructed an

SSD curve based on 21 NOEC values (from 21 studies), all of which relied on reproductive endpoints. In these studies, reproductive effects in nine fish species, which had been exposed to E2, were investigated (Caldwell et al. 2012). Moreover, in China, PNECs for chlorophenols have been developed by using the assessment factor (AF) and SSD methods (Jin et al. 2011a, b). Because little other data on PNECs for EDCs was found in the literature, we undertook this review to derive PNEC values for selected EDCs.

EDCs are toxic to reproductive and endocrine regulator systems and act via a special toxic mechanism. Although they are unlikely to cause lethality at concentrations observed in the environment, some EDCs (e.g., E2 and EE2) can cause adverse effects on the reproductive and endocrine systems of aquatic organisms, even at relatively small concentrations. Therefore, the procedure for deriving PNECs for the EDCs might benefit from considering parameters or endpoints that were different from certain other organic chemicals. Traditionally, both long-term protection PNEC values, called the "criterion continuous concentration" (CCC), and short-term protection values, called the "criterion maximum concentration" (CMC), have been developed for certain environmental contaminants (USEPA 1998). Deriving an acceptable PNEC values for EDCs requires focus on the critical endpoints (viz., reproductive effects and endocrine-disrupting effects), rather than on endpoints that have lower sensitivity, such as lethality.

In the present study, we chose E2 as a representative estrogen-like substance on which to derive PNECs by using the SSD approach. We constructed the SSD function from information available on the aquatic toxic effects of E2. We reviewed 31 NOECs (No observed effect concentrations) that were based on reproductive endpoints for different species (representing amphibians, crustaceans, rotifers, fishes, and algae). In addition, the reproductive effects of multigenerational exposures to E2 were analyzed to compare responses of the F_0 and F_1 generations. We also briefly evaluated the occurrence of E2 residues in surface water, sediment, and STP effluents, and assessed the potential estrogenic risk for E2, in the context of the PNEC for E2.

2 Review of E2 Toxicity to Aquatic Organisms

In previous studies, multiple biological effects resulting from exposure to E2 were described. The endpoints affected by E2 included mortality, growth rates, sexual maturity, and expression of mRNA for Vtg, ER, AR, metallothionein (MT), and cytochrome P4501A (CYP1A).

Robinson et al. (2007) exposed a marine fish species, sand goby (*Pomatoschistus minutus*), to E2 for 8-months, and traced the effects of E2 on mortality, growth rates, sexual maturation, hepatic VTG mRNA expression, and reproductive success. Results were that an exposure level of 97 ng E2/L significantly inhibited male sexual maturation, reduced egg fertility, induced male VTG mRNA expression, and delayed spawning. An exposure level of 669 ng E2/L increased mortality, adversely

affected hematological parameters and impaired reproductive activity, and delayed sexual maturation (Robinson et al. 2007).

Exposure to E2 caused testis–ova in male roche (*Rutilus rutilus*) that lived downstream of a STP (Jobling et al. 1998). Vtg, a precursor of egg yolk normally produced in females, was observed in the blood of male rainbow trout (*Oncorhynchus mykiss*) that were caged in areas downstream of the STP (Harries et al. 1996). VTG induction as an indicator of estrogenic effects differs in sensitivity among studies. For example, VTG induction was observed in male Japanese medaka that were exposed to E2 at 29.3 ng/L, whereas other reproductive effects, such as decreases in fecundity, fertility, and gonadal somatic index, occurred at an exposure of 463 ng E2/L (Kang et al. 2002). In contrast, when sheepshead minnows (*Cyprinodon variegatus*) were exposed to E2, plasma VTG induction was not as sensitive as other reproductive effects (Cripe et al. 2009). Moreover, several studies have demonstrated that VTG induction is reversible, and bears no relationship to long-term effects on health or reproductive performance (Brion et al. 2004; Mills et al. 2003; Nash et al. 2004). It has also been reported that exposure to environmentally relevant concentrations of E2 (<10 ng/L) during early life stages can alter sexual differentiation and fecundity of Japanese medaka (*Oryzias latipes*) (Nimrod and Benson 1998).

E2 inhibited expression of mRNA for CYP1A in juvenile and adult grey mullet (Cionna et al. 2006). Kim et al. (2008) reported that CYP1A was not as strongly expressed in male Japanese medaka liver, after the fish were exposed to E2. Exposure to E2 also reduced the expression of mRNA for CYP1A in cultured rainbow trout hepatocytes, but expression of mRNA for this trait was still up-regulated from exposure to other xenobiotics in the effluents (Navas and Segner 2001). Various studies have shown that E2 (and other estrogenic compounds) are capable of inhibiting the expression of MT in some fishes (Huang et al. 2012; Costa et al. 2010; Gerpe et al. 2000; Olsson et al. 1995).

Expression of mRNA for Vtg after exposure to E2 has been demonstrated to occur in several male fishes, including mosquitofish (*Gambusia holbrooki*), zebrafish (*Danio rerio*), and Japanese medaka (Leusch et al. 2005; Tong et al. 2004; Huang et al. 2012). In teleosts, expression of mRNA for hepatic ERα is significantly up-regulated by E2 exposure (Sabo-Attwood et al. 2004; Esterhuyse et al. 2010). In response to E2 exposure, the auto-regulation of ERα in liver is a common feature of teleosts that has been attributed to transcription of Vtg in the liver of mature females (Pinto et al. 2006). During an 84-day exposure, the NOEC for E2 on mosquitofish (*Gambusia affinis*), was 100 ng/L, based on maturation of the gonopod; however, this value was 20 ng/L, when based on frequency of sexual activity (Doyle and Lim 2002, 2005).

Exposure of Japanese medaka to 463 ng/L E2 for 3 weeks resulted in reduced fecundity and fertility; however, Japanese medaka (*Oryzias latipes*) were unaffected when exposed to 227 ng/L E2. By comparison, treating Japanese medaka with 817 ng E2/L for 2 weeks resulted in production of fewer eggs (Shioda and Wakabayashi 2000). The sexual behavior of male Japanese medaka was suppressed after exposure to 3 and 30 μg E2/g BW(body weight)/day in the diet for 2 weeks (Oshima et al. 2003). The phenotypic sex of Japanese medaka was reversed when eggs were microinjected with 2.1 ng E2 per egg (Edmunds et al. 2000). When

Japanese medaka were exposed to concentrations <2.86 ng E2/L, no effect on sexual differentiation, induction of Vtg, or any other reproductive impairment effect was observed. However, Vtg was induced in male Japanese medaka when they were exposed to a level of 8.94 ng E2/L (Seki et al. 2002, 2005). Alternatively, estrogen-responsive genes were abnormally expressed when Japanese medaka were exposed to 10 ng E2/L (Yamaguchi et al. 2005; Chen et al. 2008). Based on these studies, we suggest that a NOEC of 8.94 ng E2/L for Japanese medaka is appropriate.

When early life stages of the zebrafish (*Danio rerio*) were exposed to 54 ng E2/L, the sex ratio was significantly altered and Vtg was significantly induced (Holbech et al. 2006). Brion et al. (2004) recommended a NOEC ranging from 5 to 25 ng/L, based on gonadal development and induction of Vtg endpoints. The EC_{10} (concentration expected to cause a 10% effect) was 15.4 ng E2/L, based on a logistic regression of Vtg induction (Rose et al. 2002). Van der Ven et al. (2007) exposed adult zebrafish for 21 days to E2 at levels of 27.2, 87, and 272 ng/L, and their offspring were exposed to the same concentration for another 42 days. In males of the parental generation, Vtg production increased significantly at the dose of 87 ng/L. Results for the F1 progeny included decreased survival, increased body length and weight, Vtg-related edema and kidney lesions, and inhibited spermatogenesis at the 272 ng/L exposure level (van der Ven et al. 2007). Based on these results, the NOEC for zebrafish was determined to be 15.4 ng E2/L.

Multiple effects of exposure to E2 on male fathead minnow (*Pimephales promelas*) have been reported (Brian et al. 2007; Parks et al. 1999; Seki et al. 2006). When fathead minnows were exposed to E2 for 19 day, the calculated EC_{10} values, based on egg production, hematocrit of males and females, and plasma alkaline-labile phosphorous, were 6.6, 52.5, 562, 36.3 ng E2/L, respectively. The EC_{50} (concentration expected to cause a 50% effect) for inducing Vtg in males was 251 ng E2/L, whereas no Vtg induction plateau was observed in females (Kramer et al. 1998). The lowest-observed effect concentration (LOEC) of E2, based on induction of Vtg in male fathead minnows, was 28.6 ng E2/L (Seki et al. 2006). From evaluating these results, we suggest a NOEC of 6.6 ng E2/L for fathead minnow, based on reproductive endpoints.

When tadpoles of African clawed frogs (*Xenopus laevis*) were exposed to 27.2 ng E2/L for 4 weeks, slight abnormalities in the histology of the gonad were observed, although the sex of the frogs was not reversed (Kramer et al. 1998). However, when African clawed frogs were exposed to 74 ng E2/L, adverse effects were observed, such as fewer sperm cells, inhibition of meiotic division of germ cells, more lipid droplets (i.e., storage compartments for the sex steroid hormone precursor cholesterol), and lower plasma T concentrations (Hecker et al. 2005). Based on these results, a NOEC of 27.2 ng E2/L is suggested for tadpoles of the African clawed frog.

When juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to E2 for 14 days, a dose of 14 ng E2/L was the threshold for inducing Vtg protein, Vtg mRNA, vitelline envelope protein (VEP)β and VEPγ, whereas, VEPα was induced at a dose of 4.8 ngE2/L (Thomas-Jones et al. 2003). When female rainbow trout were exposed to E2 for 14 days, a dose–response for inducing plasma Vtg was observed; the LOEC for inducing plasma Vtg was 8.9 ng E2/L (Thorpe

et al. 2000). After being exposed to E2 at concentrations of ≥1 ng/L for 35 days, the semen volume obtained per male rainbow trout was significantly reduced, and after 50 days, sperm density and fertility were also reduced (Lahnsteiner et al. 2006). Based on this information, we recommend a NOEC of 1 ng E2/L for rainbow trout.

3 Data Selection, Evaluation, and Analysis

3.1 Data Selection and Evaluation

Information on effects of E2 on freshwater organisms was collected from the ECOTOX database (http://cfpub.epa.gov/ecotox/), individual research papers, and government reports. Toxicity threshold values for E2, expressed as NOEC, were derived for several effect endpoints, including sexual differentiation, gonad development, and sex ratio (Table 1). The accuracy, reliability, and relevance of these data were evaluated by standard methods (Klimisch et al. 1997; Bureau 2003). We classified data from the literature into four levels of reliability (Classes 1–4):

1. Reliable without restrictions
   Data were obtained from studies that were performed according to valid and/or internationally accepted testing guidelines (i.e., adherence to Good Laboratory Practice (GLP) was preferred), or in which test parameters adhered to specific (national) testing guidelines (GLP preferred) or met a comparable standard.

Table 1 List of toxicity study data for 17-[beta]-estradiol (E2) on aquatic species from the literature

Class	Test species	NOEC[a] (ng/L)	Endpoint	Klimisch code	References
Amphibian	*Xenopus laevis*	27.24	Sex differentiation	1	Oka et al. (2006)
Crustacean	*Macrobrachium rosenbergii*	1,000,000	Gonad development	1	Wu et al. (2007)
	Eurytemora affinis	4,000	Reproductive output	1	Forget-Leray et al. (2005)
	Tigriopus japonicus	100	Sex ratio	1	Marcial et al. (2003)
	Neocaridina denticulata	10,000	Egg production	2	Huang et al. (2006)
	Marsupenaeus japonicus	980.64	Vtg induction	1	Yano and Hoshino (2006)
	Daphnia magna	100,000	Sex differentiation	1	Brennan et al. (2006)
Alga	*Melosira varians*	80,000	Enhancing cell growth	1	Julius et al. (2007)

(continued)

Table 1 (continued)

Class	Test species	NOEC[a] (ng/L)	Endpoint	Klimisch code	References
Rotifer	*Brachionus calyciflorus*	10	Mictic rate	1	Ke et al. (2008)
Fish	*Danio rerio*	15.4	Vtg[b]	1	Rose et al. (2002)
	Leuciscus idus	100	Vtg	2	Allner et al. (1999)
	Poecilia reticulata	30	GSI[c]	1	Nielsen and Baatrup (2006)
	Ambystoma macrodactylum	272.4	Day of hatch	1	Ingermann et al. (1999)
	Oncorhynchus tshawytscha	250	Gonadal feminization	1	Nakamura (1984)
	Oncorhynchus KETA	500	Sex differentiation	2	Nakamura (1984)
	Salmo trutta	100	Vtg	1	Sherry et al. (1999)
	Pimephales promelas	6.6	Egg production	1	Kramer et al. (1998)
	Oncorhynchus mykiss	1	Semen volume	1	Lahnsteiner et al. (2006), Routledge et al. (1998)
	Thymallus thymallus	1	Number of males give semen	1	Lahnsteiner et al. (2006)
	Rutilus rutilus	10	Plasma levels of Vtg	1	Routledge et al. (1998)
	Carassius carassius	30	Vtg induction	1	Zhang et al. (2008a)
	Carassius auratus	5	Vtg induction	1	Zhang et al. (2008b)
	Cyprinus carpio	100	Quality of milt	2	Gimeno et al. (1998)
	Gobiocypris rarus	5	Vtg induction	1	Liao et al. (2009)
	Misgurnus anguillicaudatus	500	Vtg	1	Lv et al. (2006)
	Acanthogobius flavimanus	20	UCH[d]-mRNA expression	2	Mochida et al. (2003)
	Gambusia holbrooki	20	Sexual activity	1	Doyle and Lim (2002)
	Tanichthys albonubes	500	Vtg	1	Yang et al. (2011)
	Oryzias javanicus	16	Reproduction	1	Imai et al. (2005)
	Oryzias latipes	2.86	Vtg in male	1	Doyle and Lim (2005)
	Cyprinodon variegatus	40	Reproductive rate	1	Cripe et al. (2009)

[a] *NOEC* no observed effect concentrations
[b] *Vtg* vitellogenin
[c] *GSI* gonadosomal index
[d] *UCH* ubiquitin C-terminal hydrolase

2. Reliable with restrictions
   Data were obtained from studies (most not performed according to GLP), in which the test parameters documented were not wholly consistent with a specific testing guideline. However, the study data were scientifically acceptable and were well documented, even if not performed according to standard guidelines.
3. Not reliable
   Data were obtained from the literature or from reports in which conflicting information existed in the measuring system, or in which organisms/test systems, or exposure routes were nonrelevant, or unacceptable methods or documentation were used.
4. Not assignable
   Data were obtained from studies that did not give sufficient experimental details, or were found only in short abstracts or in secondary literature (books, reviews, etc.).

Only data classified as "1" or "2" were used in constructing the SSD or to determine a PNEC for E_2. If there were more than one datum for multiple reproductive endpoints for a species, the most sensitive value was selected as the final one for that species. If there were more than one datum for the same reproductive endpoint for a species, the geometric mean of the combined values were used as the final toxicity value for that species.

3.2 Derivation of a PNEC

The SSD approach, which represents the variation in sensitivity among species to a contaminant, was used as a statistical extrapolation method to derive a PNEC of E2 in the study. The basic assumption of the SSD approach is that species are randomly selected for analysis and are representative of the entire range of species sensitivities in a given ecosystem. The SSD for a pollutant is constructed from available NOEC values for all species, and then the threshold, which is usually denoted as the HC_5 (pollutant concentration hazardous to 5% of the species), is determined by comparing the pollutant concentration with a predetermined cumulative probability (Van Straalen and van Rijn 1998). The HC_5 value was selected because the concentration of E2 associated with the HC_5 would protect 95% of the species tested (Caldwell et al. 2008). The median value determined by the HC_5 was selected as the PNEC (Europea Commission 1996).

The qualified NOEC values were assigned correlative orders from 1 to N after being ranked, and the cumulative probability for each species is calculated from (1):

$$P = \frac{R}{(N+1)} \tag{1}$$

where: R is the rank of a species in the data series, and N is the total number of examined species (Hall et al. 1998; Schuler et al. 2008).

Given that the SSD method assumes that the selection of the species is random and is representative of the entire range of possible sensitivities among species, the NOEC values (or their logarithmic values) for the examined species should be first checked for normality, and then transformed to approximate a normal probability density function, when necessary (CCME 2007).

Several models are available for fitting distributions of toxicological data, such as the log-normal, log-logistic, Gaussian, and Burr Type III distributions (Wagner and Løkke 1991; Shao 2000). None of these models, however, allow all types of toxicological data to be perfectly fitted. Therefore, parameters (e.g., the adjusted coefficient of determination (r^2) residual sum of squares (RSS) and F value) can be used to compare the suitability of models for a given data set that will determine the optimum model.

4 Selecting NOECs and Establishing PNEC Values

4.1 NOECs of E2 Based on Reproductive Endpoints

Information on E2 reproductive effects of 31 species, which had been evaluated according to the Klimisch Criteria (Table 1), was chosen for constructing a SSD from which the HC_5 was derived. Theoretically, with a total of 31 species the resolution of the SSD would be 3.1%. Since this is less than the 5% chosen to estimate the threshold for effects on species, the HC_5 is an appropriate parametric estimator. The NOEC values for E2 were based on reproductive endpoints, and ranged from 1 ng E2/L (rainbow trout, and grayling, *Thymallus thymallus*) to 1,000,000 ng E2/L (*Decapoda palaemonidae*, *Macrobrachium rosenbergii*). In addition, although 6 NOEC values were selected to represent multigeneration studies, these were insufficient for deriving an HC_5. Therefore, only the first generation (F_0) and the second generation (F_1) results were compared to assess the relative E2 response sensitivities of F_0 and F_1 generations. The two sets of NOECs obtained from F_0 and F_1 generations were both log-normally distributed, so the values were log-transformed (Kolmogorov–Smirnov statistic, F_0: 0.418; F_1: 0.503). Below, we compare the multiple endpoints that existed for some species, and indicate what data were ultimately selected.

Some trans-generational effects were observed in multigenerational exposures to E2. Lifecycle exposure of fish to E2 significantly decreased the production of embryos in the F_1 and F_2 generations; this occurred at concentrations lower than those affecting the F_0 generation, which emphasizes the importance of evaluating estrogenic chemical effects on reproduction through at least two (F_0 and F_1) generations (Cripe et al. 2009). Adverse effects were observed on F_0 individuals, but not F_1 individuals, when they were exposed to comparable concentrations (Marcial et al. 2003; Brennan et al. 2006; Cripe et al. 2009; Ke et al. 2008). A summary of available data on multigenerational toxic effects is given in Table 2. Data were insufficient to meet the requirements for deriving a PNEC. Therefore, when more data become available in the future, E2's potential to produce trans-generational effects should be reevaluated.

Table 2 List of available NOEC values for E2 on aquatic species exposed during the F_0 and F_1 generations

Test species	NOEC for F_0 (ng/L)	Endpoint for F_0	NOEC for F_1 (ng/L)	Endpoint for F_1	Klimisch code	References
Tigriopus japonicus	100	Sex maturity	10	Sex ratio	1	Marcial et al. (2003)
Danio rerio	25	Reproductive performance	100	Sex differentiation	1	Brion et al. (2004)
Oryzias latipes	2.86	Vtg in male	8.66	Vtg in male	1	Seki et al. (2005)
Daphnia magna	400,000	Survival	200,000	Survival	1	Brennan et al. (2006)
Cyprinodon variegatus	40	Reproductive rate	10	Infertile eggs	1	Cripe et al. (2009)
Brachionus calyciflorus	10	Mictic rate	1	Survival	1	Ke et al. (2008)

4.2 PNEC Obtained from SSD

After reviewing, classifying and assessing the data available from various studies, reproductive effects were determined to be the most sensitive and critical assessment endpoints for E2. Subsequently, an SSD was constructed by using NOEC values, based on reproductive endpoints that were reported in 31 studies (Table 1). This information was used to derive a PNEC to protect F_0 individuals from the effects of E2. There are several frequency distributions that could be used to describe the data, and subsequently to interpolate and extrapolate the data. These distributions include the Allometric (Power Law model), Exponential Decay, Gaussian, and Sigmoidal models. Several parameters, such as residual sum of squares and r^2, can be used to judge the goodness of fit of a function to the actual data (Table 3). After all possible functions were evaluated, the logistic function was chosen to construct an SSD for fitting the NOEC values of E2 (Fig. 1). The selection of the logistic function was based on the fact that it resulted in the least residual sum of squares and greatest r^2 for the 31 NOEC values. After the SSD was fitted by the logistic function, an HC_5 value of 1.46 ng E2/L for aquatic organisms was derived. The HC_5 values, derived by using other functions, were similar to those derived from the logistic function. Thus, the choice of a theoretical function did not introduce a significant error into the assessment. The PNEC was generally calculated from $HC_5/2$, which was suggested by Stephan and his colleagues (Stephan et al. 1985). Thus, we recommended a PNEC of 0.73 ng E2/L (a half of HC_5 derived in the study) for protecting aquatic organisms from chronic and full-life cycle exposures to E2.

Moreover, the 6 NOEC values for E2 obtained from multigeneration exposure studies (specifically from F_1 and F_0) were also fitted by the logistic function (Table 4). Although the F_1 generation of zebrafish and Japanese medaka were less

Table 3 PNEC values for 17-[beta]-Estradiol(E_2) as calculated by using different models

Model	Formula	Parameters	R^2	Residual sum of squares	$Y=0.05$	PNEC (ng/L)
Allometric model	$y=ax^b$	$a=0.3262$ $b=0.6942$	0.9011	0.0070	$X=0.0671$	0.58
Exponential Decay model	$y=y_0+Ae^{-x/t}$	$y_0=1.1899$ $A=-1.2488$ $t=2.9318$	0.9623	0.0033	$X=0.2677$	0.93
Gaussian model	$y = y_0 + Ae^{-\frac{(x-x_c)^2}{2w^2}}$	$y_0=-1.3425$ $X_C=4.9122$ $W=4.6579$ $A=2.3104$	0.9767	0.0021	$X=0.2251$	0.84
Sigmoidal model	$y = \frac{A_1 - A_2}{1+(x/x_0)^p} + A_2$	$A_1=0.0461$ $A_2=1.0108$ $X_0=1.7907$ $P=2.3217$	0.9923	0.0006	$X=0.1668$	0.73

PNEC predicted no-effect concentration

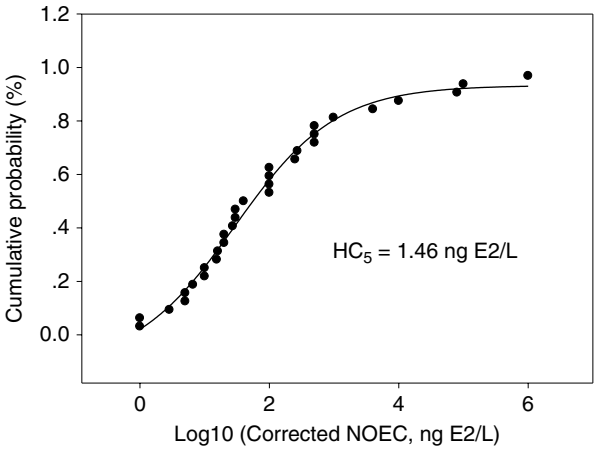

Fig. 1 The distribution of species sensitivity data for the toxicity of E2. Fifth centile of species sensitivity distribution (HC_5, ng E2/L) is presented with two-sided 90% confidence limits that are given in *parenthesis*. The goodness of fit test by using the Anderson–Darling and Kolmogorov–Smirnov test was accepted. *NOEC* no observed effects concentration

sensitive to E2 than was the F_0 generation, we observed a trend that individuals of the F_1 generation were more sensitive than those of the F_0 generation (Fig. 2). The two fitted curves for the F_1 and F_0 generation intersected at $X=2$, $Y=0.714$, and after that they were both stabilized. Therefore, for nearly 71.4% of aquatic species, F_1 individuals were more sensitive than F_0 individuals when exposed to E2 at concentrations less than 100 ng E2/L. Individuals of both the F_0 and F_1 generations would be at reproductive risk when exposed to a concentration greater than 100 ng E2/L. Because these F_1 aquatic organisms were so sensitive to the effects of E2, regulators should consider additional measures or standards to protect them.

Table 4 Comparison of fitted data results for F_0 and F_1 generation organisms

	F_0	F_1
Model	Logistic	
Formula	$y = \frac{A_1 - A_2}{1+(x/x_0)^p} + A_2$	
Parameters	$A_1 = 0.1475$	$A_1 = 0.1384$
	$A_2 = 0.8648$	$A_2 = 0.8578$
	$X_0 = 1.4883$	$X_0 = 1.1328$
	$P = 4.1855$	$P = 2.7759$
Residual sum of squares	0.0009	0.0332
R^2	0.9869	0.7676

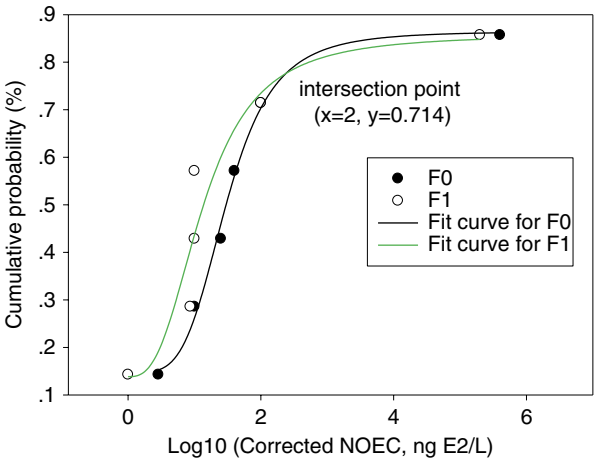

Fig. 2 The distribution of species sensitivity data for E2 to F_0 generation and F_1 generation organisms. The filled and unfilled dots represent the toxicity data of F_0 generation and F_1 generation, respectively. The *black curve* and the *green curve* represent the fitted curve for F_0 and F_1 generation, respectively

5 Discussion

5.1 Reasonableness of PNECs

The SSD method for statistically deriving water quality criteria (WQC) was first proposed to bridge the gap between dose–response data of single-species toxicity and risk assessment for populations, communities, and ecosystems (Kooijman 1987). Thereafter, this method was improved (Aldenberg and Slob 1993; Newman et al. 2000; Wagner and Løkke 1991), and was finally adapted as a standard guideline for ecological risk assessment by the US Environmental Protection Agency (USEPA 1998). The SSD approach assumes that the sensitivity among species can be adequately described by using a specified statistical distribution, such as the normal (Wagner and Løkke 1991; Aldenberg and Jaworska 2000), logistic

(Kooijman 1987; Aldenberg and Slob 1993), triangular (Stephan et al. 1985), or Weibull (Caldwell et al. 2008) probability functions, or by using distribution-free, nonparametric methods (Ling 2004; Newman et al. 2000). The advantage of the SSD approach is that it allows researchers to determine which species are most likely to be affected by an agent by estimating the HC_5. When the HC_5 was compared with other estimates of thresholds and PNEC values, it was found that the HC_5 corresponded to the concentration of chemicals that did not have any statistically significant effects on population or communities.

As an effective method to characterize variation in sensitivity to chemicals among species, the SSD method has been used not only to assess risk or develop WQC for aquatic species (Caldwell et al. 2008; Schuler et al. 2008), but also to confirm quality criteria to protect top predators from residues in soils (Jongbloed et al. 1996; Traas et al. 1996). Because data for toxicity of contaminants to wildlife are generally insufficient for constructing an SSD, using the SSD approach to assess wildlife risks has not been widely accepted. However, SSDs have been constructed for predicting the toxicity threshold value of 23 chemicals to wildlife, by incorporating interspecies toxicity correlation models (Awkerman et al. 2008). A specified effect level, such as the proportion of species expected to respond to a particular exposure for a specific measurement endpoint, can be determined to protect most of species by constructing an SSD curve. In summary, the SSD is considered to be an appropriate approach for deriving a PNEC value for E2.

The accuracy or reasonableness of a PNEC derived from a SSD is likely to depend upon the quantity of data and the particular data selected. In the present study, the Klimisch classification system was applied to evaluate the quality of the data used in to construct the SSD curve. A steady SSD can be constructed at a sample size of 10–15 data points (Wheeler et al. 2002), and an accurate PNEC value can be obtained from a SSD created for 15 or more species (Awkerman et al. 2008). Hence, the quantity of data was unlikely to affect the stabilization of the SSD or accuracy of the PNEC, as long as the quantity of stringent data encompasses at least 15 species. In this study, 31 species from 3 phyla and 8 families met the requirements proposed by USEPA for constructing an SSD. The number and diversity of taxa for which data are available ensure the stabilization of the SSD curve and allowed us to obtain an accurate and reasonable PNEC value. Even if one of the NOEC values is changed, the PNEC value will not be significantly influenced.

Several mathematical models including the Probabilistic, Allometric (Power Law model), Exponential Decay, Gaussian, and Sigmoidal could be used to describe the data and could subsequently be used for interpolation and extrapolation. After evaluating the fitness of all the possible functions by several parameters, such as residual sum of squares, and r^2, the logistic model was determined to be the best one for fitting the 31 NOECs addressed in this paper. The logistic model has been deemed by some authors to be a more appropriate model for deriving the PNEC because of its shape and curvature (Knoben et al. 1998). The advantages of the logistic model are not only its fitting goodness but also its mathematical simplicity. The simple least squares regression can be applied to probit and log-transformed data, and confidence intervals can be calculated from assumptions of the normal distribution (Wheeler et al. 2002).

Toxicity data representing different endpoints exhibit different potencies for E2. For example, when Japanese medaka were exposed to E2 and body length was used as the endpoint, the LOEC value was 1,000 ng E2/L (Metcalfe et al. 2001); when the total number of eggs from F_0 was used as the endpoint, the LOEC value was 463 ng E2/L (Kang et al. 2002); when the sex ratio of F_1 generation was used as the endpoint, the LOEC value was 8.66 ng E2/L (Ke et al. 2008). However, when induction of Vtg was used as the endpoint, the LOEC was 0.94 ng E2/L (Seki et al. 2006). The greatest LOEC value for Japanese medaka responding to E2 was 1,000-fold greater than the least LOEC. Some other effects, such as lethality to crustaceans and fish, growth of amphibian tadpoles, and development of copepod, were reported in the literature, and the NOEC or LOEC values associated with them were generally at mg/L level (Forget-Leray et al. 2005; Hogan et al. 2006; Rang et al. 2003; Hirano et al. 2004; Kashiwada et al. 2002). Thus, it can be concluded that the reproductive endpoint is most sensitive for assessing the effect of E2 on aquatic organisms.

5.2 Comparison to Other PNECs for E2

The HC_5 value derived in the present study was 1.46 ng E2/L, which is close to the HC_5 of 1.5 ng E2/L derived by using the SSD that was constructed from 77 in vivo NOECs by Zhao et al. (2011a). In Zhao's study, the selected NOECs were based on reproductive and many other endpoints (i.e., body length, body weight, and survival ratio), while in the present study, only reproductive endpoints proved to be more sensitive were selected for constructing the SSD curve.

The PNEC value was 0.73 ng E2/L (1/2 of the HC_5), which is consistent with other E2 PNEC values derived by the European Union (0.4 ng/L) for protecting aquatic life (European Union 2011). Caldwell and his colleagues recommended a slightly higher PNEC for E2 (2 ng E2/L), which was derived from an SSD curve constructed from 21 in vivo NOECs (Caldwell et al. 2012). The difference may be attributed to the species taxa selection in constructing the SSD curve. In Caldwell's study, only 21 NOECs of fishes were selected to construct the SSD curve, while in the present study, 31 NOECs from five taxa, including amphibians, crustaceans, rotifers, fishes, and algae, were chosen for constructing the SSD curve. The quality and quantity of the available data in the present study not only meet the requirement of representing 8 families from 3 different phyla as recommended by USEPA (USEPA 1986), but also satisfy the requirement of 30–50 data points for a stable SSD curve (Wheeler et al. 2002). From the perspective of protecting aquatic species rather than fishes only, 0.75 ng E2/L may be more reasonable than 2 ng E2/L, and 0.75 ng E2/L may be more protective for aquatics organisms. The PNEC of 17-[alpha]-Ethinylestradiol (EE2), a synthetic estrogen with high estrogenic potency, whose estrogenic equivalent factor was about two times that of E2 in some in vitro studies (Johnson and Sumpter 2001; Zhao et al. 2011a), was reported to be 0.35 ng E2/L to protect 95% of species; the PNEC for EE2 was derived from NOEC values based on reproductive effects from 39 papers in 26 species (Versteeg et al.

1999; Caldwell et al. 2008). The PNEC value for EE2 was consistent with its estrogenic potency being higher than that of E2 (Versteeg et al. 1999). The PNEC value for E2, derived in the present study, was consistent with the PNEC for EE2 recommended by Caldwell, when their estrogenic equivalent factors (EEFs) were incorporated. Using such comparative data could generate a new method for deriving PNECs for other estrogenic substances. In further study, EEFs or toxicity equivalent factors (TEFs) might be used as a modification factor in confirming PNECs for some toxicity data-poor chemicals. For example, if we need to derive a PNEC value for a chemical, whose toxicity data do not meet the requirement of PNEC derivation, we can refer to a PNEC value of another chemical with a similar toxicity model or toxicity mechanism or consider their EEFs or TEFs to confirm the PNECs.

5.3 Limitations of the SSD Approach

Although the SSD method is useful for assessing the range of sensitivities among species for deriving WQC and chemical risk assessment, this method has limitations. Some of these limitations are related to the properties of probability distributions. Since there is no zero (0) or 100% on a probability scale, it is impossible to establish a PNEC that would affect 0% of species, or provide 100% protection of all species. When the SSD approach is used, two sources of uncertainty need to be considered: The first is the relationship between the LOEC and NOEC. There is never a discrete boundary between LOEC and NOEC for biological phenomena. NOEC values are dependent on both author judgment and the experimental study design used to generate the NOEC. Specifically, both the range between designed doses and the number of designed doses determine the achievable resolution in establishing LOEC and NOEC values. Uncertainty that is inherent in establishing the NOEC is transmuted to estimations of the EC_5, which results in uncertainties associated with deriving the PNEC. Considering the LOEC to NOEC ratios of species, a value of 2.0 was assigned to the LOEC to NOEC correction factor (Jongbloed et al. 1996).

The second source of uncertainty, when using the SSD approach, lies in the gap between extrapolations from laboratory tests to field conditions (Forbes and Forbes 1993; Smith and Cairns 1993). Under field conditions, the actual exposure dose is generally less than the theoretical exposure dose, as a result of faster dissipation and reduced bioavailability of the test chemical. Alternatively, under laboratory conditions, exposures are kept constant and the bioavailable fraction is nearly 100%. These differences have been demonstrated in various studies done under field, or laboratory conditions or in a mesocosm system (Giesy et al. 1999). Furthermore, the adverse effects of chemicals can be mitigated by adaptive responses of organisms, not only at the individual and population levels, but also at the community level. In ecosystems, there is functional redundancy among species. Theoretically, each species cannot occupy exactly the same ecological niche. That is, they cannot have overlaps in feeding guilds; for example, if one species is affected or even removed from the ecosystem, another species can accomplish the function of the lost species.

By using the SSD approach, researchers can identify the most sensitive species, which can then be compared to economically important species, endangered species, or ecological keystone species. Thus, the use of the HC_5 value should not be blind, but rather should be used as part of a risk assessment and risk management strategy conducted by experienced professionals. Subsequently the results need to be communicated to the public by experienced professional communicators of risk. Uncertainty factors have been used to correct toxicity data from laboratory tests, and have been used to account for differences in metabolic rate, caloric content of food, and food assimilation efficiency between laboratory and wild species (Traas et al. 1996). Moreover, some authors have used a statistical procedure, which is more scientifically defensible, to estimate uncertainty factors so as to obtain more precise uncertainty factors and criteria (Calabrese and Baldwin 1994; Dourson and Parker 2007; Gaylor and Kodell 2000). Therefore, when the SSD approach is used to derive PNECs for chemicals to protect wildlife, much attention should be focused on how to bridge the extrapolation gap between laboratory and field testing.

5.4 Risk Assessment of Ambient Concentrations in China

Generally, E2 concentrations in the environment are quite small (generally less than 10 ng/L) (Table 5). The greatest concentration of E2 reported for surface water was 200 ng/L, which was reported in the USA. In China, E2 was detected in the Pearl River, Yangtze River, and Liao River at concentrations ranging from ND-7.5, 6-24 and ND-7.4 ng/L, respectively. In Great Britain, the greatest reported concentration of E2 in surface water was 17 ng E2/L, while in Italy, the greatest reported concentration was 36 ng E2/L. E2 was also detected in sediment samples from several countries or regions, including Japan, the USA, and Great Britain at ng/g concentrations. The greatest E2 concentration detected in sediments was 4.8 ng E2/g, which was reported in Japan. The greatest concentration of E2 (64 ng E2/L) occurred in Canada in effluents of domestic STPs, whereas domestic effluent levels from STPs in Great Britain ranged from 2.7 to 48.0 ng E2/L.

Potential estrogenic risk for E2 in surface waters was assessed by ranking risk quotients (RQ), which is the ratio of ambient E2 concentration to E2 PNEC value. Risk was ranked by utilizing common risk ranking criteria: $RQ < 0.1$ represents minimal risk, $0.1 \leq RQ < 1$ represents median risk, and $RQ \geq 1$ represents the greatest risk class (Hernando et al. 2006). According to these criteria, risks posed by estrogenic substances occurring in surface water of many countries including Japan, the USA, Great Britain, and Italy would be classified in the greatest risk class. If other estrogenic chemicals are considered, the risks posed in these countries would be greater.

In China, risks posed by estrogenic compounds could occur in some regions of the Pearl River, the Liao River, the Yangtze River, and the Yellow River. The E2 equivalent concentration (EEQ) (i.e., the sum of all individual compound concentration values multiplied by the corresponding estradiol equivalency factors (EEFs)), was used when we assessed the risk of other estrogenic chemicals in the aquatic

Table 5 Occurrence of E2 residues in surface water, sediment, and sewage treatment plant (STPs) effluents as reported in the cited studies

	Surface water (ng/L)		Sediment (ng/g)		STPs effluents (ng/L)	
Location	Concentration	References	Concentration	References	Concentration	References
Asia						
China					ND to 4.8 ng/L	Liu et al. (2012)
Pearl River	ND[a] to 7.5	Zhao et al. (2009)				
Yellow River	ND	Wang et al. (2012)				
Yangtze River	6–24 ng/L	Ping (2011)				
Liao River	ND to 7.4 (1.0)	Wang et al. (2011)	<LOQ[b]	Wang et al. (2011)		
Japan	ND to 12.3	Furuichi et al. (2004)	<0.1–4.8	Hashimoto et al. (2005)	0.05–2.63	Nakada et al. (2007)
	0.4–1.7	Hashimoto et al. (2005)				
Oceania						
Australia	ND to 1.2 (0.19)	Hohenblum et al. (2004)			1–4.2	Ying et al. (2008)
	0.54–3.77 (1.54)	Ying et al. (2009)				
America						
USA	0–4.5	Zhang et al. (2007)	0.16–0.45 (0.3)	Schlenk et al. (2005)	ND to 1.0	Esperanza et al. (2007)
	ND to 200 (160)	Kolpin et al. (2002)			<LOD to 3.7 (0.9)	Snyder et al. (1999)
Canada					<LOD to 64 (6)	Ternes et al. (1999)
					<1–7.4	Lee et al. (2004, 2005)
Europe						
Britain	ND to 7.1 (3.0)	Xiao et al. (2001)	<0.03–1.20	Labadie and Hill (2007)		
	<0.1	Boyd et al. (2004)	<LOQ to 4	Liu et al. (2004)		
	<LOD to 17	Liu et al. (2004)				
Italy	<1.0–36	Pojana et al. (2007)			0.35–3.5 (1.0)[a]	Baronti et al. (2000)
Germany	0.15–3.6 (0.6)	Kuch and Ballschmiter (2001)			<LOD to 3	Ternes et al. (1999)
The Netherlands					<0.1–5.0	Belfroid et al. (1999)

[a]*ND* not detected
[b]*LOQ* limit of quantification

environment. By comparing E2 residues to the PNEC (0.73 ng/L) value, 12 sites (wet season) and 21 sites (dry season) of 21 sample sites have the potential to cause estrogenic effects on some aquatic organisms; high risks existed at three sites (the EEQs were >10 ng/L) in the Liao River (Wang et al. 2011). At more than 50% of sample sites in the Pearl River system the EEQs were greater than 0.73 ng/L; this means that adverse effects from estrogenic compounds in these regions could occur (Zhao et al. 2011a). In comparison to the Liao and the Pearl River systems, the Yellow River poses a lower risk from the presence of the EDCs. Of 15 sites sampled in the Yellow River, only one site had an EEQ higher than 0.73 ng/L (Wang et al. 2012). Estrogenic effects of surface water and sediments from the Liao, Yellow, and Pearl Rivers were also assessed by using an in vitro bioassay (YES: yeast estrogen screen). Results were highly consistent with those based on chemical analysis (Zhao et al. 2011a, b; Wang et al. 2011, 2012).

6 Summary

Contamination of the aquatic environment by EDCs has received considerable attention from scientists, government officials, and the public. E2, one of the EDCs with high estrogenic effect, has the potential to cause multiple endocrine-disrupting effects, even at small concentrations. In the present review, the toxicity of E2 to aquatic organisms was reviewed. Results of published studies show that, for aquatic species, reproductive effects were the most sensitive endpoint for E2 exposure.

Although the risks posed by EDCs have caused much attention, the research on the WQC for EDCs is still at the initial stage. It has been suggested in several reports that the PNEC can be regarded as the most appropriate reference value for developing WQC for the EDCs. The SSD method was applied to derive PNECs that were based on reproductive effects endpoints. In the present review, 31 NOECs, based on reproductive effect endpoints for different species, were selected to construct the curve. The PNEC value was determined to be 0.73 ng E2/L, which could protect the biodiversity of aquatic ecosystems. Moreover, 6 NOECs for multigeneration species were also analyzed in anticipation of sensitivity comparison between the F_0 and the F_1 generations. When multiple generations of aquatic species were exposed to concentrations no greater than 100 ng E2/L, nearly 71.4% of the F_1 generation individuals were more sensitive to the effects of E2 than those of the F_0 generation. This result indicated that different generations of the same species may respond differently to EDCs exposure. Individuals of the F_1 generation were slightly more sensitive than those of the F_0 generation, in general. Therefore, protecting the F_1 generation of aquatic organisms is particularly important when WQC values for the EDCs are established.

Considering the toxic effects of EDCs on reproduction, long-term toxic effects (viz., full-life cycle study and the most sensitive life stage) should be used in setting WQC. Unfortunately, the NOECs of E2 for multigeneration species did not meet the requirement of PNEC derivation for protecting the F1 generation. Therefore, further research results are needed on the F1 generation of aquatic species to provide more insight into what constitutes adequate protection for aquatics lives.

In the present review, the PNEC values derived in the study were compared to the PNEC values developed by others, and the results showed that they were highly consistent. In addition, we also compared the PNEC value for E2 to the PNEC value for EE2, a similar estrogen, and the result was also highly consistent when their EEFs were considered. These comparisons affirmed that the method we used for deriving the PNEC value of E2 was reasonable and the PNEC values we derived were acceptable for protecting aquatic organisms. By comparing the PNEC values we calculated to actual E2 concentrations in the natural water environment, we found that E2 in surface waters may pose high risks in many countries, especially China, Japan, the USA, Great Britain, and Italy.

Acknowledgements This work was jointly supported by the National Basic Research Program of China (2008CB418200) and National Natural Science Foundation of China (41261140337, 40973090, U0833603, 41130743).

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