

Ecological Risk of Nonylphenol in China Surface Waters Based on Reproductive Fitness

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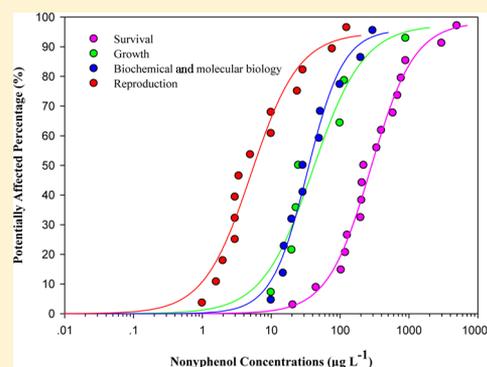
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Supporting Information

ABSTRACT: It has been recognized that ecological risk assessment based on traditional endpoints of toxicity are unable to provide adequate protection because some chemicals may affect reproductive fitness of aquatic organisms at much lower concentrations. In this paper, predicted no effect concentrations (PNECs) for 4-nonylphenol (NP) were derived based either on endpoints of survival, development, and growth or on some nonlethal biomarkers of reproduction, biochemical and molecular biology data. The PNECs derived from reproductive lesion ranged from 0.12 to 0.60 $\mu\text{g NP L}^{-1}$, which was significantly lower than those derived from other endpoints. An assessment of ecological risks posed by NP to aquatic organisms in surface waters of China was conducted based on concentration levels of NP in 16 surface waters of 4 major river basins and PNECs derived from reproductive fitness by a tiered ecological risk assessment (ERA). The results showed that 14.2% and 76.5% of surface waters in China may have ecological risks resulting from reproductive fitness if the thresholds of protection for aquatic organisms were set up as 5% (HC5) and 1% (HC1), respectively. The risks were significantly greatest in the Yangtze River Basin than in other major river basins. In comparison with the risks assessed based on traditional endpoints, such as 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 sublethal effects of reproduction, were appropriate.



1. INTRODUCTION

There have been increasing detections of hormones, hormone mimics, and other endocrine-disrupting substances in water, some of which have been found to affect reproductive fitness of aquatic organisms. Prominent among these endocrine disrupting chemicals (EDCs) are the alkylphenols and, in particular, 4-nonylphenol (NP), which is widely a degradation product of nonionic surfactants, such as nonylphenol ethoxylates (NPEs), that are found in detergents, emulsifiers, pesticides, lubricants, and oil additives, all of which are commonly used in daily life.¹ NP has been found worldwide in wastewater discharges,

sewage-treatment plant effluents, natural water, and sediments.^{1–3} Field surveys have revealed that NP occurs at mg L^{-1} concentrations in aquatic environments^{4,5} and has been detected in watersheds and drainage areas of China at relatively great concentrations.^{6–8} Because NP and NPEs are ubiquitous, persistent, easily bioaccumulated, and have the potential to

Received: August 25, 2013

Revised: December 15, 2013

Accepted: December 16, 2013

Published: December 16, 2013

cause toxic effects of aquatic organisms, these chemicals have attracted attention in the past few years. Nonylphenol has been referred to in the list of priority substances in the Water Frame Directive and in the third draft Working Document on Sludge of the EU.⁵ Previous investigations have shown that NP was estrogenic both *in vivo* and *in vitro*.^{9–12} NP can affect reproduction of teleost fishes. Notably, NP induces production of the female-specific, egg-yolk precursor vitellogenin (VTG) in livers of males, is associated with testis-ova, and decreases fecundity and fertility.^{12–16} Due to the potential for toxicity of NP to aquatic organisms, analyzing the ecological risk of NP is crucial to protection of aquatic organisms and the surface waters of China.

An important step in ecological risk assessment of chemicals is determination of the maximum concentration at which the ecosystem is protected, i.e., the predicted no-effect concentration (PNEC). PNECs are usually derived based on a laboratory toxicity test (especially for chronic) using well-defined protocols on a limited number of species.¹⁷ Despite the numerous toxicity data of NP available on fish, *Daphnia* and algae, no final decision was made regarding PNEC derivation for NP since its specific mode of action to aquatic organisms is still unclear. International organizations such as the USEPA and EU as well as other governmental agencies have issued various assessment reports on the risk management of NP. However many of them were mainly focused on the toxicity of NP to aquatic organisms and derived a toxicity threshold with assessment factor.^{18–20} USEPA have derived a criterion continuous concentration (CCC) of $6.59 \mu\text{g L}^{-1}$ for protection of aquatic life using acute to chronic ratios (ACRs) (also called application factors, AFs).²¹ However, it has been demonstrated that NP affects the reproductive system of aquatic organisms at a concentration of $1 \mu\text{g L}^{-1}$ more or less.²² Use of ACRs has been criticized.^{23–26} In some cases, average ACRs might be inadequate to extrapolate accurately from acute to chronic value.^{27,28} In the EU risk assessment report on nonylphenol of 2001 a no observable effects concentration (NOEC) of $0.33 \mu\text{g/L}$ was derived, based on the endocrine disruptive potential of NP on fresh water fish.²⁹ Lin et al. suggested that PNEC values of 4-NP ranging between 0.82 and $2.10 \mu\text{g/L}$ affect medaka population growth.³⁰ However, the evaluation data was mainly concentrated on toxicity of single fish species and lacks consideration to other aquatic organisms.

Survival, development, and growth are traditional measurement endpoints in ecotoxicology. Because these effects can be readily linked to population-concentration effects, they are favored for derivation of PNECs that protect aquatic organisms. Some researchers have indicated that traditional methods for assessment of effects with standard assessment endpoints of toxicity are unable to provide adequate protection of aquatic organisms because some contaminants have been found to affect reproductive fitness at concentrations less than those based on survival and growth.³¹ Nevertheless, nonlethal biomarkers such as vitellogenin (VTG), secondary sexual characteristics, gonadosomatic index (GSI), concentrations of steroids in blood plasma, and gonadal histology are considered “measurement endpoints” in assessment of risks of endocrine-disrupting chemicals (EDs) to aquatic organisms.³²

In the present study, predicted no effect concentrations (PNECs) of NP were derived, based either on endpoints of survival, development, and growth or on some nonlethal biomarkers of reproduction, biochemical, and molecular biology data. In addition, an assessment of the risks posed by

NP to aquatic organisms in surface waters of China was conducted by comparing PNECs derived based on reproductive fitness to concentrations of NP by applying a probabilistic ecological risk assessment (PERAs), which qualified and quantified ecological risks through exposure and effect probability distributions. Probabilistic assessments are considered to be an improvement on the hazard quotient (HQ) approach and, thus, recommended for high tiers in the ecological risk assessment (ERA) process.³³ Because PERAs can better describe the likelihood of exceeding the effect thresholds and the risk of adverse effects,³⁴ this approach has been adopted by a number of researchers.^{35–39}

2. MATERIALS AND METHODS

2.1. Data Collection and SSD Generation. Toxicity data for NP were collected from existing toxicity databases (e.g., ECOTOX Database, <http://cfpub.epa.gov/ecotox/>) and published in the literature and government documents following the principles of accuracy, relevance, and reliability.^{31,40} For acute toxicity data, selected measurement endpoints were the median lethal concentration (LC_{50}) or median effect concentration (EC_{50}) based on immobility for animals and biomass or growth for plants. For chronic toxicity data, NOECs were calculated from the available literature. When a NOEC was not available, MATC or LOEC or EC_x was used. In the case of multiple data on the same endpoint and species, the geometric mean was used.

Toxicity data for effects of NP on aquatic organisms were divided into four categories in accordance with different endpoints as follow: *Mortality data* consider the effect of lethality, livability, and behavioral inhibition to aquatic organisms. *Growth data* consider the effect of development and growth inhibition to aquatic organisms. *Reproduction data* consider the effect of fecundity, fertilization rate, hatchability, VTG, gonadosomatic index, gonadal histology, and multiple generation effect to aquatic organisms. *Biochemical and molecular biology data* consider the effect of induction or inhibition of enzyme activities, up-regulation of the expression of stress proteins, changes in RNA or DNA incidences of mutagenicity, or carcinogenicity. Toxicity data used for the Species Sensitivity Distributions (SSD) calculation are reported in the Supporting Information (Table S1).

SSDs were constructed by fitting cumulative probability distributions that plotted the concentration associated with eliciting a particular response from a particular species as a function of rank-assigned centile.^{41–43} Usually, a point estimate known as the HC_5 (hazardous concentration for 5% of species) is calculated from the SSD. This concentration is predicted to exceed no more than 5% of species effect concentrations. In this study, a log-normal distribution model was fitted to different endpoint data points of NP, and the fit of the model was evaluated using the Kolmogorov–Smirnov test. HC_5 values were then derived by use of the ETX 2.0, RIVM software packages, and final PNECs were derived by dividing the HC_5 by an application factor of 5.0.⁴⁴ The factor 5 was chosen based on the amount of supporting evidence, such as non-native species data, multispecies data present, and the presence of information based on field data. Sensitivity distributions were compared using the two-sample Kolmogorov–Smirnov test using the SPSS Version 17 software.

2.2. Exposure Concentration of NP. Samples from 16 surface waters, including reservoirs and rivers that supply water to local waterworks, were collected between March 2010 and

July 2010 (Table 1). The study area covered four out of the seven main river systems of China. Samples were collected in

Table 1. Site Information and Exposure Concentration of Nonylphenol

site	type	coordinate	concn ^b (ng L ⁻¹)
Liao River ^a			
S1	reservoir	124.101 E, 41.886 N	30.05 ± 3.97
S2	reservoir	125.404 E, 41.292 N	54.27 ± 7.34
Hai River ^a			
S3	reservoir	116.840 E, 40.490 N	109.22 ± 12.37
Yangtze River ^a			
S4	river	106.449 E, 29.597 N	168.25 ± 5.48
S5	river	106.554 E, 29.570 N	100.21 ± 7.97
S6	river	106.529 E, 29.508 N	123.58 ± 10.29
S7	river	118.694 E, 31.994 N	280.19 ± 17.67
S8	river	118.798 E, 32.142 N	288.75 ± 21.48
S9	river	118.717 E, 32.049 N	212.39 ± 14.63
S10	lake	120.223 E, 31.517 N	232.73 ± 14.65
S11	reservoir	121.357 E, 31.492 N	230.84 ± 16.52
S12	river	121.308 E, 30.974 N	259.63 ± 14.21
S13	reservoir	121.710 E, 31.420 N	224.13 ± 18.57
Pearl River ^a			
S14	reservoir	114.603 E, 23.794 N	58.33 ± 4.23
S15	reservoir	113.259 E, 23.807 N	85.16 ± 7.45
S16	reservoir	114.149 E, 22.571 N	72.65 ± 4.96

^aRiver system. ^bMean ± standard deviation ($n = 3$).

precleaned amber glass bottles. Prior to sample collection, the bottles were washed three times with water samples. To minimize contamination of samples, throughout sample collection and processing, use of personal care items and pharmaceuticals were discouraged. Samples were stored at 4 °C prior to treatment and were treated and prepared within 48 h.

Water samples were filtered through prebaked glass fiber filters to remove insoluble materials and extracted using the SPE method. Water samples for quantification of 4-NP were spiked with BPA-d16 following published procedures.⁸ Concentrations were determined by use of an Agilent 6890 gas chromatograph equipped with an Agilent MSD 5975 mass spectrometer (USA). System control and data acquisition were achieved by use of ChemStation Software (USA).⁸ All data generated from the analysis were subject to strict quality control procedures. Recoveries of spikes of NP averaged 87.7% and were within the acceptable criteria. Limit of detection (LOD) and limit of quantitation (LOQ) of the target compounds were based on the standard derivations (SD) of seven replicates of spiked water at the concentration of 5 ng NP L⁻¹. LOD was defined as three times SD, and LOQ was defined as nine times SD. The LOD and LOQ for source water were 0.10 to 0.65 ng NP L⁻¹ and 0.20 to 1.3 ng NP L⁻¹, respectively.

2.3. Ecological Risk Assessment of NP. The probabilistic ecological risk assessment (PERA) was performed by using the Probabilistic Risk Assessment Tool (PRAT).^{33,45} 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 curves (JPCs), which describes the probability of a particular set of exposure conditions occurring relative to the number of taxa that would be affected.^{38,39,45} 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.⁴⁵ In addition for comparison ecological risks posed by NP among basins, deterministic HQs were also calculated by dividing measured concentrations in the environment (MECs) with PNECs.

3. RESULTS AND DISCUSSION

3.1. Predicted No Effect Concentrations of NP. Toxicity data used for the Species Sensitivity Distributions (SSD) calculation are reported in the Supporting Information (Table S1). A total of 17 toxicity data based on lethality and behavioral effects for aquatic species were collected for NP, including 6 fishes and 11 invertebrates. Concentrations for *mortality* ranged from 20.7 to 5,000, with a median of 761.1 $\mu\text{g NP L}^{-1}$. Seven chronic NOEC values, based on the effect on development and growth, with 2 data points for fishes, 4 for invertebrates, and 1 for hygrophytes, respectively. The range of magnitudes of effects on *growth* was from 10.0 to 901, with a median of 170.7 $\mu\text{g NP L}^{-1}$. Fourteen results based on *reproduction* including fecundity, rate of fertilization, hatchability, and effects on multiple generations, included 5 fishes, 8 invertebrates, and 1 planktonic alga. The NOECs (or EC_x) ranged from 1.0 to 125, with a median of 21.24 $\mu\text{g NP L}^{-1}$. In addition, a total of 11 toxicity data were collected based on *biochemical and molecular biology* endpoints, with concentrations ranging from 10.0 to 300, with a median of 74.6 $\mu\text{g NP L}^{-1}$. Parameters of species sensitivity distributions for NP based on different endpoints are shown in Table 2.

Toxicity data for NP based on various endpoints were determined, based on results of the Kolmogorov–Smirnov test ($p < 0.05$) to meet the assumption normally for application of parametric statistics. The calculated HC₅s for each measurement endpoint ranged from 0.60 to 30.03 $\mu\text{g L}^{-1}$ (Table 2). The final PNECs were calculated as the derived HC₅ with a 50% uncertainty divided by a factor 5. The PNEC derived from *reproduction* data was lesser than those derived based on *growth* or *biochemical and molecular biology* data, and the PNEC derived from survival data was the greatest.

Table 2. Parameters of Species Sensitivity Distributions for Nonylphenol Based on Different Endpoints^a

endpoint	N	mean	SD	K–S test for normality	HC ₅ with 50% CI ($\mu\text{g L}^{-1}$)	PNEC ($\mu\text{g L}^{-1}$)
survival	17	761.6	1295	0.5224	30.03 (9.81–62.91)	6.01
growth	7	170.7	324.7	0.7711	3.77 (0.29–13.02)	0.75
biochemical and molecular biology	11	74.6	93.1	0.6142	6.43 (1.88–13.19)	1.29
reproduction	14	21.24	36.1	0.7701	0.60 (0.15–1.43)	0.12

^aN refers to number. SD refers to standard deviation. HC₅ refers to hazardous concentration for 5% of species. PNEC refers to predicted no-effect concentration.

The sensitivity distribution for NP based on various endpoints demonstrated that effects on *reproduction* occurred at lesser concentrations than those based on *lethality*, *growth*, or *biochemical and molecular biology* (Figure 1) and were

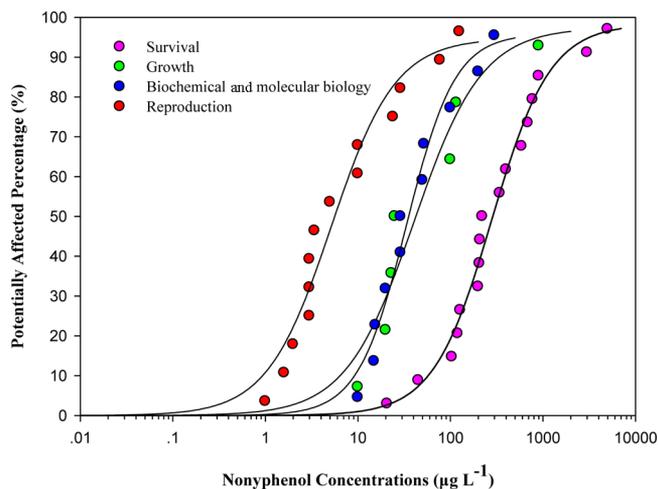


Figure 1. Species sensitivity distribution of nonylphenol based on different tested endpoints.

significantly different from data survival ($k_s = 2.186$, $n_1 = 14$, $n_2 = 17$, $p < 0.001$) and growth ($k_s = 1.409$, $n_1 = 14$, $n_2 = 7$, $p = 0.039$). PNECs based on reproductive fitness occurred at lesser concentrations than other test endpoints. Previous investigations have shown that NP was estrogenic and affects teleost reproduction.^{9–12} This conclusion is consistent with the results of the present assessment.

An important step in ecological risk assessment of chemicals is calculation of the PNEC. Derivation of PNEC values in the EU risk assessment procedure uses either the application of an assessment factor of 10–1000 on the least NOEC or the HC_5 (based in the SSD approach) divided by a safety factor.⁴⁴ Assessment factors are recognized as a conservative approach for dealing with uncertainty in assessing risks posed by chemicals.²³ In this assessment, a PNEC of $0.12 \mu\text{g NP L}^{-1}$ derived by use of the SSD approach with a 50% certainty based on reproduction data was recommended as the maximum concentration at which the aquatic organisms is protected when exposure to NP. The PNEC were numerically less than those deriving based on single fish species but in the same order of magnitude.^{29,30} However, this result is lesser by a factor of 50 than the criteria continuous concentration (CCC) of $6.59 \mu\text{g L}^{-1}$ for protection of aquatic derived by use of ACRs.²¹ Use of ACRs has been criticized, since average ACRs can be inadequate for extrapolation from acute to chronic values.^{27,28} In addition, USEPA developed the CCC for NP mostly based on survival and growth, since no information was available for effects on reproduction. Sensitivity of taxa was greater when reproduction was used as the measurement endpoint. Therefore, the PNEC derived based on reproductive toxicity in present study can better protect aquatic life from exposure to NP. Similarly, Caldwell et al. collected NOECs for reproductive effects from 39 papers in 26 species, resulting in a HC_5 of 0.35 ng L^{-1} for EE2. Reproduction of fish was identified as the most sensitive endpoint in aquatic species. The result is 100 or more times less than concentrations ($0.13 \mu\text{g L}^{-1}$) derived from traditional test endpoints with application of assessment factors.³¹

3.2. Probabilistic Ecological Risk Assessment of NP.

The site information and concentrations of NP in surface waters from China is shown in Table 1. NP was detected in all 16 samples, with concentrations in the range of $30.05\text{--}288.75 \text{ ng L}^{-1}$. For the different watersheds and drainage regions, NP was more frequently detected at greater concentrations in the Yangtze River watershed. Compared to results of previous studies in both source and surface waters, these are similar to those determined in the present study. Concentrations of NP in source waters were as great as 130, 134, 338, and 890 ng L^{-1} in the USA, Germany, Greece, and Austria, respectively.^{46–49} NPs and NPEOs have been classified in the European Union as a hazard to safety to humans and the environment.⁵⁰ In the USA, these compounds have been removed from laundry detergents. Nevertheless, these compounds have not been effectively restricted in China. So, concentrations of NP found in various Chinese rivers were often greater than those from other regions.^{7,51}

The ecological risk assessment of NP was achieved by applying a tiered ERA approach consisting of simple deterministic methods to probabilistic methods for risk characterization, concentrations of NP from nationwide surface water monitoring program, and toxicity data for different endpoints from previous published literature.

The HQs of NP in Chinese surface waters are shown (Figure 2). Risk was assessed and comparing PNECs based on various

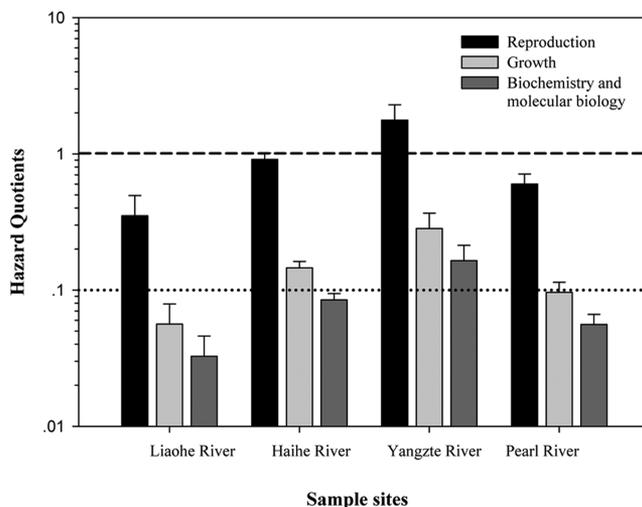


Figure 2. HQs calculated by dividing averages concentrations by PNEC based on different tested endpoints of nonylphenol.

endpoints to mean concentrations in each surface water. For assessments using PNEC derived from reproduction data, the HQs were more than 0.3 in all sample areas and even exceeded 1.0 in the Yangtze River watershed. Based on the PNEC derived from other toxicity data, the HQs were generally less than 0.1 except for the Yangtze River watershed. The results indicated NP posed a potential risk of reproductive system damage to aquatic organisms in Chinese surface water, especially in the Yangtze River.

Joint probability curves (JPCs) resulting from direct comparison of exceedance probability function (EXF, calculated as $100 - \text{cumulative distribution function (CDF)}$) and SSD offer a better representation of the overall risk (Figure 3). The x -axis of the JPC represents the intensity of effects, while the y -axis represents their probability. Each point on the curve

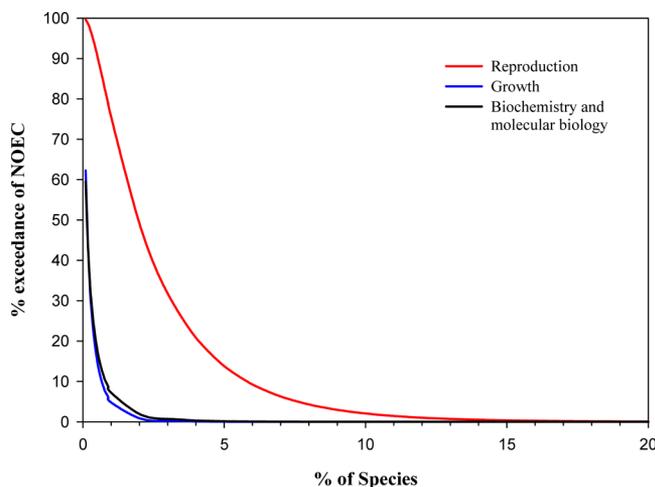


Figure 3. Joint probability curves for ecological risk of nonylphenol in surface water from China.

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.⁴⁵ In the present study, probabilities of exceeding the reproductive toxicity NOEC for 1 to 5% of the species ranged from 14.2 to 76.5% for NP. The results of the JPC analysis indicate that NP posed a significant risk on aquatic organisms' reproductive systems.

From the results of this study, NP posed a significantly greater risk to reproduction of aquatic organisms than did other measurement endpoints, such as growth and survival. For the different watersheds and drainage regions, NP posed a potential risk of reproductive system damage to aquatic organisms in Chinese surface water, especially in the Yangtze River. Methods ranging from deterministic to probabilistic methods are usually recommended for ecological risk assessment, with the probabilistic approach serving to refine the risk estimated after more conservative lower tier approaches. Results of probabilistic ERAs can provide useful information for risk managers and decision makers. However, hazard quotients, especially the single-value estimates, still have the advantage of readily identifying chemicals that have a likelihood of producing effects on species. The single-value estimate method is useful as a screening tool that can help to focus risk assessments. The initial estimations found in the first tier of the present study, which predicted relatively large HQs, suggested that a generalized potential risk for the whole area could not be excluded. The higher tier approaches allowed the estimation of the proportional risk of measured NP concentrations to aquatic species in Chinese surface waters.

3.3. Uncertainty Analysis. Uncertainty in ERA is inevitable even when higher-tier methods are used. Sources of uncertainty in the present study include the following: the variability of nonylphenol concentration within a single body of water, the ecological relevance of the toxicity data, and the risk characterization model. In particular, information on spatial and temporal variation in concentrations of NP was limited, especially for a nationwide monitoring. To more accurately describe exposures, further information needs to be collected to describe concentrations of NP at various spatial and temporal scales.

In the present study, toxicity values based on different tested endpoints for aquatic species from different trophic concentrations were used to decrease uncertainty associated with species-specific responses and to better mimic real-world scenarios of long-term exposure. It is commonly thought that different ecosystems contain different biological constituents, and a concentration threshold that would be harmless in one ecosystem may lead to irreversible toxic effects on others. The potential use of toxicity data for non-native species to derive WQC is controversial, due to the fact that it is sometimes questioned whether criteria based on species from one geographical region provide appropriate protection for species in a different region.^{52,53} However, this uncertainty could not be resolved previously in large part due to the paucity of toxicity data applicable for local species. Comparisons of sensitivities between native and non-native species for limited chemicals, such as 2,4-dichlorophenol and pentachlorophenol,^{17,54} revealed that values derived by use of non-native species values were protective of native species when a safety factor was included. It indicates that data for organisms from different geographic regions can be used in estimating PNEC for a preliminary ecological risk assessment when lacking of site-specific toxicity data. When conducting ERAs for chemicals that can modulate the neuroendocrine system, information on chronic effects including subtle effects on reproduction should be used to derive thresholds to which to compare predicted environmental concentrations. However, there is limited information of this type available, especially for local species, especially for site-specific ERAs. So, reproductive toxicities data for endocrine disruptors for site-specific species are critically needed in order to produce more accurate ecological risk assessments.

NP is a degradation product of nonylphenol ethoxylates (NPE), which is used in detergents found in cleaning products, pesticides, and personal care products. This breakdown process produces a number of chemicals with structures intermediate between NPE and NP. Because these related chemicals come from the same parent compound as NP (i.e., the original NPE detergent), they will always be present in the mixture and always accompany NP in aquatic environments. Mixtures may be much more toxic than NP alone. Because insufficient data exist to reliably analyze mixtures, then a safety factor (five in present study) was introduced to take into account related chemicals. Also, the lack of consensus on ERA methods precluded effective comparison among calculated risks made by different risk assessors. Standard methods should be developed and employed by researchers and public agencies to evaluate ecological risk.

The results of the study upon which we report here demonstrate the use of the probabilistic ERA approach based on comparing PNECs derived based on reproductive fitness to concentrations of NP in Chinese surface water. In this study, PNECs for NP were derived based either on endpoints of survival, development and growth or on some nonlethal biomarkers of reproduction, biochemical and molecular biology data. *Reproduction data* was more sensitive than other test endpoints for exposure of NP to aquatic organisms based on comparison both PNEC and sensitivity distribution. For NP, applications of more traditional assessment endpoints were less protective than application of PNECs based on nontraditional EDC endpoints. In addition, NP was found to have potential ecological risk in some Chinese surface waters. Risks were greatest in the Yangtze River Basin, which were significantly

greater than those for other basins. The use of NP might affect the aquatic organisms, and corresponding measures should be taken to minimize ecological risk posed by NP, especially in the Yangtze River Basin.

■ ASSOCIATED CONTENT

● Supporting Information

Toxicity data for NP were collected from existing toxicity databases (e.g., ECOTOX Database, <http://cfpub.epa.gov/ecotox/>), published in the literature, and government document follow the principles of accuracy, relevance, and reliability. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was financially supported by National Natural Science Foundation of China (21307165) and National Major S&T Program for Water Pollution Control and Treatment (2013ZX07502001). Prof. Giesy was supported by the Canada Research Chair program, a Visiting Distinguished Professorship in the Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, the 2012 "High Level Foreign Experts" (#GDW20123200120) program, funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University and the Einstein Professor Program of the Chinese Academy of Sciences. We also would like to thank Dr. Weiwei Jiang (National Engineering Research Center of Urban Water Resources, China) for the assistance with chemical analysis.

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Table S1 Summary of toxicity data for exposure of aquatic species to nonylphenol

Test species	Time (days)	Endpoint	Measurement	Con. ($\mu\text{g/L}$)	Reference	Klimisch code
<i>Chironomus riparius</i>	1	LC50	Mortality	688	1	2
<i>Daphnia magna</i>	2	EC50	Mortality	104	2	1
<i>Daphnia magna</i>	7	LC50	Mortality	120	3	2
<i>Hyalella azteca</i>	4	EC50	Intoxication	20.7	2	1
<i>Homarus americanus</i>	4	LC50	Mortality	200	4	2
<i>Americamysis bahia</i>	4	LC50	Mortality	45	5	2
<i>Crangon septemspinosa</i>	4	LC50	Mortality	400	4	2
<i>Neomysis integer</i>	4	LC50	Mortality	590	6	2
<i>Oncorhynchus mykiss</i>	4	LC50	Mortality	221	2	1
<i>Pimephales promelas</i>	4	LC50	Mortality	128	2	1
<i>Salmo salar</i>	4	LC50	Mortality	900	4	2
<i>Lepomis macrochirus</i>	4	LC50	Mortality	209	2	1
<i>Anodonta cataracta</i>	6	LC50	Mortality	5000	4	2
<i>Mytilus edulis</i>	4	LC50	Mortality	3000	7	2
<i>Lumbriculus variegatus</i>	4	LC50	Mortality	342	2	1
<i>Physa virgata</i>	15	LC50	Mortality	774	2	1
<i>Xiphophorus helleri</i>	4	LC50	Mortality	206	8	2
<i>Xenopus laevis</i>	14	NOEC	Development	25.0	9	2
<i>Daphnia magna</i>	21	NOEC	Growth	116	2	1
<i>Americamysis bahia</i>	14	NOEC	Growth	10	10	2
<i>Chironomus tentans</i>	2	NOEC	Growth	100	11	2
<i>Oncorhynchus mykiss</i>	91	NOEC	Growth	23.1	2	1
<i>Lemna minor</i>	4	NOEC	Growth	901	2	1
<i>Xiphophorus helleri</i>	60	LOEC	Growth	20	8	2

<i>Daphnia magna</i>	21	NOEC	Reproduction	24	3	2
<i>Daphnia magna</i>	21	NOEC	Reproduction	77.3	2	1
<i>Daphnia magna</i>	21	EC10	Reproduction	2.0	12	2
<i>Brachionus calyciflorus</i>	4	NOEC	Reproduction	5	13	2
<i>Ceriodaphnia dubia</i>	7	NOEC	Reproduction	125	14	1
<i>Americamysis bahia</i>	14	NOEC	Morphology	3	10	2
<i>Oryzias latipes</i>	90	NOEC	Reproduction/ gonadal development	10	15	1
<i>Oryzias latipes</i>	100	NOEC	Population	29	15	1
<i>Scenedesmus subspicatus</i>	3	EC10	Population	3	12	2
<i>Brachionus calyciflorus</i>	4	NOEC	Population	10	13	2
<i>Neomysis integer</i>	4	NOEC	Reproduction	1	6	2
<i>Pimephales promelas</i>	42	NOEC	Reproduction/ fertilization	1.6	2	1
<i>Oncorhynchus mykiss</i>	94	NOEC	Reproduction/ vitellogenin	3.4	16	1
<i>Oryzias latipes</i>	100	LOEC	Morphology	3	15	1
<i>Neomysis integer</i>	4	NOEC	Biochemistry	100	6	2
<i>Psetta maxima</i>	21	LOEC	Biochemistry	29	17	2
<i>Gadus morhua</i>	21	LOEC	Biochemistry	29	17	2
<i>Pelophylax nigromaculatus</i>	45	NOEC	Biochemistry	200	18	2
<i>Cyprinus carpio</i>	70	NOEC	Biochemistry	15.5	19	1
<i>Salmo salar</i>	7	LOEC	Genetics	15	20	2
<i>Oryzias latipes</i>	7	LOEC	Genetics	20	15	1
<i>Rivulus marmoratus</i>	4	NOEC	Genetics	300	21	2
<i>Gobius niger</i>	3	LOEC	Genetics	50	22	2
<i>Xiphophorus helleri</i>	3	LOEC	Genetics	52	8	2
<i>Chironomus riparius</i>	1	NOEC	Genetics	10	1	2

Selection Criteria

Toxicity data for NP were collected from existing toxicity databases (e.g. ECOTOX Database, <http://cfpub.epa.gov/ecotox/>), published in the literature, and government documents following the principles of accuracy, relevance and reliability. The terms reliability, adequacy, and relevance are defined as follows^{23,24}:

Reliability considers the completeness of the reported test methodology in comparison to accepted, standardized methodology. For data to be considered reliable, the experimental procedure and results must be properly described as well as sufficiently clear and plausible and must support the findings.

Relevance considers whether the data, the test procedures, or both are appropriate to assess the reported effect or end point.

Adequacy considers the usefulness of the data. When there is more than one set of data for an effect or end point, the most reliable and relevant data are used to describe the effect or end point.

Four categories of reliability are proposed by Klimisch²⁴:

Reliable without restriction (Level 1): studies described in the literature or reports that were conducted according to, or on the basis of, generally valid or international or national accepted testing guidelines (preferably performed according to *GLP*), or studies in which all parameters described are closely related/comparable to a guideline method.

Reliable with restrictions (Level 2): studies described in the literature or reports that may not be performed according to *GLP*, and not all test parameters comply with a specific testing guideline, but are sufficient to accept the data. This includes investigations that cannot be subsumed under a testing guideline(s), but that are nevertheless well-documented and scientifically acceptable.

Not reliable (Level 3): studies described in the literature or reports that include procedures that are in conflict with the proper performance of the test procedure or ascribed test guideline. This includes interferences between the analytical procedure and the test substance, the use of organisms or test systems that are not relevant to the

exposure, the use of a test procedure that is not acceptable, and documentation that is not convincing to the expert judge.

Not assignable (Level 4): studies described in the literature or reports that do not provide sufficient experimental details and that are only listed in short abstracts or secondary literature.

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