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Toxic Pollutants in China

Study of Water Quality Criteria

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ISSN 2191-5547 ISSN 2191-5555 (electronic)
SpringerBriefs in Environmental Science
ISBN 978-94-017-9794-8 ISBN 978-94-017-9795-5 (eBook)
DOI 10.1007/978-94-017-9795-5

Library of Congress Control Number: 2015931805

Springer Dordrecht Heidelberg New York London

Printed on acid-free paper

Springer Science+Business Media B.V. Dordrecht is part of Springer Science+Business Media (www.springer.com)

Preface

Water quality criteria (WQC) form the scientific basis for the development of water quality standards (WQSs). Developed countries have established a relatively mature WQC system. For example, the United States, Canada, the Netherlands, Australia, and New Zealand have all published a number of documents and technical guidelines on WQC. Research into WQC has played an important role in the determination of WQSs and other aspects of environmental management, such as environmental monitoring, environmental impact assessment, and environmental risk assessment.

Fragmentary studies in WQC have been undertaken in China for about 30 years. There has been no systematic research in China leading to the establishment of a mature WQC system. However, despite this, there has been some progress on WQSs over the last 30 years. Various WQSs have been issued to protect surface water, seawater, fisheries waters, irrigation water, groundwater, and drinking water. These standards were derived from and/or based on relevant standards or criteria from developed countries or international organizations. However, the geographic, regional, eco-environmental, and socioeconomic characteristics in China are different from those of developed countries—including ecosystem structures and functions, sensitive aquatic organisms, economic conditions, living habits, priority pollutants, etc. Since the criteria derivations are influenced by all the above factors, it is questionable whether the current standards provide appropriate protection to the ambient water environment in China.

In order to deal with increasingly serious environmental pollution and to improve environmental management, the Chinese government has recently confirmed "scientifically establishing criteria" to be a national target. Some major projects have been set to support systematic WQC study in China. Currently, various WQC are being studied intensively in China, including aquatic life, biological, sediment quality, and human health criteria. Moreover, due to the existing pollution situation and its management requirements, the Chinese government and scientists are paying more attention to the development of freshwater WQC than they are to seawater WQC.

This short book introduced some WQC studies for toxic pollutants in China, including nitrobenzene, PAHs, triclosan, and so on. We hope the results of our research could provide valuable information for establishment of Chinese WQSs.

Contents

Chapter 1
Development of Water Quality Criteria for Toxic Organic Pollutants

Zhenguang Yan, Jiang-yue Wu, Xiao-nan Wang and Ya-hui Zhang

Abstract Nitrobenzene, phenanthrene (PHE), benzo[a]pyrene (BaP), triclosan (TCS), and perfluorooctane sulfonic acid (PFOS) are toxic organic pollutants in water ecosystem. However, there is still rare study on water quality criteria (WQC) in China for them. By gathering published toxicity data and conducting toxicity test of them with Chinese aquatic species, the dataset of these pollutants were obtained. Then, the method of species sensitivity distributions (SSD) developed by United States Environmental Protection Agency (USEPA) was mainly applied to derive criterion maximum concentration (CMC) and criterion continuous concentration (CCC), respectively. For nitrobenzene, CMC and CCC are 0.018 mg/L and 0.001 mg/L, respectively. For PHE, CMC and CCC are 0.0514 mg/L and 0.0186 mg/L, respectively. For BaP, CMC and CCC are 0.73 μg/L and 0.38 μg/L, respectively. For TCS, CMC and CCC are 0.009 mg/L and 0.002 mg/L, respectively; for PFOS, CMC and CCC are 32.24 μg/L and 4.56 μg/L, respectively. Besides, SSD differences between native and non-native species were compared to decide which species are more sensitive to a certain pollutant.

Keywords Toxic organic pollutants · Water quality criteria (WQC) · Criterion maximum concentration (CMC) · Criterion continuous concentration (CCC) · Species sensitivity distributions (SSD)

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Z. Yan and Z. Liu (eds.), *Toxic Pollutants in China*,
SpringerBriefs in Environmental Science, DOI 10.1007/978-94-017-9795-5_1

1.1 Development of Aquatic Life Criteria for Nitrobenzene in China

1.1.1 Nitrobenzene

1.1.1.1 Introduction

Water quality criteria (WQC) are the levels of individual pollutants, water quality characteristics or descriptions of conditions of a water body which will generally protect the designated use(s), and water quality standards (WQSs) (USEPA 2003). These definitions showed that the WQC formed the basis for water quality standards (WQSs). Both WQC and WQS play an important role in environmental management. The requirement for a WQC to protect environmental resources was recognized in the 1970s in the USA and Europe. So far, developed countries have well established a WQC system. In China, the study on WQC is urgent because of the revised WQSs mainly from foreign WQC or WQSs (Xia and Zhang 1990). For example, the current Chinese WQS for nitrobenzene to protect surface water (0.017 mg/L) was copied from the American current human health criteria of nitrobenzene (USEPA 2009). However, the existing WQS in China may be over- or under protective for aquatic ecosystems in China due to the differences in many physical, chemical, and biological factors between the USA and China which can affect the toxicity of a substance in aquatic organisms. Nitrobenzene is produced for industrial use by the nitration of benzene with nitric and sulfuric acids and is widely used in production of dyes and pesticides. It was the principal compound involved in the Songhuajiang accident in 2007, one of the largest water pollution accidents in China. Despite the published toxicity data of nitrobenzene, the aquatic life criteria for nitrobenzene in China have not been established. This study performed six acute toxicity tests using Chinese native species, including yellow head catfish (*Pelteobagrus fulvidraco*), common carp (*Carassius auratus*), Chinese brown frog (*Rana chensinensis*), mud snail (*Cipangopaludina cahayensis*), oriental river prawn (*Macrobrachium nipponense*), and water flea (*Daphnia magna*). Two chronic tests with *D. magna* and *C. auratus* were performed, too. Data analysis applying all available toxicity data of nitrobenzene enabled the derivation of the criterion maximum concentration (CMC) and criterion continuous concentration (CCC).

1.1.1.2 Materials and Methods

1. **Collection of published ecotoxicity data for nitrobenzene**

The published ecotoxicity data of nitrobenzene were gathered from the ECOTOX database (http://cfpub.epa.gov/ecotox), the US WQC for nitrobenzene (USEPA

1980) and other sources. The data were analyzed based on guidelines for aquatic life criteria (USEPA 1985).

2. **Test chemicals and organisms**

Analytical grade nitrobenzene (99 % purity) was from Beijing Chemical Reagent Company. Tap water was dechlorinated with activated carbon and used as dilution water for all tests. The measured quality parameters of dilution water were the following: pH 7.2 ± 0.5, dissolved oxygen (DO), 7.0 ± 0.4 mg/l, total organic carbon, 0.020 mg/l, and hardness, ($CaCO_3$), 136 mg/l.

Data on at least eight families of aquatic animals drawn from three different phyla and one aquatic plant are required in the derivation of WQC (USEPA 1985). In addition to the published ecotoxicity data, six resident aquatic species in China were chosen to produce toxicity data by performing acute and chronic ecotoxicity tests. They included the following: *P. fulvidraco*, *C. auratus*, *M. nipponense*, *R. chensinensis, Cipangopaludina chinensis,* and *D. magna*. Prior to the toxicity tests, all the organisms were acclimatized to the dilution water during a minimum of seven days. All toxicity tests were carried out according to ASTM standard guidelines (ASTM 1993a, b, c).

3. **Determination of nitrobenzene**

The concentration of nitrobenzene in the solutions of each group at the beginning and end of the experiments was measured. Briefly, the solutions were first filtered through a 0.45-μm filter membrane and followed by high performance liquid chromatography (HPLC) detection, using an L-7000 system (Hitachi, Tokyo, Japan), a C18 column (150 mm × 4.6 mm, particle size 5 μm; Elite, Dalian, China), mobile phase of methanol and water (50:50, v/v) with a flow rate of 1.0 mL/min. The column temperature was 25 °C, a loading volume of 10 μL and nitrobenzene had a good ultraviolet (UV) detection at 262 nm, and a retention time of 5 min. Measured/nominal concentration was 92.30–99.19 %. The variability of nitrobenzene concentration was controlled in 20 %, in compliance with the requirement of the toxicity text.

4. **General test conditions**

All tests were static-renewal whereby test solutions were totally replenished at 24 h intervals. The standard conditions were three replicate test containers with ten organisms, at different concentrations, solvent control, and blank control. All tests were undertaken at a light:dark photoperiod of 12:12 h. Test organisms were not fed during the acute test periods. Test chambers were immersed in a water bath to maintain the water temperature at 22 ± 2 °C. Temperature, DO, and pH were measured in test chambers daily in the acute toxicity tests and once a week in chronic tests. Biological observations were performed once daily.

5. ***P. fulvidraco*** **acute toxicity test**

The fish were from Quanxin Farm, Huzhou, Zhejiang Province. The mean wet weight was 0.05 ± 0.01 g. Fish were exposed to test solutions in 2-L glass beaker

for 96 h. Measured exposure concentrations in the definitive test were 0 (control), 79.7, 96.9, 114.8, 138.6, and 164.3 mg/L nitrobenzene, respectively.

6. ***C. auratus*** **acute toxicity test**

The fish were purchased from Lukou Farm, Nanjing, Jiangsu Province. The mean wet weight of the fish was 4.0 ± 0.8 g. Fish were exposed to test solutions in 40-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0, 92.4, 110.8, 130.2, 161.4, and 195.3 mg/L nitrobenzene, respectively.

7. ***Tadpole (R. chensinensis)*** **acute toxicity test**

The tadpoles of *R. chensinensis* were from in-house cultures at the environmental laboratory of Nanjing University, with the mean wet weight of 0.047 ± 0.008 g. The temperature of the test chambers was maintained at 18–20 °C. They were exposed to test solutions in 2-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0, 50.6, 67.6, 88.4, 114.5, 150.0, 198.4, and 260.8 mg/L nitrobenzene, respectively.

8. ***C. cahayensis*** **acute toxicity test**

C. cahayensis were from Xuanwu Lake, Nanjing, Jiangsu Province with the mean wet weight of 0.48 ± 0.13 g. The organisms were exposed to test solutions in 2-L glass aquaria for 96 h. Nominal exposure concentrations were 0, 99.7, 125.6, 150.2, 174.8, 190.5, 221.5, and 202.8 mg/L nitrobenzene, respectively.

9. ***M. nipponense*** **acute toxicity test**

The shrimp were from Lukou Farm, Nanjing, Jiangsu Province and had a mean wet weight of 0.16 ± 0.04 g. They were exposed to test solution in 2-L glass aquaria for 96 h. Nominal concentrations in the definitive test were 0, 0.0194, 0.0316, 0.0506, 0.0800, 0.1305, and 0.2088 mg/L nitrobenzene, respectively.

10. ***D. magna*** **acute toxicity test**

The daphnia were from in-house cultures at environmental laboratory of Nanjing University. The daphnia (<24 h age) were exposed to test solution in 100-ml beakers for 48 h. Nominal concentrations in the definitive test were 0, 40.9, 45.2, 50.6, 54.1, 59.7, and 66.3 mg/L nitrobenzene, respectively.

11. ***D. magna*** **chronic toxicity test**

Twenty-one day survival-reproduction tests using neonates of *D. magna* (<24 h age) were carried out in 100-ml beakers. The daphnia were fed with green algae (*Scenedesmus obliquus*) at a cell concentration of 10^5 cell/ml test solution. The survival, growth, and reproduction were recorded each day. The endpoints include the time to first brood, number of broods, the 21-d body length, and number of exuviations. Nominal concentrations in the definitive test were 0, 0.63, 1.25, 2.5, 5.0, 10.0, and 20.0 mg/L nitrobenzene, respectively, with six replicate test containers each containing one test organism.

12. ***C. auratus* chronic toxicity test**

The used fish was the same with the acute toxicity test. Twenty-one day short-term chronic toxicity test was conducted in 40-L glass aquaria. They were fed with a commercial diet. The endpoints include survival, growth, and body weight. Nominal exposure concentrations in the definitive test were 0, 1.0, 2.0, 4.0, 8.0, and 16.0 mg/L nitrobenzene, respectively.

13. **Data analysis**

Probit was employed to calculate 48 h-LC_{50}, 96 h-LC_{50} values and corresponding 95 % confidence intervals depending on the raw data distribution. Data on chronic tests were determined as the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) for the most sensitive biological endpoint in each test.

The USEPA guidelines for aquatic life criteria (USEPA 1985) and a species sensitivity distribution (SSD) method (Van Vlaardingen et al. 2004) exploited by the National Institute for Public Health and the Environment (RIVM) were employed to derive the WQC for nitrobenzene. The data processing software is OriginLab 7.5.

1.1.1.3 Results

1. **Published toxicity data of nitrobenzene to freshwater organisms**

Published toxicity data of nitrobenzene to freshwater organisms were collected and calculated. As shown in Table 1.1, there were acute toxicity data on 13 genera, which include nine genera of Chinese resident species, alien species, guppy, rainbow trout, and bluegill are extensively cultured in China, while swordtail and zebrafish are standard toxicity test species. The chronic toxicity data of nitrobenzene are not enough (Table 1.2). Apart from non-native species, there is only daphnia with chronic toxicity data. In addition, toxicity data on six genera of freshwater alga were collected (Table 1.3). No toxicity data of nitrobenzene were described toward other freshwater plants.

2. **Results of toxicity tests of six native aquatic organisms**

Toxicity of nitrobenzene to *P. fulvidraco*, *C. auratus*, *R. chensinensis* (tadpole), *C. cahayensis*, *M. nipponense,* and *D. magna* was tested and the results were shown in Table 1.4. All control mortality rates were less than 7 %. Results of acute toxicity tests showed that the most sensitive species to nitrobenzene was *M. nipponense*, followed by *D. magna*, and the least sensitive species was the tadpole of *R. chensinensis* with a 96 h-LC_{50} of 161.9 mg/L. *C. auratus* and *C. cahayensis* were less sensitive than *P. fulvidraco*. As shown in Table 1.5, the results of the chronic test with *D. magna* showed a consistence with previous studies (Kuhn 1988; Kuhn et al. 1989), where the

Table 1.1 Acute toxicity of nitrobenzene to freshwater animals

Species	Endpoint	ρ(nitrobenzene)/(mg/L)	References
Cyprinus carpio	96 h-LC_{50}	1.907	Yen et al. (2002)
Oryzias latipes	48 h-LC_{50}	141.4[c]	Zhou et al. (2007)
Oryzias latipes	48 h-LC_{50}	1.8	Yoshioka et al. (1986)
Gobiocypris rarus	48 h-LC_{50}	133	Zhou et al. (2007)
Culex pipiens	48 h-LC_{50}	70	Canton et al. (1985)
Daphnia magna	48 h-LC_{50}	16.3	Wang and Lv (2004)
Daphnia magna	48 h-EC_{50}	73	Wang et al. (2003a, b)
Daphnia magna	48 h-EC_{50}	34.6	Ramos et al. (1998)
Daphnia magna	48 h-LC_{50}	27	LeBlanc (1980)
Daphnia magna	48 h-LC_{50}	62	Canton et al. (1985)
Daphnia magna	48 h-EC_{50}	35	Canton et al. (1985)
Daphnia magna	48 h-LC_{50}	33	Maas-Diepeveen and Leeuwen (1986)
Daphnia magna	48 h-EC_{50}	33	Deneer et al. (1989)
Daphnia magna	48 h-EC_{50}	58.7	CRAES (2006)
Daphnia carinata	48 h-LC_{50}	39.8	Lv et al. (2004)
Lymnaea stagnalis	96 h-LC_{50}	64.5	Ramos et al. (1998)
Limnodrilus hoffmeisteri	48 h-LC_{50}	116.12	Liu et al. (2008)
Carassius auratus	48 h-LD_{50}	126.1	Li et al. (2007)
Poecilia reticulate[a]	96 h-LC_{50}	135	Ramos et al. (1998)
Oncorhynchus mykiss[a]	96 h-LC_{50}	24.23	Castano et al. (1996)
Lepomis macrochirus[a]	96 h-LC_{50}	43	Buccafusco et al. (1981)
Xiphophorus helleri[b]	96 h-LC_{50}	120.9	Lu and Shen (2002)
Xiphophorus helleri[b]	96 h-LC_{50}	121	Wang et al. (2003a, b)
Xiphophorus helleri[b]	96 h-LC_{50}	141	CRAES (2006)
Danio rerio[b]	96 h-LC_{50}	92	Schafer et al. (1993)
Danio rerio[b]	96 h-LC_{50}	112.5	Wellens (1982)

[a]Alien species extensively cultured in China
[b]Standard toxicity test species
[c]Abandoned data on insensitive life stage of *Oryzias latipes* in WQC development

Table 1.2 Chronic toxicity of nitrobenzene to freshwater animals

Species	Endpoint	ρ(nitrobenzene)/(mg/L)	References
Daphnia magna	21 d-NOEC	13	Kuhn (1988)
Daphnia magna	21 d-NOEC	2.6	Kuhn et al. (1989)
Danio rerio[a]	14 d-NOEC	5	Schafer et al. (1993)

[a]Standard toxicity test species

Table 1.3 Toxicity of nitrobenzene to freshwater plants

Species	Endpoint	ρ(nitrobenzene)/(mg/L)	References
Euglena gracilis	96 h-EC_{50}	119.78	Zhu and Deng (2006)
Scenedesmus obliquus	96 h-EC_{50}	16.5	Wang et al. (2003a, b)
Scenedesmus obliquus	96 h-EC_{50}	18.6	CRAES (2006)
Scenedesmus quadricauda	96 h-EC_{50}	40	Bringmann and Kuhn (1959)
Chlorella pyrenoidosa	72 h-NOEC	9.2	Ramos et al. (1999)
Chlorella pyrenoidosa	96 h-EC_{50}	18	Maas-Diepeveen and Leeuwen (1986)
Chlorella pyrenoidosa	72 h-EC_{50}	28	Ramos et al. (1999)
Chlorella pyrenoidosa	72 h-LOEC	16	Ramos et al. (1999)
Chlorella pyrenoidosa	96 h-EC_{50}	17.8	Deneer et al. (1989)
Selenastrum capricornutum	96 h-EC_{50}	24	Bollman et al. (1989)
Selenastrum capricornutum	96 h-EC_{50}	43	USEPA (1980)
Selenastrum capricornutum	96 h-EC_{50}	44.1	USEPA (1978)
Selenastrum capricornutum	96 h-EC_{50}	42.8	USEPA (1978)
Pseudokirchneriella subcapitata	24 h-EC_{50}	100	USEPA (1978)
Pseudokirchneriella subcapitata	48 h-EC_{50}	53.1	USEPA (1978)
Pseudokirchneriella subcapitata	72 h-EC_{50}	51.6	USEPA (1978)
Pseudokirchneriella subcapitata	96 h-EC_{50}	36.6	USEPA (1978)
Pseudokirchneriella subcapitata	96 h-EC_{50}	35.5	USEPA (1978)
Pseudokirchneriella subcapitata	96 h-EC_{50}	20.79	Bollman et al. (1989)
Pseudokirchneriella subcapitata	96 h-EC_{50}	23.78	Bollman et al. (1989)
Pseudokirchneriella subcapitata	96 h-NOEC	3.2	USEPA (1978)
Skeletonema costatum	96 h-EC_{50}	10.3	USEPA (1978)
Scenedesmus quadricauda	8-d LOEC	33	Bringmann and Kuhn (1978)

values ranged from 3.54 to 14.14 mg/L depending on various endpoints. The results of the *C. auratus* chronic toxicity test showed that the chronic value of nitrobenzene was 5.65 mg/L (Table 1.6). The body length and weight of the fish have no significant differences between the control group and the test group.

Table 1.4 Acute toxicity of nitrobenzene to six resident aquatic organisms

Species	Exposure time (h)	Functions	R^2	p	LC_{50}/(mg/L)[a]
P. fulvidraco	96	$y = 11.391x - 16.448$	0.969	<0.05	76.22 (71.17–80.41)
C. auratus	96	$y = 6.651x - 8.814$	0.956	<0.05	119.1 (108.4–130.3)
R. chensinensis	96	$y = 4.992x - 6.080$	0.924	<0.05	161.9 (144.0–193.4)
C. cahayensis	96	$y = 8.227x - 11.563$	0.989	<0.01	103.0 (88.1–112.3)
M. nipponense	96	$y = 2.940x + 9.162$	0.921	<0.01	0.039 (0.031–0.048)
D. magna	48	$y = 12.867x - 17.135$	0.955	<0.01	52.61 (50.59–55.15)

[a]Linear regression analysis of acute toxicity data was shown in supplementary information

Table 1.5 Chronic toxicity of nitrobenzene to *D. magna*

Endpoint	NOEC/(mg/L)	LOEC/(mg/L)	Chronic value/(mg/L)
Time to first brood	2.50	5.00	3.54
Number of broods	5.00	10.00	7.07
21-d body length	10.00	20.00	14.14
Number of exuviations	10.00	20.00	14.14

Table 1.6 Chronic toxicity of nitrobenzene to *C. auratus*

Endpoint	NOEC/(mg/L)	LOEC/(mg/L)	Chronic value/(mg/L)
Survival	4.0	8.0	5.65
Body length	–	–	–
Body weight	–	–	–

3. Nitrobenzene criteria derivation

By an analysis of all the toxicity data for nitrobenzene, species mean acute values (SMAVs) were calculated as GMAVs, which were calculated as a geometric mean of the SMAVs. The species acute-chronic ratios (SACRs) were calculated as a ratio of acute and chronic values. Ranked GMAVs with SACRs were listed in Table 1.7.

ACMC value of 0.018 mg/L nitrobenzene was obtained by dividing FAV (final acute value, 0.036 mg/L nitrobenzene) by two (USEPA 1985). The final acute-chronic ratio (FACR) was calculated to be 18.60 as geometric means of the three SACRs (21.08, 20.35, and 15.00). The FCV (final chronic value, 0.001 mg/L) was obtained by dividing the FAV by the FACR, and the lower value between FCV and FPV (final plant value, 3.2 mg/L) was selected as the CCC (0.001 mg/L). A CMC of 0.836 mg/L for nitrobenzene was also obtained by the use of a software tool ETX 2.0 (exploited by RIVM) to assess the ecological risk, (Van Vlaardingen et al. 2004). This value was higher than the CMC (0.018 mg/L) calculated according to the USEPA guidelines.

Table 1.7 Ranked GMAVs with SACRs

Rank	GMAVs/(mg/L)[a]	SMAVs/(mg/L)	Species	SACRs
17	161.9	161.9	*Rana chensinensis*	–
16	135	135	*Poecilia reticulate*	–
15	133	133	*Gobiocypris rarus*	–
14	127.29	127.29	*Xiphophorus helleri*	–
13	119.1	119.1	*Carassius auratus*	21.08
12	116.12	116.12	*Limnodrilus hoffmeisteri*	–
11	103.3	103.3	*Cipangopaludina cahayensis*	–
10	101.73	101.73	*Danio rerio*	20.35
9	76.22	76.22	*Pelteobagrus fulvidraco*	–
8	70	70	*Culex pipiens*	–
7	64.5	64.5	*Lymnaea stagnalis*	–
6	43	43	*Lepomis macrochirus*	–
5	39.39	38.99	*Daphnia magna*	15.00
		39.8	*Daphnia carinata*	–
4	24.23	24.23	*Oncorhynchus mykiss*	–
3	1.907	1.907	*Cyprinus carpio*	–
2	1.8	1.8	*Oryzias latipes*	–
1	0.039	0.039	*Macrobrachium nipponense*	–

[a]Normal at 0.05 level (checked by Shapiro–Wilk test)

1.1.1.4 Discussion

In this study, six Chinese freshwater organisms were selected to provide complementary data to the published toxicity data of nitrobenzene. Among them, *P. fulvidraco, R. chensinensis* (tadpole), *C. cahayensis,* and *M. nipponense* have no toxicity data, when *C. auratus* and *D. magna* were tested to validate reported literature toxicity data. The results showed that the toxicity data of nitrobenzene to *C. auratus* and *D. magna* were consistent with previous studies (Kuhn 1988; Kuhn et al. 1989; Li et al. 2007). Toxicity data of three alien species and several standard test species were also applied in the derivation of WQC (Table 1.1). These species were selected because the three alien species are all cultured extensively in China and are therefore suitable species for WQC development when toxicity data are insufficient. Besides, the toxicity data of local species are critical for the derivation of WQC. Previous to the white paper "Aquatic Life: Criteria for Contaminants of Emerging Concern" issued by USEPA (OW/ORD Emerging Contaminants Workgroup 2008), the US guidelines of aquatic life criteria prescribed that only the data of resident species could be used in the calculation of criteria (USEPA 1985). Some study showed that there was no significant difference in the species sensitivity to 2,4-dichlorophenol between native species and non-native species (Jin et al. 2011). The difference between the toxicity data of native and non-native species for deriving WQC deserves future study.

All the gathered acute toxicity data of nitrobenzene are shown in Table 1.7. There are 17 genera in total, 10 fish species included. The most sensitive species to

nitrobenzene is the oriental river prawn, followed by the Chinese brown frog. The extensive SSD of fish results in no significant trend from the existing data.

Because a chronic toxicity test is hard to be conducted, the available chronic toxicity data of nitrobenzene are insufficient to get a WQC. FCV can be obtained by dividing FAV by FACR when the chronic data are insufficient (USEPA 1985). Thus, acute and chronic toxicity data on *C. auratus*, *D. rerio,* and *D. magna* were used to calculate FACR (Tables 1.5 and 1.6).

Generally, except the FCV and FPV statistics, a final residue value (FRV) should be also considered due to high bioaccumulation in the derivation of CCC. However, the FRV was neglected in this study when a previous study (USEPA 1980) showed that nitrobenzene is poorly bioaccumulated.

Two methods to derive the CMC in this study showed that the CMC value derived by the SSD method of RIVM is higher than that described in the US guidelines. This could be explained by this fact that the oriental river prawn is very sensitive to nitrobenzene, and the method of the US guidelines is more relative on the sensitive toxicity data than the RIVM method. The more flexible WQC for nitrobenzene derived in this study may be properly used in regions with fewer shrimp. The CMC and CCC values of nitrobenzene in this study will provide useful data to calculate national and site-specific WQC for nitrobenzene. More endemic aquatic species should be tested to develop a site-specific WQC, especially when the endemic biological composition is significantly different from regions where the WQC have already been established. When water-effect ratio procedure (USEPA 1994) is suggested to be a useful method for quickly deriving the site-specific WQC for nitrobenzene, these additional studies will increase future confidence in the derived criteria values of nitrobenzene.

1.1.1.5 Conclusions

76.22, 119.1, 161.9, 103.0, and 0.039 mg/L are the 96 h-LC_{50} of nitrobenzene for *P. fulvidraco*, *C. auratus*, *R. chensinensis* (tadpole), *C. cahayensis,* and *M. nipponense*, respectively, and 52.61 mg/L is the 48 h-LC_{50} for resident *D. magna* in China. 3.54 and 5.65 mg/L are the chronic values of nitrobenzene for *D. magna* and *C. auratus*, respectively. 0.018 mg/L and 0.001 mg/L are the CMC and CCC of aquatic life criteria for nitrobenzene in China, respectively, with shrimp potentially being the limited factor to the WQC of nitrobenzene.

1.2 Study of Water Quality Criteria for Two Kinds of PAHs

1.2.1 Phenanthrene

1.2.1.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) belong to a class of organic contaminants with two or more fused aromatic rings and can be found in all environmental media (Nadal et al. 2004). Phenanthrene, CAS#85-01-8, is a priority PAHs and

has been shown to have high toxicity to marine diatoms, gastropods, mussels, crustaceans, and fish (Zhang et al. 2014). Phenanthrene (PHE) has been detected in surface water worldwide in recent years, and it has already become the subject of various scientific fields (Callen et al. 2013; Juhasz et al. 2014; Qiao et al. 2006). Previous studies showed that PHE is toxic to aquatic organisms (Meier et al. 2013; Stringer et al. 2012), so it may impose risks to the ambient aquatic environment. However, comprehensive ecological risk assessment of PHE is not able to perform; the reason is the absence of WQC for PHE.

WQCs are the maximum concentrations of pollutants in aquatic environments that they are presumed to not affect organisms and their functions after long-term or short-term exposures (USEPA 2003). WQC as the scientific measures used in water quality standards have played an important role in the management of aquatic environments, especially in China. The establishment of appropriate criteria has become an important area of investigation worldwide (Paul et al. 2008; Yang et al. 2012). There were some toxicity data of PHE available on fish and algae, but few other taxonomic levels were tested, especially for native species in China. So, WQC could not be derived for PHE. Systematic WQC studies are getting more and more attention in recent years in China. Meanwhile, US Environmental Protection Agency (USEPA) guidelines and other SSD methods have been used to derive WQC for some toxicants with an emphasis on using Chinese native species (Wang et al. 2014a, b; Yan et al. 2012b).

In this study, 8 representative and widespread native species were used to 8 acute toxicity tests and 3 chronic tests, and the WQC of PHE was used according to the USEPA guidelines. The aquatic species from different taxonomic levels (3 phyla and 7 families) were selected, which included two Cyprinidae fishes (*Rhodens sinensis* and *Pseudorasbora parva*), a Cobitidae fish (*Misgurnus anguillicaudatus)*, a planktonic crustacean (*D. magna*), a benthic crustacean (*M. nipponense*), an annelid (*Limnodrilus hoffmeisteri*), an insect (*Chironomus plumosus*), and an amphibian (*Rana limnocharis*). Moreover, the SSD method of Holland National Institute for Public Health and the Environment (RIVM) (Van Vlaardingen et al. 2005) and the log-logistic SSD method were both applied to validate the results. Furthermore, the difference of sensitivity between native and non-native species was compared.

The objectives of this work were to (1) provide a supplement to PHE toxicity database, (2) derive WQC for PHE. This work could provide useful information for environmental risk assessment and pollution management for PHE in ambient aquatic environment.

1.2.1.2 Materials and Methods

1. **Collection of published ecotoxicity data for PHE**

The published ecotoxicity data of PHE were collected from the ECOTOX database (http://cfpub.epa.gov/ecotox), the CNKI (http://www.cnki.net), and ELSEVIER (http://www.sciencedirect.com). The data were screened and analyzed according

to the US guidelines for aquatic life criteria (USEPA 1985). The keywords were Phenanthrene, aquatic life, acute toxicity, and chronic toxicity.

2. **Test chemicals and organisms**

PHE, $C_{14}H_{10}$, ≥98 % purity (HPLC), was purchased from J&K Chemical Company. According to the USEPA guidelines, data on at least eight families of aquatic animals drawn from three different phyla and one aquatic plant are required in the derivation of WQC. In our study, in addition to the published ecotoxicity data, eight resident aquatic species in China were chosen for the acute and chronic toxicity tests.

3. **General test conditions**

All tests were static-renewal whereby test solutions were totally replenished at 24 h intervals. Dechlorinated tap water, with activated carbon, was used for dilution in tests. Measured chemical parameters of dilution water were as follows: pH 7.2 ± 0.5, dissolved oxygen (DO) 7.3 ± 0.5 mg/L, total organic carbon 0.02 mg/L, and hardness as $CaCO_3$ 192 ± 0.1 mg/L. The toxicity tests were conducted in three replicates (with each containing 10 organisms) at assigned concentrations, solvent control (DMSO), and blank control. All tests were undertaken at a light:dark photoperiod of 12:12 h.

Test organisms were not fed during the acute test periods. Test chambers were immersed in a water bath adjusted to maintain the water temperature at 20 ± 2 °C, unless otherwise noted. Temperature, DO, and pH were measured in test chambers daily in the acute toxicity tests and at least once a week in the chronic toxicity tests. Biological observations were performed at least once daily. All toxicity tests were conducted according to ASTM standard guidelines (ASTM 1993a, b, c, d; Gaikowski et al. 1999; Yin et al. 2003).

In the acute toxicity test, 48-h-EC_{50} (effective concentration in 50 % of the test organisms over 48 h) for *D. magna* and 96-h-LC_{50} (lethal concentration in 50 % of the test organisms over 96 h) for other aquatic animals were used as main endpoints. In the chronic toxicity test, 21-d-EC_{10} (effective concentration in 10 % of the test organisms over 21 days) for *D. magna* and 28-d-EC_{10} for fishes were used.

4. **Acute toxicity tests**

(a) ***R. sinensis* acute toxicity test**
The fish were obtained from Large Forest Market, Haidian, Beijing. The mean wet weight and length of the fish was 0.30 ± 0.05 g and 4.0 ± 0.5 cm. Fish were exposed test solutions in 5-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 1.30, 1.80, 2.30, 3.00, 3.90, 5.10, and 6.6 mg/L PHE, respectively.

(b) ***M. anguillicaudatus* acute toxicity test**
The fish were obtained from Chaolai Market, Chaoyang, Beijing. The mean wet weight and length of the fish were 0.70 ± 0.05 g and 6.0 ± 0.5 cm, respectively. Fish were exposed to test solutions in 10-L

glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.00 mg/L PHE, respectively.

(c) ***D. magna*** **acute toxicity test**
D. magna (<24 h age) was obtained from State Environmental Protection Key Laboratory of Ecological Effect and Risk Assessment of Chemicals, Chinese Research Academy of Environmental Sciences. The species were exposed to 100 mL test solution in 150-mL beakers for 48 h. Nominal concentrations in the definitive test were 0.00, 0.14, 0.20, 0.27, 0.38, 0.480, 0.54, and 0.75 mg/L PHE, respectively.

(d) ***M. nipponense*** **acute toxicity test**
The shrimp were obtained from Large Forest Market, Haidian, Beijing. The mean wet weight and length of the fish were 0.2 ± 0.05 g and 3.0 ± 0.2 cm, respectively. Shrimp were exposed to test solutions in 2-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.60, 0.70, 0.90, 1.20, 1.60, 2.00, and 2.60 mg/L PHE, respectively.

(e) ***L. hoffmeisteri*** **acute toxicity test**
The Huo Fu tubifex were obtained from Large Forest Market, Haidian, Beijing. The mean wet weight and length of the tubifex were 0.05 ± 0.01 g and 1.5 ± 0.2 cm, respectively. Tubifex were exposed test solutions in 10 mL test solutions in glass culture dish for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.60, 0.70, 0.90, 1.20, 1.60, 2.00, and 2.60 mg/L PHE, respectively.

(f) ***P. parva*** **acute toxicity test**
The fish were obtained from Chaolai Market, Chaoyang, Beijing. The mean wet weight and the length of the fish were 0.25 ± 0.02 g and 2.5 ± 0.2 cm, respectively. Fish fry were exposed to test solutions in 5-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.28, 0.37, 0.48, 0.62, 0.81, 1.05, and 1.37 mg/L PHE, respectively.

(g) ***C. plumosus*** **acute toxicity test**
The midges were obtained from Chaolai Market, Chaoyang, Beijing. The mean wet weight of the organism was 0.03 ± 0.01 g. The species were exposed to 25 mL test solutions in glass culture dish for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.28, 0.37, 0.48, 0.62, 0.81, 1.05, and 1.37 mg/L PHE, respectively.

(h) ***R. limnocharis*** **acute toxicity test**
The tadpoles were obtained from Olympic Forest Park, Chaoyang, Beijing. The mean body length of the tadpoles was 1.60 ± 0.20 cm. Tadpoles were exposed to test solutions in 2-L beakers for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.38, 0.46, 0.55, 0.66, 0.79, 0.95, and 1.24 mg/L PHE, respectively.

5. ***Chronic*** **toxicity tests**

(a) ***R. sinensis*** **chronic toxicity test**
The fish used in the chronic toxicity test were the same as in the acute toxicity test. Twenty-eight-day short-term chronic toxicity tests were conducted. The fish were fed with brine shrimp at a rate of 0.1 % body weight twice daily. Endpoints observed included survival, growth, and body weight. Nominal exposure concentrations in the definitive test were 0.00, 0.35, 0.46, 0.60, 0.78, 1.01, and 1.32 mg/L PHE, respectively.

(b) ***M. anguillicaudatus*** **chronic toxicity test**
Twenty-eight-day short-term chronic toxicity tests were conducted. The fish were fed with brine shrimp at a rate of 0.1 % body weight twice daily. Endpoints observed included survival, growth, and body weight. Nominal exposure concentrations in the definitive test were 0.00, 0.42, 0.55, 0.72, 0.94, 1.22, and 1.59 mg/L PHE, respectively.

(c) ***D. magna chronic toxicity test***
Twenty-one-day survival-reproduction tests using neonates of *D. magna* (<24 h age) were conducted in 150-mL beakers filled with 100 mL test solutions. The daphnia were fed once every day with green algae (*S. obliquus*) that had a cell concentration of 1.0×10^5 cells/mL in the test solution. The survival, growth, and reproduction were recorded daily. The endpoints include the time to first brood, number of broods, the 21-d body length and number of exuviations. Nominal concentrations in the definitive test were 0.00, 0.03, 0.04, 0.05, 0.06, 0.08, 0.10, 0.13, 0.17, and 0.22 mg/L PHE, respectively. The toxicity tests were carried out in ten replicates with each containing one organism.

6. **Chemical analysis**

Briefly, the samples were filtered through a 0.45-μm filter membrane and determined by an Agilent 7,890 GC equipped with 5,975 mass selective detector (MSD) under full scanning mode (FSM), scan range 35–550 amu. A DB-5 silica-fused capillary column (30 m × 0.25 mm × 0.25 μm) was used with helium as the carrier gas at a constant flow rate of 1 ml/min. Split injection of 1 μl at split ratio 10:1 of the sample was conducted with an auto sampler. The GC oven temperature was programmed from 50 to 300 °C at 8 °C/min and kept for 5 min. The injector and detector temperatures were 280 and 300 °C, respectively. Mass spectra were acquired at the electron ionization (EI) mode with an electron multiplier voltage of 906 eV. Before sample analysis, the instrument was tuned daily with decafluorobiphenyl phosphine (DFTPP). PHE in the samples was identified by the retention time and the abundance of quantification ions/confirmation ions with respect to authentic PHE standards. Automated library searching was performed using the National Institute of Standards and Technology (NIST) Mass Spectral Database. Quantization was performed using the five-point calibration curve for individual components. Detection limits were 1.4–3.8 ng/L for PHE. Following the

establishment of a response to a known concentration, the result of measured/nominal concentration was 93.14–102.58 %. For quality control, the concentrations of PHE from test groups of the minimum and the maximum concentrations were detected, and the variation was within 20 %, in compliance with the requirement of the toxicity test guidelines. Because the concentrations of all test groups were not detected, all subsequent toxicity results were expressed based on the nominal concentrations of PHE (Bouloubassi and Saliot 1991; Karacık et al. 2013).

Laboratory quality control procedures include analyses of method blanks (solvent), spiked blanks (standards spiked into solvent), matrix spikes/matrix spike duplicates, and sample duplicates. Instrument stability and response were checked using NIST standard solutions. The instruments were calibrated daily with calibration standards and the relative percent differences between the five-point calibration, and the daily calibrations were <20 % for all of the target analyses. The recoveries for surrogate standards fell within a fairly narrow range, 81.89 ± 18.17 % (phenanthrene-d_{10}).

7. **Statistical analysis and SSD generation**

Probit methodology was employed to calculate the 48-h-EC_{50}, 96-h-LC_{50} values and corresponding 95 % confidence intervals. Data of the chronic toxicity tests were fit into linear regressions (with *y* axis as percentage of responses and *x* axis as the common logarithm of PHE concentrations), and the EC_{10} values for all chronic endpoints were calculated.

Three procedures, the USEPA guidelines for WQC, the software ETX2.0 (Orvos et al. 2002) exploited by the RIVM, and the log-logistic SSD method were used to derive the WQC for PHE. SPSS 20.0 (IBM, Armonk, NY) and Origin 8.0 (OriginLab, Northampton, MA) were used for data analysis.

1.2.1.3 Results and Discussion

1. **Results of toxicity tests of eight native aquatic organisms**

Acute toxicity values of PHE to 8 aquatic species are shown in Table 1.8. No mortality was observed in the control groups and the solvent control groups.

Results of acute toxicity tests using eight aquatic species (Table 1.8) showed that *D. magna* with a 96-h-LC_{50} of 0.275 mg/L was the most sensitive species to PHE followed by *C. plumosus, P. parva, R. limnocharis, L. hoffmeisteri*, *M. nipponense,* and *R.sinensis* with decreasing sensitivity, while the least sensitive species was *M. anguillicaudatus* with a 96-h-LC_{50} of 3.684 mg/L. Results of this study indicate that PHE is highly toxic to native freshwater aquatic organisms especially the planktonic crustacean. It was reported that the 48-h-LC_{50} of PHE on water flea *D. magna* was 0.230 mg/L (Black et al. 1983). This is close to the result of our study (0.275 mg/L). In addition, previous study found that demersal fish *M. anguillicaudatus* is sensitive to some organochlorine pesticide (Wang et al. 2013a, b), while *M. anguillicaudatus* is not sensitive to PHE. That is, species observed

Table 1.8 Acute toxicity of PHE to eight resident aquatic organisms

Species	Exposure time (h)	Functions	R^2	p	LC_{50}/(mg/L)
R. sinensis	96	$y = 3.5541x - 7.1086$	0.9917	<0.01	2.550 (2.429–2.671)
M. anguillicaudatus	96	$y = 2.5550x - 4.1122$	0.9727	<0.01	3.684 (3.571–3.797)
D. magna	48	$y = 2.7539x - 1.7168$	0.9850	<0.01	0.275 (0.199–0.351)
M. nipponense	96	$y = 4.9140x - 9.9069$	0.9468	<0.01	1.079 (0.983–1.175)
L. hoffmeisteri	96	$y = 3.6980x - 5.7350$	0.9315	<0.01	0.799 (0.702–0.896)
P. parva	96	$y = 2.8940x - 2.9237$	0.9529	<0.01	0.547 (0.485–0.622)
C. plumosus	96	$y = 3.1371x - 3.3590$	0.9261	<0.01	0.462 (0.390–0.559)
R. limnocharis	96	$y = 4.1552x - 6.6347$	0.9044	<0.01	0.631 (0.523–0.718)

to be sensitive species to specific chemicals were also significantly different (Yan et al. 2013; Zhang et al. 2010). Among different species, the aquatic invertebrates of annelid and insect were more sensitive than the fishes, and the benthic fishes were the least sensitive species.

Chronic toxicity values of PHE to 3 aquatic species are shown in Table 1.9. The chronic data cannot be compared with previous studies due to lack of resident toxicity data in China.

Results of our study and previous studies using non-native species (Table 1.10) were compared. Previous studies reported that 96-h-LC_{50} for fish *Lepomis macrochirus*, *Oncorhynchus mykiss,* and *Cyprinodon variegatus* were 0.234, 0.375, and 0.478 mg/L, respectively (Call et al. 1986; Moreau et al. 1999), and this was not in accordance with native species. In this study, we found that 48-h-LC_{50} for planktonic crustacean *D. magna* was 0.275 mg/L. It is in agreement with previous studies which reported that toxicity value for planktonic crustacean *D. pulex*

Table 1.9 Chronic toxicity of PHE to three resident aquatic organisms

Species	Endpoints	Functions	R^2	p	EC_{10}/(mg/L)
R. sinensis	Longevity (*d*)	$y = 3.0980x - 4.5453$	0.9930	<0.01	0.435
M.anguillicaudatus	Longevity (*d*)	$y = 3.3827x - 5.5225$	0.9743	<0.01	0.540
D. magna	Longevity (*d*)	$y = 0.6883x + 0.8547$	0.8971	<0.05	0.191
	Time to first brood (*d*)	$y = 0.3410x + 0.8649$	0.9572	<0.01	0.337
	Number to first brood (*n*)	$y = 0.8538x + 1.3222$	0.9315	<0.01	0.147
	Total number of spawning (*n*)	$y = 0.6488x + 1.2801$	0.9203	<0.01	0.060
	Number of broods (*n*)	$y = 1.7450x + 1.5623$	0.9364	<0.01	0.471

Table 1.10 Acute toxicity data of PHE to non-native aquatic organisms

Rank	Species	LC_{50}/EC_{50}(mg/L)	Time (days)	References
1	*A. bahia*	0.027	4	Miller et al. (1988)
2	*G. pseudolimnaeus*	0.126	4	Call et al. (1986)
3	*L. macrochirus*	0.234	4	Call et al. (1986)
4	*D. pulex*	0.350	2	Geiger and Buikema Jr (1982)
5	*O. mykiss*	0.375	4	Call et al. (1986)
6	*L. variegatus*	0.419	4	Call et al. (1986)
7	*C. variegatus*	0.478	4	Moreau et al. (1999)
8	*N. arenaceodentata*	0.600	4	Rossi and Neff (1978)
9	*B. calyciflorus*	1.098	1	DellaGreca et al. (2001)
Algae	*L. minor*	0.658	4	Hailing-Sorensen et al. (1996)

was 0.350 mg/L (Evans and Nipper 2008; Jin et al. 2011; Louati et al. 2013). We found that 96-h-LC_{50} for native crustacean *M. nipponense* was 1.079 mg/L, it was 40-fold higher than that of non-native crustacean *Americamysis bahia* which was 0.027 mg/L (Lussier et al. 1999). Call et al. reported that the 96-h-EC_{50} of PHE on annelid *L. variegatus* was 0.419 mg/L, and this was lower than the value in our study that the 96-h-EC_{50} of PHE on *L. hoffmeisteri* was 0.799 mg/L (Call et al. 1986). The acute toxicity of the insect *C. plumosus* and amphibian *R. limnocharis* cannot be compared with previous studies due to lack of toxicity data for resident and non-native freshwater species.

For chronic test, Call et al. reported that 21 days NOEC for the survival of *D. Magna* was 0.163 mg/L, whereas the 21-d-EC_{10} for total number of spawning was 0.057 mg/L and no mortality was observed in all concentration groups in this study, which indicated that total number of spawning is a more sensitive endpoint than survival (Call et al. 1986). The 28-d-EC_{10} for growth of fish *R. sinensis* was 0.435 mg/L, and previous studies reported that 32 days NOEC for survival of *O. mykiss* was 0.066 mg/L PHE (Call et al. 1986). Similarly, as mentioned in the above paragraph, the planktonic crustacean *D. magna* is also the most sensitive species in the chronic tests in this study.

In general, the sensitivities of the native species tested in this study were not similar to those reported in previous studies of non-native species.

5. Comparison of SSD between native and non-native taxa

Davies et al. (1994) brought up the question about the feasibility of using toxicity data of species from one geographical region to assess the ecological risk posed to species in a different region. Moreover, differences in the sensitivity of cold-water, temperate, and tropical fish species have been reported (Allen and Hansen 1996; Dyer et al. 1997). In this study, the difference of SSD based on native and non-native species was compared (Chapman et al. 2006; Cheung et al. 2007; Yan et al. 2012a). Since there has been few information on the toxicity of PHE to native and non-native species, comparison could only be conducted on the SSDs constructed

Table 1.11 Ranked GMAVs with SACRs

Rank	Species	SMAVs/(mg/L)	GMAVs/(mg/L)	SACRs
1	*Hydra* sp.	0.096	0.096	
2	*D. magna*	0.275	0.251	4.18
		0.230		
3	*C. plumosus*	0.462	0.462	
4	*P. parva*	0.547	0.547	
5	*R. limnocharis*	0.631	0.631	
6	*L. hoffmeisteri*	0.799	0.799	
7	*M. nipponense*	1.079	1.079	
8	*R. sinensis*	2.550	2.550	5.86
9	*M.anguillicaudatus*	3.684	3.684	6.82
Duckweed	*L. minor*	0.658	0.658	

from acute toxicity data of native (Table 1.11) and non-native (Table 1.10) species (Aldenberg and Jaworska 2000; Newman et al. 2000; Wheeler et al. 2002).

Based on the comparison in this study (Fig. 1.1), the overall SSD curves were non-native taxa, all taxa, and native taxa from left to right; it indicated that the non-native freshwater organisms with acute exposures to PHE were more sensitive than the native species in China. The HC_5s were 0.0780, 0.0785, and 0.0854 mg/L, respectively.

Although there were differences for HC_5 between native and non-native taxa, the comparison of SSD showed that there was no statistically significant difference (Pearson's correlation test: pc = 0.948, $n1 = 9$, $n2 = 9$, $p < 0.01$). Considering the lack of toxicity data for insect and amphibian in non-native taxa, the difference was also not significant (pc = 0.830, $n1 = 7$, $n2 = 9$, $p = 0.021$) when removing the two taxa toxicity values (Diamond et al. 2006; Dyer et al. 2006).

Similarly, sensitivities among North American and European taxa with different geographic distributions to a series of pollutants have been shown to be not statistically different (Dyer et al. 1997; Feng et al. 2012). Moreover, the difference between SSDs of Australian and non-Australian organisms to endosulfan was not significant. Jin et al. (2011) also reported that there was no statistically significant difference

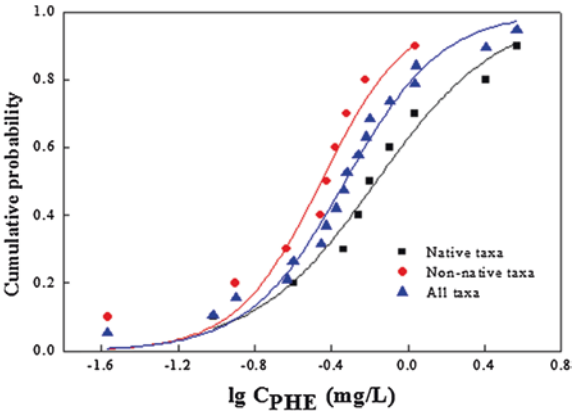

Fig. 1.1 Species sensitivity distribution of native, non-native, and all species toxicity data for PHE

between SSDs of native and non-native species when exposed to 2,4-dichlorophenol. In addition, in this study, there was no statistically significant difference between the sensitivity of native and total species (Fig. 1.1) as well (pc = 0.817, $n1 = 9$, $n2 = 18$, $p = 0.007$) (Grimmer et al. 1997). Previous study found that natural history, habitat type, and geographical distribution of the species used to construct the SSD did not have a significant influence on the assessment of hazard, and this was in accordance with this study (Frampton et al. 2006; Hose and Van den Brink 2004).

6. **WQC derivation**

The USEPA guidelines of WQC prescribed that only the toxicity data of resident species could be used in the calculation of criteria. In this study, using ten species toxicity values (Table 1.10), three methods were used to derive the aquatic life WQC of PHE including USEPA guidelines, software ETX 2.0 (exploited by RIVM) (Unger et al. 2007; Van Vlaardingen et al. 2004) and log-logistic (SSD) method (Paul et al. 2008; Raimondo et al. 2007). Using the methods described in USEPA guidelines, the SMAVs and the genus mean acute values (GMAVs) were calculated. The species acute-chronic ratios (SACRs) were calculated as a ratio of acute and chronic values. Ranked GMAVs with SACRs are listed in Table 1.10. A CMC of 0.033 mg/L PHE was obtained by dividing FAV (final acute value, 0.066 mg/L PHE) by two. The FACR was calculated as geometric means of the three SACRs (4.18, 5.86 and 6.82), which was 5.51. The final chronic value (FCV; 0.012 mg/L) was obtained by dividing the FAV by the FACR. Final plant value (FPV) was the toxicity data of *L. minor*, calculated to be 0.658 mg/L. The lower value between FCV and FPV was selected as the CCC 0.012 mg/L.

In addition, the values of HC_5 derived based on the ETX 2.0 and the constructed log-logistic SSDs were 0.102 and 0.0854 mg/L, respectively. Therefore, the CMCs of PHE developed with the two SSD methods were 0.051 and 0.0427 mg/L when the factor is 2, which were in the same order of magnitude with the CMC (0.033 mg/L) that calculated according to the USEPA guidelines.

Some environmental pollutants, such as ammonia and heavy metals, have a lot of toxicity data for the derivation of their WQC (Allen and Hansen 1996; Augspurger et al. 2003). However, few toxicity data of PHE from previous study have been available (Mitra et al. 2000). As is well known, the certainty of derived WQC can be improved with increasing amounts of toxicity data. So, it is essential to acquire toxicity data of more native species, especially sensitive species. Meanwhile, the toxicity of PHE may be affected by pH, hardness, temperature, and organic matter in water body (Wang et al. 2013a, b). Therefore, the influences of regional water quality characteristics on the toxicity of PHE and subsequently on the WQC of this compound should be investigated in the future (Juhasz et al. 2014).

1.2.1.4 Conclusions

This study is a contribution in the assessment of the effect of PHE in the aquatic environment. Toxicity values of 8 acute and 3 chronic tests for 8 native species from 3 phyla and 7 families were obtained in this study, among which planktonic

crustacean *D. magna* was the most sensitive species, and the aquatic invertebrates of annelid and insect were more sensitive than the fishes. Furthermore, the CMC derived using USEPA guidelines is similar as the results derived from RIVM ETX2.0 and the log-logistic SSD method, the CMC and CCC of WQC for PHE are 0.0514 and 0.0186 mg/L, respectively.

1.2.2 Benzo[a]pyrene

1.2.2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) with two or more fused aromatic rings belong to a class of organic contaminants and can be found in all environmental media (Nadal et al. 2004). In recent years, Benzo[*a*]pyrene (BaP, CAS#50-32-8), as a priority PAH, has been detected at high levels in surface water worldwide and reported to pose potential risks to ambient aquatic environment (Meier et al. 2013; Stringer et al. 2012).

WQC are the maximum concentrations of pollutants in aquatic environments that presumed to not affect organisms and their functions after long-term or short-term exposure (USEPA 2003). As the scientific measures used in water quality standards, WQC have played an important role in the management of aquatic environments in China, and the establishment of appropriate criteria have become an important area of investigation worldwide (Paul et al. 2008; Yang et al. 2012). In China, no WQC for BaP is available as there is lack of toxicity data for native species.

In this study, 8 acute toxicity tests and 3 chronic tests were conducted for 8 representative and widespread native species, and the WQC of BaP for freshwater aquatic life was derived using a battery of toxicity data according to the USEPA guidelines. The aquatic species from different taxonomic levels (from 3 phyla and 8 families), including a Siluridae fish (*Silurus asotus*), a Cyprinidae fish (*Cyprinus flammans*), a Cobitidae fish (*M. anguillicaudatus*), a planktonic crustacean (*D. magna*), a benthic crustacean (*M. nipponense*), an annelid (*L. hoffmeisteri*), an insect (*C. plumosus*), and an amphibian (*R. limnocharis*). Moreover, the SSD method of Holland National Institute for Public Health and the Environment (RIVM) (Van Vlaardingen et al. 2005) and the log-logistic SSD method were both applied to validate the results. Furthermore, by using risk quotient (RQ) to assess the site-specific ecological risk in Taihu Lake, the results indicated that the BaP might pose risk to local aquatic species in Meilianghu Area.

The objectives of this work were to: (1) provide a supplement to BaP toxicity database, and (2) derive WQC for BaP.

1.2.2.2 Materials and Methods

1. **Collection of published ecotoxicity data for BaP**

The published ecotoxicity data of BaP were collected from the ECOTOX database (http://cfpub.epa.gov/ecotox), TOXNET Database (http://toxnet.nlm.nih.gov),

GEO Database (www.ncbi.nlm.nih.gov/geo), the CNKI (http://www.cnki.net), and ELSEVIER (http://www.sciencedirect.com). The data were screened and analyzed according to the USEPA guidelines for aquatic life criteria (USEPA 1985). The keywords were "Benzo[*a*]pyrene," "aquatic life," "acute toxicity," and "chronic toxicity."

2. **Test chemicals and organisms**

BaP, $C_{20}H_{12}$, ≥98 % purity (HPLC), was purchased from J&K Chemical Company.

According to the USEPA guidelines, data on at least eight families of aquatic animals drawn from three different phyla and one aquatic plant are required in the derivation of WQC. In this study, in addition to the published ecotoxicity data for BaP, eight resident aquatic species in China were chosen for the acute tests and chronic toxicity tests.

3. **General test conditions**

All tests were static-renewal whereby test solutions were totally replenished at 24 h intervals. Dechlorinated tap water treated with activated carbon was used for dilution in toxicity tests. Measured chemical parameters of dilution water were as follows: pH 7.2 ± 0.5, dissolved oxygen (DO) 7.3 ± 0.5 mg/L, total organic carbon 0.02 mg/L, and hardness as $CaCO_3$ 192 ± 0.1 mg/L. The toxicity tests were conducted in three replicates (with each containing 10 organisms) at assigned concentrations, solvent control, and blank control. All tests were undertaken at a light:dark photoperiod of 12:12 h.

Test organisms were not fed during the acute test periods. Test chambers were immersed in a water bath with water temperature maintained at 20 ± 2 °C, unless otherwise noted. Temperature, DO, and pH were measured in test chambers daily. Biological observations were performed at least once daily. All toxicity tests were conducted according to American Society for Testing and Materials (ASTM) standard guidelines (ASTM 1993a, b, c, d; Gaikowski et al. 1999; Yin et al. 2003). 48-h-EC_{50} (effective concentration in 50 % of the test organisms over 48 h) for *D. magna* and 96-h-LC_{50} (lethal concentration in 50 % of the test organisms over 96 h) for other aquatic animals were used as test endpoints.

4. **Acute toxicity tests**

All the organisms tested in this study were obtained from Chaolai and Large Forest aquaculture companies. Prior to toxicity tests, all test organisms were acclimated to general test conditions (see above Sect. 2.2) for a minimum of 7 days. *D. magna* (<24 h age) was obtained from in-house cultures at our chemical laboratory of Chinese Research Academy of Environmental Sciences. Acute toxicity tests of BaP to eight resident aquatic organisms are shown in Table 1.12.

5. **Chronic toxicity tests**

(a) ***C. flammans* chronic toxicity test**

The fish used in the chronic toxicity test were the same as in the acute toxicity test. Twenty-eight-day short-term chronic toxicity tests were conducted. The fish were fed with brine shrimp at a rate of 0.1 % body weight twice daily. Endpoints observed included survival, growth, and body

Table 1.12 Acute toxicity tests of BaP to eight resident aquatic organisms

Species	Wet weigh (g)	Length (cm)	Time (h)	Exposure concentrations (μg/L)
C. flammans	0.30 ± 0.02	2.5 ± 0.2	96	0.00, 0.46, 0.58, 0.76, 1.00, 1.30, 1.70, 2.20
S. asotus	0.40 ± 0.05	3.0 ± 0.5	96	0.00, 2.20, 2.90, 3.70, 4.80, 6.30, 8.20, 10.70
M. anguillicaudatus	0.70 ± 0.05	6.0 ± 0.5	96	0.00, 12.8, 16.7, 21.7, 28.3, 36.7, 47.8, 62.1
D. magna			48	0.00, 16.1, 20.2, 0.27, 0.38, 0.48, 0.54, 0.75
M. nipponense	0.25 ± 0.05	3.0 ± 0.2	96	0.00, 0.60, 0.70, 0.90, 1.20, 1.60, 2.00, 2.60
L. hoffmeisteri	0.05 ± 0.01	1.5 ± 0.2	96	0.00, 0.60, 0.70, 0.90, 1.20, 1.60, 2.00, 2.60
C. plumosus	0.03 ± 0.01	1.0 ± 0.2	96	0.00, 0.28, 0.37, 0.48, 0.62, 0.81, 1.05, 1.37
R. limnocharis	0.20 ± 0.02	1.6 ± 0.2	96	0.00, 0.38, 0.46, 0.55, 0.66, 0.79, 0.95, 1.24

weight. Nominal exposure concentrations in the definitive test were 0.00, 3.84, 5.00, 6.50, 8.45, 11.00, 14.28, and 18.56 μg/L BaP, respectively.

(b) ***M. anguillicaudatus*** **chronic toxicity test**
Twenty-eight-day short-term chronic toxicity tests were conducted. The fish were fed with brine shrimp at a rate of 0.1 % body weight twice daily. Endpoints observed included survival, growth, and body weight. Nominal exposure concentrations in the definitive test were 0.00, 7.00, 8.70, 11.31, 14.70, 19.11, and 24.85 μg/L BaP, respectively.

(c) ***D. magna*** **chronic toxicity test**
Twenty-one-day survival-reproduction tests using neonates of *D. magna* (<24 h age) were conducted in 150-mL beakers filled with 100 mL test solutions. The daphnia were fed once every day with green algae (*S. obliquus*) that had a cell concentration of 1.0×10^5 cells/mL in the test solution. The survival, growth, and reproduction were recorded daily. The endpoints include the time to first brood, number of broods, the 21-d body length, and number of exuviations. Nominal concentrations in the definitive test were 0.00, 0.70, 0.91, 1.18, 1.54, 2.00, and 2.60 μg/L BaP, respectively. The toxicity tests were carried out in ten replicates with each containing one organism.

6. **Chemical analysis**

Please see Sect. 1.2.1.2 (6).

7. **Statistical analysis and SSD generation**

Please see Sect. 1.2.1.2 (7).

1.2.2.3 Results and Discussion

(1) Results of toxicity tests of eight native aquatic organisms

Acute toxicity values of BaP to 8 aquatic species are shown in Table 1.13. No mortality was observed in the control groups and the solvent control groups.

Results of acute toxicity tests using eight aquatic species (Table 1.13) showed that *D. magna* with a 96-h-LC_{50} of 1.298 μg/L was the most sensitive species to BaP followed by *L. hoffmeisteri*, *C. plumosus*, *C. flammans*, *S. asotus, R. limnocharis,* and *M. nipponense* with decreasing sensitivity, while the least sensitive species was *M. anguillicaudatus* with a 96-h-LC_{50} of 29.98 μg/L. Results of this study indicate that BaP is highly toxic to native freshwater aquatic organisms especially the planktonic crustacean. It had been reported that the 48-h-LC_{50} of BaP on water flea *D. magna* was 1.5 μg/L (Black et al. 1983), which is consistent with the result of our study (1.298 μg/L). In addition, previous study reported that demersal fish *M. anguillicaudatus* was sensitive to some organochlorine pesticides (Wang et al. 2013a, b), while it was not sensitive to BaP. That is, species observed to be sensitive to specific chemicals were also significantly different (Oliveira et al. 2010; Yan et al. 2013; Zhang et al. 2010). In general, the aquatic invertebrates (e.g., annelid and insect) are more sensitive than the fishes, and the benthic fishes are the least sensitive species (Wang et al. 2013a, b).

Chronic toxicity values of BaP to 3 aquatic species are shown in Table 1.14. The chronic data cannot be compared with previous studies due to lack of resident toxicity data in China.

2. WQC deriving

The USEPA guidelines of WQC prescribed that only the toxicity data of resident species could be used in the calculation of criteria. In this study, using ten species

Table 1.13 Acute toxicity of BaP to eight resident aquatic organisms

Species	Exposure time (h)	Functions	R^2	p	LC_{50}/(μg/L)
C. flammans	96	$y = 1.6097x + 4.1009$	0.9422	<0.01	3.626 (3.517–3.804)
S. asotus	96	$y = 2.0478x + 4.7683$	0.9324	<0.01v	5.000 (4.881–5.237)
M. anguillicaudatus	96	$y = 1.1701x + 3.2723$	0.9498	<0.01	29.98 (27.43–31.17)
D. magna	48	$y = 2.8499x - 1.6745$	0.9769	<0.01	1.298 (1.174–1.406)
M. nipponense	96	$y = 2.1227x + 3.1271$	0.9491	<0.01	7.632 (7.155–7.848)
L. hoffmeisteri	96	$y = 1.6399x + 4.6474$	0.9651	<0.01	1.642 (1.498–1.769)
C. plumosus	96	$y = 1.2792x + 4.6587$	0.9296	<0.01	1.851 (1.694–1.987)
R. limnocharis	96	$y = 9.1498x - 1.5991$	0.9893	<0.01	5.264 (5.101–5.423)

Table 1.14 Chronic toxicity of BaP to three resident aquatic organisms

Species	Endpoints	Functions	R^2	p	EC_{10}/(μg/L)
C. flammans	Longevity (*d*)	$y = 5.7681x + 5.1211$	0.9855	<0.01	0.960
M.anguillicaudatus	Longevity (*d*)	$y = 9.3612x - 2.5461$	0.9717	<0.01	8.681
D. magna	Longevity (*d*)	$y = 7.5714x - 1.3980$	0.9608	<0.01	0.300
	Time to first brood (*d*)	$y = 7.2891x - 1.7987$	0.9219	<0.01	0.345
	Number to first brood (*n*)	$y = 5.4690x + 2.2566$	0.9203	<0.01	0.374
	Total number of spawning (*n*)	$y = 9.8461x - 3.2369$	0.9364	<0.01	0.369
	Number of broods (*n*)	$y = 5.7681x + 5.1211$	0.9855	<0.01	0.960

toxicity values (Table 1.15), three methods were used to derive the aquatic life WQC of BaP including USEPA guidelines, software ETX 2.0 (exploited by RIVM) (Unger et al. 2007; Van Vlaardingen et al. 2004) and log-logistic (SSD) method (Paul et al. 2008; Raimondo et al. 2007). Using the methods described in USEPA guidelines, the SMAVs and the GMAVs were calculated. The SACRs were calculated as a ratio of acute and chronic values. Ranked GMAVs with SACRs are listed in Table 1.15. A CMC of 0.73 μg/L BaP was obtained by dividing FAV (final acute value, 1.46 μg/L BaP) by two. The FACR was calculated as geometric means of the three SACRs (3.78, 3.45, and 4.33), which was 3.84. The final chronic value (FCV; 0.38 μg/L) was obtained by dividing the FAV by the FACR. Due to lake of FPV in previous studies, FCV was selected as the CCC 0.38 μg/L.

In addition, the values of HC_5 derived based on the ETX 2.0 and the constructed log-logistic SSD were 1.79 and 1.68 μg/L, respectively (Fig. 1.2). Therefore, the CMCs of BaP developed with the two SSD methods were 0.90 and 0.84 μg/L when the factor is 2, which were in the same order of magnitude with the CMC (0.73 μg/L) that calculated according to the USEPA guidelines.

Some environmental pollutants, such as ammonia and heavy metals, have a lot of toxicity data for the derivation of their WQC (Allen and Hansen 1996; Augspurger et al. 2003). However, few toxicity data of BaP from previous study have been available (Mitra et al. 2000). As is well known, the certainty of derived WQC can be improved with increasing amounts of toxicity data. So, it is essential to acquire

Table 1.15 Ranked GMAVs with SACRs

Rank	Species	SMAVs/(μg/L)	GMAVs/(μg/L)	SACRs
1	*D. magna*	1.298	1.298	4.33
2	*L. hoffmeisteri*	1.642	1.642	
3	*C. plumosus*	1.851	1.851	
4	*C. flammans*	3.626	3.626	3.84
5	*S. asotus*	5.000	5.000	
6	*R. limnocharis*	5.264	5.264	
7	*M. nipponense*	7.632	7.632	
8	*M. anguillicaudatus*	29.98	29.98	3.45

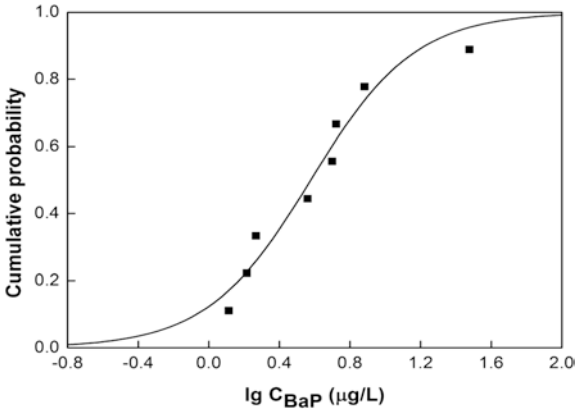

Fig. 1.2 Species sensitivity distribution of 8 native species toxicity data for BaP

toxicity data of more native species, especially sensitive species. Meanwhile, the toxicity of BaP may be affected by pH, hardness, temperature, and organic matter in water body (Wang et al. 2013a, b). Therefore, the influences of regional water quality characteristics on the toxicity of BaP and subsequently on the WQC of this compound should be investigated in the future (Juhasz et al. 2014).

1.2.2.4 Conclusions

This study is a contribution in the assessment of the effect of BaP in the aquatic environment. Toxicity values of 8 acute and 3 chronic tests for 8 native species from 3 phyla and 8 families were obtained in this study, among which planktonic crustacean *D. magna* was the most sensitive species, and the aquatic invertebrates of annelid and insect were more sensitive than the fishes. Furthermore, the CMC derived using USEPA guidelines is similar as the results derived from RIVM ETX2.0 and the log-logistic SSD method, and the CMC and CCC of WQC for BaP are 0.73 and 0.38 μg/L, respectively. Furthermore, by using risk quotient (RQ) to assess the site-specific ecological risk in Taihu Lake, the results indicated that the BaP might pose risk to local aquatic species in Meilianghu Area.

1.3 Study of Water Quality Criteria for Emerging Pollutants

1.3.1 Triclosan (TCS)

1.3.1.1 Introduction

Personal care products (PCPs) are broadly used in everyday life and can be found in all environmental media (Amorim et al. 2010; Ternes et al. 2004). The possible effects of PCPs on nontarget organisms are of great concern worldwide

(Daughton and Ternes 1999; Kümmerer 2004; Veldhoen et al. 2006). Among the PCPs, triclosan (TCS, CAS#3380-34-5) is a broad spectrum antimicrobial agent used in a variety of personal care products including soaps, deodorant, and toothpaste (McAvoy et al. 2009; Price et al. 2010). Water environment is of great concern of government and the society, and TCS has been detected in surface water worldwide in the recent years (Ciniglia et al. 2005; Dayan 2007; Benotti et al. 2008; Kasprzyk-Hordern et al. 2008; Kim et al. 2009a, b; Zhao et al. 2009; Dougherty et al. 2010; Zhao et al. 2010; Ramaswamy et al. 2011; Wang et al. 2011; Venkatesan et al. 2012). Moreover, toxicity tests showed that TCS is toxic to aquatic species (Ishibashi et al. 2004; Orvos et al. 2009; Yang et al. 2009; Palenske et al. 2010); so, it may impose a potential risk to the ambient water environment. However, comprehensive ecological risk assessment of TCS is hard to perform due to the absence of WQC for TCS. WQC is an important basis for the development of water quality standards (WQSs), risk assessment, and so on (USEPA 2003).

Although there were some toxicity test of TCS available on fish and algae, few taxonomic levels were used, especially for Chinese native species. Therefore, there was no WQC value derived for TCS. Recently, systematic WQC studies are getting more attention in China, and US Environmental Protection Agency (USEPA) guidelines and other SSD methods have been used to derive WQC for some pollutants with Chinese native species (Jin et al. 2011; Yan et al. 2012a, b; Yang et al. 2012; Wang et al. 2014a, b).

In this study, acute toxicity test and some chronic test were conducted for 9 Chinese native species, and the WQC of TCS for freshwater aquatic life was derived using these toxicity data according to the USEPA guidelines. The aquatic species were selected from 3 phyla and 7 families, included 1 planktonic crustacean (*D. magna*), 1 benthic crustacean (*Neocaridina denticulata sinensis*), 1 insect (*C. plumosus*), 1 annelid (*L. hoffmeisteri*), 1 amphibian (*R. limnocharis*), and 4 fishes (*P. parva*, *C. auratus*, *M. anguillicaudatus,* and *Tanichthys albonubes*). Additionally, the log-logistic SSD method and the method of Holland National Institute for Public Health and the Environment (RIVM) (Van Vlaardingen et al. 2004) were both used to validate the results. Moreover, the difference of sensitivity between native and non-native species was compared. This work could provide valuable information for environmental risk assessment and pollution management imposed by TCS in ambient water environment.

1.3.1.2 Materials and Methods

1. **Collection of toxicity data of TCS**

The published toxicity data of TCS were collected from the ECOTOX database (http://cfpub.epa.gov/ecotox), the CNKI (http://www.cnki.net) and ELSEVIER (http://www.sciencedirect.com).

2. **Test organisms and chemicals**

Data on at least eight families of aquatic animals drawn from three different phyla and one aquatic plant are required in the derivation of WQC according to the USEPA guidelines (USEPA 1985). In this study, nine native aquatic species in China were chosen for the acute and chronic toxicity tests. Before the toxicity tests started, all test organisms were acclimatized to the dilution water during a minimum of 7 days. All toxicity tests were conducted strictly according to the ASTM guidelines (ASTM 1993a, b, c).

TCS, $C_{12}H_7Cl_3O_2$, ≥97 % purity (HPLC), was purchased from Sigma Aldrich.

3. **General test conditions**

Dechlorinated tap water was used in all toxicity tests. The measured quality parameters of tap water were as follows: total organic carbon 0.02 mg/L, pH 8.00 ± 0.20, dissolved oxygen (DO) 8.30 ± 0.30 mg/L, and hardness as $CaCO_3$ 190 ± 0.10 mg/L. All tests were static-renewal whereby test solutions were totally changed per 24 h. Treatment groups, solvent control (DMSO) group, and blank control group were included, and three replicate test containers each containing 10 organisms were used. A light:dark photoperiod of 12:12 h was used for all the tests. Test organisms were not fed during the acute test. A water bath was used to maintain the water temperature at 22 ± 2 °C, unless otherwise noted. Temperature, DO, and pH were measured daily in the acute toxicity tests and at least once a week in the chronic toxicity tests. Biological observation was performed at least once daily.

Endpoints of 48-h-EC_{50} (effective concentration in 50 % of the test organisms over 48 h) for *D. magna* and 96-h-LC_{50} (lethal concentration in 50 % of the test organisms over 96 h) for other aquatic animals were used in the acute toxicity test. Endpoints of 21-d-EC_{10} (effective concentration in 10 % of the test organisms over 21 days) for *D. magna* and 30-d-EC_{10} (effective concentration in 10 % of the test organisms over 30 days) for fishes were used in the chronic toxicity test. In this study, for fry growth, the specific growth rate (SGR) was used because it is less dependent on the initial size of the fish and the time between measurements than the other endpoint such as relative growth rate (RGR) (Mallett et al. 1997). The SGR was calculated as ((ln(final mass) − ln(initial mass)) × 100)/day of exposure (Crossland 1985).

4. **Acute toxicity tests**

D. magna (<24 h age) was obtained from in-house cultures at our laboratory of Chinese Research Academy of Environmental Sciences (CRAES). Other species tested in this study were obtained from Chaolai Market, Chaoyang, Beijing.

(a) **Acute toxicity test of *D. magna***

D. magna was exposed to 100 mL test solution in 150-mL beakers for 48 h. Nominal concentrations in the acute toxicity test were 0.00, 0.23, 0.28, 0.33, 0.40, 0.48, 0.58, and 0.69 mg/L TCS, respectively.

(b) **Acute toxicity test of *M. anguillicaudatus***
The mean wet weight of the fish was 0.68 ± 0.05 g. Fish were exposed to test solutions in 20-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.02, 0.03, 0.04, 0.07, 0.10, 0.15, and 0.23 mg/L TCS, respectively.

(c) **Acute toxicity test of *C. auratus***
The mean wet weight of the fish was 4.00 ± 0.80 g. Fish were exposed to test solutions in 50-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 1.35, 1.49, 1.64, 1.80, 1.98, 2.18, 2.40, and 2.64 mg/L TCS, respectively.

(d) **Acute toxicity test of *R. limnocharis***
The mean body length of the tadpoles was 1.60 ± 0.20 cm. Tadpoles were exposed to test solutions in 2-L beakers for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.27, 0.32, 0.38, 0.46, 0.55, 0.66, 0.79, and 0.95 mg/L TCS, respectively.

(e) **Acute toxicity test of *T. albonubes***
The mean wet weight of the fish was 0.02 ± 0.01 g. Fish fry were exposed to test solutions in 2-L beakers for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.75, 0.79, 0.83, 0.87, 0.91, 0.96, and 1.00 mg/L TCS, respectively.

(f) **Acute toxicity test of *C. plumosus***
The mean wet weight of the organism was 0.03 ± 0.01 g. The species were exposed to 25 mL test solutions in glass culture dish for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 2.07, 2.27, 2.50, 2.75, 3.03, 3.33, 3.66, 4.03, and 4.43 mg/L TCS, respectively.

(g) **Acute toxicity test of *P. parva***
The mean wet weight of the fish was 0.20 ± 0.02 g. Fish fry were exposed to test solutions in 5-L glass aquaria for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.02, 0.03, 0.04, 0.06, 0.09, 0.13, and 0.20 mg/L TCS, respectively.

(h) **Acute toxicity test of *N. denticulata sinensis***
The mean wet weight of the crustacean was 0.02 ± 0.01 g. Shrimp were exposed to test solutions in 2-L beakers for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.59, 0.65, 0.72, 0.79, 0.87, 0.95, and 1.05 mg/L TCS, respectively.

(i) **Acute toxicity test of *L. hoffmeisteri***
The mean body length of the tubifex was 3.00 ± 0.50 cm. The species were exposed to 25 mL test solutions in glass culture dish for 96 h. Nominal exposure concentrations in the definitive test were 0.00, 0.36, 0.55, 0.82, 1.23, 1.84, 2.76, 4.14, and 6.21 mg/L TCS, respectively.

5. **Chronic toxicity tests**

(a) **Chronic toxicity test of *D. magna***
Three weeks survival-reproduction tests using neonates of *D. magna* (<24 h age) were conducted in 150-mL beakers filled with 100 mL test solutions. The daphnia were fed once a day with green algae (*S. obliquus*) that had a cell concentration of 1.0×10^5 cell/mL in the test solution. The survival, growth, and reproduction were recorded daily. The endpoints included the time to first brood, number to first brood, number of broods, and total number of spawning. Nominal concentrations in the definitive test were 0.00, 0.02, 0.02, 0.03, 0.04, 0.05, 0.07, 0.09, 0.12, 0.15, and 0.20 mg/L TCS, respectively, with ten replicate test containers each containing one organism.

(b) **Chronic toxicity test of *T. albonubes***
Thirty-day short-term chronic toxicity tests were conducted. The fish were fed with brine shrimp at a rate of 0.1 % body weight twice daily. Endpoints observed included survival, growth, and body weight. Nominal exposure concentrations in the definitive test were 0.00, 0.08, 0.10, 0.12, 0.14, 0.17, and 0.20 mg/L TCS, respectively.

(c) **Chronic toxicity test of *M. anguillicaudatus***
The fish used in the chronic toxicity test were the same as in the acute toxicity test. Thirty-day short-term chronic toxicity tests were conducted. The fish were fed with brine shrimp at a rate of 0.1 % body weight twice daily. Endpoints observed included survival, growth, and body weight. Nominal exposure concentrations in the definitive test were 0.00, 3.00, 5.00, 7.00, 10.00, 15.00, and 23.00 μg/L TCS, respectively.

6. **Chemical analysis**

The concentration of TCS in the solutions of each group at the beginning and end of the 24 h period was analyzed. Briefly, the solutions were filtered through a 0.45-mm filter membrane and followed by HPLC detection, using an LC-10AD system (SHIMADZU, Tokyo, Japan), a ODS column (150 mm × 4.6 mm, particle size 5 μm; Shim-pack CLC-ODS, SHIMADZU, Japan), mobile phase of methanol and water (90:10, v/v) with a flow rate of 1.0 mL/min. The column temperature was 40 °C, a loading volume of 10 μL and ultra violet (UV) detection was at 230 nm. TCS had a retention time of 5 min. Following the establishment of a response to a known concentration, the result of measured/nominal concentration was 93.14–102.58 %. The variability of TCS concentration was less than 20 %, in compliance with the requirement of the toxicity text guidelines. Therefore, all subsequent toxicity results were expressed on the nominal concentrations of TCS.

7. **Statistical analysis and SSD generation**

Probit methodology was used to calculate the 48-h-EC_{50}, 96-h-LC_{50} values and corresponding 95 % confidence intervals. As for chronic toxicity test, the EC_{10} for the most sensitive biological endpoint in each test was estimated.

Three procedures, the USEPA guidelines (USEPA 1985), the log-normal software ETX2.0 (Van Vlaardingen et al. 2004) exploited by the RIVM, and the log-logistic SSD method (Newman et al. 2000; Wheeler et al. 2002; Feng et al. 2013), were used to calculate the WQC for TCS. The data analysis softwares were SPSS 20.0 and OriginLab 8.0.

1.3.1.3 Results

1. **Toxicity tests of 9 native aquatic organisms and published toxicity data**

Toxicity values of TCS to 8 aquatic species and an endangered species were shown in Tables 1.16 and 1.17. No mortality was observed in the control groups and the solvent control groups. Results of acute toxicity tests showed that *M. anguillicaudatus* with a 96-h-LC_{50} of 0.045 mg/L was the most sensitive species

Table 1.16 Acute toxicity of TCS to nine resident aquatic species

Species	Functions	R^2	p	LC_{50}/EC_{50} (mg/L)
P. parva	$y = 1.0416x + 3.0744$	0.9417	<0.01	0.071 (0.029–0.169)
C. auratus	$y = 9.9154x + 2.3766$	0.9880	<0.01	1.839 (1.649–2.050)
M. anguillicaudatus	$y = 2.6102x + 0.6783$	0.9515	<0.01	0.045 (0.027–0.077)
T. albonubes	$y = 20.128x - 54.352$	0.9871	<0.01	0.889 (0.845–0.934)
D. magna	$y = 6.9004x - 12.449$	0.9833	<0.01	0.338 (0.278–0.410)
N. denticulata sinensis	$y = 10.952x - 26.625$	0.9832	<0.01	0.772 (0.700–0.852)
C. plumosus	$y = 10.276x - 30.564$	0.9572	<0.01	2.890 (2.667–3.131)
L. hoffmeisteri	$y = 2.6515x - 3.779$	0.9765	<0.01	2.046 (1.399–2.994)
R. limnocharis	$y = 4.8031x - 8.0358$	0.9795	<0.01	0.518 (0.436–0.615)

Table 1.17 Chronic toxicity of TCS to three resident aquatic species

Species	Endpoints	Functions	R^2	p	EC_{10} (mg/L)
M. anguillicaudatus	Growth	$y = 1.5822x + 2.221$	0.9394	<0.01	0.009
T. albonubes	Growth	$y = 5.2638x - 6.4808$	0.9839	<0.01	0.087
D. magna	Time to first brood (d)	$y = 1.5045x - 2.3882$	0.9730	<0.01	0.045
	Number to first brood (n)	$y = 4.6941x - 3.9019$	0.9271	<0.01	0.042
	Total number of spawning (n)	$y = 1.6896x + 1.2411$	0.9571	<0.01	0.029
	Number of broods (n)	$y = 2.5486x - 1.1721$	0.9086	<0.01	0.083

Table 1.18 Acute toxicity data of TCS to non-native aquatic species

Rank	Species	EC_{50}/LC_{50} (mg/L)	Time (h)	References
1	*B. woodhousii*	0.152	96	Palenske et al. (2010)
2	*C. dubia*	0.168	48	Orvos et al. (2009)
3	*X. laevis*	0.259	96	Yang et al. (2009)
4	*P. promelas*	0.260	96	Orvos et al. (2009)
5	*O. mykiss*	0.288	96	USEPA (2000)
6	*A. blanchardii*	0.367	96	Palenske et al. (2010)
7	*L. macrochirus*	0.370	96	Orvos et al. (2009)
8	*T. platyurus*	0.470	24	Kim et al. (2009a, b)
9	*R. sphenocephala*	0.562	96	Palenske et al. (2010)
10	*O. latipes*	0.602	96	Ishibashi et al. (2004)
Algae	*S. subspicatus*	0.001	96	Orvos et al. (2009)
Algae	*N. pelliculosa*	0.019	96	Orvos et al. (2009)

to TCS followed by *P. parva*, *D. magna*, *R. limnocharis*, *N. denticulata sinensis*, *T. albonubes*, *C. auratus*, and *L. hoffmeisteri*, while the least sensitive species was *C. plumosus* with a 96-h-LC_{50} of 2.890 mg/L.

Results of our study and previous studies using non-native species (Table 1.18) were compared. In this study, we found that 96-h-LC_{50} for amphibian *R. limnocharis* was 0.518 mg/L, and the 48-h-EC_{50} of TCS on *D. magna* was 0.338 mg/L. Previous studies reported that 96-h-LC_{50} for fish *Pimephales promelas*, *L. macrochirus,* and *Oryzias latipes* were 0.260, 0.370, and 0.602 mg/L, respectively (Ishibashi et al. 2004; Orvos et al. 2009).

Chronic toxicity test showed that the 21-d-EC_{10} for total number of spawning was 0.029 mg/L and no mortality was observed in all concentration groups. The 30-d-EC_{10} for growth of fish *T. albonubes* and *M. anguillicaudatus* was 0.087 and 0.009 mg/L.

2. Comparison of SSD between native and non-native taxa

Davies et al. (1994) reported that using toxicity data of species from one geographical region to assess the hazard posed to species in a different region have been questioned. Moreover, differences in the sensitivity of cold-water, temperate, and tropical fish species have been reported (Dyer et al. 1997). In this study, the difference of SSD based on native and non-native species was compared. Since there has been little information on the toxicity of TCS to native and non-native species, only SSD constructed from acute toxicity data of native (Table 1.19) and non-native (Table 1.18) species was compared.

Based on the comparison in this study (Fig. 1.3), the SSD curve of native species below 0.15 (HC_{15}, hazardous concentration for the 15 % of species) was shifted to the left of non-native species. A low HC_5 (hazardous concentration for the 5 % of species) indicated that Chinese freshwater organisms with acute exposures to TCS were more sensitive than the non-native species at the tail of SSD.

Table 1.19 Ranked GMAVs with SACRs according to USEPA guideline

Rank	Species	SMAVs (mg/L)	GMAVs (mg/L)	SACRs	References
1	*M. anguillicaudatus*	0.045	0.045	5.00	In this study
2	*P. parva*	0.071	0.071	–	In this study
3	*D. magna*	0.338	0.338	11.66	In this study
4	*R. limnocharis*	0.518	0.518	–	In this study
5	*N. denticulata sinensis*	0.772	0.772	–	In this study
6	*T. albonubes*	0.889	0.889	10.22	In this study
7	*X. helleri*	1.470	1.470	–	Liang et al. (2013)
8	*C. auratus*	1.839	1.839	–	In this study
9	*L. hoffmeisteri*	2.046	2.046	–	In this study
10	*C. plumosus*	2.890	2.890	–	In this study
Algae	*Chlorella* spp.	0.065			Wu et al. (2009)
Algae	*S. capricornutum*	0.092			Liao (2012)

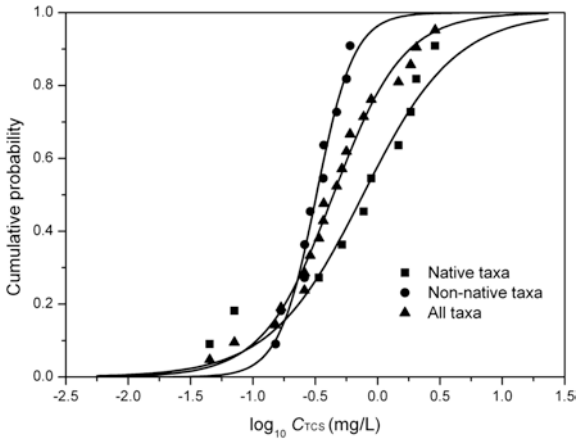

Fig. 1.3 Species sensitivity distribution of native, non-native, and total species toxicity data for TCS

However, the HC_{50} (hazardous concentration for the 50 % of species) for native taxa was higher than that for non-native taxa.

The result of the comparison of SSD between all the native and non-native taxa showed that there was no statistically significant difference (Kolmogorov–Smirnov test: $ks = 1.342$, $n1 = 10$, $n2 = 10$, $p = 0.06$). Considering the lack of toxicity data for Annelid and insect in non-native taxa, the difference was also not significant ($ks = 1.054$, $n1 = 8$, $n2 = 10$, $p = 0.22$) when removing the two taxa toxicity values. Moreover, in our study, there was no statistically significant difference between the sensitivity of native and total species as well ($ks = 0.78$, $n1 = 10$, $n2 = 20$, $p = 0.59$).

3. Aquatic life criteria derivation

The USEPA guidelines of aquatic life criteria prescribed that only the toxicity data of resident species could be used in the calculation of criteria (USEPA 1985). In

this study, using 10 species toxicity values (Table 1.19), 3 methods were used to derive the aquatic life WQC of TCS including USEPA guidelines, software ETX 2.0 (exploited by RIVM) and log-logistic (SSD) method.

Using the methods described in USEPA guidelines, the SMAVs and the GMAVs were calculated. The SACRs were calculated as a ratio of acute and chronic values. Ranked GMAVs with SACRs were listed in Table 1.19. A CMC of 0.009 mg/L TCS was obtained by dividing final acute value (FAV, 0.017 mg/L TCS) by two. The FACR was calculated as geometric means of the three SACRs (10.22, 11.66 and 5.00) to be 8.41. The final chronic value (FCV, 0.002 mg/L) was obtained by dividing the FAV by the FACR. Final plant value (FPV) was the toxicity data of *Chlorella* spp. and *Selenastrum capricornutum*, calculated to be 0.065 and 0.092 mg/L. The lower value between FCV and FPV was selected as the CCC, i.e., 0.002 mg/L.

In addition, the values of HC_5 derived based on the ETX 2.0 and the constructed log-logistic SSD were 0.054 and 0.060 mg/L, respectively. Therefore, the CMCs of TCS developed with the two SSD methods were 0.027 and 0.030 mg/L when the factor is 2, which were in the same order of magnitude with the CMC (0.009 mg/L) calculated according to the USEPA guidelines.

1.3.1.4 Discussion

1. **Toxicity tests of 9 native aquatic organisms**

Results of this study indicate that TCS is highly toxic to native freshwater aquatic organisms. Liang et al. (2013) reported that the 96-h-LC_{50} of TCS on fish *X. helleri* was 1.47 mg/L. This is consistent with our study (0.889–1.839 mg/L for fishes except 0.045 mg/L for *M. anguillicaudatus* and 0.071 mg/L for *P. parva*). Previous study also reported that *P. parva* is sensitive to brominated flame retardant TBBPA (Tetrabromobisphenol A). Yang et al. (2012), and Wang et al. (2013a, b) reported that *P. parva* is sensitive to organic pollutants, especially to pesticides. So, it might be a sensitive species to antimicrobial agent, too. In addition, our previous study found that demersal fish *M. anguillicaudatus* is sensitive to some organochlorine pesticide. Brausch and Rand also indicated that TCS could affect benthic animals due to its transfer characteristics (Brausch and Rand 2011). Therefore, TCS might have special toxic modes of action on *M. anguillicaudatus* and *P. parva.* Among different species, the fishes were more sensitive than the aquatic invertebrates of Annelid and insect, and the insect was the least sensitive species. The chronic data and the other acute toxicity value cannot be compared with previous studies due to lack of resident toxicity data in China.

The 96-h-LC_{50} of *P. promelas*, *L. macrochirus,* and *O. latipes* were 0.260, 0.370, and 0.602 mg/L, respectively, (Ishibashi et al. 2004; Orvos et al. 2009); this was in accordance with our study. The 96-h-LC_{50} for amphibian *R. limnocharis* was 0.518 mg/L, and it is in agreement with previous studies that toxicity values for amphibian *Xenopus laevis*, *Acris blanchardii*, *Bufo woodhousii,* and *Rana*

sphenocephala were 0.259, 0.367, 0.152, and 0.562 mg/L, respectively (Yang et al. 2009; Palenske et al. 2010). Orvos et al. (2009) reported that the 48-h-EC_{50} of TCS on planktonic crustacean *D. Magna* and *C. dubia* were 0.390 and 0.168 mg/L, and this was in accordance with our study. In general, the sensitivities of the native species tested in this study were similar to those reported in previous studies. The acute toxicity of the shrimp *N. denticulata sinensis*, the insect *C. plumosus,* and the annelid *L. hoffmeisteri* cannot be compared with previous studies due to lack of toxicity data for native and non-native freshwater species. For chronic test, Orvos et al. (2009) reported that 21 days NOEC for the survival of *D. Magna* was 0.2 mg/L, whereas the 21-d-EC_{10} for total number of spawning was 0.029 mg/L in this study, which indicated that endpoint of total number of spawning is more sensitive than survival. For chronic test of fishes, previous studies reported that the 21 d LOEC (lowest observed effect concentration) for growth of fish *O. latipes* (Kim et al. 2009a, b) and 96 d LOEC for survival of *O. mykiss* (Orvos et al. 2009) were 0.2 and 0.071 mg/L TCS, whereas 30-d-EC_{10} for growth of fish *T. albonubes* was 0.087 mg/L, and 30-d-EC_{10} for growth of fish *M. anguillicaudatus* was 0.009 mg/L in this study. Similarly, as mentioned in the above paragraph, demersal fish *M. anguillicaudatus* is also the most sensitive species in the chronic tests in this study.

Previous studies reported that the 96-h-EC_{50}s for algae *Chlorella* spp., *S. capricornutum, S. subspicatus,* and *N. pelliculosa* were 0.065, 0.092, 0.001, and 0.019 mg/L, respectively (Orvos et al. 2009; Wu et al. 2009; Liao 2012). Therefore, compared with other taxonomic levels, the algal was the most sensitive taxa and the algal growth was affected at concentrations less than 1 μg/L. High TCS sensitivity in algae is likely due to TCS antibacterial characteristics, by uncoupling of oxidative phosphorylation (Newton et al. 2005), membrane destabilization (Franz et al. 2008), or disruption of lipid synthesis through the FabI (fatty acid synthesis) and FASII (enoyl acyl carrier protein reductase) pathways (Lu and Archer 2005) which are similar between algae and bacteria (Coogan et al. 2007).

2. **Comparison of SSDs**

The reason for differences of HC_5 and HC_{50} derived from SSD of native and non-native species might be the lack of toxicity data for Annelid and insect in non-native taxa, while the two taxa were demonstrated to be tolerance to TCS in our study (2.046 mg/L for *L. hoffmeisteri*, 2.890 mg/L for *C. plumosus*). Although there were differences for HC_5 and HC_{50} between native and non-native taxa, the comparison of SSD showed that there was no statistically significant difference (Kolmogorov–Smirnov test: $ks = 1.342$, $n_1 = 10$, $n_2 = 10$, $p = 0.06$; $ks = 1.054$, $n_1 = 8$, $n_2 = 10$, $p = 0.22$ (removing the Annelid and insect toxicity values). Similarly, sensitivities among North American and European taxa with different geographic distributions to a series of pollutants have been shown to be no statistically significant difference (Dyer et al. 1997; Maltby et al. 2002). Moreover, the difference between SSDs of Australian and non-Australian organisms to endosulfan was not significant (Hose and Van den Brink 2004). Jin et al. (2011) also reported that there was no statistically significant difference between SSDs of native and non-native species when exposed to 2,4-dichlorophenol. Previous study found that natural history, habitat

type, and geographical distribution of the species used to construct the SSD did not have a significant influence on the assessment of hazard (Hose and Van den Brink 2004; Maltby et al. 2005), and this was in accordance with our present study.

3. **Aquatic life criteria for TCS**

Due to the wide use of the TCS, the levels of TCS in aquatic environments ranged from ng/L to μg/L (Kolpin et al. 2002; Ciniglia et al. 2005; Ramaswamy et al. 2011), therefore its risk to the aquatic organisms cannot be ignored. The potential environmental risk of TCS could be assessed by using RQ according to the European technical guidance document (TGD) (EC 2003). In China, the highest concentration for TCS reported so far in surface water was 478 ng/L in Shijing River (Zhao et al. 2010), and it is lower than the CCC of 0.002 mg/L in this study. The CCC of this study might provide useful information in site-specific ecological risk assessment because our study and previous studies both indicated that there was no significant difference between the sensitivity of native and non-native species. So, it is possible to use non-native species toxicity data for site-specific ecological risk assessment of TCS. Taking into account that the higher concentrations detected so far in surface waters for TCS was 5,160 ng/L in India (Kolpin et al. 2002; Ramaswamy et al. 2011) and 2,300 ng/L in US streams (Kolpin et al. 2002), and the CCC was 0.002 mg/L, the RQ values were calculated to be 2.58 and 1.15 which indicated that the TCS might pose risk to the aquatic species in these places.

1.3.1.5 Conclusions

This study is a contribution in the assessment of the effect of TCS in the aquatic environment. Toxicity values of 9 acute and 3 chronic tests for 9 native species from 3 phyla and 7 families were obtained in this study, in which demersal fish *M. anguillicaudatus* was the most sensitive species, and the fishes were more sensitive than the aquatic invertebrates of Annelid and insect, and the insect was the least sensitive species. Comparing the SSDs, there was no significant difference between native species and non-native species. It indicates that toxicity data from different geographic region can be used in site-specific ecological risk assessment of TCS. Furthermore, the CMC derived using USEPA guidelines is similar with the results of RIVM ETX2.0 and the log-logistic SSD method, and the CMC and CCC of aquatic life criteria for TCS are 0.009 and 0.002 mg/L, respectively.

1.3.2 Study on the Water Quality Criteria of PFOS in China

1.3.2.1 Introduction

Perfluorinated compounds (PFCs) have been widely applied in the commercial and industrial products such as active ingredients in textiles, food containers and upholstery and surfactants in fire fighting foams, floor polishes, and shampoos

(Giesy and Kannan 2002). Due to the moderate water solubility, PFCs have globally distributed in the aquatic environment (Saito et al. 2003; So et al. 2004; Skutlarek et al. 2006; Pistocchi and Loos 2009) and the environmental organisms (Taniyasu et al. 2003; Kannan et al. 2005). Perfluorooctane sulfonic acid (PFOS), as one of the major PFCs and an important precursor to other PFCs, was one of the most frequently detected pollutants in water bodies of China (Jin et al. 2009; Hu et al. 2011; Yang et al. 2011). In the Yangtze River Estuary, PFOS were detected with maximum concentrations of 703 ng/L in the river water (Pan et al. 2009). It showed that the concentration of PFOS was up to 99 ng/L in the Pearl River (So et al. 2007). The mean concentrations of PFOS in Huai River and Taihu Lake were 4.7 ng/L and 15 ng/L, respectively (Yu et al. 2013).

From laboratory toxicity studies, the PFOS is known to be moderately acute and slightly chronically toxic to aquatic organisms, in general. Based on the acute and chronic toxicity data of PFOS, it is necessary to evaluate the potential risk of PFOS in aquatic ecosystem. OECD (2002) and the United Kingdom (Brooke et al. 2004) had published the environment hazard or risk assessment reports of PFOS and its salts. The environment risk limits (ERLs) of RIVM (Moermond et al. 2010) and the WQC in USA Minnesota Pollution Control Agency (Stevens and Coryell 2007) had already been developed. In addition, the predicted no-effect concentrations (PNECs) of PFOS for aquatic ecosystem were available with different approaches (Qi et al. 2011).

In China, the study of WQC had a late start. Originally, it was only based on the collection of data from abroad and discussion with the derivation methods of WQC. With the growing water pollution and little success on water pollution repair, China had recently attached increasing importance to studies designed to develop a national WQC system (Wu et al. 2010). The WQC of Zn,Cd, Cr(6+), benzene, nitrobenzene, pentachlorophenol(PCP), 2,4-dichlorophenol (2,4-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP) were discussed in China's regional (Lei et al. 2009; Wu et al. 2012). As one of the new types of persistent organic pollutants (POPs), PFOS has been raised more concerns in China. However, the WQCs of PFOS in the freshwater have not been derived due to the scarcity of the site-specific toxicity data. According to the methods and procedures of surface WQC established by USEPA, this study was to derive the WQC of PFOS in the aquatic environment using native aquatic organisms in China.

1.3.2.2 Materials and Methods

1. **Criteria for selecting the toxicological data of PFOS**

An overview of acute and chronic toxicity data for PFOS is given in Tables 1.20 and 1.21. The tested freshwater organisms for PFOS include macrophytes, algae, crutaceans, mollusca annelida, amphibians, and fish. The toxicological data were collected from all available materials. PFOS acid, the potassium salt of PFOS, and the other salts such as lithium and tertraethylammonium salt in the test were

selected in this study. All toxicity data were required for clear toxicological endpoint, of which the data of non-native species should be rejected and the introduced exotic species such as rainbow trout adopted. In addition, the data reported in the recent references were employed to derive the WQC of PFOS. The detailed selection criteria for the toxicity data were evaluated according to the USEPA guidelines (USEPA 1985).

For the species with different endpoints in the same duration, the most sensitive endpoint was chosen; for one species with many data on the same endpoint, the geometric mean was calculated.

According to the method of deriving the WQCs of USEPA (USEPA 1985), the minimum dataset was required at least three phyla and eight different families. The available data on acute toxicity of PFOS could meet the requirements for the acute criterion-criteria maximum concentration (CMC) and criterion-criteria continuous concentration (CCC). The FACR calculated by dividing the FAV was employed to derive FCV. The data requirement for the FACR comprised of at least three different families, a fish, an invertebrate, and one acutely sensitive species.

2. **Toxicity test with fish**

Acute toxicity with medaka *O. latipes* at three months old of PFOS was investigated with a period of 96 h static assays, following the protocols of OECD 203(OECD 1992). Chronic toxicity test with a period of 14 days semi-static assays on *Gobiocypris rarus* at six months old followed the method developed by the protocols of OECD 204(OECD 1992). The potassium salt of PFOS ([CF3][C7F14SO3-K]) was purchased from J&K chemical company (purity 98 %). The water used in the experiment was the dechlorinated tap water with hardness of 187 mg $CaCO_3$/L, pH 8.26, and temperature 23 ± 1 °C.

3. **Acute toxicity test with *O. latipes***

The test medaka was exposed with 2 L test solutions in the natural light. Five concentrations and one control with three parallels were assigned to seven fish in each glass beaker. Nominal concentrations of PFOS in the assay were 57.5, 66.13, 76.04, 87.45, and 100.57 mg/L after a preliminary test with concentration range of 50–100 mg/L. Water quality parameters in the test solution including pH, dissolved oxygen, and temperature were measured at each day during the test.

4. **Chronic toxicity test with *G. rarus***

The test rare minnow were exposed with two liters test solutions in the natural light. Each treatment with five concentrations and one control at three replications was assigned ten fish in each glass beaker. Theoretical concentrations of PFOS in assay were 0.05, 0.1, 0.5, 1, and 5 mg/L after an acute test with concentration range of 1–30 mg/L. Water quality parameters in the test solution including pH, dissolved oxygen, and temperature were measured at before and after renewal during the test.

5. **Chemical analysis**

Analyses of the concentrations of test solutions were performed using an Agilent 1,200 HPLC equipped with an Agilent 6,310 ion trap mass spectrometric detection (HPLC/MS) operated in negative electrospray ionization (ESI) mode. The analyte was separated on a Zorbax Eclipse Plus C_{18} column (2.1 i.d. 150 × length mm) with 2.5 mM ammonium acetate and acetonitrile as mobile phase in the volume ratio of 70:30 % at a flow rate of 0.2 mL/min. A total of 10μL was automatically injected and the oven temperature was 40 °C. Chromatograms were recorded using Auto MSn mode. PFOS was quantified with monitoring transitions ion at m/z = 499. The ion source working parameters were as follows: Nebulizer was 20.0 psi; flow of the dry gas was 9.0 L/min; dry temperature was 350 °C; target mass was 499 m/z; compound stability was 100 %; trap drive level 100 %; and optimize was normal.

Samples at the highest and the lowest concentrations with two parallels collected at test initiation and 96 h had measured values from 86.2 to 115 % and 88.7 to 98.2 % of nominal concentration after dilution, respectively. The limit of quantification (LOQ) was determined with 1 μg/L. Test results were analyzed with probit analyses using SPSS 16.0 software for Windows and the value of 96 h LC_{50} and 95 % confidence interval were determined.

1.3.2.3 Results

1. **The toxicity data for freshwater species**

Thirty-six acute data and 46 chronic data were collected to calculate the WQC of PFOS in Tables 1.20 and 1.21. Of all the toxicity data, native freshwater species were used to derive the WQC of PFOS and non-native species such as *Unio complanatus* (Drottar and Krueger 2000c), *X. laevis* (Palmer and Krueger 2001), *Rana pipiens* (Ankley et al. 2004), and *P. promelas* (Drottar and Krueger 2000g) were abandoned in this study. Similarly, the questionable data in the report of Minnesota, USA, for deriving the surface WQC on PFOS (Stevens and Coryell 2007) were excluded.

As shown in Table 1.20, data on the acute toxicity of PFOS to aquatic organism mainly included macroalgae, crustaceans, insects, amphibians, and fish. The acute toxicity of PFOS on macroalgae (*Lemna gibba*) has been reported. The values, based on frond number and biomass, were 59.1 and 31.1 mg/L, respectively. Several studies on the acute toxicity of PFOS have been conducted with the cladoceran *D. magna* which is known to be a representative species. In these acute toxicity studies, based on mortality and immobility as endpoints, the range of LC_{50} or EC_{50} values was determined to be from 37.4 to 169 mg/L. Several acute toxicity studies with PFOS have been conducted on fish including *O. mykiss*, *L. macrochirus*, *O. latipes,* and *G. rarus.* Of the freshwater fish exposures, the *L. macrochirus* was the most sensitive species with a 96-h LC_{50} of 7.8 mg/L.

Table 1.20 Acute toxicity data of PFOS for freshwater species

Trophic level	Species	Duration	Endpoint	EC_{50}/LC_{50} (mg/L)	References
Macroalgae	*Lemna gibba* G3	7 days	Frond number	108 (46–144)	Desjardins et al. (2001a)
	Lemna gibba	7 days	Frond number	59.1 (51.5–60)	Desjardins et al. (2001a)
	Lemna gibba	7 days	Biomass (wet weight)	31.1 (22.2–36.1)	Boudreau et al. (2003a)
Microalgae	*Pseudokirchneriella subcapitata*	96 h	Growth (cell density)	68 (63–70)	Drottar and Krueger (2000d)
	Pseudokirchneriella subcapitata	96 h	Inhibition of growth	121 (110–133)	Drottar and Krueger (2000d)
	Pseudokirchneriella subcapitata	96 h	Growth (cell density)	48.2 (45.2–51.1)	Boudreau et al. (2003a)
	Pseudokirchneriella subcapitata	96 h	Growth (chlorophyll a)	59.2 (50.9–67.4)	Boudreau et al. (2003a)
	Pseudokirchneriella subcapitata	72 h	Growth (cell density)	35.0 (34.2–35.5)	Rosal et al. (2010)
	Navicula pelliculosa	96 h	Growth (cell density)	263 (217–299)	Sutherland and Krueger (2001)
	Navicula pelliculosa	96 h	Inhibition of growth	305 (295–316)	Sutherland and Krueger (2001)
	Chlorella vulgaris	96 h	Growth (cell density)	81.6 (69.6–98.6)	Boudreau et al. (2003a)
	Chlorella vulgaris	96 h	Growth (chlorophyll a)	88.1 (71.2–104)	Boudreau et al. (2003a)
	Anabaena flos-aquae	96 h	Inhibition of growth rate	176 (169–181)	Desjardins et al. (2001b)
Crustaceans	*Daphnia magna*	48 h	Immobility/mortality	67.2	Boudreau et al. (2003a)
	Daphnia magna	48 h	Immobility/mortality	61	Drottar and Krueger (2000a)
	Daphnia magna	48 h	Immobility/mortality	63	Li (2009)
	Daphnia magna	48 h	Immobility/mortality	37.4	Ji et al. (2008)
	Daphnia magna	48 h	Immobility/mortality	58	OECD (2002)
	Daphnia pulicaria	48 h	Mortality	169	Boudreau et al. (2003a)
	Daphnia pulicaria	48 h	Immobility	134	Boudreau et al. (2003a)
	Moina macrocopa	48 h	Immobility/mortality	18	Ji et al. (2008)
	Neocaridina denticulate	96 h	Mortality	10	Li (2009)
Insects	*Chironomus tentans*	10 days	Growth	>0.150	MacDonald et al. (2004)
	Chironomus tentans	10 days	Survival	0.0872 (0.0755–0.0988)	MacDonald et al. (2004)

(continued)

Table 1.20 (continued)

Trophic level	Species	Duration	Endpoint	EC_{50}/LC_{50} (mg/L)	References
Mollusca	*Physa acuta*	96 h	Mortality	178	Li (2009)
	Perna viridis	96 h	Mortality	68.3	Wang et al. (2012)
Platyhelmintes	*Dugesia japonica*	96 h	Mortality	17	Li (2008)
	Dugesia japonica	96 h	Mortality	23	Li (2009)
Annelida	*Lumbriculus variegatus*	96 h	Mortality	5.6	Stevens and Coryell (2007)
Amphibians	*Rana nigromaculata*	96 h	ELS	81	Su et al. (2012)
	Rana nigromaculata	96 h	The embryo development	51	Ren et al. (2012)
	Pseudacris crucifer	96 h	Mortality	38	Stevens and Coryell (2007)
Fish	*Oncorhynchus mykiss*	96 h	Mortality	22	Palmer et al. (2002)
	Lepomis macrochirus	96 h	Mortality	7.8	OECD (2002)
	Oryzias latipes	96 h	Mortality	87.73	The present study
	Gobiocypris rarus	96 h	Mortality	10.525	The present study

Table 1.21 Chronic toxicity data of PFOS for freshwater species

Trophic level	Species	Duration	Endpoint	NOEC/LOEC (mg/L)	References
Algae	*Chlorella vulgaris*	96 h	Growth (cell density)	8.2 (6.4–13.0)	Boudreau et al. (2003a)
	Chlorella vulgaris	96 h	Growth (chlorophyll a)	9.6 (7.6–16.5)	Boudreau et al. (2003a)
	Navicula pelliculosa	96 h	Growth (cell density)	150	Sutherland and Krueger (2001)
	Navicula pelliculosa	96 h	Inhibition of growth	206	Sutherland and Krueger (2001)
	Pseudokirchneriella subcapitata	96 h	Growth (cell density))	42	Drottar and Krueger (2000d)
	Pseudokirchneriella subcapitata	96 h	Inhibition of growth	42	Drottar and Krueger (2000d)
	Pseudokirchneriella subcapitata	96 h	Growth (cell density)	5.3 (4.6–6.8)	Boudreau et al. (2003a)
	Pseudokirchneriella subcapitata	96 h	Growth (chlorophyll a)	16.6 (8.5–28.1)	Boudreau et al. (2003a)
Cyanobacteria	*Anabaena flos-aquae*	96 h	Inhibition of growth rate	93.8	Desjardins et al. (2001b)
Macroalgae	*Lemna gibba* G3	7 days	Frond number	15.1	Desjardins et al. (2001a)
	Lemna gibba	7 days	Frond number	29.2	Desjardins et al. (2001a)
	Lemna gibba	7 days	Biomass (wet weight)	6.6 (4.5-13.6)	Boudreau et al. (2003a)
	Myriophyllum sibiricum	42 days	Biomass (dw)	2.9	Hanson et al. (2005)
	Myriophyllum sibiricum	42 days	Root length (cm)	0.3/2.9	Hanson et al. (2005)
	Myriophyllum spicatum	42 days	Biomass (dw)	11.4/32.3	Hanson et al. (2005)
	Myriophyllum spicatum	42 days	Root length (cm)	11.4/32.3	Hanson et al. (2005)
Crustaceans	*Daphnia magna*	21 days	Survival	12	Drottar and Krueger (2000f)
	Daphnia magna	21 days	Reproduction	12/24	Drottar and Krueger (2000f)
	Daphnia magna	21 days	Growth	12	Drottar and Krueger (2000f)
	Daphnia magna	21 days	Adult survival	25	Boudreau et al. (2003a)
	Daphnia magna	21 days	Days to first brood	25	Boudreau et al. (2003a)
	Daphnia magna	21 days	Number of young/adult or brood	25/50	Boudreau et al. (2003a)
	Daphnia magna	21 days	Adult survival	5.3 (2.5–9.2)	Boudreau et al. (2003a)
	Daphnia magna	21 days	Number of young/brood	1.25/2.5	Ji et al. (2008)

(continued)

Table 1.21 (continued)

Trophic level	Species	Duration	Endpoint	NOEC/LOEC (mg/L)	References
	Daphnia magna	21 days	Number of broods/adult	≥5	Ji et al. (2008)
	Moina macrocopa	7 days	Adult survival	1.25	Ji et al. (2008)
	Moina macrocopa	7 days	Days to first brood	≥5	Ji et al. (2008)
	Moina macrocopa	7 days	Number of young/brood	0.3125/0.625	Ji et al. (2008)
	Moina macrocopa	7 days	Number of brood/adult	0.3125	Ji et al. (2008)
Insects	*Chironomus tentans*	36 days	Total emergence	<0.0023	MacDonald et al. (2004)
	Chironomus tentans	20 days	Growth	0.022/0.095	MacDonald et al. (2004)
	Chironomus tentans	20 days	Survival	0.095	MacDonald et al. (2004)
	Chironomus tentans	10 days	Growth	0.049	MacDonald et al. (2004)
	Chironomus tentans	10 days	Survival	0.049	MacDonald et al. (2004)
	Enallagma cyathigerum	120 days	Foraging success	0.01	Van Gossum et al. (2009)
	Enallagma cyathigerum	15 days	Hatching	1.0	Bots et al. (2010)
	Enallagma cyathigerum	20 days	Larval survival	0.1	Bots et al. (2010)
	Enallagma cyathigerum	120 days	Larval survival	0.01	Bots et al. (2010)
	Enallagma cyathigerum	120 days	Metamorphosis	<0.01	Bots et al. (2010)
Fish	*Oryzias latipes*	14 days	Reproduction	0.1/1.0	Ji et al. (2008)
	Oryzias latipes	14 days	Survival of larvae	<0.01	Ji et al. (2008)
	Gobiocypris rarus	14 days	Survival	1/5	The present study

As shown in Table 1.21, many studies have been conducted to determine the toxicity of PFOS to aquatic species including microalgae, macroalgae, crustaceans, insects, and fish. Reported 96 h-NOEC values for freshwater microalgae (growth endpoint as measured by cell density) ranged from 5.3 to 150 mg/L. The chronic toxicity of PFOS was evaluated for two macrophytes, *Lemna gibba,* and *M. Spicatum*. For *M. Spicatum,* the NOEC values of 2.9 and 0.3 mg/L were based on biomass and root length, respectively. Life cycle tests with *D. magna* have been conducted to evaluate the chronic toxicity of PFOS to freshwater aquatic crustaceans. When the measured test endpoints were the survival and reproduction, 21 days NOEC values were ranged from 5.3 to 25 mg/L. However, as the other crustaceans of *Moina macrocopa,* 7 days NOEC values were ranged from 0.3125 to 5 mg/L based on the same endpoints with *D. magna*. Thus, using survival as the endpoint, *M. macrocopa* was more sensitive than *D. magna*. Chronic fish toxicity data, from a 14 days prolonged toxicity test, are available for *O. latipes* and *G. rarus*. The NOEC and LOEC based on reproduction for *O. latipes* were determined to be 0.1 and 1.0 mg/L. When survival was evaluated as the test endpoint, *G. rarus* was more sensitive fish species than *O. latipes.*

Of all the acute and chronic toxicity data, the midge *Chironomus tentans* was the most sensitive species to PFOS of all the species. In the acute toxicity to *C. tentans*, the EC_{50} value of 0.0872 mg/L for PFOS was observed under static-renewal conditions for a 10 days period. A 20 days NOEC of 0.0217 mg/L for growth and 20 days LOEC of 0.0023 mg/L for the total emergence in the life cycle were obtained (MacDonald et al. 2004). In comparison with other freshwater species, the toxicity of PFOS to *C. tentans* was about two oders of magnitude higher.

Of all the acute toxicity in the studies of fish medaka might be the least sensitive species exposed to PFOS in this study. The 96 h LC_{50} of *O. latipes* based on endpoint of mortality was 87.73 mg/L (95 % confidential interval of 84.23–91.94 mg/L), which was sevenfold of the value of *Oncorhynchus mykiss* ($96LC_{50}$ = 11.93 mg/L) (Palmer et al. 2002), ninefold of *P. promelas* ($96LC_{50}$ = 9.1 mg/L) (Drottar and Krueger 2000b), and 11fold of *L. macrochirus* ($96LC_{50}$ = 7.80 mg/L) (OECD 2002).

2. **WQC for PFOS in the freshwater**

Using the approach for deriving national WQC of USEPA, the FAV of PFOS on freshwater organisms was calculated to be 64.48 μg/L. And then, CMC equal to FAV/2 was 32.24 μg/L. The FAV value was derived mainly using the four least acute toxicity values of aquatic species. The toxicity data of the most sensitive species *C. tentans* were about 64 times lower than that of the next most sensitive species *L. variegatus.* Consequently, the FAV value derived in this study would be protective of most freshwater organisms.

As shown in Table 1.21, the chronic toxicity database could not meet the least dataset requirement of three families of eight families. Then, it could not calculate the FCV with the same approach as FAV. Consequently, the FAV was derived with dividing the FAV by the FACR. According to the guideline (USEPA 1985), a chronic value (ChV) equal to the geometric mean of the lower limit (NOEC) and

the upper limit (LOEC) was divided from a SMAV to calculate the ACR for one (NOEC) and the upper limit (LOEC) were divided from a SMAV to calculate the ACR for one species. The geometric mean of ACR for many data on the same endpoint was obtained for one species. Lastly, the FACR was the geometric mean of all ACRs of all selected species.

Based on the available acute and chronic toxicity data of PFOS, five species including *O. latipes*, *M. macrocopa*, *C. Tentans*, *D. Magna, and G. rarus* were selected to calculate the FACR. The LOEC and NOEC values for the most sensitive endpoint are shown in Table 1.21. For *O. latipes*, the SMAV value from the acute data was 87.73 mg/L, and the ChV was calculated as 0.32 mg/L, which resulted in an ACR of 277.43. For *M. macrocopa*, the SMAV value from the acute data was 18.00 mg/L, and the ChV was calculated as 0.44 mg/L, which resulted in an ACR of 40.73. For *C. tentans*, the SMAV value from the acute data was 0.09 mg/L, and the ChV was calculated as 0.05 mg/L, which resulted in an ACR of 1.92. For *D. magna*, the SMAV value from the acute data was 56.19 mg/L, and the ChV was obtained 10.20 from calculated the geometric mean of 16.97, 35.36, and 1.77 mg/L in three studies based on reproduction, which resulted in an ACR of 5.51. For *G. rarus,* the SMAV value from the acute data was 10.52 mg/L, the ChV was calculated based on 14d survival toxicity test as 2.23 mg/L, which resulted in an ACR of 4.72. Based on the five ACR values, the FACR for PFOS was obtained as 14.13. Then, FCV based on the freshwater organisms for PFOS was calculated as 4.56 μg/L.

The chronic toxicity data for freshwater plant were screened to calculate the FPV for PFOS. The study of the alga *Myriophyllum* sp. was selected because the biological and ecological important endpoints and the concentrations of PFOS in the test were measured (Beach et al. 2006; Giesy et al. 2010). As shown in Table 1.21, the NOEC and LOEC data for *myriophyllum sibiricum* were the lowest values of respective 0.3 and 2.9 mg/L of all the freshwater plants in the study. The ChV was calculated as 0.93 mg/L, which was deemed as the FPV of PFOS. The value of FPV for PFOS was higher than that of FCV, and then the CCC for PFOS was 4.56 μg/L.

The final residue value was not derived due to no sufficient data on the tissue residue in the aquatic organism such as fish. In the study of estimating the residue level of bluegill sunfish, 87 mg PFOS/kg was regarded as the critical tissue concentration which was not expected to cause acute toxicity in fish (Drottar et al. 2001). Similar to the results in this study, Minnesota Pollution Control Agency was not taken into account in the derivation of the WQCs of PFOS.

1.3.2.4 Discussion

Various environmental limits for PFOS had been derived to protect the aquatic organisms from adverse effects of PFOS in freshwater. In the USA, Stevens and Coryell (Stevens and Coryell 2007) derived surface water quality criteria for PFOS for the Minnesota Pollution Control Agency. The final FAV for PFOS is 170 μg/L.

The maximum criterion (MC) was calculated by dividing the final FAV by 2. Thus, the maximum (MC) is 85 μg/L. They calculated an LC_{50} of 170 μg/L for 10-days survival of *C. tentans* which is divided by an acute/chronic ratio (ACR) of 9.12, resulting in a chronic quality standard (CC) of 18.6 μg/L. It was about three times higher of CMC (32.24 μg/L) and four times higher than the CCC (4.56 μg/L) in this study, respectively. However, the WQCs for PFOS in the freshwater environment had been calculated according to USEPA Great Lakes Initiative (GLI) (USEPA 1995). A CMC was calculated for PFOS by dividing the FAV (42 μg/L) by 2, and this resulted in a value of 21 μg/L. The CCC was determined as the lower value between the FCV (5.1 μg/L) and the FPV (2,300 μg/L), thus the CCC is 5.1 μg/L (Giesy et al. 2010). The CMC value was lower than that of 32.24 μg/L and the CCC value was similar to that of 4.56 μg/L.

From the above, it can be concluded that the WQCs for freshwater that are derived in this study are lower than those obtained by others. One reason is that different approaches are available to derive WQCs values. The GLI comprises with a two-tiered methodology (Tier I and Tier II), in which, the Tier I procedure is the same as the method in the calculation of WQCs in the USEPA guideline (USEPA 1985), and the Tier II procedure is suitable for the derivation of WQCs with limited toxicity data available. For the Minnesota Pollution Control Agency, the minimum requirement of eight species studies had not been met; there were no acceptable data for an aquatic insect. Therefore, the GLI Tier II method was used to calculate the criteria. For the US freshwater environment, the data on acute toxicity of PFOS met the GLI species requirements for using GLI Tier I methodology. The other reason is due to the toxicity data in which considerable effects are observed at concentrations below the lowest NOEC reported so far.

However, with the 36 days NOEC value of lower than 2.3 μg/L for emergence of *C. tentans* (MacDonald et al. 2004), the chronic criteria (18.6, 5.1, 4.56 μg/L) in these studies were obviously not protective. Furthermore, it was reported that severe effects would caused on fish and insects at the concentration of 10 μg/L (Moermond et al. 2010), which also demonstrated that the chronic criteria was not considered sufficiently protective.

In 2004, the Predicted No-effect Concentrations (PNECs) of PFOS for the aquatic environment with four different methods were set to be ranged from 0.61 to 6.66 μg/L (Qi et al. 2011). The PNECs of 0.61 and 4.0 μg/L were calculated respective in terms of FCV and FAV/FACR according to USEPA guideline (USEPA 1985), which were comparable with the FCV in this study. However, the toxicity dataset in the study (Qi et al. 2011) included the species of *Danio rerio*. The toxicity data of 0.01 mg/L based on the endpoint of mRNA expression was not adequate for the calculation of PNEC. Except the PNEC value of 0.61 μg/L, the CCC for PFOS derived in this study was lower than those obtained by other methods. In addition, the PNEC in the risk evaluation report on PFOS was 25 μg/L (Brooke et al. 2004) by applying the assessment factor of 10 to the NOEC of 0.25 mg/L for the saltwater species mysid (*Mysidopsis bahia*) (Drottar and Krueger 2000e). The PNEC value was highly underprotective for many species such as *Enallagma cyathigerum* (Bots et al. 2010) and *C. tentans* (MacDonald et al. 2004).

In the Netherlands, National Institute for Public Health and the Environment published the ERLs with negligible concentration (NC), maximum permissible concentration (MPC), maximum acceptable concentration for ecosystems (MAC_{eco}), and serious risk concentration for ecosystems (SRC_{eco}) for PFOS {Moermond et al. 2010 #134} (Moermond et al. 2010). Of all ERLs, the value of MAC_{eco} of 36 μg/L based on the acute toxicity data was in the same order of magnitude with the value of CMC, although it was deemed to be mainly theoretical. The calculations of MPC, NC, and SRC_{eco} had been taken food chain transfer into account, which were similar to the WQCs based on the human health of USEPA. Then, it could not be compared directly with the values in this study.

In addition, the microcosm studies of exposure of zooplankton to PFOS were reported in the test duration of 35-d. In a static conditions test of 30-L outdoor aquaria of PFOS, zooplankton microcosm communities consisted of *Cyclops canthocamptus staphylinus*, *C. strenuous*, *C. diaptomus*, *D. magna*, *Keratella quadrata*, *Phyllopoda* sp., *Echninorhynchus* sp., *Ostracoda* sp., total *Rotifera* sp., other macrophytes, and invertebrates. Total zooplankton population had reduced 90–100 % at 30 mg/L after 7 days exposure and similar reduction at 10 mg/L after 14 days. The results showed that *Ephemeroptera* sp. were completely absent at 10 mg/L and significantly decreased at 1 mg/L after 35 days exposure (Sanderson et al. 2002). Natural zooplankton microcosm communities of 92 species were exposed to PFOS using 12,000-L outdoor aquaria in the static test period. It was found that an overall community shifted from a larger zooplankton dominated community to a community dominated by Rotifers. The 35 days NOEC of the structure community was at concentration of 3 mg/L (Boudreau et al. 2003b). In another microcosm study of PFOS, zooplankton communities were tested using both indoor and outdoor static aquaria. The outdoor microcosm LOEC (community) value for PFOS was 10–30 mg/L, which was higher than that of 1–10 mg/L in indoor microcosm after 35 days exposure (Sanderson et al. 2003). The lowest toxicity effect of LOEC of 1 mg/L in the three studies on the microcosms, which was much higher than the CCC value in this study. Consequently, the chronic criterion of PFOS could be protective of most of aquatic organisms in the microcosm.

1.3.2.5 Conclusions

The WQCs for PFOS using the native aquatic organisms in China were derived in freshwater with the criteria maximum concentration (CMC) of 32.24 μg/L and criteria continuous concentration (CCC) of 1.73 μg/L depending on the toxicity data of aquatic organisms. The preliminary results of the WQCs for PFOS in the freshwater could be used as reference values to further decision for developing environmental quality standards and studies on the potential risk evaluation of PFOS for aquatic ecosystem in China.

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Chapter 2
Development of Emergency Water Quality Standard for Typical Heavy Metals with Chinese Resident Ecotoxicity Data

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Abstract Environmental pollution emergency of heavy metals has posed serious ecological risks in China. However, local emergency water quality standards (WQSs) are not yet established. In the present study, local ecotoxicity data of six heavy metals, Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Hg^{2+} and Cr^{6+}, were collected and screened. The suitability of four species sensitivity distribution (SSD) methods assumed to be used to derive the WQSs was evaluated by data analysis. Then, the methodology of emergency WQSs was established with the principles of SSD and ecological risk assessment, and the tiered emergency WQSs values of the six heavy metals were derived with the established methodology. Finally, a case analysis was demonstrated with the developed cadmium emergency WQSs and risk grade definition. The results may provide technical references for response to environmental pollution emergency.

Keywords Emergency · Water quality criterion · Water quality standard · Ecological risk assessment · Heavy metals

2.1 Introduction

Water quality standards (WQSs) form the foundation of discharge standards of pollutants and protection of ambient water environment quality. They can be divided into long-term exposure WQSs and short-term (emergency) exposure WQSs. The latter meant to estimate severe effects and to protect most species against lethality during intermittent and transient events (e.g., spill events to

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Z. Yan and Z. Liu (eds.), *Toxic Pollutants in China*,
SpringerBriefs in Environmental Science, DOI 10.1007/978-94-017-9795-5_2

aquatic-receiving environments, infrequent releases of short-lived/non-persistent substances.). In contrast, long-term exposure guidelines are meant to protect against all negative effects during indefinite exposures (CCME 2007). The technical system of long-term exposure WQSs have been established maturely in developed countries, such as the criterion continuous concentration (CCC) of the United States (USEPA 1985), the water quality guidelines issued by the Canadian Council of Ministers of the Environment (CCME 1991), the predicted no effect concentration of the European Union (ECB 2003), the trigger values of Australia and New Zealand (ANZECC and ARMCANZ 2000) and the negligible concentration (NC), the maximum permissible concentration (MPC), and the serious risk concentration (SRC) issued by the Netherland (Traas 2001).

The emergency WQSs were studied earlier in the United States. In the American water quality criteria (WQC) document issued in 1968, also called "Green Book" (National Technical Advisory Committee to the Secretary of the Interior 1968), the criterion maximum concentration (CMC) was proposed to deal with the acute exposure of pollutants, and the concept was still in use today in the United States (USEPA 2009). In recent years, many countries strengthen the study on the emergency WQSs. For example, the Netherlands issued the revised guidance for the derivation of environmental risk limits in 2007. In the guidance (Van Vlaardingen and Verbruggen 2007), in addition to the NC, MPC, and SRC, a new concept of maximum acceptable concentration for ecosystem (MAC_{eco}) was proposed to protect the aquatic ecosystem against acute toxic effects exerted by exposure to short-term peak concentrations or against acute effects of transient exposure peaks.

After decades of development, China has established relatively mature long-term exposure WQSs to protect the quality of surface water, groundwater, marine water, and so on. However, emergency WQSs are not yet developed, not even studied. On the other hand, at present, China has entered the period of high risk of pollution accident, and unexpected environment pollution events of various pollutants, especially heavy metals, occurred often. Fox example, recently, the serious sudden accident of pollution of cadmium taking place in the Longjiang River in Guangxi Province has caused tens of tons of adult fish and more than one million of fry to death (Xinhua News Agency reported). The emergency WQSs are needed urgently in China to assess the ecological risk posed by heavy metals in emergency pollution accidents. This study collected and screened the acute ecotoxicity data of six heavy metals and established the methodology of emergency WQSs with the principle of species sensitivity distribution (SSD) and ecological risk assessment. And, the tiered emergency WQSs for six typical heavy metals were derived. The results can provide valuable information to the environmental management of sudden pollution accident of heavy metals.

2.2 Materials and Methods

2.2.1 Collection of Published Acute Ecotoxicity Data of Six Heavy Metals

The published acute toxicity data of Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Hg^{2+}, and Cr^{6+} to aquatic animals were collected from the ECOTOX database (http://cfpub.epa.gov/ecotox), TOXNET Database (http://toxnet.nlm.nih.gov), the China National Knowledge Infrastructure (www.cnki.net), and other open literatures. The data were screened according to the guidelines for deriving national WQC for the protection of aquatic organisms and their uses in the United States (USEPA 1985). Unqualified data with unsuitable exposure time, unusual diluted water, unscientific experimental design, and relatively insensitive life stages were not selected. Data of non-Chinese species were also abandoned. As for the test endpoints, the 48 h-LC_{50} or EC_{50} for daphnia or larvae of midge, and 96 h-LC_{50} or EC_{50} for fish, mollusks, shrimp, and other organisms were chosen.

2.2.2 Evaluation of the Suitability of Four SSD Methods

In order to obtain the optimal model, the suitability of several SSD methods assumed to be used to develop the methodology of emergency WQSs was evaluated. The hazardous concentrations for 5 % of the species (HC_5) were calculated according to four SSD methods that are based on log-triangle (USEPA 1985), log-normal (Van Vlaardingen et al. 2004), log-logistic (Aldenberg and Solb 1993), and BurrIII function (Hose and Van den Brink 2004), respectively. The model that gained a suitable HC_5 value was chosen to derive the pollutant concentration corresponding to different affected fractions of species.

2.2.3 Establishment of Methodology of Tiered Emergency WQSs

The methodology of tiered emergency WQSs was developed with the principle of SSD and ecological risk assessment. The SSD curve was fitted by the desirable SSD method that screened out in the above procedure. The tiered ecological risks were defined according to different affected fractions of species, and the corresponding pollutant concentrations were calculated by the fitting function. Then, the tiered emergency WQSs were developed on the basis of the tiered pollutant concentration and a correction factor.

2.2.4 Data Analysis and Development of Emergency WQSs of Six Heavy Metals

The data were analyzed using the PASW statistics 18. The normality of the data was checked by Kolmogorov–Smirnov test. Statistical significances were considered to be significant at $p \leq 0.05$. The species acute toxicity data were used to generate the SSD curve. If a species has more than one toxicity datum, the species mean acute value (SMAV) was used instead, and it is equal to the geometric average of all the qualified toxicity data of the species. According to the methodology established above, the emergency WQSs for the six heavy metals were developed. Finally, a case analysis of sudden cadmium pollution in Longjiang River, Guangxi Province, in 2012 was demonstrated with the derived emergency WQSs of cadmium.

2.3 Results

2.3.1 Freshwater Species Sensitivity of the Six Heavy Metals

Published acute toxicity data of six heavy metals to freshwater organisms were collected and screened, and the results were shown in the supporting materials. Qualified toxicity data of 45 species for Cd^{2+}, 54 species for Cu^{2+}, 26 species for Pb^{2+}, 26 species for Zn^{2+}, 47 species for Hg^{2+}, and 30 species for Cd^{2+} were obtained. The normality of these ecotoxicity data was analyzed by Kolmogorov–Smirnov test, and the results showed that they are all acceptable.

The statistic characteristics of the qualified data were analyzed, and the results were shown in Table 2.1. We can see that the data of Cu^{2+} are sufficient and the data of Pb^{2+} and Zn^{2+} are relatively insufficient. Fortunately, they all meet the minimum toxicity data requirement of SSD generation [ten data for fitting of one SSD curve (Wheeler et al. 2002)]. In terms of the average value, Cu^{2+} and Hg^{2+} have higher toxicity, while Pb^{2+} and Cr^{6+} have lower toxicity to freshwater

Table 2.1 Statistic characteristics of toxicity data of the six heavy metals

Heavy metals	Sample number	Minimum (μg/L)[a]	Maximum (μg/L)[a]	Average (μg/L)	SD
Cd^{2+}	45	0.15	4.76	2.85	1.07
Cu^{2+}	54	−0.80	4.46	1.82	0.89
Pb^{2+}	26	1.80	5.84	3.78	1.23
Zn^{2+}	26	1.93	4.85	3.40	0.91
Hg^{2+}	47	−0.52	4.23	2.00	1.01
Cr^{6+}	30	0.48	5.54	3.61	1.49

[a]The data have been transformed by common logarithm

organisms. The standard deviation (SD) of the data set of Cr^{6+} (1.49) is the highest, and that of Cu^{2+} (0.89) is the lowest.

As for the sensitivity of freshwater organism to the six heavy metals, the most sensitive and insensitive species to Cd^{2+} are *Salmotrutta* (LC_{50} = 1.40 μg/L) and *Branchiurasowerbyi* (LC_{50} = 58,020 μg/L), respectively. Except 1 fish and 1 rotifer, in the 10 most sensitive organisms to Cd^{2+}, the other 8 are all crustaceans. The most sensitive and insensitive species to Cu^{2+} are *Tubifextubifex* (LC_{50} = 0.16 μg/L) and *Sinopotamonhenanense* (LC_{50} = 28,610 μg/L), respectively. Except the tubificid worm*Tubifextubifex*, in the 10 most sensitive organisms to Cu^{2+}, the other 9 are all crustaceans. The most sensitive and insensitive species to Pb^{2+} are *Ceriodaphniadubia* (LC_{50} = 63.8 μg/L) and *Sinopotamonhenanense* (LC_{50} = 692,090 μg/L). The 10 most sensitive organisms to Pb^{2+} contain 5 crustaceans, 4 fish, and 1 shrimp, indicating the diversity of species sensitivity to this heavy metal. The most sensitive and insensitive species to Zn^{2+} are *Ceriodaphnia reticulate* (LC_{50} = 85.4 μg/L) and *Ranacatesbeiana* (LC_{50} = 70,000 μg/L), respectively. Except 1 fish and 1 rotifer, in the 10 most sensitive organisms to Zn^{2+}, the other 8 are all crustaceans. The most sensitive and insensitive species to Hg^{2+} are *Ictaluruspunctatus* (LC_{50} = 0.30 μg/L) and *Ranatigrina* (LC_{50} = 17,165 μg/L), respectively. Except 2 fish, in the 10 most sensitive organisms to Hg^{2+}, the other 8 are all crustaceans. The most sensitive and insensitive species to Cr^{6+} are *Diaphanosomabrachyurum* (LC_{50} = 3.00 μg/L) and *Cyprinuscarpio* (LC_{50} = 346,700 μg/L). The 10 most sensitive organisms to Cr^{6+} are all crustaceans. So, the SSD of the six heavy metals suggested that crustaceans are the most sensitive species to heavy metals.

2.3.2 The Suitability of the Four SSD Methods Assumed to Be Used to Develop the Methodology

In order to screen the desired construction method of SSD curve, four SSD fitting methods were adopted to analyze the acute toxicity data of the six heavy metals to acquire the HC_5 values. The results were shown in Table 2.2, and it suggested that different SSD method produced different HC_5 values. Generally, the values derived with "SSD-RIVM" and "SSD-AU & NZ" methods were relatively higher, while the "SSD-EU" method was relatively stringent.

2.3.3 Establishment of the Methodology of Tiered Emergency WQSs

The methodology of tiered emergency WQSs was developed with the principle of SSD and ecological risk assessment. Different affected fraction of the aquatic

Table 2.2 Comparison of the derived HC_5 values of the four SSD fitting methods

Fitting methods	Fitting functions	HC_5 (μg/L)					
		Cd^{2+}	Cu^{2+}	Pb^{2+}	Zn^{2+}	Hg^{2+}	Cr^{6+}
SSD-USA[2]	Log-triangular	4.32	3.32	88.54	90.14	0.86	7.28
SSD-EU[11]	Log-logistic	0.72	0.26	10.28	23.19	0.54	0.74
SSD-RIVM[16]	Log-normal	12.09	2.19	54.26	75.71	2.16	13.40
SSD-AU & NZ[12]	BurrIII	9.42	3.22	110.26	115.31	1.16	1.34

The "SSD-AU & NZ" method was chosen as a desirable fitting method for its moderate derived values to develop the methodology of the emergency WQSs

organism corresponds to different ecological risk level caused by the pollutant. In the Netherlands, if the affected fraction of the aquatic species reaches 50 %, the risk level caused by the pollutant is considered serious (Traas 2001). On the other hand, if the affected fraction of the aquatic species is less than 5 %, the ecological risk posed by the pollutant can be ignored generally (USEPA 1985). Taking the above principles as references, four grades of risk levels were designed in this study according to different affected fractions of the aquatic species, and the corresponding four grades of emergency WQSs were derived. As shown in Fig. 2.1, they are "4th grade (IV)" (serious risk, the affected fraction is greater than 50 %), "3rd grade (III)" (apparent risk, the affected fraction is greater than 30 %), "2nd grade (II)" (some risk, the affected fraction is greater than 15 %), and "1st grade (I)" (potential risk, the affected fraction is greater than 5 %). The *X* value in Fig. 2.1 indicates the hazardous concentration (Shcheglov et al. 1990) of the pollutant, and WQS equals the HC value divided by the correction factor that was

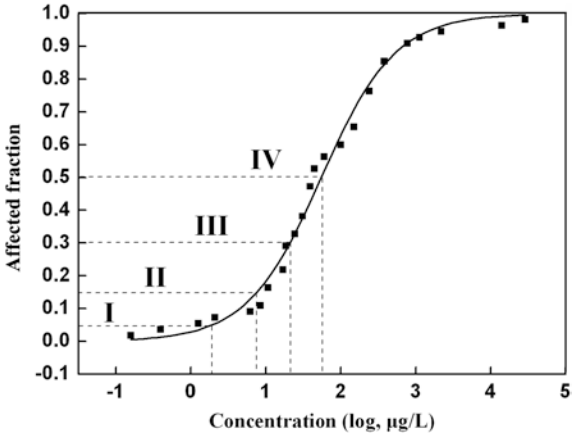

Fig. 2.1 Sketch map of SSD curve fitting. "*I*", "*II*", "*III,*" and "*IV*" in the figure represent the four-grade WQSs and is corresponding to the four grades of ecological risk levels: *I* (potential risk, the affected fraction is greater than 5 %), *II* (some risk, the affected fraction is greater than 15 %), *III* (apparent risk, the affected fraction is greater than 30 %), and *IV* (serious risk, the affected fraction is greater than 50 %)

generally assumed to be 1–5 (van Vlaardingen and Verbruggen 2007). Because the uncertainty of the ecological risk rises with the increase of concentration of pollutant, the correction factor value was set to be 5, 4, 3, and 2, corresponding to the four grades of WQSs, IV, III, II, and I, respectively.

In addition, the emergency duration time and frequency of the derived WQSs in this study were designated "3 h" and "not more than one time pre-three years" according to the technical guidelines of WQC in the USA (USEPA 1985; Jin et al. 2009). They were proposed according to the results of related scientific research that concerned the toxic effects of pollutant to individual species and ecosystem.

2.3.4 Derivation of the Tiered Emergency WQS for Heavy Metals

The tiered emergency WQS for the six heavy metals was derived according to the established methodology. The calculated SSD parameters were shown in Table 2.3, and the results were shown in Fig. 2.2 and Table 2.4.

2.3.5 Case Analysis with the Developed Emergency WQSs of Cadmium

A sudden cadmium environmental pollution was occurred in Longjiang River, Guangxi Province, in China in 2012. The reported peak value of cadmium concentration exposed in the river in the accident was 400 μg/L, and after emergency disposal, it decreased to about 125 μg/L (Xinhua News Agency reported). In terms of the emergency WQSs of cadmium developed in this study, the 400 μg/L of cadmium may pose serious ecological risks (risk grade: IV), and most of the regional aquatic species are threatened, while the 125 μg/L of cadmium may pose apparent ecological risks (risk grade: III), and the affected aquatic species is greater than 30 % (Table 2.4; Fig. 2.2). The affected organisms contained shrimps, some sensitive fish, e.g., the bighead fish, and some aquatic invertebrates, such as hydras,

Table 2.3 The SSD parameters calculated with "SSD-AU & NZ" method

Heavy metals	Fitting functions	Calculated SSD parameters		
Cd^{2+}	BurrIII	1122.381 (*b*)	0.745 (*c*)	0.834 (*k*)
Cu^{2+}	BurrIII	24.957 (*b*)	0.791 (*c*)	1.662 (*k*)
Pb^{2+}	ReWeibull	21.158 (α)	0.415 (β)	
Zn^{2+}	ReWeibull	38.224 (α)	0.536 (β)	
Hg^{2+}	BurrIII	599.347 (*b*)	1.496 (*c*)	0.320 (*k*)
Cr^{6+}	BurrIII	217341.131 (*b*)	3.834 (*c*)	0.065 (*k*)

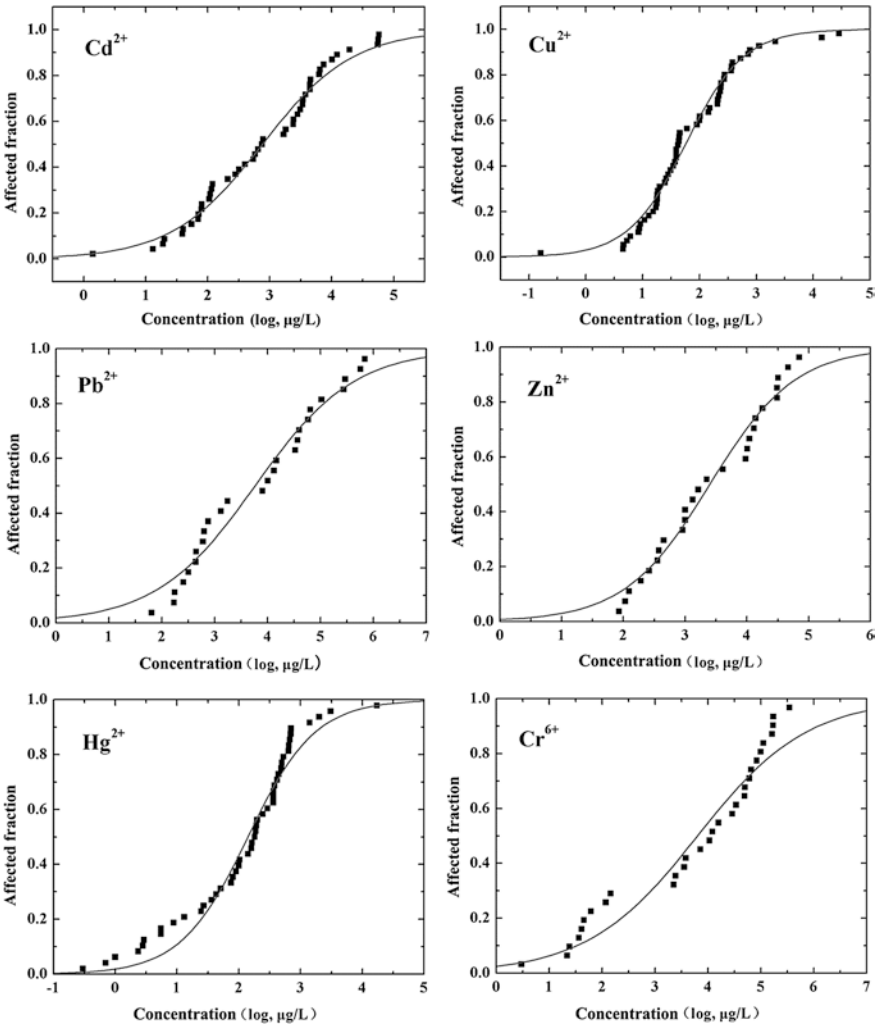

Fig. 2.2 SSD fitting of six heavy metals. The *solid square* in the figure indicates the acute ecotoxicity data of different heavy metals

Table 2.4 The four-grade emergency WQSs of the heavy metals

Grades	Risks	Affected fraction (%)	Emergency WQSs (μg/L)					
			Cd^{2+}	Cu^{2+}	Pb^{2+}	Zn^{2+}	Hg^{2+}	Cr^{6+}
IV	Serious	50	158	11.5	746	353	30.6	2,702
III	Apparent	30	58.1	5.80	247	157	12.3	436
II	Some	15	20.5	3.20	110	90.1	3.80	36.2
I	Potential	5	4.71	1.61	55.1	57.7	0.580	0.67

daphnia, and rotifers. Basically, after emergency disposal, there were no risks to some insensitive species, such as common carp, loach, amphibians, oysters, and crabs with the time limitation of 3 h.

2.4 Discussions

WQSs play important role in water environment management. They can be divided into long-term exposure WQS and emergency WQS (USEPA 1985). Generally, the value of the former is lower, and they are determined according to the results of WQC study, risk assessment, and cost–benefit analysis (USEPA 1994), while the value of the latter is higher, and they are developed according to the results of WQC study and risk assessment (Van Vlaardingen and Verbruggen 2007; Sloof 1992).

As for the minimum toxicity data requirement for deriving the WQC, the USEPA prescribed that at least three phyla and eight families should be used in the calculation, and the details of the aquatic animals are contained in the aquatic life criteria guidelines (USEPA 1985). The emergency WQS were divided into two sorts, A and B, in the WQS guidelines issued by the CCME (2007). The WQS of sort A should be developed by the SSD method with sufficient toxicity data at least from three fish (including One Salmonidae fish and One non-Salmonidae fish), three aquatic or semi-aquatic invertebrate (including one pelagic crustacean), and if possible, aquatic plants and amphibian. When the toxicity data are insufficient, the assessment factor (AF) method was recommended to derive the WQS of sort B. In the guidance for derivation of environmental risk limits issued by the Netherlands (Van Vlaardingen and Verbruggen 2007), according to different situations, three methods, the AF method, the SSD method, and simulation of ecosystem, were recommended to develop the emergency WQS, respectively. In the guidance, at least three acute toxicity data of three kinds of different trophic level, such as algae, daphnia, and fish, are required to be used in the AF method, and at least eight acute toxicity data of aquatic organisms, including 6 aquatic animals, 1 algae, and 1 higher plant, are required to be used in the SSD method. Two kinds of vertebrate, including 1 fish, should be contained in the 6 aquatic animals. In the present study, sufficient toxicity data were collected for all the six heavy metals, and the data quantity is qualified for all the SSD methods.

There are several popular SSD methods for derivation of the WQSs, and all of them were accepted by the Organization for Economic Cooperation and Development (OECD 1992; Posthuma et al. 2002). These SSD methods were evaluated in the present study with calculation of the HC_5 value. Through comparison analysis, the "SSD-AU & NZ" method produced relatively moderate results for all the six heavy metals and was chosen to derive the WQS in this study. The derived emergency WQS was set to four grades (I, II, III, and IV) in this study. The correct factors for the HC values were set to be 2, 3, 4, and 5 for the four grades of risks due to the increasing risks with the higher pollutant concentrations, and they still need to be validated in field study or management of emergency environmental accident.

According to the methodology of the emergency WQSs, the duration time is 3 h. In an accident of emergency environmental pollution, when the exposure time is beyond 3 h, the posed ecological risk could be increased. How to assess the increased risks in an emergency is worth study. Before a perfect theory being proposed, at least some aquatic organisms can be taken as biological indicators for risk assessment. For example, in the above case analysis of the accident of the Longjiang River cadmium pollution, according to Fig. 2.2, we can know that when the sensitive freshwater shrimp is hard to survive, the pollutant can be considered to have posed some ecological risks, and the death of bighead fish and amphibians indicates apparent and serious risks, respectively.

Generally, the ecotoxicity of heavy metals can be affected by some water quality parameters, such as hardness, temperature, and pH. So a perfect WQS should be developed according to different regional water conditions. Moreover, the water quality conditions in different basins or regions in China are of high diversity. WQSs should be developed according to different ecoregions to facilitate risk assessment, ecoregion protection, and environmental management in emergency accident. The derived WQSs in the present study may be improved in these aspects in the future.

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Chapter 3
Study of Species Sensitivity Distribution for Pollutants

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Abstract Species sensitivity of different taxonomic groups from China and America for 8 priority pollutants including nitrobenzene, 2,4,6-trichlorophenol, 2,4-dichlorophenol, parathion, As(III), Cr(VI), Hg, and Cd was compared. The results showed that there was no significant difference between Chinese and American taxa. Results from species sensitivity distribution (SSD) curves of vertebrates and invertebrates for heavy metals including Cu, Hg, Cd, Cr(VI), Pb, and Zn showed that invertebrate taxa were more sensitive to each heavy metal than vertebrates. Copper was the most toxic to vertebrate, followed by Hg, Cd, Zn, and Cr(VI). The hazardous concentrations for 5 % of the species affected (HC_5) were derived to determine the criteria value. The HC_5 values of the six heavy metals were sorted in the following order: Zn > Pb > C r > Cd > Hg > Cu, indicating their toxicities in opposite order. Gene expression effect toxicity data of three metals were also analyzed to decide the rank of species sensitivity. The results showed that different metals may have different order for acute, chronic, and gene effect data. Besides, the interspecies correlation estimation (ICE) model can use the initial toxicity estimate for one species to produce correlated toxicity values for multiple species. The ICE model can usually be utilized to develop SSD and HC_5. The results from phenanthrene and benzo[*a*]pyrene (BaP) showed that the ICE model was verified as a valid approach for generating SSDs with limited toxicity data to derive WQC for phenanthrene benzo[*a*]pyrene (BaP) and possibly other toxicants if applicable.

Keywords Water quality criteria (WQC) · Species sensitivity distribution (SSD) · Gene effect data · Interspecies correlation estimation (ICE) · Heavy metals

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Z. Yan and Z. Liu (eds.), *Toxic Pollutants in China*,
SpringerBriefs in Environmental Science, DOI 10.1007/978-94-017-9795-5_3

3.1 Regional Comparative Studies on Species Sensitivity Distribution

3.1.1 Introduction

The species sensitivity distribution (SSD) method is widely used in risk assessment procedures (Solomon et al. 1996; Versteeg et al. 1999a, b) and the development of water quality criteria (WQC) (ANZECC and ARMCANZ 2000; Wheeler et al. 2002a). One of the purposes of SSD method is to calculate the environmental concentration of a pollutant that protects most species in the environment. Usually, a point estimate of the 95 % protection level (Van Straalen and Van Rijn 1998) or the HC_5 (the hazardous concentration for 5% of species) is used for this purpose. SSD curves are constructed by fitting a cumulative distribution function to a plot of species toxicity data against rank-assigned percentiles (Van Straalen and Denneman 1989; Aldenberg and Slob 1993; Wheeler et al. 2002a). The cumulative distribution function used in the SSD method of Europe and the United States is often lognormal (Wagner and Løkke 1991; European Commission 2011) or log-logistic (Aldenberg and Slob 1993), while that in Australia and New Zealand is the Burr Type III function (Shao 2000). From each of these cumulative distribution functions, the HC_5 values are calculated; it is known as the final acute values (FAVs) or final chronic values (FCVs) in the USA (Suter 2002). SSD method is dependent upon available datasets and can differ in type of volume of toxicity data, taxonomic diversity, and distribution (Wheeler et al. 2002a; Maltby et al. 2005a, b). A plenty of toxicity data from various taxonomic groups can derive a robust HC_5. Therefore, recommendations of minimum volume of toxicity data necessary for meaningful HC_5s of different distribution methodology vary in different countries (Feng et al. 2012a, b). Eight toxicity values of species from 3 phyla and 8 families have been considered a sufficient number to derive WQC in the United States (Stephen et al. 1985). Similar data volume and taxa requirements have also been applied in the WQC guidelines of other countries (ANZECC and ARMCANZ 2000; ECB 2003; CCME 2007; Van Vlaardingen and Verbruggen 2007; European Commission 2011).

Generally speaking, SSDs are constructed using measured toxicity values of aquatic species. However, it is difficult to obtain such toxicity data because a number of toxicity tests are limited by toxicity test method, time, expense, and species availability, especially for wildlife and endangered species. So, for most existing and emerging substances, this type of data is lacking (Sijm et al. 2001). Moreover, in most countries, HC_5 values are used to calculate WQC for pollutants based on native species data or site-specific data (Stephen et al. 1985; ANZECC and ARMCANZ 2000; Yin et al. 2003a, b). The potential use of non-native toxicity data for handling local problems is controversial and leaves one to question whether WQC based on species from one geographical region provide appropriate protection for species in another region (Davies et al. 1994). However, this argument cannot be resolved appropriately mainly because of the lack of toxicity data applicable for native species and the paucity of studies on such problems. Therefore, it is important to study whether it is feasible to use toxicity data of non-native species to derive WQC to protect native species.

In China, systematic WQC research has drawn increasing concern in recent years, and SSD methods with HC_5 values have been used to calculate WQC for limited pollutants with an emphasis on using native species (Jin et al. 2011; Yan et al. 2012b; Yang et al. 2012; Wang et al. 2014c). However, the comparison of SSDs based on native Chinese species and species from other regions is rarely studied.

In this study, eight priority pollutants both in China and the United States were selected due to the lack of suitable native ecotoxicology data and the priority management of priority pollutants in China. The priority pollutants were 2,4,6-trichlorophenol, 2,4-dichlorophenol, nitrobenzene, parathion, mercury (Hg), chromium (Cr(VI)), arsenic (As(III)), and cadmium (Cd). In addition, comparative investigations on SSDs of different taxa between China and the USA were carried out. The purposes of the present study were (1) to determine the differences of sensitivity of each taxonomic group between China and the United States and (2) to discuss whether toxicity data of species from the USA can be used in calculating criteria to protect species in China. This study could provide useful information for site-specific risk assessment and environmental management.

3.1.2 Materials and Methods

1. *Data collection*
   The published acute toxicity data of nitrobenzene, 2,4,6-trichlorophenol, 2,4-dichlorophenol, parathion, As(III), Cr(VI), Hg, and Cd were collected from the China National Knowledge Infrastructure (http://www.cnki.net), US EPA WQC document for parathion, As(III), Cr(VI), Hg and Cd (US EPA 1996), the ECOTOX database (http://cfpub.epa.gov/ecotox), and other sources. Species were selected based on whether they are (1) introduced for economic reasons and now widely exist in China or (2) native to China. The same principles were used for American species toxicity data when there was no WQC document. All toxicity data were screened and analyzed according to guidelines for WQC (Stephen et al. 1985). Test organisms of these toxicity data were categorized as either invertebrates or vertebrates, and each group was analyzed separately, including Chinese and/or American species. Toxicity data were limited to acute toxicity test with exposure periods of 48 h for cladocerans and 96 h for others, such as effective concentration (EC_{50}) and lethal concentration (LC_{50}) values.
2. *Data analysis*
   Various cumulative distribution functions have been used to fit SSDs (Erickson and Stephan 1988; Wagner and Løkke 1991; Aldenberg and Jaworska 2000; Van der Hoeven 2001; Chen 2004; Hose and Van den Brink 2004). In the present study, just one method was used to make the comparisons feasible and statistically meaningful. The log-logistic distribution was used for it often fits the toxicity data well (Kooijman 1987; Newman et al. 2000; Wheeler et al. 2002a; Feng et al. 2012a, b). The cumulative distribution function is as follows:

$$y = 1/\left(1 + \exp\left((P1 - x)/P2\right)\right)$$

where x is the mean of the $\log_{10}$-transformed LC_{50} or EC_{50} values; y is the cumulative probability of species, defined as (the order of the data point)/(1 + total number of data points); $P1$ is the parameter representing the intercept; and $P2$ is the parameter representing the slope of the curve.

The log-logistic distribution method was fitted to toxicity data points and evaluated using the chi-square goodness-of-fit test with the adjusted coefficient of determination R^2 in the software OriginLab 8.0 (USA, Origin Lab Company).

Statistical analyses of the difference of SSD for total species, vertebrates, or invertebrates between China and the USA were compared using the Mann–Whitney test and two-sample Kolmogorov–Smirnov test in the SPSS software (SPSS 20.0 for Windows). The Mann–Whitney test (M-W test) and two-sample Kolmogorov–Smirnov test (K-S test) are nonparametric methods that can be used to test whether two samples came from the same distribution. The two-sample Kolmogorov–Smirnov test (K-S test) has been used to compare the difference between SSDs in previous studies (Maltby et al. 2005a, b; Jin et al. 2011, 2012; Wang et al. 2013, 2014a, b). Additionally, hazardous concentrations for 5 % (HC_5) and 50 % of the species (HC_{50}) were calculated according to the log-logistic SSD method and compared between Chinese and American taxa.

3.1.3 Results

1. *Toxicity data and SSD construction*
   A total of 20, 16, 17, 32, 14, 29, 47, and 49 acute toxicity values for Chinese species were collected for nitrobenzene, 2,4,6-trichlorophenol, 2,4-dichlorophenol, parathion, As(III), Cr(VI), Hg, and Cd, respectively (Table 3.1). All the species toxicity data were divided into invertebrate and vertebrate taxa. Moreover, 13, 16, 13, 38, 16, 34, 33, and 50 acute toxicity values for the American taxa were found for the respective pollutants from WQC documents, ECOTOX, and other literature. The species included insects, annelids, benthic crustaceans, planktonic crustaceans, fish, amphibians, and so on (see supporting information).
   The results showed that the log-logistic distribution fits the data points of most taxonomic groups well, with high R^2 values of different taxonomic groups both in China and the USA (R^2: 0.82 − 0.99, $p < 0.01$). However, the distribution did not fit the data points of nitrobenzene for American vertebrates and 2,4-dichlorophenol for American invertebrates ($p > 0.05$) (Table 3.1, Fig. 3.2).
2. *Comparison of SSDs for total species*
   In this study, SSDs based on the total species were compared between Chinese and American taxa (Tables 3.2, 3.3; Fig. 3.1). The results showed that compared with the SSDs of total American species, the SSD curves of Cr(VI) and Hg, and SSD curves of As(III) and nitrobenzene for Chinese species below HC_{20} were shifted to the left, which indicated that Chinese species were more sensitive (Fig. 3.1). As for nitrobenzene, the lower tails of both Chinese and American species curves did not fit well, and species appeared to have similar

Table 3.1 Number of data values and goodness of fit of different taxonomic groups for eight pollutants

Pollutants	Taxonomic group	n	R^2	p
Nitrobenzene	Total Chinese species	20	0.88	<0.01
	Chinese invertebrate	8	0.83	<0.05
	Chinese vertebrate	12	0.82	<0.01
	Total American species	13	0.94	<0.01
	American invertebrate	6	0.96	<0.01
	American vertebrate	7	0.69	<0.10
2,4,6-trichlorophenol	Total Chinese species	16	0.98	<0.01
	Chinese invertebrate	8	0.95	<0.01
	Chinese vertebrate	8	0.95	<0.01
	Total American species	16	0.97	<0.01
	American invertebrate	6	0.94	<0.01
	American vertebrate	10	0.96	<0.01
2,4-dichlorophenol	Total Chinese species	17	0.96	<0.01
	Chinese invertebrate	6	0.88	<0.05
	Chinese vertebrate	11	0.96	<0.01
	Total American species	13	0.93	<0.01
	American invertebrate	5	0.85	<0.10
	American vertebrate	8	0.91	<0.01
Parathion	Total Chinese species	32	0.96	<0.01
	Chinese invertebrate	18	0.95	<0.01
	Chinese vertebrate	14	0.94	<0.01
	Total American species	38	0.93	<0.01
	American invertebrate	23	0.96	<0.01
	American vertebrate	15	0.97	<0.01
As(III)	Total Chinese species	14	0.94	<0.01
	Chinese invertebrate	8	0.83	<0.05
	Chinese vertebrate	6	0.94	<0.01
	Total American species	16	0.91	<0.01
	American invertebrate	9	0.88	<0.01
	American vertebrate	7	0.93	<0.01
Cr(VI)	Total Chinese species	29	0.94	<0.01
	Chinese invertebrate	16	0.93	<0.01
	Chinese vertebrate	13	0.98	<0.01
	Total American species	34	0.85	<0.01
	American invertebrate	17	0.94	<0.01
	American vertebrate	17	0.98	<0.01

(continued)

Table 3.1 (continued)

Pollutants	Taxonomic group	n	R^2	p
Hg	Total Chinese species	47	0.97	<0.01
	Chinese invertebrate	25	0.99	<0.01
	Chinese vertebrate	22	0.97	<0.01
	Total American species	33	0.98	<0.01
	American invertebrate	25	0.98	<0.01
	American vertebrate	8	0.94	<0.01
Cd	Total Chinese species	49	0.99	<0.01
	Chinese invertebrate	32	0.98	<0.01
	Chinese vertebrate	17	0.95	<0.01
	Total American species	50	0.94	<0.01
	American invertebrate	30	0.94	<0.01
	American vertebrate	20	0.89	<0.01

n is the number of data values of each taxonomic group; R^2 is the adjusted coefficient of determination; p is the significance level of the adjusted coefficient of determination, R^2

sensitivity above HC_{25}. On the contrary, the SSD curves for American species were shifted to the left compared with the SSD curves of 2,4,6-trichlorophenol, 2,4-dichlorophenol, parathion, and Cd for Chinese species (Fig. 3.1). The comparison showed that the HC_5 values of total Chinese species were similar to those of total American species (difference: −146.87 to 64.83 %, Table 3.3) except parathion and Cr(VI). The HC_{50} values of total Chinese species were similar to those of total American species for all eight toxicants (difference: −217.63 to 79.73 %, Table 3.3). Results of the Mann–Whitney test ($p = 0.109$–1.000) and two-sample Kolmogorov–Smirnov test (ks = 0.411–1.059, $p = 0.212$–0.996) showed that the SSDs for total Chinese species and American species were not significantly different for all eight pollutants.

3. *Comparison of SSDs for Chinese and American taxonomic groups*
   In the present study, SSDs based on toxicity data of invertebrates were compared between Chinese and American taxa (Tables 3.2, 3.3; Fig. 3.2). Similar comparisons were conducted for vertebrates. Compared with the SSD curves of nitrobenzene, As(III), Cr(VI), Hg, and Cd for American invertebrates, the SSD curves for Chinese species were shifted slightly to the left (Fig. 3.2). As for vertebrates, the SSD curves of Chinese species for parathion, As(III), Cr(VI), and Hg were shifted to the left, too (Fig. 3.2). The results showed that HC_5 and HC_{50} values of Chinese invertebrates and American invertebrates were similar, except for parathion (Table 3.3). As for vertebrates, HC_5 and HC_{50} values between the two countries were similar except HC_5 for nitrobenzene and Cd. Moreover, the results of comparison of sensitivity distributions for Chinese and American invertebrates showed that there was no significant difference for all eight pollutants (M-W test: $p = 0.109$–1.000; K-S test: ks = 0.298–1.113, $p = 0.168$–1.000). The difference for vertebrates was not significant, too. (M-W test: $p = 0.360$–0.847; K-S test: ks = 0.401–0.835, $p = 0.488$–0.997).

Table 3.2 Comparison between different taxonomic groups from China and the USA using the two-sample Kolmogorov–Smirnov test and Mann–Whitney test

Pollutants	Taxa	ks	*p* (K-S test)	*p* (M-W test)
Nitrobenzene	Total	0.411	0.996	0.924
	Invertebrate	0.526	0.945	0.622
	Vertebrate	0.401	0.997	0.837
2,4,6-trichlorophenol	Total	0.707	0.699	0.423
	Invertebrate	0.309	1.000	1.000
	Vertebrate	0.738	0.648	0.360
2,4-dichlorophenol	Total	0.436	0.991	0.732
	Invertebrate	0.298	1.000	1.000
	Vertebrate	0.514	0.955	0.600
Parathion	Total	0.953	0.324	0.147
	Invertebrate	1.113	0.168	0.109
	Vertebrate	0.423	0.994	0.847
As(III)	Total	0.830	0.497	0.570
	Invertebrate	0.543	0.930	0.815
	Vertebrate	0.642	0.804	0.628
Cr(VI)	Total	1.059	0.212	0.264
	Invertebrate	0.834	0.490	0.345
	Vertebrate	0.835	0.488	0.805
Hg	Total	0.693	0.723	0.494
	Invertebrate	0.849	0.468	0.210
	Vertebrate	0.606	0.857	0.801
Cd	Total	0.581	0.889	0.685
	Invertebrate	0.984	0.288	0.269
	Vertebrate	1.106	0.173	0.044

ks is a test statistic used to determine the significance level p (K-S test), $p > 0.05$ means the difference between distributions is not significant; p (M-W test) represents the significance level, and $p > 0.05$ means the difference between distributions is not significant

3.1.4 Discussion

1. *SSD construction*
   In the present study, we found that the log-logistic distribution fits the toxicity data of different taxonomic groups in China and the USA well according to the goodness-of-fit test (R^2: 0.82–0.99, $p < 0.01$) (Table 3.1). Similarly, previous studies reported that log-logistic distribution often fits the toxicity data best (Kooijman 1987; Newman et al. 2000; Wheeler et al. 2002a). But, it does not work when the toxicity data are insufficient (Table 3.1).
2. *Comparison of SSD*
   In this work, HC_5 values as well as SSD of vertebrates were compared with invertebrates in both China and the USA (Table 3.3, Fig. 3.2). Sensitivity of invertebrates to 2,4-dichlorophenol, parathion, As(III), Cr(VI), Hg, and Cd was

Table 3.3 HC_5 and HC_{50} values of different taxonomic groups and comparisons between Chinese and American taxa

Toxicant	Taxonomic group	HC_5a	Difference (%)	HC_{50}a	Difference (%)
Nitrobenzene	Total Chinese species	8.86	−53.38	56.51	−6.93
	Total American species	13.59		60.43	
	Chinese invertebrate	11.45	−57.11	51.01	−9.76
	American invertebrate	17.99		55.99	
	Chinese vertebrate	4.48	95.98	57.30	38.28
	American vertebrate	0.18		35.36	
2,4,6-trichlorophenol	Total Chinese species	0.91	64.83	3.56	25.84
	Total American species	0.32		2.64	
	Chinese invertebrate	1.06	12.26	3.49	−6.30
	American invertebrate	0.93		3.71	
	Chinese vertebrate	0.51	80.39	3.48	45.11
	American vertebrate	0.10		1.91	
2,4-dichlorophenol	Total Chinese species	0.96	34.37	5.36	11.19
	Total American species	0.63		4.76	
	Chinese invertebrate	0.44	22.72	3.88	−11.85
	American invertebrate	0.34		4.34	
	Chinese vertebrate	1.52	53.94	6.37	19.93
	American vertebrate	0.70		5.10	

(continued)

Table 3.3 (continued)

Toxicant	Taxonomic group	HC_5a	Difference (%)	HC_{50}a	Difference (%)
Parathion	Total Chinese species	0.22	90.90	222.37	79.73
	Total American species	0.02		45.07	
	Chinese invertebrate	0.01	−900.00	32.23	90.69
	American invertebrate	0.10		3.00	
	Chinese vertebrate	132.86	−99.36	984.60	−7.97
	American vertebrate	264.88		1063.11	
As(III)	Total Chinese species	264.05	−26.97	12295.32	21.97
	Total American species	335.29		9593.34	
	Chinese invertebrate	28.04	−151.03	5717.42	34.39
	American invertebrate	70.39		3751.11	
	Chinese vertebrate	5949.86	−41.21	23016.54	19.65
	American vertebrate	8402.05		18492.69	
Cr(VI)	Total Chinese species	4.15	−905.06	5478.86	−217.63
	Total American species	41.71		17402.83	
	Chinese invertebrate	0.39	−5.12	317.12	−315.17
	American invertebrate	0.41		1316.59	
	Chinese vertebrate	8014.93	−193.58	63794.02	9.22
	American vertebrate	23530.48		57912.19	

(continued)

Table 3.3 (continued)

Toxicant	Taxonomic group	HC_5a	Difference (%)	HC_{50}a	Difference (%)
Hg	Total Chinese species	3.52	−146.87	139.95	−36.86
	Total American species	8.69		191.54	
	Chinese invertebrate	0.49	−630.61	64.50	−165.45
	American invertebrate	3.58		171.22	
	Chinese vertebrate	29.87	−140.91	250.81	10.75
	American vertebrate	71.96		223.84	
Cd	Total Chinese species	4.39	54.44	644.40	19.31
	Total American species	2.00		519.96	
	Chinese invertebrate	3.07	9.44	235.30	−112.83
	American invertebrate	2.78		500.80	
	Chinese vertebrate	384.17	99.85	3836.45	86.68
	American vertebrate	0.57		510.67	

[a] The unit of As(III), Cr(VI), Hg, Cd, and parathion is μg/L, the unit of nitrobenzene, 2,4,6-trichlorophenol, and 2,4-dichlorophenol is mg/L. The difference was calculated by the function $(HC_{5\ \text{Chinese taxa}}\text{-}HC_{5\ \text{American taxa}})/HC_{5\ \text{Chinese taxa}} \times 100\ \%$

higher than that of vertebrates in both China and the USA. On the contrary, sensitivity of invertebrates was lower than that of vertebrates to 2,4,6-trichlorophenol and nitrobenzene. This was similar with previous study that reported the need to derive SSDs for taxonomic groups separately for toxicants because of the different and specific toxic modes of action (Maltby et al. 2002); the study showed a significant difference in the sensitivity of vertebrate and invertebrate groups for atrazine, diuron, and 2,4-D. But, there was no significant difference in the sensitivity of vertebrate and invertebrate groups for simazine (Maltby et al. 2002). The results of present work indicated that 2,4-dichlorophenol, parathion, As(III), Cr(VI), Hg, and Cd might have similar toxic modes of action on invertebrates and vertebrates, while 2,4,6-trichlorophenol and nitrobenzene might have different toxic modes of action. The reason for this difference requires further investigation. Therefore, the toxic mode of action of toxicants as well as the sensitivity of different taxonomic groups should be taken into account when deriving site-specific WQC.

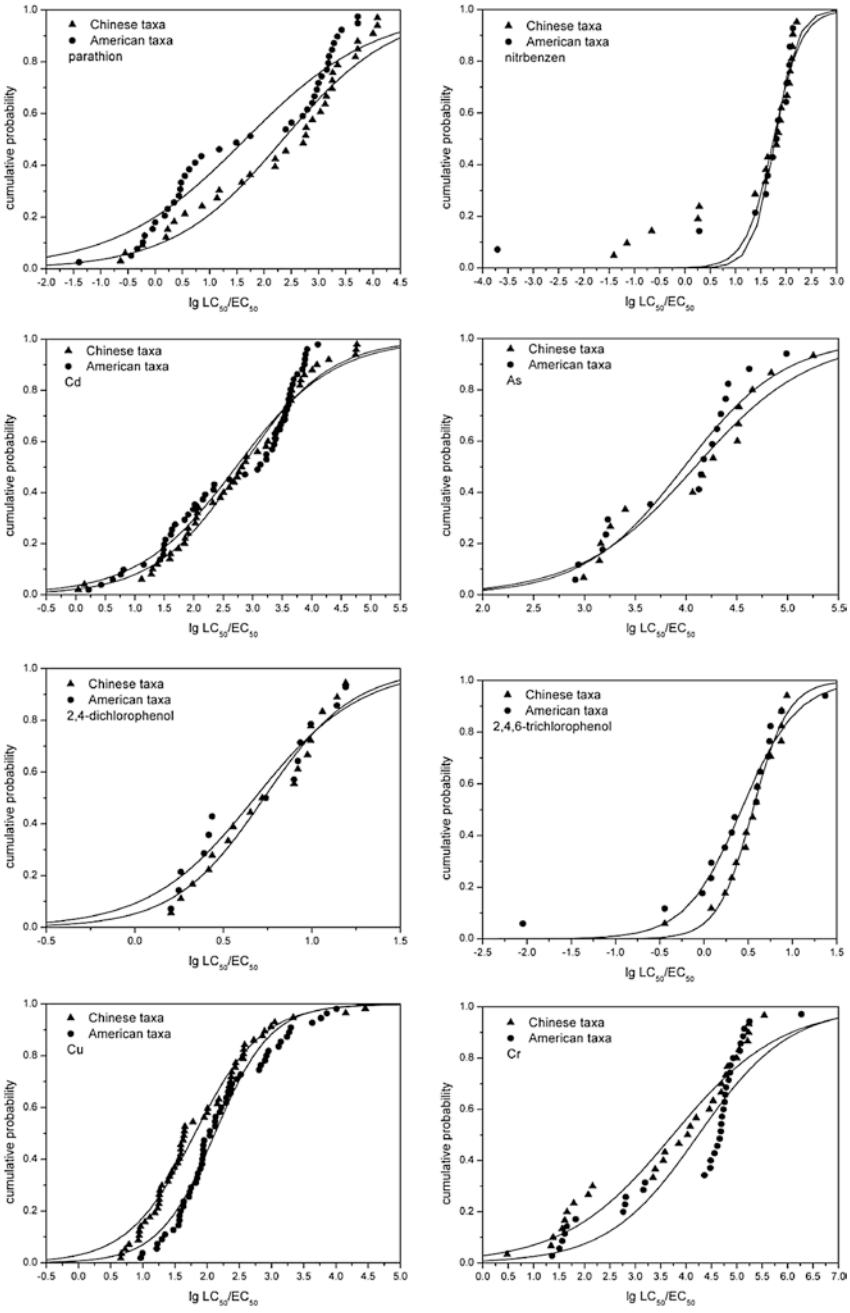

Fig. 3.1 Species sensitivity distribution of total species from China and the USA for nitrobenzene, parathion, 2,4,6-trichlorophenol, 2,4-dichlorophenol, As(III), Cr(VI), Hg, and Cd

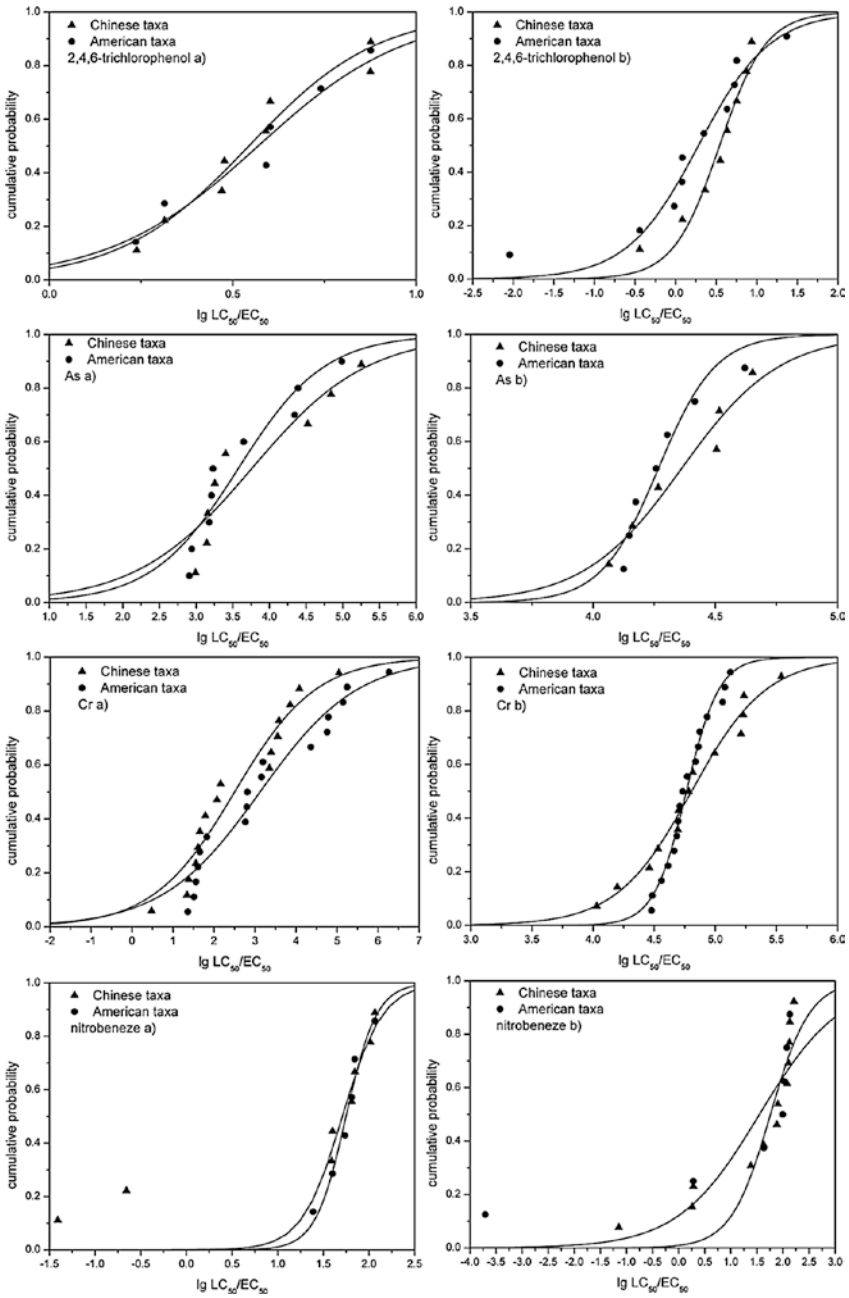

Fig. 3.2 SSD of different taxonomic groups from China and the United States. Letter a stands for the SSD curve derived from Chinese and American invertebrates for nitrobenzene, parathion, 2,4,6-trichlorophenol, 2,4-dichlorophenol, As(III), Cr(VI), Hg, and Cd; letter b stands for the SSD curve derived from Chinese and American vertebrates for the same toxicants

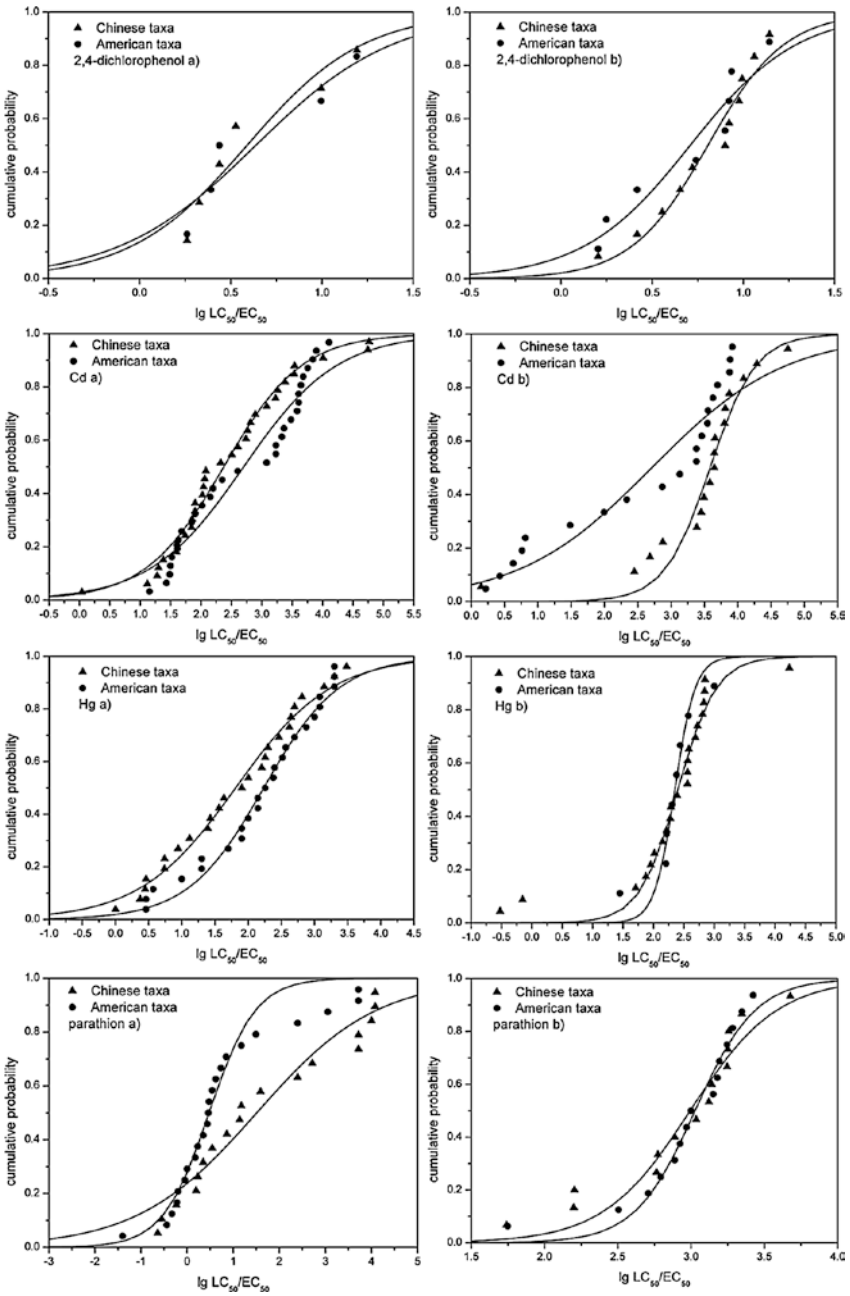

Fig. 3.2 (continued)

The SSD curves of nitrobenzene, As(III), Cr(VI), and Hg for total Chinese species were shifted to the left of those for total American species, resulting in lower HC_5 and HC_{50} values, except for HC_{50} of As(III) (Table 3.3, Fig. 3.1). The results were similar with previous study that showed HC_5 values derived from European species were lower than HC_5 values derived from North American species (Maltby et al. 2002). As for nitrobenzene (Fig. 3.1), species from China and the USA appeared to have similar sensitivity above HC_{25}, but the lower tail of both curves did not fit well. Additionally, there were almost two times more available data for Chinese species than American species, and this might cause the difference in sensitivity. On the contrary, the HC_5 and HC_{50} values for Chinese taxa to 2,4,6-trichlorophenol, 2,4-dichlorophenol, parathion, and Cd were higher (Fig. 3.1).

Comparison of the HC_5 and HC_{50} values between Chinese and American taxa showed that they were within an order of magnitude except HC_5 for parathion and Cr(VI) (Table 3.3). Previous studies reported that HC_5 values within an order of magnitude were acceptable in deriving WQC (Dyer et al. 2008; Feng et al. 2012a, b). The order of magnitude differences of HC_5 for parathion and Cr(VI) was mainly because of the most sensitive species: *Diaphanosoma brachyurum* for Cr(VI) in China and *Orconectes nais* for parathion in the United States. Comparison of SSDs showed that there was no statistically significant difference for total species between China and the USA for all eight pollutants (M-W test: $p = 0.109–1.000$; K-S test: ks $= 0.411–1.059$, $p = 0.212–0.996$) (Table 3.2). Previous studies found there was no significant difference in SSDs for Chinese and American (non-Chinese) species for pentachlorophenol and zinc (Jin et al. 2012; Feng et al. 2013a, b), and this was in accordance with this study. Additionally, previous studies showed no significant difference in SSDs between Australian and non-Australian organisms exposed to endosulfan (Hose and Van den Brink 2004). Moreover, Maltby et al. (2002) reported similar sensitivities among North American and European taxa with different geographic distributions. Other study also found that there was no significant difference in the acute toxicity of malathion, lindane, or carbaryl on tropical or temperate fish (Dyer et al. 1997).

In this study, the SSDs based on Chinese and American vertebrates were compared. The SSDs of parathion showed lower HC_5 and HC_{50} values for Chinese vertebrates, while SSDs of As(III), Cr(VI), and Hg showed lower HC_5 but higher HC_{50} values. It was opposite for nitrobenzene, 2,4,6-trichlorophenol, and 2,4-dichlorophenol, resulting in higher HC_5 and HC_{50} values for Chinese vertebrates. However, the differences of HC_5 and HC_{50} values between the two countries were small and within an order of magnitude except HC_5 values of vertebrates for Cd and nitrobenzene. As for invertebrates, results showed that

the SSD curves of nitrobenzene, Cr(VI), and Hg for Chinese invertebrates were shifted to the left of the American invertebrates, resulting in lower HC_5 and HC_{50} values. The SSDs of parathion and As(III) showed lower HC_5 but higher HC_{50} values for Chinese invertebrates. Previous studies reported that the difference of HC_5 values within an order of magnitude was acceptable in deriving WQC (Dyer et al. 2008; Feng et al. 2012a, b). The order of magnitude differences of HC_5 values was mainly because of the most sensitive species, *Oncorhynchus mykiss*, for nitrobenzene in the American vertebrate group, and there were many toxicity data from Salmonidae fishes that were very sensitive to Cd in the American vertebrate group. Additionally, there was no significant difference in the sensitivity distributions for vertebrates and invertebrates between China and the USA for all eight pollutants (vertebrates: M-W test: $p = 0.360–0.847$, K-S test: $ks = 0.401–1.106$, $p = 0.173–0.997$; invertebrates: M-W test: $p = 0.109–1.000$, K-S test: $ks = 0.298–1.113$, $p = 0.168–1.000$) except the sensitivity distribution of vertebrates for Cd (Table 3.2). This was mainly because these cadmium-sensitive Salmonidae fishes are native species in the USA but non-native species in China. This was in accordance with previous study that showed no significant difference was observed in sensitivity distribution of invertebrate arthropods between temperate and tropical areas for chlorpyrifos and fenitrothion, or between Europe and America (Maltby et al. 2002). Moreover, previous study also showed that there was no significant difference in sensitivity distribution of arthropods and fish between Australia and non-Australia exposed to endosulfan (Hose and Van den Brink 2004).

3.1.5 Conclusion

In the present study, the acute SSD of total species, vertebrates, and invertebrates from China and the USA for eight priority pollutants was constructed, and the differences of sensitivity distributions were compared between the two countries. The results showed that the log-logistic SSD method fits the toxicity data of different taxonomic groups in China and the USA well. The results indicated that 2,4-dichlorophenol, parathion, As(III), Cr(VI), Hg, and Cd might have similar toxic modes of action on invertebrates and vertebrates, while 2,4,6-trichlorophenol and nitrobenzene might have different toxic modes of action. Comparison of the sensitivity distributions and HC_5 and HC_{50} values showed that there was no statistically significant difference between Chinese and American species. Therefore, the use of toxicity data from America or another place could be feasible in emergencies or other situations. This finding provides useful information in site-specific WQC derivation.

3.2 Species Sensitivity Analysis of Heavy Metals to Freshwater Organisms

3.2.1 Introduction

Heavy metal pollution is one of the major causes of the poor freshwater quality globally (Liu et al. 2009; Montuori et al. 2013; Sekabira et al. 2010). Source of the heavy metal pollutants is mainly human activities such as industrial discharge, agricultural drainages, vehicle emissions, and domestic wastes, which have all posed serious risks to human and water bodies (Adnano 1986; Moore and Ramanamoorthy 1984; Sekhar et al. 2003). For example, several contamination accidents caused by cadmium (Cd) and Cr happened in China recently. Some heavy metals such as Zn and Cu are essential elements for the physiological activity of living organisms including human beings. Other ones such as Hg and Cr are not required for metabolic activities and may cause damage to aquatic organisms instead. It has been proved that the existence of heavy metals in ecosystems becomes dangerous for organisms when the concentration surpasses the natural background in water (Lopa and Adhikari 2006). Due to their characterizations of being persistent, non-degradable, toxic, bioconcentrated and biomagnified, heavy metals have been paid more attentions compared to other pollutants, and they can be transferred to the human body via food chain and therefore pose serious threats to the ecosystem (Gavrilescu 2004). Because a certain pollutant may generate different toxic effects in various organisms (Maltby et al. 2005a, b), there is a growing need to assess the risks to different aquatic organisms posed by the heavy metals .Freshwater species consist of vertebrates and invertebrates. A diverse range of fish, reptiles, and amphibians make up vertebrates with fish being dominant. Crustaceans, mollusk, and worms are a major part of invertebrates in which cladoceran species take up a large proportion. Previous studies revealed that the toxicity mechanism of heavy metals is different according to different species at various trophic levels (Amiard et al. 2006; Thierry et al. 2009). Screening of sensitive test organisms is a crucial prerequisite for WQC derivation, and some related studies have been conducted (Wang et al. 2014a, b; Cai et al. 2014; Zheng et al. 2014). *Daphnia magna* and *Hyalella azteca* are standard test organisms for invertebrates, and for fish, *Pimephales promelas* was chosen as the standard species. However, these standard test species showed different degree of sensitivity to different pollutants. *D. magna* is not always the most sensitive species; for example, it is less susceptive to neonicotinoids compared to insects (Rubach et al. 2010). The purpose of the study is to better understand taxonomic differences in species sensitivity.

Analysis of the SSD is based on cumulative probability distributions of multiple species to heavy metals and using a statistical or empirical distribution function of responses to show the sensitivity variation of species toward a pollutant (Posthuma et al 2002). This method was first proposed by Kooijman (1987), and latterly, something new was introduced into the method in subsequent studies (Aldenberg and Slob 1993; Newman et al. 2000; Posthuma et al. 2002; Wagner and Løkke 1991). SSD method has been widely used to evaluate the ecological risk posed by heavy metals (Brix et al. 2001). SSD is also used to calculate the concentration at which a specified proportion of species will be not in protection, referred to as the hazardous concentration (HC) for *p* % of species (HCp) (Newman et al. 2000). The most frequently estimated HCs is the HC_5, at the concentration of which 95 % of species is not affected (USEPA. Environmental Protection Agency 2004; Dyer et al. 2006). Meanwhile, the percentile of species associated with a certain concentration can be used to evaluate the toxicity of a specific heavy metal and also the potential affected species.

Numerous studies have showed that heavy metals have direct impact on freshwater organisms (Priel and Hershfinkel 2006; Birungi et al. 2007; Johnson et al. 2007). However, studies on toxicity comparison of various heavy metals have less been investigated and current researches related used only one or a few chemicals and species (Li et al. 2012; Zhang et al. 2014). It was necessary to evaluate the toxicities of heavy metals on various taxa species and the sensitivities of different taxa species to each heavy metal. In this study, we tried to compare SSDs of six typical heavy metals constructed, expecting some new significant findings.

In this study, acute toxicity data of copper (Cu), mercury (Hg),cadmium (Cd), hexavalent chromium (Cr(VI)), lead(Pb), and zinc (Zn) were collected. Based on the toxicology data, SSD curves were individually constructed by a log-logistic model for various taxonomic groups from different trophic levels. Sensitivity of different taxa species exposed to a given heavy metal was sorted to compare the sensitivity variance. Moreover, comprehensive comparison on SSD of different trophic levels was performed to assess ecological risk of six typical heavy metals to aquatic organisms. The present study aimed to explore the relationship between the species sensitivity of taxonomic taxa and the toxicity of heavy metals.

3.2.2 Materials and Methods

1. *Ecotoxicity data collection and screening*
   Ecotoxicity data of six heavy metals, Hg, Cu, Cr(VI), Cd, Pb, and Zn were obtained from the ECOTOX database (http://cfpub.epa.gov/ecotox/) and some literature databases such as Web of Science, Scopus, and Scirus. The key words

used during the search included "mercury," "chromium," "cadmium," "lead," "copper," "zinc," "heavy metals," "ecotoxicity," "aquatic organisms," etc.
The collected literature data were screened according to the following screening criteria (Stephen et al. 1985): acute toxicity data indicators of LC50 and EC50; the exposure time for daphnia and chironomid larve is 48 h, and that for other aquatic animals is 96 h; chronic toxicity data were not included because of insufficiency for SSDs construction; and the toxicity data of the species in more sensitive life stages. If toxicity data difference for one species exceed 10 times, abandon the outliers; experiment conditions (e.g., temperature, oxygen, and particulate matter concentration) should be carefully controlled; concentrations of test sample must be measured at the beginning and end of the experiment, and the actual concentrations should be in the range of effective concentration, that is, not deviating from the nominal concentrations by more than 20 %. Also, obtaining of the qualified data should be complying with scientific test principles, including setting of control group and implementation of quality control.

2. *Data analysis*
In the situation where more than one toxicological data were obtained for only one species, the geometric mean value was calculated and used as the estimate for that species. The estimated value was referred as species mean acute value (SMAV). The SMAVs were fitted using SSD method (Van Vlaardingen and Verbruggen 2007), and after that, the species sensitivity was analyzed. There has been many cumulative distribution functions used to fit SSDs (Erickson and Stephan 1988; Wagner and Løkke 1991; Aldenberg and Jaworska 2000; Van der Hoeven 2001; Chen 2004; Hose and Van den Brink 2004). In the present study, in order to obtain more feasible and statistically meaningful results, we used only log-logistic distribution method, because it always provides the best overall fit to toxicity datasets (Kooijman 1987; Newman et al. 2000; Wheeler et al. 2002a; Versteeg et al. 1999a, b) and more conservative HC_5s (Forbes and Calow 2002). The equation is as follows:

$$y = 1/(1 + \exp((P1 - x)/P2))$$

In the equation y is the cumulative probability of species, defined as (the order of the data point)/(1 + total number of data points), x stands for the mean value of the log10-transformed LC_{50} or EC_{50} and $P1$ and $P2$ are parameters representing the intercept and the slope of the curve, respectively. The distribution model was fitted to toxicity data points and assessed by the chi-square goodness of fittest with the adjusted coefficient of determination R^2 (Adj-R^2), using the software OriginLab 8.0 (USA, Origin Lab Company).

The difference of SSDs for total species, invertebrates, and vertebrates was analyzed and compared using the SPSS software (SPSS 20.0 for Windows) with the two-sample K-S test and MW. And then, HC_5 and HC_{50} were calculated according to the method from Aldenberg and Jaworska (2000).

3.2.3 Results

1. *Data Collection and SSD Construction*
   In the present study, we collected the acute toxicity data of Cd, Cu, Pb, Zn, Hg, and Cr(VI), and the quantities of the aquatic species were 45, 54, 26, 26, 47, and 30, respectively (Table 3.4). The acute toxicity datasets for Cu, Cd, and Hg were larger than that for the other heavy metals in size. Nearly 50 % of vertebrates and invertebrates were fish and cladoceran, respectively. To research the toxicities of six heavy metals on aquatic organisms from different trophic

Table 3.4 Data quantity and goodness of fit of different taxonomic groups for six heavy metals

Heavy metals	Taxa group	Data quantity (n)	Adj-R^2	p
Cd	Total	45	0.98	<0.01
	Fish	12	0.92	<0.01
	Cladoceran	10	0.93	<0.01
	Vertebrate	16	0.95	<0.01
	Invertebrate	29	0.97	<0.01
Cu	Total	54	0.98	<0.01
	Fish	14	0.97	<0.01
	Cladoceran	19	0.99	<0.01
	Vertebrate	19	0.98	<0.01
	Invertebrate	35	0.96	<0.01
Pb	Total	26	0.97	<0.01
	Fish	11	0.92	<0.01
	Cladoceran	7	0.97	<0.01
	vertebrate	11	0.92	<0.01
	Invertebrate	15	0.98	<0.01
Zn	Total	26	0.97	<0.01
	Fish	6	0.9	<0.01
	Cladoceran	13	0.96	<0.01
	Vertebrate	9	0.94	<0.01
	Invertebrate	17	0.98	<0.01
Hg	Total	47	0.97	<0.01
	Fish	15	0.95	<0.01
	Cladoceran	11	0.95	<0.01
	Vertebrate	22	0.97	<0.01
	Invertebrate	25	0.99	<0.01
Cr	Total	30	0.94	<0.01
	Fish	9	0.94	<0.01
	Cladoceran	12	0.89	<0.01
	Vertebrate	13	0.98	<0.01
	Invertebrate	17	0.94	<0.01

p is the significance level of the adjusted coefficient of determination (R^2)

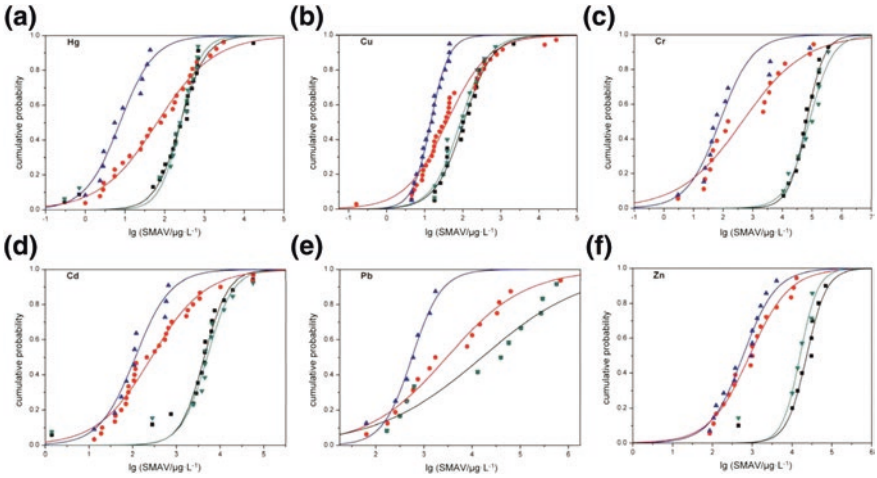

Fig. 3.3 Species sensitivity distribution of different taxonomic groups species for Cd, Cu, Pb, Zn, Hg, and Cr(VI), *triangle* stands for cladoceran, *circle* for invertebrates, *rectangle* for vertebrates, and *inverted triangle* for fish

levels, SSD curves of invertebrates, cladoceran, and vertebrates, fish were constructed and shown in Fig. 3.3. The results indicated that the log-logistic distribution fit most of the taxa data points, with Adj-R^2 of different taxa changing from 0.89 to 0.99 ($p < 0.01$) (as shown in Table 3.1).

In the SSDs cures of the five heavy metals except Hg, invertebrates showed to be more vulnerable than vertebrates, and the concentrations posing risk to most sensitive species differed by one to three orders of magnitude, 63.8 μg L^{-1} for *Ceriodaphnia dubia* to Pb compared with 170 μg L^{-1} for *Cyprinus carpio* at a minimum and 3 μg L^{-1} for *Diaphanosoma brachyurum* to Cr(VI) compared with 10,700 μg L^{-1} for *Aristichthys nobilis* at a maximum. As to Hg, *Ictalurus punctatus* with the concentration of 0.3 μg L^{-1} and *Carassius auratus* with 0.7 μg L^{-1} were both more sensitive than the most sensitive invertebrate *Moinamacrocopa* with 1 μg L^{-1}. Furthermore, for the five heavy metals except Cu, the most sensitive vertebrates and invertebrates were fishes and cladoceran, while the most sensitive species for Cu was *Tubifex tubifex* which belonged to annelid instead of cladoceran.

2. *Individual species sensitivity of freshwater organisms to heavy metals*

The SSDs of a particular heavy metal constructed by each group were compared to evaluate the sensitivity of different trophic levels. On the whole, SSD curves of all heavy metals showed that the SSD curves of invertebrate species were shifted left from those of vertebrate, indicating the invertebrate species was more susceptible than vertebrate species. The sensitivities differed from one to three orders of magnitude, and only invertebrates were slightly higher than vertebrates for Cu. It is noteworthy that for some heavy metals such as Cu and Cr(VI), crossing of SSD curves of invertebrates and vertebrates occurred at higher concentration. In particular to Hg, vertebrate was more sensitive when the exposure concentration exceeded a certain high concentration. As to Pb, the crossing happened at lower

concentration. Moreover, it was obvious that cladoceran was more sensitive than to a given heavy metal except for Pb. The significance level was analyzed, and the results are shown in Table 3.5. *P* values were mostly less than 0.05, indicating there was a great difference between distributions except for Hg and Pb.

The points of 5 and 50 % should be paid attention to because the former ascertain the safety of 95 % species below the corresponding concentration and the latter means the majority of species were endangered. Thus, values of HC_5 and HC_{50} for six heavy metals were calculated based on the SSD curves, and the results are shown in Table 3.6. In general, for the five heavy metals except for Pb, the HC_5 derived from invertebrates was all lower than that of vertebrates with several orders of magnitude. The results demonstrated that the heavy metals were more toxic to invertebrate species than to vertebrate species. In other words, at a certain concentration, more invertebrates were sensitive than vertebrates. It was found that invertebrates were more sensitive than cladoceran to heavy metals except slightly less sensitive to Hg. However, HC_5 values for invertebrate were lower than other taxa groups, indicating invertebrate were more sensitive to heavy metals except Pb; the sensitivities

Table 3.5 Comparison of species sensitivities to heavy metals for different taxa groups

Heavy metals	Taxa groups	ks	*p*(K-S test)	*p* (M-W test)
Cd	Invertebrate	0.64	0	0.001
	Vertebrate			
	Fish	0.833	0.001	0.001
	Cladoceran			
Cu	Invertebrate	0.4	0.039	0.066
	Vertebrate			
	Fish	0.684	0.001	0
	Cladoceran			
Pb	Invertebrate	0.412	0.231	0.281
	Vertebrate			
	Fish	0.636	0.063	0.085
	Cladoceran			
Zn	Invertebrate	0.778	0.002	0
	Vertebrate			
	Fish	0.833	0.007	0.003
	Cladoceran			
Hg	Invertebrate	0.389	0.058	0.092
	Vertebrate			
	Fish	0.867	0	0.001
	Cladoceran			
Cr	Invertebrate	0.824	0	0
	Vertebrate			
	Fish	0.917	0	0
	Cladoceran			

ks is a test statistic parameter used to indicate the significance level; *p* represents the significance level; $p > 0.05$ means the difference between distributions is not significant

Table 3.6 The calculated HC_5 and HC_{50} values of vertebrate, fish, invertebrate, cladoceran, and total species for six heavy metals

	Heavy metals	Cu ($\mu g\ L^{-1}$)	Hg ($\mu g\ L^{-1}$)	Cd ($\mu g\ L^{-1}$)	Cr ($\mu g\ L^{-1}$)	Zn ($\mu g\ L^{-1}$)	Pb ($\mu g\ L^{-1}$)
HC_5	Total species	1.82	3.52	5.34	5.58	23.1	10.3
	Vertebrate	8.06	75.2	695	8,014	4,251	10.0
	Fish	6.92	42.3	698	1,997	3,492	10.0
	Invertebrate	0.940	0.49	4.26	0.350	28.3	12.1
	Cladoceran	2.80	0.39	7.19	1.78	346	71.6
HC_{50}	Total species	57.9	140	760	6,337	2,507	5,814
	Vertebrate	105	251	4,304	63,794	23,339	16,082
	Fish	86.6	250	5,008	79,122	15,913	16,082
	Invertebrate	38.2	64.5	268	444	853	3,002
	Cladoceran	14.4	7.43	114	76.5	585	569

are generally ranked in the following order: invertebrate > cladoceran > fish > vertebrate. The maximum HC_5 values varied from 9 times to more than 20,000 times of the minimum. However, this general order was found to be inconsistent for a gradient of increasing concentrations. For example, the HC_{50} values of cladoceran were ahead of those of other invertebrate, and vertebrate on the whole lower than fish for Cd and Cr. Therefore, the sensitivity of different taxonomic groups and the toxic action mode of toxicants should be taken into consideration for the ecological risks assessment of heavy metals.

3. *Comparison of toxicity of six heavy metals against the same taxa group*
From the viewpoint of a certain trophic level, the SSD curves of six heavy metals against the same taxa group were gathered and also compared. As shown in Fig. 3.4a and b, generally, the curves of Cu and Hg were shifted left and Cd and Zn in the middle and Cr on the right. Particularly, the SSD curves of Pb intersected with those of the rest, crossing the curves of Cu and Hg below the cumulative probability of 0.10 (HC_{10}), that of Cd at about 0.30 (HC_{30}), and that of Zn and Cr(VI) above HC_{50} successively. In particular at the lower concentration, the curves of Pb were shifted left from that of Cu, indicating more vertebrates or fish faced the threat. The HC_5 values of the six heavy metals on vertebrates could be ranked in an ascending order: Cu, Hg, Cd, Zn, and Cr(VI),with the lowest HC_5 value 8.06 $\mu g\ L^{-1}$.The HC_5 values of Cr(VI) were found to be over 100 times higher than those of Cu. When the exposure concentration of Pb was below 10 $\mu g\ L^{-1}$, more vertebrate and fish could be affected even than that exposed to Hg and Cu. When the exposure concentration of Cd came up to 100 $\mu g\ L^{-1}$, vertebrate and fish were not affected. While with the concentration up to 1,000 $\mu g\ L^{-1}$, the sensitivity of the vertebrate and fish increased rapidly. The HC_5 and HC_{50} values of Pb ranked the second and the fourth place, respectively, and HC_{50} was about four times of the third Cd, showing less toxicity. The sensitivity (Table 3.6; Fig. 3.4b) followed in a descending order (HC_5:Cu > Pb > Hg > Cd > Zn > Cr, HC_{50}: Cu > Hg > Cd > Pb > Zn > Cr).

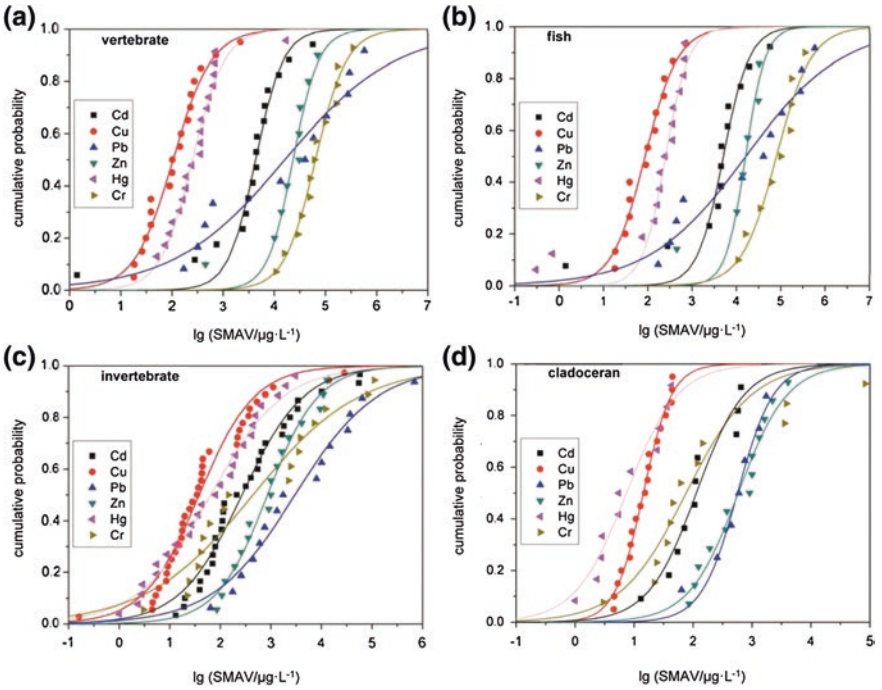

Fig. 3.4 SSD curves for vertebrates, fish, invertebrates, and cladoceran exposed to different heavy metals

For invertebrate, most of curves crossed with each other, especially in lower concentration, indicating the sensitivities below and above intersecting points followed different trends. For example, the SSD curve of Cr(VI) was shifted to the left from that of Hg and Cu at lower concentration, which indicated the invertebrate was most sensitive to Cr(VI). Interestingly, the situation of obviously crossing the SSD curves of Pb to vertebrates did not occur. Zn was more toxic to invertebrates than Pb at higher concentration. Instead of Pb, the SSD curves of Cr(VI) crossed with that of Cu and Hg below 0.20 (HC_{20}), that of Cd at about 0.40 (HC_{40}), and that of Zn above HC_{60} successively. When the exposure concentration of Cr(VI) was below 1 $\mu g\ L^{-1}$, more invertebrate and cladoceran could be affected even than exposed to Hg and Cu. When exposure concentration of Cd was below 1 $\mu g\ L^{-1}$, invertebrate and cladoceran were not affected. While when the concentration reached up to 10 $\mu g\ L^{-1}$, invertebrate and cladoceran became increasingly sensitive. In terms of invertebrates, the toxicity of each heavy metal was generally higher, with HC_5 values all lower than 30 $\mu g\ L^{-1}$. HC_{50} values were mostly lower than 1 mg L^{-1} except for those of Pb. For cladoceran, the HC_5 and HC_{50} values showed similar trends (HC_5: Hg > Cr > Cu > Cd > Pb > Zn, HC_{50}: Hg > Cu > Cr > Cd > Pb > Zn).

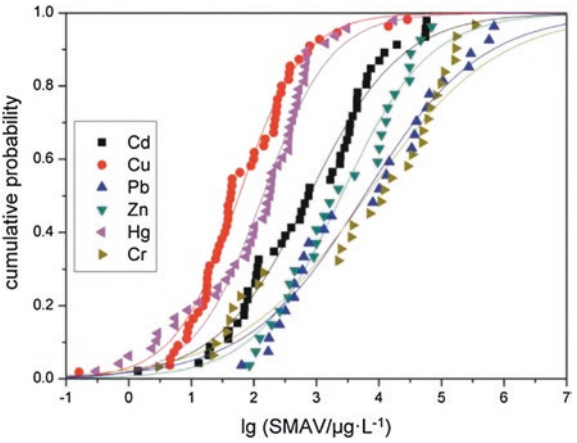

Fig. 3.5 SSD curves for total species exposed to different heavy metals

4. *Comparison of SSDs of six heavy metals for total species*
   Figure 3.5 shows the SSD curves of six heavy metals, which were constructed based on the total species. According to the SSDs, the sensitivity relationship between individual taxa species and total species was investigated for all heavy metals. Interestingly, the situation of obviously crossing the SSD curves for Pb and Cr(VI) for individual group did not appear. Cd showed a moderate toxicity among all heavy metals. The SSD curves of Zn shifted to the left of Cr and Pb at higher concentration, indicating the toxicity of Zn was largely higher. The acute HC_5 values for Hg, Cd, Cr(VI), Pb, and Zn were determined to be 1.82, 3.52, 5.34, 5.58, 10.27, and 23.13 $\mu g\ L^{-1}$, respectively. All these six heavy metals showed high toxicity to freshwater organisms. The sensitivity of total species to these heavy metals ranked following the order of Cu > Hg > Cd > Cr > Zn > Pb for HC_5, Cu > Hg > Cd > Zn > Cr > Pb for HC_{50}. When the exposure concentration was below 1 $\mu g\ L^{-1}$, no significant differences among the toxicities of Cu, Cd, Cr(VI), Zn, and Pb were observed except for Hg which was slightly more toxic. However, as the exposure concentration increased to 10 $\mu g\ L^{-1}$, the ecological risks of Hg and Cu to aquatic organisms rose rapidly. In brief, the difference was not significant when the data of total species were taken into consideration.
5. *The potential affected fractions of different groups at certain concentration*
   PAF of the different trophic levels at a certain concentration of the heavy metals reflects the degree of the lack of protection. As showed in Table 3.7, at the concentration of 10 $\mu g\ L^{-1}$, Cu could affect 18.3 % of total species, Hg 10.8 %, Cd 27.8 %, Cr 15 %, Zn 22.4 %, and Pb 4.9 %. 6.3 % of vertebrates were affected by Cu and 5 % by Pb; other heavy metals could not cause damage to the vertebrates at this concentration. The situation of fish was similar to that of vertebrates. Therefore, when the exposure concentration of heavy metals such as Hg and Cu reached 10 $\mu g\ L^{-1}$, PAFs of invertebrates (including cladoceran) varied from 24.5 to 57.4 %, indicating their high toxicity. When the exposure concentration came up to 1,000 $\mu g\ L^{-1}$, 91.9, 82.8, and 63.4 % of total species were threatened by Cu, Hg, and Zn, respectively, which indicated the three heavy metals were most toxic. The

Table 3.7 Predicted PAF values of the heavy metals under various concentrations

Heavy metals	Concentration/(μ g /L)	Total	Vertebrate	Fish	PAF invertebrate	Cladoceran
Cu	0.1	–	–	–	0.9	–
	1	3.1	0.5	0.5	5.2	0.8
	10	18.3	6.3	7.5	25.6	34.2
	100	61.4	48.6	54.2	68.3	97.1
	1,000	91.9	93	94.5	93.1	–
Hg	0.1	–	–	–	–	1.2
	1	1.9	–	–	7.5	11.9
	10	10.8	1.15	0.5	24.5	57.4
	100	41.6	32.5	17.9	68.3	93.1
	1,000	82.8	87.6	90.9	93.1	99.3
Cd	1	1.9	–	–	–	–
	10	27.8	–	–	8.8	6.9
	100	23.1	0.2	0.29	33.3	93.1
	1,000	54.1	8.7	8.3	71.9	91
	10,000	82.2	79.6	73.7	92.9	99.2
Cr	1	6.3	–	–	7.6	3.2
	10	15	–	–	17.4	16.9
	100	31.6	–	–	35.1	55.2
	1,000	45.2	0	0.8	58.3	88.5
	10000	76	6.7	9.3	78.3	97.8
	10,0000	89.3	94.5	77.2	90.3	–
Zn	1	–	–	–	0.3	–
	10	22.4	–	–	2.1	1.4
	100	33.1	–	–	13.6	26
	1,000	63.4	0.4	0.5	53.4	63.6
	10,000	70.5	18.8	20.3	89.3	95.1
	100,000	91	92.6	97.3	98	–
Pb	10	4.9	5	5	4.6	0.3
	100	11.7	11.7	11.7	14	7.8
	1,000	30.6	24.8	24.8	35.8	56.1
	10,000	56.3	45.3	45.3	65.4	98.3
	100,000	78.9	67.5	67.5	86.7	–

adverse effects of Cr and Pb should also be paid attention to because of their PAFs up to 45.2 and 30.6 %, respectively.

3.2.4 Discussion

According to the principle of SSD, both acute and chronic toxicology data can be used in the construction of SSD curves. Chronic toxicity data are more ecologically important

because freshwater aquatic organisms are usually exposed in low concentration of pollutants for a long time. However, we used only acute data in this study, because acute data are more easily available to construct SSD curves for most pollutants (Wang et al. 2008). Chronic toxicity data are often insufficient and cannot meet the data requirements of SSD construction (Wheeler et al. 2002a; Hose and Van den Brink 2004).

Various species showed different sensitivity to heavy metals. From the present study, invertebrates appeared to be more sensitive than vertebrate. That may be because the skin of fish and amphibian can isolate the pollutants and protect the organisms from the toxic damage to some extent (Harri et al. 1979), while some crustacean and insects molt in certain life stage and would be more sensitive to chemicals just after molting (Hanazato 2001). Besides, the sensitivity degrees to these heavy metals may be also due to the different patterns of exposure and accumulation. The result is in compliance with the previous finding (Li et al. 2012).

The toxicity data of all heavy metals to aquatic organisms showed that *Daphnia magna* was among the most sensitive invertebrate, consistent with previous studies (Von der Ohe and Liess 2004; Wu et al. 2012). Particularly, cumulative probability of *Daphnia magna* is above 60 % in SSD curves of Cu. Statistical analysis also showed that invertebrates were more susceptive than vertebrates and cladocera were more vulnerable than fish, just as shown in Table 3.5. However, p is more than 0.05 from comparing invertebrates with vertebrates to Pb or Hg, which proved that invertebrates were less sensitive than vertebrates on the whole. The main reason was that invertebrate species were more tolerant at higher concentrations of Hg although more invertebrate species were affected at lower concentrations. As to Pb, the reason for that was due to the lack of sufficient species from various trophic levels. The datasets were suggested to be more than ten according to previous report (Wheeler et al. 2002a).

SSD curves and HC_5 values are usually used to derive WQC for toxicants (Stephen et al. 1985; Wang et al. 2013, 2014a, b). According to the methodologies of WQC derivation, the HC_5 is considered to be the concentration aiming to protect more than 95 % of total species. So HC_5 value can be used as a reference concentration to assess the toxicity of pollutants. However, the present study showed that even at HC_5 several species were still in danger because their cumulative probability is below 0.05 in SSD curves by total species (Fig. 3.5). On the other hand, these species should not be considered as indicators for risk assessment associated with heavy metal. It was worthy of mentioning that there was a great difference between the HC_5 values of all heavy metals for total species and that derived from each of four taxonomic groups. For example, HC_5 for total species was higher than that for vertebrate, fish, and cladoceran, indicating that several species belong to invertebrate but not cladoceran. Actually, only *Tubifex tubifex* belonging to annelid was threatened at the HC_5 for total species.

In the present study, Hg and Cu exhibited the greatest toxicities and should be given greater attentions. Mercury was found to be most toxic to cladoceran, belonging to arthropod. And *Moina macrocopa* was the most sensitive invertebrate to Hg and still unprotected when exposed in a concentration above HC_5. The results were in accordance with previous study which showed that in the lower taxonomic levels, the sensitivity of arthropod is higher than fish in China (Li et al. 2012). In Figs. 3.4 and 3.5, Cu showed greater toxicity than most of other heavy metals, and that may be because Cu is an essential metal to the normal physiology

of crustaceans. On the contrary, Cd is generally not a necessary element for metabolism (Valavanidis and Vlachogianni 2010) and showed less toxicity than Cu ranking in the middle of SSD in our study.

According to the SSD curves of heavy metals in Fig. 3.4, the toxicity profiles were classified as being highly toxic, moderately toxic, low toxic, and lesser toxic within the whole concentration range. Cr, Hg, and Cu were classified as being highly toxic to invertebrates, with HC_5 values differing from 0.35 to 0.94 $\mu g\ L^{-1}$. Cu and Hg were classified as being moderately toxic to vertebrates (including fish), with HC_5 values from 6.92 to 10 $\mu g\ L^{-1}$, and also Cd was classified as being moderately toxic to invertebrates with HC_5 values between 4.26 and 7.19 $\mu g\ L^{-1}$. Zn and Pb were classified as being less toxic to invertebrates with HC_5 values from 12.05 to 345 $\mu g\ L^{-1}$. Zn and Cr were classified as being less toxic to vertebrates with HC_5 values above 1,000 $\mu g\ L^{-1}$. However, based on the HC_5 for total species, Cu, Hg, Cd, and Cr should be classified as being moderately toxic with HC_5 values from 1.82 to 5.58 $\mu g\ L^{-1}$, but Pb and Zn were classified as being less toxic with HC_5 values being 10.27 and 23.13 $\mu g\ L^{-1}$, respectively. So Cu was the most toxic heavy metal and showed great ecological risk. The toxic differences of the heavy metals to taxonomic groups would account for why SSD curves of Pb and Cr by individual taxa group crossed that of other metals but did not occur in the SSD curves by total species.

In fact, PAF could reflect the ecological risks of different heavy metals to some extent. When exposure concentration was 1 $\mu g\ L^{-1}$, Cr and Hg showed ecological risks with PAFs from 0.3 to 7.6 % among invertebrates. However, Cd, Cu and Hg, Zn, and Cr(VI) exceeded the threshold of 10 % (PAF) at the exposure concentration of 10 $\mu g\ L^{-1}$. When exposure concentration came up to 1,000 $\mu g\ L^{-1}$, Cu and Hg could exert great effect on most of the aquatic organisms.

However, the present study lays further emphasis on the fact that it is necessary to investigate the toxicity of heavy metals to various trophic levels. Comprehensive comparisons demonstrated the species sensitivity was important during the WQC derivation and risk assessment of each heavy metal. Without the species sensitivity analysis, overprotection or lack of protection on taxa species may well occur under the WQC threshold of a given heavy metal. Therefore, it should be given priority to evaluate the sensitivity of various trophic levels during the derivation of WQC and development of water quality standard in the future. Besides, when the ecological risk assessments are performed, ignorance on the differences will lead to overestimation or underestimation of the risk. Furthermore, more efforts should be put on the difference analysis of taxa species sensitivity to varying pollutants, not limited to typical heavy metals. Only in this way, more effective measures from governments could be carried out to protect the aquatic ecosystems.

3.2.5 Conclusions

In the present study, the toxicity of six typical heavy metals to vertebrates and invertebrates was investigated. In general, sensitivities of invertebrates to six heavy metals were higher than that of vertebrates. The ecological risk of all heavy metals

posed to cladoceran was higher than to fish. However, when closer examination between vertebrate and invertebrate species dataset was performed, consistent differences in species sensitivity to main heavy metals were found, such that invertebrates were considered to be more susceptive. A prior consideration should be given to species with high sensitivity to heavy metals in order to allocate more efforts toward relevant target species.

The toxicities of six heavy metals to the same species were also evaluated. Overall, Cu was the most toxic to vertebrates, followed by Hg, Cd, Zn, and Cr. The toxicity of Pb should be paid more attention because its SSD constructed by vertebrates crossed with that of other metals. When the comprehensive dataset including vertebrates and invertebrates was available, Cu proved to be more toxic than the rest. The crossing of SSD curves for Pb and Cr disappeared. Generally, HC5 derived from invertebrate was lower than that from vertebrates. Toxicities of the six heavy metals were ranked in the following order: Cu > Hg > Cd > Zn > Pb > Cr.

3.3 Preliminary Analysis of Species Sensitivity Distribution Based on Gene Expression Effect

3.3.1 Introduction

WQC play an important role in ambient water environmental management. The species sensitivity distribution (SSD) is one of the popular methods of several approaches to derive WQC values, and it was proposed independently by scientists from Europe and the United States in the 1970s and the 1980s (Posthuma et al. 2002). The SSD curve is constructed based on different functions, such as log-triangle function (US EPA 2004), log-logistic function (Aldenberg and Slob 1993), or lognormal distribution (Aldenberg and Jaworska 2000). The WQC value is determined to protect certain proportion of aquatic organisms by fitting the ecotoxicity data of one pollutant to various organisms, combined with the theory of ecological risk assessment. Nowadays, SSD curves are constructed by mainly using the ecotoxicity data at the individual level. For example, the half lethal concentration (LC_{50}) and half effect concentration (EC_{50}) are usually used to calculate acute WQC (Yan et al. 2011), while chronic WQC are calculated by the no observed effect concentration (NOEC) and low observed effect concentration (LOEC) (Yan et al. 2010). However, the toxic effect data in other biological tissue levels are seldom applied in the derivation of WQC. Under certain circumstances, they are occasionally used as the validation data (Stephen et al. 1985).

Given the wide migration and distribution of environmental pollutants, for a long time, the extensive ecotoxicological studies (Markert et al. 2003) were carried out to accumulate a large number of ecotoxicological data at molecular, cellular, tissue, individual, population, community, or ecosystem levels including in-depth analysis of ecological environment harm of environmental pollutants. The “omics”

technologies were used in the field of ecotoxicological studies sincethe 1990s (Aardema and MacGregor 2002). "Ecotoxicogenomics" is characterized with the toxic effects of environmental pollutants or chemical substances on living organisms at the gene level and has become a hot topic in research (Neumannand Galvez 2002). The impact of environmental toxicants on gene expression is generally believed to be more sensitive than the effect indicators at the individual level, and stress response of gene expression effect endpoint to environmental toxicants should be much earlier than survival indicators of individual organisms and reproductive effect indicators. The effect of gene expression under pollutant stress has become the important subject of early warning study of environmental risks (Pennie et al. 2004; Thomas et al. 2001; Snell et al. 2003; Bartosiewicz et al. 2001). And these data also could be possibly applied in the WQC derivation. However, some arguments about the role of gene expression effect data in early warning of environmental risks existed (Forbes et al. 2006; Menzel et al. 2009).

In order to evaluate the application of gene expression effect data in the risk assessment, Fedorenkova et al. 2010 selected cadmium with the most abundant genetic toxicity data as an example to carry out a comparative analysis of acute, chronic toxicity data and gene expression effect data at biological individual level by using toxicity database (e-toxBase) of the National Institute for Public Health and the Environment (RIVM) and the published data, and results showed that the sensitivity of gene expression effect on cadmium was unexpectedly greater than the acute toxicity indicators and significantly lower than the chronic toxicity indicators. Therefore, according to the authors, it is not certainly the most sensitive toxicological endpoint and is not certainly the gene expression effect at the molecular level. Because of the uncertainty of data analysis in that paper, its conclusion was doubted (Van Straalen et al. 2010). This study analyzed the sensitivity of acute, chronic, and gene expression effect data of cadmium based on more abundant data including ECOTOX Toxicity Database (http://cfpub.epa.gov/ecotox/), TOXNET Database (http://toxnet.nlm.nih.gov), GEO Database (www.ncbi.nlm.nih.gov/geo), CNKI (www.cnki.net), and the published data. Meanwhile, copper and zinc toxicity data were used to verify the findings of cadmium. The application of gene expression effect data in environmental risk evaluation and WQC development was also discussed in the paper.

3.3.2 Materials and Methods

1. *Data collecting and screening*
   Toxicity data of heavy metals including cadmium, copper, and zinc on aquatic organisms were collected through the above-mentioned databases and the published literature data in Web of Science, Scopus, Scirus, and other literature databases; "cadmium," "copper," "zinc," "heavy metals," "ecotoxicity," "gene expression," "gene chips," "genomics," "proteomics," "transcriptomics," "risk assessment," "risk analysis," "ecological risk," etc, as key words, were searched. And the cited references in the searched literatures were showed in the supplement.

The collected literature data were screened based on the following screening principle: acute toxicity data indicators of LC_{50} and EC_{50}, the exposure time from 2 to 4 days and longer period; chronic toxicity data indicators of NOEC and LOEC, the exposure time of more than 7 days and longer period data in different exposure time; the toxicity data of more sensitive life stages in the toxicity data of the same species at different life stages; and in gene expression effect data, the sensitive data were in priority to the exposure time. To unify analysis and comparison, the minimal pollutant concentration causing gene expression effect was considered as LOEC of gene expression effect.

2. *Data analysis*
The collected and screened data were sorted and analyzed. For several similar toxicity data of the same endpoints of the same species, the geometric mean values were calculated. The sorted toxicity data were fitted with the logarithm-logistic function to establish SSD curve. The function equation is $y = 1/(1 + \exp((\alpha - x)/\beta))$ (Awkerman et al. 2008). The hazardous concentration for 5 % of the species (HC_5) was obtained by the literature methods (Aldenberg and Jaworska 2000; Awkerman et al. 2008).

3.3.3 Results

1. *Toxic effects of heavy metal on gene expression*
Three kinds of heavy metals (cadmium, copper, and zinc), the extensive collected acute, chronic, and genetic effects of toxicity data included the acute toxicity data of 82 species and chronic of 12 species for cadmium, acute toxicity data of 118 species, and chronic of 81 species for copper, acute toxicity data of 81 species, and chronic of 15 species for zinc.
As is shown in Table 3.8, the collected gene expression effect data were significantly less than the acute and chronic toxicity data, dealing with a total of 19 aquatic organisms and including 18 species of cadmium, 7 species of copper, and 3 species of zinc, and the metal of most abundant gene expression toxicity data is cadmium. The 18 species included 5 species of fishes, suggesting the large impact of environmental pollutants on fishes. According to the available data at the gene level of cadmium, *Oryzias javanicus* was the most sensitive species and the least sensitive species was *Tigriopus japonicus* (the data of the two species had 50,000-time difference). For copper, the most sensitive species was *Daphnia magna* and the least sensitive species was *Carassius auratus* (the data of the two species had 50-time difference), while for zinc, the sensitivity data of *Daphnia magna* and *Crassostrea gigas* had 10-time difference.
In gene expression toxicity data of 19 species, the group numbers of exposure concentration were not the same. Only one group of exposure concentration was arranged in 14 references. In one reference, there were five groups of exposure concentrations. And in all the other references, exposure concentration group numbers were less than 5. Compared with standard toxicity test methods (acute

Table 3.8 Gene expression effects of aquatic organisms induced by cadmium, copper, and zinc

Heavy metals	Species	Exposure concentration (μg/L)	Exposure time	LOEC (μg/L) (exposure time)	References
Cd	*Tetrahymena thermophila*	495, 989, 2,473, 3,956, 4,946	1 h	3,956 (1 h)	(Yu et al. 2005)
	Tigriopus japonicus	5,000, 10,000, 15,000	96 h	5,000 (96 h)	(Lee et al. 2008)
	Daphnia magna	6, 20, 37	24 h	6 (24 h)	(Connon et al. 2008)
	Chironomus tentans	200, 2,000, 20,000	24 h	200 (24 h)	(Lee et al. 2006)
	Anadara granosa	250	4, 8, 12, 16 d	250 (4 d)	(Chan et al. 2002)
	Mytilus edulis	200	4, 21 d	200 (4 d)	(Lemoine et al. 2000)
	Crassostrea virginica	50	48 h	50 (48 h)	(Ivanina et al. 2009)
	Carassius auratus	50, 500, 2,500	12, 24, 36 h	50 (12 h)	(Liu et al. 2010)
	Platichthys flesus	50	1, 2, 4, 8, 16 d	50 (1 d)	(Williams et al. 2008)
	Cyprinus carpio	9.4, 105, 480	3 h, 42 h, 7d, 28 d	9.4 (3 h)	(Reynders et al. 2006)
	Kryptolebias marmoratus	610	6, 12, 24, 48, 96 h	610 (48 h)	(Rhee et al. 2009)
	Oryzias javanicus	0.1, 10, 1,000	24 h	0.1 (24 h)	(Woo et al. 2009)
	Takifugu obscurus	2,050	6, 12, 24, 48, 96 h	2,050 (6 h)	(Kim et al. 2008)
	Oncorhynchus mykiss	560	6 h	560 (6 h)	(Vergani et al. 2009)
	Mytilus gallo-provincialis	200	2, 9 d	200 (2 d)	(Zorita et al. 2007)
	Laeonereis acuta	100, 1,000	14 d	100 (14 d)	(Sandrini et al. 2006)
	Danio rerio	1.9, 9.6	7, 21 d	1.9 (7 d)	(Gonzalez et al. 2006)
	Crassostrea gigas	5.32	14 d	5.32 (14 d)	(Marie et al. 2006)

(continued)

Table 3.8 (continued)

Heavy metals	Species	Exposure concentration (μg/L)	Exposure time	LOEC (μg/L) (exposure time)	References
Cu	*Daphnia magna*	2, 4	24 h	2 (24 h)	(Watenabe et al. 2007)
	Eriocheir sinensis	50, 100, 500, 1,000	7 d	50 (7 d)	(Ren 2010)
	Carassius auratus	100, 1,000, 5,000	12, 24, 36 h	100 (12 h)	(Liu et al. 2010)
	Tigriopus japonicus	4.74	6, 12, 24 h	4.74 (6 h)	(Ki et al. 2009)
	Oncorhynchus mykiss	19.2	6, 12, 24, 48 h	19.2 (24 h)	(Walker et al. 2008)
	Danio rerio	8, 15	21 d	8 (21 d)	(Craig et al. 2009)
	Mytilus gallo-provincialis	40	2, 9 d	40 (2 d)	(Zorita et al. 2007)
Zn	*Daphnia magna*	100, 200, 500, 1000	24 h	100 (24 h)	
	Oncorhynchus mykiss	915.46	6, 12, 24, 48 h	915.46 (24 h)	(Walker et al. 2008)
	Crassostrea gigas	994.5	14 d	994.5 (14 d)	(Marie et al. 2006)

or chronic) (SEPA 2002), less exposure concentration was arranged in gene expression toxicity tests. From the experimental results, the minimal exposure concentration was often LOEC of gene expression effect in the group of exposure concentrations. Whether there was gene expression effect when the designed concentration was lower than the minimal concentration of pollutants was unknown. According to international standard test methods of LOEC derivation, the pollutant concentration arrangement should be further improved to derive scientific LOEC of gene expression effect. In addition, exposure time in gene effect study varied widely from a minimal value of 1 h (*Tetrahymena thermophila*) up to 7 days (*Eriocheir sinensis*). Considering the early warning indicators, exposure time was not used as the screening indicator while screening data.

2. *Data comparison of acute, chronic, and gene expression effects*
   To compare the sensitivity difference among acute, chronic, and gene expression effect data, sensitivity distribution fittings were carried out on the data of cadmium, copper, and zinc according to logistic function. The results showed in Fig. 3.6 that the gene expression effect data of zinc were too few (only three data), and distribution fitting was not carried out. The slopes of SSD curves of cadmium and copper were relatively small, while that of zinc SSD curve was relatively large. This meant that the sensitivity of most different aquatic organisms for zinc was relatively close and that for cadmium and copper was different. Sorting sequence of cadmium sensitivity data was chronic > gene > acute,

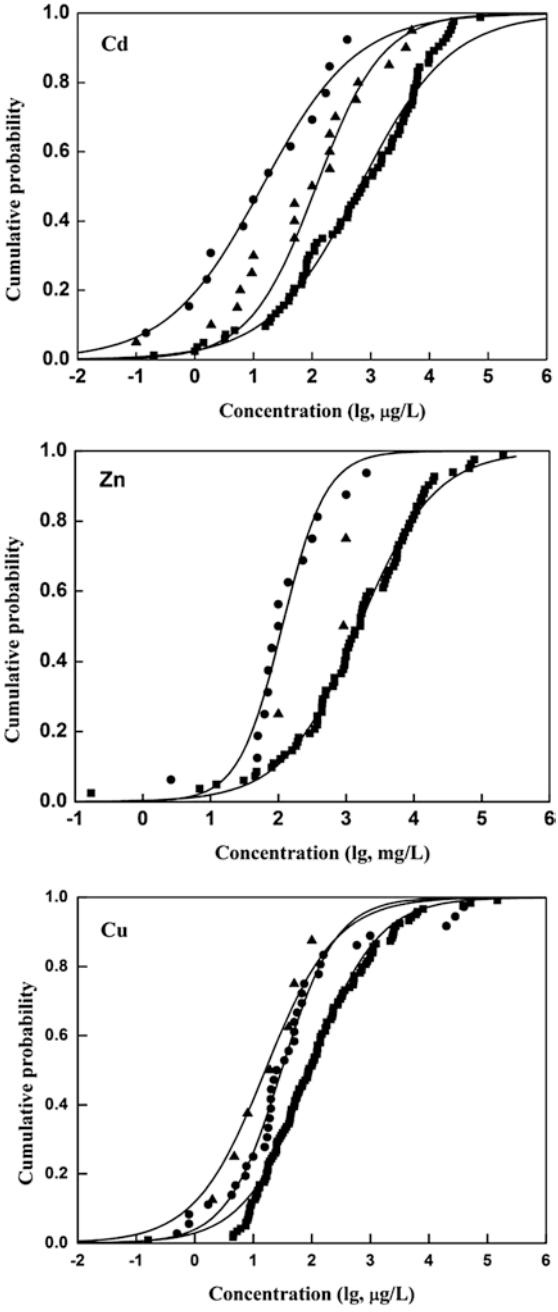

Fig. 3.6 SSD curve of acute, chronic, and gene expression effect data of aquatic organisms for cadmium, copper, and zinc. *square* represented the acute toxicity data; *circle* represented chronic toxicity data; *triangle* represented gene expression effect data. Curve was fitted through logistic function

and gene toxicity data of copper and zinc were not enough. Zinc sensitivity was similar to cadmium sensitivity. Gene expression effect for copper was more sensitive; gene expression effect sensitivity was similar to chronic data in the

Table 3.9 HC_5 of cadmium, copper, and zinc to aquatic organisms[a]

Heavy metals	Categories of data	HC_5 (μg/L)	90 % confidence interval (μg/L)
Cd	Individual-level acute	5.26	2.23–10.8
	Individual-level chronic	0.197	0.0147–0.938
	Gene expression effect	0.717	0.0907–2.91
Cu	Individual-level acute	2.98	1.70–4.82
	Individual-level chronic	0.529	0.140–1.45
	Gene expression effect[a]	/	/
Zn	Individual-level acute	16.6	7.39–32.8
	Individual-level chronic	9.09	2.36–21.6
	Gene expression effect[a]	/	/

[a] For few data are obtained ($n < 10$); data fitting is not carried out (Posthuma et al. 2002)

high-concentration range. For the three heavy metals, that acute toxicity data were the least sensitive was one common point, and the other was that the sensitivity of chronic toxicity data was not consistent with gene expression effect data. The HC_5 with 95 % protection ratio of aquatic organisms was respectively calculated to compare quantificationally acute, chronic, and gene expression effect data of the three heavy metals, respectively. The HC_5 value was important for the calculation of WQC for protecting aquatic organism (see Table 3.9). For cadmium and copper, the sensitivity of chronic effect indicators was higher than that of acute indicators. The differences between the sensitivity of acute and chronic effect indicators of cadmium and copper were 27 and 6 times, respectively, which indicated that the chronic indicator was more important than the acute indicator in routine water environment management. For zinc, the sensitivity of acute effect was triple the sensitivity of chronic effect. The difference was less than that of cadmium and copper. And there were insufficient gene expression effect data; only the data of cadmium are so abundant fitting to calculate HC_5. The results indicated that for 95 % protection ratio of aquatic organisms, the sensitivity of gene expression effect indicators was seven times more than that of acute effect indicators and three times less than that of chronic effect indicators. For copper and zinc, the gene expression effect data were too few to accurately calculate the HC_5. There were only seven gene expression effect data for copper and three gene expression effect data for zinc. Copper SSD curve was established to analyze the sensitivity trend, and zinc data were shown without the corresponding SSD curve (Fig. 3.6).

3.3.4 Discussion

With the development of ecotoxicological studies, toxic effect data in individual level showed more limitation in the derivation of WQC. The new trend of WQC was the characterization method based on broader, more microscopic or more comprehensive toxic effect indicators. Generally, the chronic toxicity effect indicator was more sensitive than acute toxicity effect indicator and also played a more important role in

routine environmental management; confirmed by this study expression, change of the genes induced by environmental pollutant stress had become one of research focuses in the ecological risk assessment as "omics" technologies were developing and applying in the environmental science field. Gene expression change at the microscopic level was generally believed to be earlier than the effect change at the cells, tissues, individuals, populations, and communities, and other levels (Pennie et al. 2004; Snell et al. 2003). Therefore, extensive references of ecotoxicology studies on the genetic level were recently published. A bridge between the microscopic warning indicators and macroscopic warning indicators was built by the genetic-level study connecting with the study at individual level and population level. Thus, the gene expression effect indicators would perform a more important function in environmental risk assessment (Connon et al. 2008; Magrinial et al. 2008; Roh et al. 2009; Spurgeon et al. 2005).

With the increase of accumulated ecotoxicology gene toxicity data, it was possible to study the relationship between the gene effect indicators and more macroscopic indicators by adopting data analysis method (SSD method). Recently, through extensive literature searching (and based on toxicity database of RIVM (e-toxBase) (Fedorenkova et al. 2010), because of its abundant genetic toxicity data, cadmium was selected as an example to analyze the relationship among acute toxicity data, chronic toxicity data, and gene expression effect data. The sensitivity of gene expression effect on cadmium was unexpectedly significantly lower than the chronic toxicity indicators. Therefore, whether gene expression effect could be used as an early warning indicator of ecologic risks or not was dubious. They also proposed that gene expression data were not enough and needed to be improved. The conclusion based on cadmium toxicity data study should be verified and improved (Fedorenkova et al. 2010). Thus, in this paper, acute, chronic, and gene expression effects were analyzed by selecting more abundant biologic toxicity data. Meanwhile, to verify the conclusion from cadmium data, copper and zinc with relatively abundant gene effect data were selected to study. The results showed that the conclusions drawn from the analysis of three effect data of cadmium were the same as that conclusion of Fedorenkova and others: The sensitivity order was as follows: "chronic > gene > acute." But gene data of copper and zinc were insufficient; the data sensitivity of zinc and cadmium was similar according to the general trend. The gene expression effect data of copper were more sensitive than chronic toxicity data, and especially, their difference was obvious in low-concentration range which played more important role in environment management. These demonstrated that gene expression effect indicators might become potential indicators of ecological risk assessment. The logicality of distribution makes it possible to apply in WQC study, although they still need to be validated in the future.

The sensitivity of gene indicators was lower than that of chronic indicators according to the data analysis of cadmium and zinc. Even for copper, compared with chronic indicators, the advantages of sensitivity of gene indicators were not very obvious. The results showed that the minimum exposure concentration was LOEC of gene expression indicators except for *Tetrahymena thermophila*, which indicated that smaller values might exist for certain in the gene expression. The current gene expression LOEC obtained from the literature was likely not the

actual LOEC. The LOEC of gene expression effect would be reduced as the lower exposure concentrations were setting up in the test; this showed that the sensitivity of gene expression effect would be improved. Another important reason was that the selected stress response genes in the references were probably not the earliest response gene to the pollutants, while there were thousands of target genes in the organism. Thus, the most sensitive response genes would be discovered by high-throughput screening of target genes using genomics technology, and then, the sensitivity of gene expression effect indicators would be properly evaluated.

In quantitative analysis of the sensitivity, HC_5 which had important reference values in deriving WQC was analyzed contrastively. And it was a pity that gene expression effect data of copper and zinc were too few to calculate HC_5. Despite all this, cadmium, copper, and zinc were the pollutants with relatively abundant gene effect data, (Fedorenkova et al. 2010) and there were no more effective gene effect data for the analysis and calculation. Because of data limitation, the principle of "minimum toxicity data requirements" was not strictly observed in HC_5 calculation (US EPA 1985; Leeuwen and Vermeire 2007), and probably derivation was carried out to compare the sensitivity. Compared with three HC_5 of cadmium [acute: 18.3 μg/L; chronic: 0.23 μg/L; gene: 0.72 μg/L (2010)] of Fedorenkova et al., acute HC_5 (5.26 μg/L) in the study was significantly lower, chronic HC_5 (0.197 μg/L) was similar, and HC_5 (0.717 μg/L) of gene effect was basically the same. These differences were related to the datasets used in the analysis. For example, acute cadmium data (82 sets of data) screened in this paper were much greater than the acute data [20 sets of data (Fedorenkova et al. 2010)] obtained from e-toxBase databases by Fedorenkova and others. It resulted in the major difference between the two acute HC_5. However, although the selected cadmium data were different, the conclusion was the same.

It was stated that gene expression effect of pollutant stress response at the molecular level had good application potential in early warning of ecological risks. But the available data were difficult to support its wide application. High-throughput screening of target genes was needed to be carried out by a variety of "omics" technologies, determining the specific target response genes of specific pollutants. And the toxicity test methods of gene expression effect endpoint need to be further improved, so that the gene expression effect would practically play an important part in early warning of ecological risks and effectively promote its application in the WQC study.

3.4 Species Sensitivity Distribution Analysis for Phenanthrene and Benzo[a]pyrene with ICE Model

3.4.1 Phenanthrene

3.4.1.1 Introduction

SSD is a principal tool for the formulation of WQC (Solomon et al. 1996; Wheeler et al. 2002a). SSD analysis is usually used to determine the hazardous concentration of a toxic chemical for five percent of the species, known as the HC_5

(Van der Hoeven 2001). Usually, HC_5 has been used to set quality objectives for the environment in Europe and the USA (Eduljee 2000; Kahru and Dubourguier 2010; Kemmlein et al. 2009). SSD depends upon available datasets and can differ in various taxonomic groups, sample sizes, and distributions (Frampton et al. 2006; Maltby et al. 2005a, b, Wheeler et al. 2002b). Recommendations on minimal sample sizes necessary for meaningful estimations depend on dataset and estimation methodology (Augspurger et al. 2003; Chapman et al. 1998). In general, aquatic toxicity data of different taxonomic levels were derived from acute toxicity tests. However, the number of test species was limited by the complexity of test procedures and species availability (especially for threatened and endangered species). Therefore, it is valuable to investigate how to predict the toxicities for nontested species using existing data.

Recently, interspecies correlation estimation (ICE) model has been developed by US EPA to fill in data gaps in SSD and is one of the most effective approaches to reduce the numbers of organisms used for experimental purposes. ICE statistical model is regarded as an alternative for estimating the toxicities of chemicals to both aquatic organisms and terrestrial wildlife using surrogate species (Dyer et al. 2006, 2008; Golsteijn et al. 2012; Raimondo et al. 2007). The entire acute toxicity dataset used in the development of aquatic Web-ICE models consisted of 5,487 test results of 180 species and 1,258 chemicals (Raimondo et al. 2010). ICE model is a log–log correlation of multiple chemical toxicity values for a pair of species that allows the toxicities for multiple species to be predicted from a single metrical acute toxicity value using a surrogate species (Zhang et al. 2010). A current and particularly useful attribute of interspecies correlations is their use to avoid the usage of threatened and endangered species. While the models are applicable to many toxicants, so far there has been no report on the suitability of ICE for polycyclic aromatic hydrocarbons (PAHs) (Bejarano and Barron 2014).

Phenanthrene (PHE, CAS#85-01-8) is a three-ring compound included in the low molecular weight PAH group, and it is considered as one of the priority pollutants. PHE has been widely detected in Chinese freshwaters, and the exposure concentration levels of PHE in Chinese freshwaters range from 45.01 to 379.28 μg/L (Callen et al. 2013; Juhasz et al. 2014; Qiao et al. 2006; Yang et al. 2011; Zhang et al. 2004, 2012; Zhou and Maskaoui 2003). When the internal dose exceeds certain content, it might cause a number of adverse effects through combination with biological macromolecules to the organisms, such as immunotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity (Simpson et al. 1996; Woodhead et al. 1999; Zakaria et al. 2002; Zuo et al. 2007). According to the number of aromatic rings, the 16 PAH compounds were divided into three groups, representing two-, and three-, four-, and five-, and six-ring PAHs (Soclo et al. 2000; Sprovieri et al. 2007). It has been shown that the proportion of two- and three-ring PAHs were higher than those with higher number of rings, and PHE poses potential risk to the aquatic species after preliminary ecological risk assessment in previous study (Viguri et al. 2002; Wang et al. 2003). However, it is hard to derive the WQC of PHE due to the lack of toxicity data, especially toxicity data for native species. Thus, it would be valuable to estimate toxicity of PHE by means of ICE

models for risk assessment (Meier et al. 2013; Stringer et al. 2012). The objectives of the present study were to determine whether ICE models could be developed to generate SSDs for PHE with acceptable deviation from the actual measurement, and to further derive WQC in China.

3.4.1.2 Materials and Methods

1. *Metrical toxicity data collection and processing*
   Metrical toxicity data of PHE were collected from ECOTOX (http://cfpub.epa.gov/ecotox) and 2 supplementary online databases CNKI (http://www.cnki.net) and ELSEVIER (http://www.sciencedirect.com). The data were screened and analyzed according to US EPA guidelines (Stephan et al. 1985). The keywords were "PAHs" "Phenanthrene," "aquatic life/organisms," "toxicity," and "ecotoxicity."
   Data were subjected to rigorous quality assurance guidelines (Yan et al. 2013). First, aquatic acute toxicity tests found within the databases or literature included data such as 48-h LC_{50} or EC_{50} for *Daphnia* and 96-h LC_{50} or EC_{50} for other species. Second, the toxicological endpoints of PHE were mainly associated with death effects, such as immobility, respiratory inhibition, and lethality. Third, the majority of exposure experiments included both flow-through and static/renewal. Finally, species existing in China were selected. All test conditions were conducted according to ASTM standard guidelines (ASTM 1993; Gaikowski et al. 1999; Yin et al. 2003).
2. *ICE dataset*
   US EPA has developed both ICE software (Raimondo et al. 2010) and additional robust ICE models for aquatic and wildlife species available from an online database (http://www.epa.gov/webice). The Web-based ICE platform was used in this study. Web-based ICE provides interspecies extrapolation models to predict acute toxicity via a user-friendly interface.
   Six reported toxicity data were screened, which included four invertebrate species (*Chironomus tentans*, *Daphnia magna*, *Daphnia pulex,* and *Hydra sp.*) and two vertebrate species (*Lepomis macrochirus* and *Oncorhynchus mykiss*). According to the principle of choosing surrogate species, three to four invertebrate and vertebrate species must be included (Dyer et al. 2006). In reference to previous research on ICE models, a kind of invertebrate species and at least two kinds of fishes from different taxonomic level were generally chosen (Awkerman et al. 2008; Zhang et al. 2010). Thus, this study selected *D. magna* (a planktonic crustacean), *L. macrochirus* (a Centrarchidae fish), and *O. mykiss* (a Salmonidae fish) as the surrogate species. *D. magna* is a standard organism, *L. macrochirus* is usually selected, and *O. mykiss* is prevalent worldwide. Acute toxicity values for the three surrogate species were 230, 234, and 375 μg/L, respectively (Call et al. 1986).
3. *Data analysis*
   Many cumulative distribution functions have been used to fit SSDs (Aldenberg and Jaworska 2000; Hose and Van den Brink 2004; Van der Hoeven 2001,

Wagner and Løkke 1991; Wang et al. 2014a; Yan et al. 2012a, b). In this study, log-logistic distribution was used (Kooijman 1987; Newman et al. 2000). The equation is as follows:

$$Y = 1/(1 + \exp((\alpha - X)/\beta)) \quad (1)$$

where Y is the cumulative probability of species, defined as "the order of the data point" divided by one plus the total number of data points; X is the log-transformed LC_{50} or EC_{50}; α is a parameter representing the location (or intercept); β is a parameter representing the slope of the curve.

Several statistic methods are provided for each model to evaluate the accuracy of the estimated values. A description of the validity of the models is provided by Raimondo et al. (2010). The criteria used to check the Web-ICE prediction are briefly listed as follows: (1) relatively low mean square error (MSE) (<0.22); (2) close taxonomic distance (53); (3) high cross-validation success rate (>85 %); (4) high degree of freedom (df > 8); (5) high R^2 value (>0.6); (6) low p values (<0.01); and (7) narrow confidence bands on the graph (Dyer et al. 2006). If there were multiple Web-ICE predictions for the same species, we should preferentially consider criteria (1) and (2) (Raimondo et al. 2007).

SPSS 20.0 (IBM, Armonk, NY) and Origin 8.0 (OriginLab, Northampton, MA) were used for data analysis. An independent t test was used to compare the means between the measured data group and the total toxicity data groups. Before comparing the differences between means, Homogeneity of variance was checked to decide which test ways should be used. Analysis of variance (ANOVA) was also performed to compare mean values among several data groups and the data with equal variance probability values less than 0.05 were considered to be statistically significant.

3.4.1.3 Results and Discussion

1. *Estimated toxicity of PHE using Web-ICE*
   Based on the aforementioned criteria, the entire aquatic animal acute toxicity records for PHE contained 15 families, 17 genus, and 19 species, including fish, invertebrates, and amphibians. The detailed information about the acute toxicity data is listed in Table 3.10.
   The data were then fitted with log-logistic distribution to construct SSD. In addition, geometric species mean acute values were calculated and displayed when more than one acute (e.g., LC_{50} or EC_{50}) value was available for each species. Acute toxicity data were ranked into order. The SPSS 18.0 (IBM, USA) software was used for data analysis.
   Statistical parameters such as coefficient of determination (R^2), mean square error (MSE), degrees of freedom (df), p value, cross-validation success rate, and taxonomic distance are important to evaluate the accuracy of model estimation. Dyer et al. (2008) conducted a comprehensive study on the application

Table 3.10 Metrical SMAVs of PHE to freshwater animals

Taxa	Phylum	Class	Species	Species mean acute values (μg/L)
Invertebrates	Cnidaria	Hydrozoa	*Hydra* sp.[a]	96
	Mollusca	Lamellibranchiata	*Lumbriculus variegatus*[a]	419
	Rotifer	Monogonta	*Brachionus calyciflorus*[a]	1,098
	Arthropoda	Crustacea	*Artemia salina*[a]	280
	Arthropoda		*Palaemonetes pugio*[a]	360
	Arthropoda		*Americamysis bahia*[b]	27.1
	Arthropoda		*Oithona davisae*[b]	521
	Arthropoda		*Daphnia magna*[a]	230
	Arthropoda		*Daphnia pulex*[a]	350
	Arthropoda		*Diporeia* sp.[a]	295
	Arthropoda		*Gammarus minus*[a]	460
	Arthropoda		*Gammaru spseudolimnaeus*[a]	126
	Arthropoda		*Hyalela azteca*[a]	437
	Arthropoda		*Chironomus tentans*[a]	490
	Arthropoda		*Neanthesaren aceodentata*[a]	600
Vertebrates	Chordata		*Micropterus salmoides*[b]	70
			Lepomis macrochirus[b]	234
			Cyprinodonvarie gatus[b]	478
			Oncorhynchus mykiss[b]	375

[a] Native species in China
[b] Introduced species to China

of interspecies correlation model to various chemical classes. To optimize the results, they only used models with high correlations ($p < 0.05$) and with at least 10 observations per species pair and slopes greater than 0.70.

Due to few toxicity metrical data of algae, we focused our work on only invertebrates and vertebrates. The species selection process in ICE has been shown to be important for predicting accurate toxicity values of chemicals. Based on the aforementioned guidelines, *D. magna*, *L. macrochirus,* and *O. mykiss* were used to estimate the toxicities for other species using the Web-ICE program. Predicted species from correlation models are presented in Table 3.11. The selected species included both native species from China, such as *Chironomus tentans* and *Carassius auratus*, and introduced species, like *O. mykiss* and *Lepomis macrochirus*. Collectively, a total of 23 species toxicity data were estimated using the 3 surrogate species by Web-ICE program (see Table 3.11).

Table 3.11 Summary of the regression parameters of surrogate-predicted species using ICE models

Surrogate species	Predicted species	Cross-validation success rate (%)	MSE	R^2	p value
Daphnia magna	*Chironomus tentans*	96.31	0.08	0.98	<0.01
	Daphnia pulex	92.85	0.12	0.82	<0.01
	Farfante penaeus duorarum	85.45	0.17	0.85	<0.01
	Gammarus fasciatus	87.55	0.11	0.46	<0.01
	Gammarus pseudolimnaeus	86.84	0.07	0.77	<0.01
Lepomis macrochirus	*Carassius auratus*	86.95	0.02	0.8	<0.01
	Lepomis cyanellus	92.85	0.14	0.9	<0.01
	Micropterus salmoides	96.87	0.11	0.92	<0.01
	Oncorhynchus kisutch	92.3	0.15	0.91	<0.01
	Oncorhynchus tshawytscha	87.33	0.20	0.9	<0.01
	Perca flavescens	94.44	0.11	0.93	<0.01
	Salvelinus fontinalis	88.88	0.20	0.88	<0.01
Oncorhynchus mykiss	*Crassostrea virginica*	85.3	0.13	0.91	<0.01
	Caecidotea brevicauda	87.5	0.18	0.84	<0.01
	Oncorhynchus gilae	97.05	0.08	0.93	<0.01
	Notropis mekistocholas	100	0.02	0.98	<0.01
	Oncorhynchus tshawytscha	100	0.06	0.97	<0.01
	Etheostoma fonticola	90.47	0.14	0.93	<0.01
	Pimephales promelas	85.71	0.17	0.82	<0.01
	Poecilia reticulata	87.49	0.23	0.91	<0.01
	Salmo salar	86.61	0.13	0.95	<0.01
	Salmo trutta	94.73	0.07	0.96	<0.01
	Salvelinus namaycush	96.15	0.08	0.93	<0.01

For *D. magna*, a total of 22 species toxicity values were estimated in the Web-ICE program. Having eliminated the poorly correlated species, five species were qualified for our estimation. They are *Chironomus tentans*, *Daphnia pulex*, *Farfantepenaeus duorarum*, *Gammarus fasciatus*, and *Gammarus pseudolimnaeus*. For *L. macrochirus*, seven species were estimated in accordance with the guidelines above. For *O. mykiss*, eleven species toxicity values were estimated; all species were fishes except for *Crassostrea virginica*.

Uncertainties in model prediction are inevitable. The uncertainty observed in the ICE prediction was associated with surrogate species selection, accuracy of the model prediction, and the selection of predicted values. Since the ICE models were developed in the USA, its application in China might not be totally suitable. For example, some taxa used in the USA might not be suitable in China, possibly due to the badly correlated relationship and non-native species

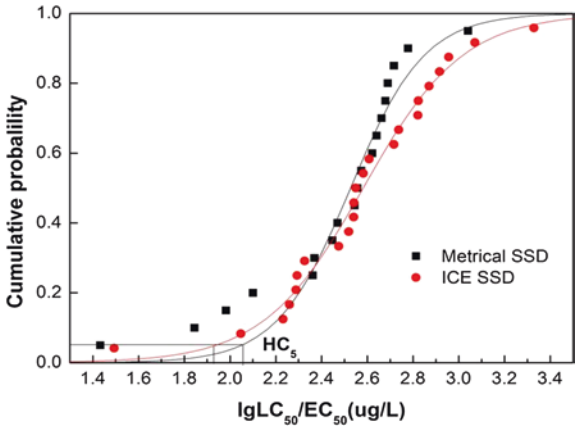

Fig. 3.7 Comparison of SSDs for metrical and ICE predicted species acute toxicity values using 3 surrogate species

(Awkerman et al. 2008). In a distributional approach, the greater the number of single species toxicity values, the lower the uncertainty about the distributional parameters and the shape of the distribution (Newman et al. 2000). Uncertainty may also be decreased by examining SSDs corresponding to their ecological context, such as freshwater versus marine, benthic versus pelagic, and invertebrate versus fish. (Forbes and Calow 2002).

2. *ICE and measure-based SSD*

   According to US EPA water quality guidelines, SSDs were created for both the metrical PHE acute toxicity data and predicted toxicity data from Web-ICE. The results showed that the two distributions fitted well, with R^2 equal to 0.946 and 0.986 for ICE- and experiment-based models, respectively (Fig. 3.7). One-way ANOVA showed that there was no significant difference between the two distributions based on ICE and the measured data.

   A qualitative comparison of species placement between various SSDs showed that some species frequently occurred in the sensitive or tolerant portions of the curve, which can lead us to rank species placements (Feng et al. 2013a, b). The smaller the cumulative probability is, the more sensitive the species is. Hence, species in the first quartile of the SSD curve were thought as the most sensitive species, while that in the following two quartiles were considered as relatively tolerant species, and that in the fourth quartile were the most tolerant species (Dyer et al. 2006). The ranking results of the predicted and the metrical species distributions showed that the most sensitive species on the basis of the ICE-based SSD curves included *Chironomus tentans*, *Daphnia pulex,* and *Gammarus fasciatus*, and the most sensitive species in the metrical SSD curves included *America mysisbahia*, *Micropterus salmoides*, *Hydra* sp., *Gammarus pseudolimnaeus,* and *Daphnia magna*. The results of the ranking clearly illustrated that although there are some species differences between the two SSD curves, the SSD models were similar when invertebrate crustaceans were dominant. With regard to the most tolerant species, there was also difference between the two distributions. The most tolerant species on the basis

of the ICE-based SSD were *Carassius auratus*, *Farfantepenaeus duorarum*, *Etheostoma fonticola,* and *Chironomus tentans* (two fishes and two invertebrates), and the relatively tolerant species were *Brachionus calyciflorus*, *Neanthesaren aceodentata*, and *Oithona davisae* (dominated by invertebrates). Dyer et al. (2008) ranked species using ICE and suggested that coldwater fish species were generally more sensitive to a wide range of chemicals with diverse modes of action. This is consistent with our study. Moreover, Tremolada et al. (2004) also concluded that trout were the most sensitive species to pesticides via ICE predicted values. That is, species observed to be sensitive species to specific chemicals were also significantly different.

Predictive toxicological models are integral to ecological risk assessment because data for most species are limited (Hose and Van den Brink 2004). Given the cost of multiple toxicity tests, ICE provides the opportunity to generate multiple toxicity values with limited costs. Web-ICE for aquatic species developed by US EPA is based primarily on North American species. Thus, it is not known whether ICE-based SSDs may be applicable to non-North American geographies, such as China. Even so, others have found that the geographic distribution of species was not a significant factor in the establishment of SSD (Maltby et al. 2005a, b). Otherwise, the selection of toxicological endpoints of chemicals might influence the SSDs. Suitable endpoints needed be selected due to the toxic effect of pollutants. For the same substances, the more sensitive endpoint should be used to construct SSDs (Feng et al. 2012a, b). Generally, the acquired data from ICE mainly contain species from North America. It is likely that some native species in China are not included in the Web-ICE dataset. However, the measured toxicity data contained both introduced species and native species from China. In order to investigate the accuracy of ICE metrical, ICE-based values were combined with the metrical toxicity data and the SSDs were constructed based on a "total" dataset (i.e., the combination of both the metrical and the predicted). The results showed that the two distributions were fitted well, with R^2 of total and measured SSD models being 0.987 and 0.991, respectively (Fig. 3.8). One-way ANOVA showed that there was no significant difference between the two distributions based on the measured and "total" data.

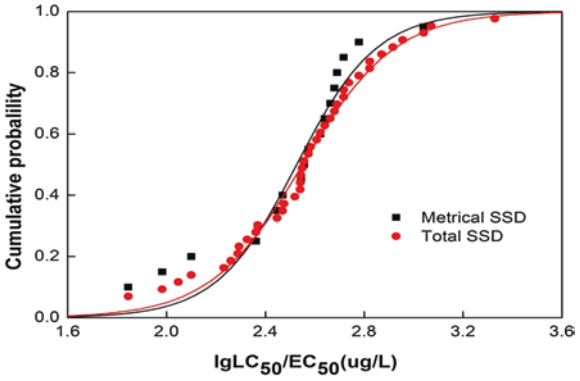

Fig. 3.8 SSDs for PHE using the metrical values and "total" values, which is combined ICE predicted with the measured data. *Dashed line* illustrated the 95 % confidence interval of metrical values and the ICE predicted values

3. *Aquatic life criteria derivation*
   The fifth percentile at the lower tail of a SSD curve (HC_5) has been widely used as the criteria value to protect aquatic life (Jin et al. 2011). In the present study, the ICE-based HC_5 and the metrical HC_5 for acute PHE toxicity data were 86.9 and 112.3 μg/L, respectively, indicating that there was no significant difference ($p = 0.08$).
   Dyer et al. (2008) found that the majority of ICE-based predictions were metrically similar to the experimental measurement. In terms of a statistical analysis and effect assessment, the comparative results implied the potential for extrapolation using ICE. Therefore, the use of ICE models to provide reasonably accurate estimates of chemical toxicity and protective criteria was encouraging, which could be used as a potential replacement for the current water quality derivation methods when sufficient empirical toxicity data are lacking.
   While there are reasonable ecological reasons to eliminate the use of non-China species for developing China-specific water quality criterion, much recent research regarding the evaluation of ecological traits (Baird et al. 2011; Baird and Van den Brink 2007; Van den Brink et al. 2011) as aspects worth of protection may provide a reconsideration of such eliminations. If traits are the target of protection, then where the species are endemic will not matter. This is certainly very important in site-specific risk assessment. We envision the development and use of ICE models that contain diverse species from all over the globe and as such would provide the most robust methodology for environmental criterion development, regardless of geography.

3.4.1.4 Conclusions

This study comprehensively evaluated the accuracy of ICE-generated SSDs by comparing ICE-based HC_5 to HC_5 derived from metrical acute toxicity values of PHE for aquatic species. The comparison of results between the ICE-based and the measurement-metrical-based SSD illustrated that there was no significant difference between the two distributions, indicating ICE was a potential option to obtain the predicted acute toxicity data for aromatic compounds. The ICE models are recommended as a valuable alternative in ecological risk assessment.

3.4.2 Benzo[a]pyrene

3.4.2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) with two or more fused aromatic rings belong to a class of organic contaminants and can be found in all environmental media (Nadal et al. 2004). In recent years, Benzo[*a*]pyrene (BaP, CAS#50-32-8), as a priority PAH, has been detected at high levels in surface water worldwide and reported to pose potential risks to ambient aquatic environment (Meier et al. 2013; Stringer et al. 2012).

WQC are the maximum concentrations of pollutants in aquatic environments that presumed to not affect organisms and their functions after long-term or short-term exposure (US EPA 2003). As the scientific measures used in water quality standards, WQC have played an important role in the management of aquatic environments in China, and the establishment of appropriate criteria has become an important area of investigation worldwide (Yang et al. 2012). In China, no WQC for BaP are available as there is lack of toxicity data for native species.

To fill in data gaps, ICE model was developed by US EPA, which is based on a log–log correlation of multiple chemical toxicity values for a pair of species (Zhang et al. 2010). The entire acute toxicity dataset used in the development of aquatic Web-ICE models consisted of 5,487 test results of 180 species and 1,258 chemicals (Raimondo et al. 2010). Appling the ICE model, chemical toxicities for multiple species (both aquatic and terrestrial) can be predicted from the data of surrogate species (Dyer et al. 2006, 2008; Golsteijn et al. 2012; Raimondo et al. 2007). The ICE model is very useful when there is lack of toxicity data, and it has become one of the most effective approaches to reduce the numbers of organisms used for experimental purposes (Callen et al. 2013; Juhasz et al. 2014). Particularly, application of the model can avoid use of threatened and endangered species. To date, the model has been proved to be applicable to many toxicants (Awkerman et al. 2008; Bejarano and Barron 2014; Raimondo et al. 2007), but there has been no report on its application to PAHs so far. To address this issue, SSDs using both experimental data and predicted data from ICE model are plotted and they were compared with each other to validate the applicability of ICE for BaP compound.

In the present study, acute toxicity tests were conducted using 8 representative and widespread native species (from 3 phyla and 8 families), including a Siluridae fish (*Silurus asotus*), a Cyprinidae fish (*Cyprinus flammans*), a Cobitidae fish (*Misgurnus anguillicaudatus)*, a planktonic crustacean (*Daphnia magna*), a benthic crustacean (*Macrobrachium nipponense*), an annelid (*Limnodrilus hoffmeisteri*), an insect (*Chironomus plumosus*), and an amphibian (*Rana limnocharis*). ICE-based predicted toxicity values were generated using three surrogate species (*Lepomis macrochirus*, *Cyprinus carpio,* and *Daphnia magna*).

The objectives of this work were to (1) provide a supplement to BaP toxicity database, (2) generate SSDs for BaP using measured acute toxicity data and ICE-based predicted values, and (3) discuss the possibility of exploring ICE models to derive WQC for BaP with acceptable deviation from the actual measurement. This work could provide useful information for environmental risk assessment and pollution management for BaP in ambient aquatic environment.

3.4.2.2 Materials and Methods

1. *Test chemicals and organisms*
   BaP, $C_{20}H_{12}$, ≥98 % purity (HPLC), was purchased from J&K Chemical Company.

According to the USEPA guidelines, data on at least eight families of aquatic animals drawn from three different phyla and one aquatic plant are required in the derivation of WQC. In this study, in addition to the published ecotoxicity data for BaP, eight resident aquatic species in China were chosen for the acute tests.

2. *General test conditions*
   All tests were static renewal whereby test solutions were totally replenished at 24-h intervals. Dechlorinated tap water treated with activated carbon was used for dilution in toxicity tests. Measured chemical parameters of dilution water were as follows: pH 7.2 ± 0.5, dissolved oxygen (DO) 7.3 ± 0.5 mg/L, total organic carbon 0.02 mg/L, and hardness as $CaCO_3$ 192 ± 0.1 mg/L. The toxicity tests were conducted in three replicates (with each containing 10 organisms) at assigned concentrations, solvent control, and blank control. All tests were undertaken at a light: dark photoperiod of 12:12 h.
   Test organisms were not fed during the acute test periods. Test chambers were immersed in a water bath with water temperature maintained at 20 ± 2 °C, unless otherwise noted. Temperature, DO, and pH were measured in test chambers daily. Biological observations were performed at least once daily. All toxicity tests were conducted according to American Society for Testing and Materials (ASTM) standard guidelines (ASTM 1993; Gaikowski et al. 1999; Yin et al. 2003a, b, c). 48-h-EC_{50} (effective concentration in 50 % of the test organisms over 48 h) for D. magna and 96-h-LC_{50} (lethal concentration in 50 % of the test organisms over 96 h) for other aquatic animals were used as test endpoints.
3. *Acute toxicity tests*
   All the organisms tested in this study were obtained from Chaolai and Large Forest aquaculture companies. Prior to toxicity tests, all test organisms were acclimated to general test conditions (see above Sect. 2.2) for a minimum of 7 days. D. magna (<24 h age) were obtained from in-house cultures at our chemical laboratory of Chinese Research Academy of Environmental Sciences. Acute toxicity tests of BaP to eight resident aquatic organisms are shown in Table 3.12.
4. *Chemical analysis*
   Please see 1.2.1.2 (6).
5. *Collection of published ecotoxicity data for BaP*
   The published ecotoxicity data of BaP were collected from the ECOTOX database (http://cfpub.epa.gov/ecotox), TOXNET Database (http://toxnet.nlm.nih.gov), GEO Database (www.ncbi.nlm.nih.gov/geo), the CNKI (http://www.cnki.net), and ELSEVIER (http://www.sciencedirect.com). The data were screened and analyzed according to the US EPA guidelines for aquatic life criteria (Stephan et al. 1985). The keywords were "Benzo[*a*]pyrene," "aquatic life," "acute toxicity," and "chronic toxicity."
6. *ICE dataset*
   US EPA has developed both ICE software (Awkerman et al. 2008) and additional robust ICE models for aquatic and wildlife species available from an online database (http://www.epa.gov/webice). The Web-based ICE platform

Table 3.12 Acute toxicity tests of BaP to eight resident aquatic organisms

Species	Wet weigh (g)	Length (cm)	Time (h)	Exposure concentrations (μg/L)
S. asotus	0.30 ± 0.02	2.5 ± 0.2	96	0.00, 0.46, 0.58, 0.76, 1.00, 1.30, 1.70, 2.20
C. flammans	0.40 ± 0.05	3.0 ± 0.5	96	0.00, 2.20, 2.90, 3.70, 4.80, 6.30, 8.20, 10.70
M.anguillicaudatus	0.70 ± 0.05	6.0 ± 0.5	96	0.00, 12.8, 16.7, 21.7, 28.3, 36.7, 47.8, 62.1
D. magna	ND	ND	48	0.00, 16.1, 20.2, 0.27, 0.38, 0.48, 0.54, 0.75
M. nipponense	0.25 ± 0.05	3.0 ± 0.2	96	0.00, 0.60, 0.70, 0.90, 1.20, 1.60, 2.00, 2.60
L. hoffmeisteri	0.05 ± 0.01	1.5 ± 0.2	96	0.00, 0.60, 0.70, 0.90, 1.20, 1.60, 2.00, 2.60
C. plumosus	0.03 ± 0.01	ND	96	0.00, 0.28, 0.37, 0.48, 0.62, 0.81, 1.05, 1.37
R. limnocharis	0.20 ± 0.02	1.6 ± 0.2	96	0.00, 0.38, 0.46, 0.55, 0.66, 0.79, 0.95, 1.24

ND not determined

was used in this study. Web-based ICE provides interspecies extrapolation models to predict acute toxicity via a user-friendly interface.

Based on geometric means from the measured database, Web-ICE was seeded with acute toxicity values for *L. macrochirus, C. carpio, and D. magna* (5, 27 and 1.5 μg/L, respectively) to predict acute toxicity values for a variety of invertebrate and vertebrate species. These three species are prevalent in China.

7. Data analysis

Please see Sect. 3.4.1.2 (3).

3.4.2.3 Results and Discussion

1. *Results of toxicity tests of eight native aquatic organisms*

Acute toxicity values of BaP to 8 aquatic species are shown in Table 3.13. No mortality was observed in the control groups and the solvent control groups.

Results of acute toxicity tests using eight aquatic species (Table 3.13) showed that *D. magna* with a 96-h-LC_{50} of 1.298 μg/L was the most sensitive species to BaP followed by *L. hoffmeisteri*, *C. plumosus*, *C. flammans*, *P. parva*, *R. limnocharis,* and *M. nipponense* with decreasing sensitivity, while the least sensitive species was *M. anguillicaudatus* with a 96-h-LC_{50} of 29.98 μg/L. Results of the present study indicate that BaP is highly toxic to native freshwater aquatic organisms especially the planktonic crustacean. It had been reported that the 48-h-LC_{50} of BaP on water flea *D. magna* was 1.5 μg/L (Black et al. 1983), which is consistent with the result of our study (1.298 μg/L). In addition, previous study reported that demersal fish *M. anguillicaudatus* was sensitive to some organochlorine

Table 3.13 Acute toxicity of BaP to freshwater organisms

Species	Exposure time	Functions	R^2	p	LC_{50} (95 % CI) (μg/L)
S. asotus	96 h	$y = 2.8499x - 1.6745$	0.9769	<0.01	5.000 (4.881–5.237)
C. flammans	96 h	$y = 1.6097x + 4.1009$	0.9422	<0.01	3.626 (3.517–3.804)
M. anguillicaudatus	96 h	$y = 1.1701x + 3.2723$	0.9498	<0.01	29.98 (27.43–31.17)
D. magna	48 h	$y = 2.0478x + 4.7683$	0.9324	<0.01v	1.298 (1.174–1.406)
M. nipponense	96 h	$y = 2.1227x + 3.1271$	0.9491	<0.01	7.632 (7.155–7.848)
L. hoffmeisteri	96 h	$y = 1.6399x + 4.6474$	0.9651	<0.01	1.642 (1.498–1.769)
C. plumosus	96 h	$y = 1.2792x + 4.6587$	0.9296	<0.01	1.851 (1.694–1.987)
R. limnocharis	96 h	$y = 9.1498x - 1.5991$	0.9893	<0.01	5.264 (5.101–5.423)

CI confidence interval

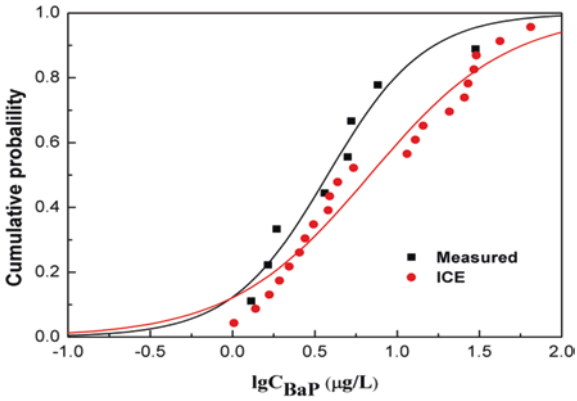

Fig. 3.9 Comparison of SSDs constructed using measured toxicity data and ICE predicted toxicity data from 3 surrogate species

pesticides (Wang et al. 2013), while it was not sensitive to BaP. That is, species observed to be sensitive to specific chemicals were also significantly different (Yan et al. 2013; Zhang et al. 2010). In general, the aquatic invertebrates (e.g., annelid and insect) are more sensitive than the fishes, and the benthic fishes are the least sensitive species (Wang et al. 2013).

Acute toxicity data were ranked in an increasing order and then fitted with log-logistic distribution to construct SSD (Fig. 3.9). Geometric species mean acute values were calculated and displayed when more than one acute (e.g., LC_{50} or EC_{50}) values were available for the same species.

2. *Estimated toxicity of BaP using Web-ICE*

Statistical parameters, such as coefficient of determination (R^2), mean square error (MSE), degrees of freedom (*df*), p value, cross-validation success rate,

and taxonomic distance, are important to evaluate the accuracy of model estimation. Dyer et al. (2008) conducted a comprehensive study on the applicability of interspecies correlation model to various chemical classes. To optimize the results, they only used models with significant correlations ($p < 0.05$) and with at least 10 observations per species pair and slopes greater than 0.70.

Due to few measured toxicity data for algae, the following discussion would be focused on invertebrates and vertebrates only. The species selection process in ICE is important for predicting accurate toxicity values of chemicals. Based on the aforementioned guidelines, *D. magna*, *L. macrochirus,* and *O. mykiss* (with LC_{50} or EC_{50} of 230, 234 and 375 μg/L, respectively) were used to estimate the toxicities for other species using the Web-ICE program. Predicted species from correlation models are presented in Table 3.14. The selected species included both native species from China, such as *C. tentans* and *C. auratus*, and introduced species, like *O. mykiss* and *L. macrochirus*. Collectively, toxicity data for a total of 23 species were estimated using the 3 surrogate species by Web-ICE program (see Table 3.14).

For *D. magna*, a total of 19 species toxicity values were estimated in the Web-ICE program. Having eliminated the poorly correlated species, five species were qualified for our estimation. They are *Gammarus pseudolimnaeus*, *Simocephalus serrulatus*, *Ceriodaphnia dubia*, *Farfantepenaeus duorarum*, and *Daphnia pulex*. For *L. macrochirus*, twelve species were estimated in accordance with the guidelines above. For *C. carpio*, five species toxicity values were estimated, and all of them were fishes.

Uncertainties in model prediction are inevitable. The uncertainty observed in the ICE prediction was associated with surrogate species selection, accuracy of the model prediction, and the selection of predicted values. In a distributional approach, the greater the number of single species toxicity values, the lower the uncertainty about the distributional parameters and the shape of the distribution (Newman et al. 2000). Uncertainty may also be decreased by examining SSDs corresponding to their ecological context, such as freshwater versus marine, benthic versus pelagic, invertebrate versus fish (Forbes and Calow 2002).

3. *Comparison of ICE and measure-based SSDs*

According to US EPA water quality guidelines, SSDs were created for both the measured BaP acute toxicity data and predicted toxicity data from Web-ICE. Based on the comparison in this study (Fig. 3.9), the lower tail of ICE-based SSD curve (below 0.10, which is equivalent to HC_{10}: hazardous concentration for the 10 % of species) was shifted to the left of measure-based SSD curve. A lower HC_5 (hazardous concentration for the 5 % of species) indicated that the species at the lower tail of ICE-based SSD curve were more sensitive to BaP than the species at the lower tail of measured-based SSD curve. In contrast, the HC_{50} (hazardous concentration for the 50 % of species) obtained from measured data was lower than that of predicted data. This is probably due to the lack of toxicity data for sensitive species (e.g., annelid and insect) in predicted data group.

The SSD curves for both measured toxicity data and ICE predicted toxicity data were fitted well with R^2 equal to 0.968 and 0.979, respectively. Although

Table 3.14 Summary of the regression parameters of surrogate-predicted species using ICE models

Surrogate species	Predicted species	Estimated toxicity (μg/L)	Cross-validation success (%)	MSE	R^2	p
Lepomis macrochirus	*Ictalurus punctatus*	26.97	86.71	0.14	0.75	<0.01
	Oncorhynchus kisutch	4.36	92.30	0.15	0.91	<0.01
	Lepomis cyanellus	12.89	92.85	0.14	0.90	<0.01
	Micropterus salmoides	3.89	96.87	0.11	0.92	<0.01
	Oncorhynchus mykiss	5.43	90.61	0.21	0.88	<0.01
	Cyprinodon variegatus	20.78	86.11	0.19	0.71	<0.01
	Perca flavescens	3.81	94.44	0.11	0.93	<0.01
	Salmo salar	4.38	100.00	0.08	0.96	<0.01
	Salvelinus fontinalis	1.93	88.88	0.20	0.88	<0.01
	Etheostoma lepidum	2.67	95.00	0.11	0.94	<0.01
	Salvelinus namaycush	14.41	86.36	0.21	0.70	<0.01
	Sander vitreus	29.29	87.80	0.16	0.73	<0.01
Cyprinus carpio	*Ameiurus melas*	30.29	100.00	0.12	0.95	<0.01
	Ictalurus punctatus	25.62	91.66	0.11	0.95	<0.01
	Pimephales promelas	42.13	100.00	0.04	0.98	<0.01
	Carassius auratus	64.86	100.00	0.07	0.96	<0.01
	Oncorhynchus mykiss	11.51	92.72	0.10	0.82	<0.01
Daphnia magna	*Gammarus pseudolimnaeus*	1.02	89.84	0.17	0.77	<0.01
	Ceriodaphnia dubia	1.76	94.50	0.15	0.80	<0.01
	Daphnia pulex	3.10	85.85	0.13	0.82	<0.01
	Simocephalus serrulatus	1.21	86.66	0.11	0.63	<0.01
	Farfantepenaeus duorarum	2.55	88.45	0.07	0.85	<0.01

there were differences in HC_5 and HC_{50} between measured and predicted data groups, the Kolmogorov–Smirnov analysis showed that there was no significant difference (Kolmogorov–Smirnov test: ks = 1.342, $n1 = 8$, $n2 = 22$, $p = 0.36$) between two fitted curves.

A qualitative comparison of species placement between various SSDs showed that some species frequently occurred in the sensitive or tolerant portions of the curve (Feng et al. 2013a, b). The smaller the cumulative probability is, the more sensitive the species is. Hence, species in the first quartile of the SSD curve were thought as the most sensitive species, while the following two quartiles were considered as relatively tolerant species, and the fourth quartile were the most tolerant species (Dyer et al. 2006). The rankings of the predicted toxicity dataset showed that the most sensitive species were *Gammarus pseudolimnaeus*, *Simocephalus serrulatus,* and *Ceriodaphnia dubia,* while the most sensitive species in the measured toxicity dataset were *D. magna* and *L. hoffmeisteri*. The results of the ranking clearly illustrated that although there are differences in species composition between the two data groups, the fitted SSD curves were similar to each other when crustaceans were dominant. With regard to the most tolerant species, there was also difference between the two distributions. The most tolerant species in predicted dataset were *Carassius auratus*, *Pimephales promelas*, *Ameiurus melas*, *Sander vitreus,* and *Ictalurus punctatus* (five fishes), but the tolerant species in measure-based dataset were *M. anguillicaudatus* and *M. nipponense* (one fish and one invertebrate). This is in accordance with the results in the study conducted by Dyer et al. (2008), in which it was suggested that coldwater fish species were generally more sensitive to a wide range of chemicals with diverse modes of action.

4. *WQC deriving*

SSDs have been used to derive WQC. For example, the fifth percentile of a SSD (HC_5) is always adopted as a criterion to protect aquatic life (Jin et al. 2011). In the present study, the ICE-based HC_5 and the measured HC_5 for acute BaP toxicity data were 0.39 and 0.51 μg/L, respectively. The criterion maximum concentrations (CMC) for BaP were 0.195 and 0.255 μg/L for ICE-based and measure-based dataset, respectively, which are obtained by dividing HC_5 values by a factor of two. The measured data-based CMC value for BaP was in the same order of magnitude as the ICE-based HC_5, indicating the good predictability of ICE model with acceptable deviation from the actual measurement.

Dyer et al. (2008) found that the majority of ICE-based predictions were metrically similar to the experimental measurement. In terms of statistical analysis and effect assessment, the comparative results implied the potential for extrapolation using ICE. Therefore, using ICE models to provide reasonably accurate estimates for chemical toxicity and to derive protective criteria was encouraging, which could be used as a potential replacement for the current water quality derivation methods when sufficient empirical toxicity data are lacking. Despite the indefiniteness, ICE can significantly reduce the number of experimental animals and thus bring economical benefit to ecological risk assessment. We expect the application of ICE will be implemented in China in the near future.

3.4.2.4 Conclusions

This study comprehensively evaluated the applicability of ICE model for BaP by comparing SSD curves and HC5 values between the ICE-based and measure-based toxicity dataset. The results showed that there was no significant difference between the two SSDs and between HC_5 values derived from the two distributions, indicating ICE could be a potential option to obtain the predicted acute toxicity data for aromatic compounds. As the ICE model was verified as a valid approach for generating SSDs with limited toxicity data for BaP, it is recommended as a valuable alternative in ecological risk assessment and the derivation of WQC in China.

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Chapter 4
Study on the Mixture Toxicity of Organophosphorus (OP) Pesticides

Ya-hui Zhang and Zhengtao Liu

Abstract Organophosphorus (OP) pesticides have been largely produced and utilized in China and have been become ubiquitous contaminants in water. For single pesticides, their individual hazard has been well established. OP pesticides, however, frequently occur as mixtures, rarely as pure compounds, in most cases and have been detected as mixtures in the water bodies. Though the concentrations of single pesticides in the environment are less than their individual security control standards, the joint toxicities of the mixtures of organic pesticides have been attracted more and more attention. To examine the mixture toxicity with various compositions, the uniform design (UD) is employed to design the mixtures, which can explore the concentration variation with few experimental efforts. Two equivalent-effect concentration mixtures are also applied to build the whole concentration–response curves.. The mixture toxicities of OP pesticides are predicted by two models, concentration addition (CA) and independent action (IA). The results showed that the joint toxicity of all the OP mixtures could be well predicted by CA. However, the model of IA tends to underestimate the joint toxicity of all OP mixtures. The mixture toxicity based on SSD approach and the model of CA is applied to assess the mixture risk of five OP pesticides in Lake Taihu. The results show that the risk quotients of OP pesticide mixtures were greater than 1. The HC5-95 % values derived for five OP pesticides are much higher than the environmental quality standards for surface water set by China. It is suggested that the mixture toxicity of OP pesticides should be taken into account when setting the water quality criteria of OP pesticides.

Keywords Mixture toxicity · Uniform design · Pesticides · Concentration addition · Independent action · Ecological risk assessment

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Z. Yan and Z. Liu (eds.), *Toxic Pollutants in China*,
SpringerBriefs in Environmental Science, DOI 10.1007/978-94-017-9795-5_4

4.1 Introduction

Two reference models of concentration addition (CA) and independent action (IA) based on the toxicity of individual chemicals have been widely applied to evaluate the mixture toxicity.

The model of CA, also called Loewe additivity (Goldoni and Johansson 2007), is based on the concept that all chemicals in the mixtures share same or similar modes and mechanisms of action (Berenbaum 1985). The model represents that every chemical of the mixtures contributes to the overall toxicity and one chemical can be replaced by another one totally or partly when the overall toxicities of the two chemicals in the mixtures are identical. CA model is initially employed to predict the joint toxicity of two chemicals, which is defined as (Formula 4.1):

$$\frac{c_1}{\mathrm{EC}x_1} + \frac{c_2}{\mathrm{EC}x_2} = 1 \tag{4.1}$$

where c_1 and c_2 are individually the concentrations of the chemicals in mixtures; $\mathrm{EC}x_1$ and $\mathrm{EC}x_2$ are the effect concentrations of the chemicals reducing $x\%$ effect in mixtures.

CA model was applied to predict and assess the joint toxicity of mixtures with multiple chemicals subsequently (Berenbaum 1985). For the mixture with n chemicals, CA is defined as

$$\sum_{i=1}^{n} \frac{c_i}{\mathrm{EC}x_i} = 1 \tag{4.2}$$

where c_i is the concentration of the ith chemical when the mixture elicits $x\%$ effect; $\mathrm{EC}x_i$ is the effect concentrations of the ith chemical which result in $x\%$ effect when applied individually. $c_i / \mathrm{EC}x_i$ is defined as the toxic unit of ith chemical in the mixture.

$$\mathrm{EC}x_{\mathrm{mix}} = \left(\sum_{i=1}^{n} \frac{p_i}{\mathrm{EC}x_i} \right)^{-1} \tag{4.3}$$

where $\mathrm{EC}x_{\mathrm{mix}}$ is the effect concentration of the mixture that elicits $x\%$ overall effect, $\mathrm{EC}x_i$ is the same as (4.2) and p_i is the ratio of the concentration of the ith component (c_i) in the mixture to the total concentration of the mixture (c_{mix}).

The CA model had been employed to predict the combined effect of multiple mixtures in the aquatic environment including the narcotic action chemicals (Hsieh et al. 2006; Nirmalakhandan et al. 1997), the phenol derivatives (Altenburger et al. 2000), polycyclic aromatic hydrocarbons (PAHs) (Brian et al. 2005; Olmstead and LeBlanc 2005), and five estrogenic chemicals to the male fathead minnows (Brian et al. 2005). For pesticides, the joint toxicities of the mixtures of 10 quinolone antibiotics (cinoxacin, enoxacin, flumequine, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, oxolinic acid, pipemidic acid, piromidic

acid) to *Vibrio fischeri* (Backhaus et al. 2000b), 18 *s*-triazine herbicides to the unicellular green alga *Scenedesmus vacuolatus* (ametryn, atraton, atrazine, cyanazine, desmetryn, dimethametryn, dipropetryn, methoprotryne, prometon, prometryn, propazine, sebuthylazine, secbumeton, simazine, simetryn, terbumeton, terbuthylazine, terbutryn) (Faust et al. 2001) to *Scenedesmus vacuolatus*, eight sulfonylurea herbicides (bensulfuron-methyl, cinosulfuron, chlorsulfuron, metsulfuron-methyl, primisulfuron-methyl, prosulfuron, rimsulfuron, triasulfuron) (Junghans et al. 2003b) to *Scenedesmus vacuolatus*, eight chloroacetanilide herbicides (butachlor, pretilachlor, acetochlor, alachlor, propachlor, metazachlor, dimethachlor, metolachlor) (Junghans et al. 2003a) to *Scenedesmus vacuolatus* could be accurately predicted by the CA model. For phenylurea herbicides, the CA and IA models had resulted in practically identical predictions for the combined effect of 12 phenylurea herbicides (buturon, chlorbromuron, chlortoluron, diuron, fenuron, fluometuron, isoproturon, linuron, metobromuron, metoxuron, monolinuron, monuron) on *Scenedesmus vacuolatus* (Backhaus et al. 2004b).

The IA model, also called Bliss additivity (Bliss 1939), is based on the assumption of the chemicals in mixtures with different or dissimilar mechanisms of action (Bliss 1939). For the mixture with two chemicals, IA is expressed as the formula (4).

$$E(c_{\text{mix}}) = E(c_1) + E(c_2) - E(c_1)E(c_2) = 1 - (1 - E(c_1))(1 - E(c_2)) \quad (4.4)$$

where $E(c_{\text{mix}})$ is the total effect caused by the total concentration of the mixture (c_{mix}), and $E(c_1)$ and $E(c_2)$ are the effect elicited by the concentration of two chemicals individually.

$$E(c_{\text{mix}}) = 1 - \prod_{i=1}^{n} (1 - E(c_i)) \quad (4.5)$$

where $E(c_{\text{mix}})$ is the total effect caused by the total concentration of the mixture (c_{mix}), and $E(c_{\text{i}})$ is the effect elicited by the concentration of the *i*th chemical (c_{i}) when applied individually.

In comparison with the CA model, IA can be an instrument for predicting the combined effect of the substances with different modes of action on the algae communities (Backhaus et al. 2004a), the freshwater algae (Faust et al. 2003; Walter et al. 2002), and the photobacterium *V. fischeri* (Backhaus et al. 2000a).

Generally, CA and IA can provide accurate predictions for the joint toxicity of the compounds with "similar" and "dissimilar" mechanisms of action, respectively. However, it is difficult to obtain the knowledge on the mechanisms of action for most pesticides in the aquatic environment, and under the realistic environment scenarios, different kinds of pesticides are occured as mixtures in most cases. For the mixture of 25 pesticides (2,4-D, aclonifen, alachlor, atrazine, bromoxynil, carbofuran, carbosulfan, chloridazon, clopyralid, cycloxydim, ethofumesate, ioxynil, isofenphos, isoproturon, isoxaflutole, lenacil, linuron, 2-methyl-4-chloro-phenoxyacetic acid, metamitron, metolachlor, pendimethalin, terbuthylazine, thifensulfuron-methyl, triasulfuron, tribenuron-methyl) including 22 pesticides with seven kinds of dissimilar mechanisms of action and three

pesticides of carbofuran, clopyralid and carbosulfan with inexplicit mechanisms of action at the levels of predicted environmental effect (PEC) could be well predicted by the CA model rather not the IA model (Junghans et al. 2006). So the CA model is a reasonable assumption for hazard assessments of mixtures of chemicals with unspecified mechanisms of action and is recommended as a precautious model for the joint toxicity of multiple pesticides in the aquatic environment.

Organophosphorus (OP) pesticides are widely existed as mixtures in the water bodies. OPs inhibiting the acetylcholinesterase enzyme have raised concerns about their potential to threaten nontarget organisms in the aquatic environment (Burkepile et al. 2000; Li and Tan 2011). The predictability of the CA and IA models for the joint toxicity of OP pesticides to *Daphnia magna* is examined based on individual toxicity of single OPs. To analyze the joint toxicity of OP pesticides with various concentration compositions, the uniform design (UD) method and the fixed-effect concentration method are employed to assign the concentration ratios of five OPs.

4.2 Materials and Methods

4.2.1 Materials

Five OP pesticides, parathion (PAR), methyl parathion (MPA), malathion (MLA), dimethoate (DIM), and dichlorvos (DIC), were purchased from Chem Service Inc. (West Chester, PA, USA) with the highest available purity (>98 %). Pesticide stock solutions are prepared in dimethyl sulfoxide (HPLC-grade, Dikma, China) by ultrasonication, stored in the dark at 4 °C, and then diluted with Milli-Q water to a certain concentration for the test.

4.2.2 Test Organism

The test organism *D. magna* Straus is cultured at 20 ± 1 °C under 16-h light and 8-h dark photoperiod in dechlorinated tap water with pH 7.8 ± 0.2 and hardness 200 mg/L (as $CaCO_3$) and fed daily with the unicellular alga *Scenedesmus subspicatus* which cultured in medium M11 and renewed three times per week. Test neonates are applied in the test containers within 24 h and their sensitivity to $K_2Cr_2O_7$ was tested periodically to ensure that they were in a proper condition for the test.

4.2.3 Experimental Design and Data Analyses

Toxicity tests of single OP pesticides and their mixture were performed in 50-mL glass beakers. Ten organisms were allocated in each container with 40-mL test

medium covered with a glass plate. Ten concentrations of one pesticide or one mixture were prepared by geometric serial dilution with reconstituted water (OECD 2004). Each concentration was carried out with three replicates, one solvent control of 2 ‰ (v/v) DMSO and one blank control. The numbers of test animals which were immobile or showed adverse effects were recorded to calculate the effect concentration (EC_x).

The observed concentration–effect (inhibition ratio) data are fitted with two functions, Logit (4.6) and Weibull (4.7), and the best fitted function is used to estimate the EC_x. The best fitted function is determined by higher relationship coefficient (R) or lower root-mean-square error (RMSE) as a criterion. The formulas of Logit and Weibull are as follows:

$$\text{Logit: } E = 1/\left(1 + \exp\left(-\alpha - \beta \log_{10}(c)\right)\right) \tag{4.6}$$

$$\text{Weibull: } E = 1 - \exp\left(-\exp\left(\alpha + \beta \log_{10}(c)\right)\right) \tag{4.7}$$

where α and β are the parameters to be estimated; E is the effect ratio of a pesticide or a mixture to *D. magna*; c is the test concentration of single pesticide or the mixture resulting in an effect of E. The α and β estimated were then used to calculate various effect concentrations such as EC_{50} and EC_5 and their 95 % confidence intervals (CIs). All the fitted procedure of the concentration–effect curves and the prediction of the joint toxicity by CA and IA were performed with the program APTox® (Assessment and Prediction for the Toxicity of chemical mixtures) developed by professor Liu et al. (2012).

Mixture experiments were conducted according to the UD (Zhang et al. 2008, 2010). Application of UD to determine the combined effect of chemicals is efficient especially when the number of the chemicals (the factors in the UD) in mixtures and the involved concentrations of the chemicals (the levels of the factors) are large (Fang et al. 1993). The experimental effort of UD linearly increases when the factors and levels add in the mixtures in comparison with those of the orthogonal design and the factorial design exponentially increasing. In this study, the UD table of U_7 (7^6) with six factors (pesticides, superscript) and seven levels (concentrations) and seven experiments (mixtures, subscript) is selected to allocate the concentration compositions in the mixtures. The EC_xs of (EC_5, EC_{10}, EC_{20}, EC_{30}, EC_{40}, EC_{50}) of single OPs are used as the level factors, and six UD mixtures denoted as Mix-UD1, Mix-UD2, Mix-UD3, Mix-UD4, Mix-UD5, and Mix-UD6 are arranged according to the front six lines of U_7 (7^6) as shown in and the percent concentration ratios (p_i %) of various OP pesticides in the mixtures are listed in Table 4.1. Due to the EC_xs in the mixture arranged following the last line of the UD table are EC_{50}, the mixture of the seventh line is deleted.

Furthermore, the equivalent-effect concentration ratio (EECR) method is also employed to determine the combined effect of the pesticide mixtures. The EC_xs of (EC_5, EC_{10}, EC_{50}) of single OPs are used to arrange three EECR mixtures of Mix-EC5, Mix-EC10, Mix-EC50 and the concentration ratios of the fixed-effect concentration ratio method are also shown in Table 4.1.

Table 4.1 The percentage concentration ratios (p_i %) of five pesticides, the observed EC_{50mix}, and the predicted EC_{50mix} and MDR by CA and IA of the pesticide mixtures

Mixtures	p_i %					Observed EC_{50mix} (mol/L)	Predicted EC_{50mix}		MDR	
	DIC	MPA	PAR	DIM	MLA		CA	IA	CA	IA
Mix-UD1	0.05	0.77	0.06	98.65	0.48	1.27×10^{-6} [1.19×10^{-6}, 1.35×10^{-6}]	1.04×10^{-6}	3.55×10^{-6}	0.83	2.81
Mix-UD2	0.11	1.79	0.21	96.97	0.92	5.98×10^{-7} [5.61×10^{-7}, 6.37×10^{-7}]	4.98×10^{-7}	1.66×10^{-6}	0.83	2.77
Mix-UD3	0.05	0.85	0.04	98.67	0.38	1.26×10^{-6} [1.13×10^{-6}, 1.38×10^{-6}]	1.13×10^{-6}	3.87×10^{-6}	0.90	3.08
Mix-UD4	0.10	1.10	0.14	98.01	0.65	6.53×10^{-7} [5.35×10^{-7}, 8.19×10^{-7}]	6.72×10^{-7}	2.29×10^{-6}	1.03	3.50
Mix-UD5	0.05	0.65	0.03	98.97	0.30	1.00×10^{-6} [9.11×10^{-7}, 1.01×10^{-6}]	1.31×10^{-6}	4.37×10^{-6}	1.31	4.37
Mix-UD6	0.08	1.10	0.09	98.31	0.41	7.81×10^{-7} [7.34×10^{-7}, 8.35×10^{-7}]	8.4×10^{-7}	2.86×10^{-6}	1.07	3.65
Mix-EC5	0.09	1.20	0.07	98.06	0.59	9.18×10^{-7} [8.55×10^{-7}, 9.88×10^{-7}]	7.86×10^{-7}	2.32×10^{-6}	0.89	2.63
Mix-EC10	0.10	1.43	0.07	97.69	0.70	8.81×10^{-7} [8.26×10^{-7}, 9.46×10^{-7}]	6.92×10^{-7}	2.32×10^{-6}	0.75	2.52
Mix-EC50	0.06	0.76	0.08	98.73	0.37	1.30×10^{-6} [1.23×10^{-6}, 1.37×10^{-6}]	1.04×10^{-6}	3.61×10^{-6}	0.80	2.78

MDR is the model deviation ratio which is defined as the ratio of the combined-effect concentration predicted by CA or IA to the effect concentration fitted by the toxicity experiment data

4.3 Results and Discussion

4.3.1 Prediction for the Joint Toxicity of the Pesticide Mixtures by CA and IA

The toxicity scatter data of three parallels of five OP pesticides are fitted to the Logit (4.6) and Weibull (4.7) functions using APTox® software. The parameters (α, β) of the best fitted function are obtained according to the best fit criterion. The optimal function for DIC, PAR, and MPA is the Logit function and for MAL and DIM is the Weibull function. The value of EC_{50} of five OPs ranged from three orders of magnitude from DIM (5.12×10^{-6} mol/L) to DIC (2.88×10^{-9} mol/L), and the toxicity order is DIC > PAR > MPA > MAL > DIM using EC_{50} as the criterion.

Based on the concentration–response data of individual OPs, the CA and IA models are applied to predict the joint toxicity of the UD and EECR mixtures. The optimal fitted concentration–response curves of the pesticide mixtures according to the UD and EECR method are shown in Figs. 4.1 and 4.2, respectively. The observed scatter point (O) of the concentration–effect data, the fitted curves (blue solid line), the 95 % CIs (dashed lines), and the predicted curves by CA (red solid line) and IA (green solid line) of Mix-UD2 selected as an example are shown in the Fig. 4.1, and Mix-EC50 is an example in Fig. 4.2.

As compared to the predicted curves of IA of Mix-UD2 and Mix-EC50, the CA curves of the two mixtures are closer to the 95 % CIs from Figs. 4.1 and 4.2, which imply a CA feature, though the CA curves are located out of the 95 % CI. The model deviation ratio (MDR) is defined as the ratio of the combined-effect concentration predicted by CA or IA to the effect concentration fitted by the toxicity experiment data (Scholze et al. 2001), which is applied to quantify the predictive accuracy of the CA and IA models. For analyzing the predictive accuracy of the models, the median-effect concentration of a mixture, EC_{50mix}, is selected as the reference point. In this study, the MDR between the observed EC_{50mix} and the predicted by CA and IA is calculated, respectively, and listed in Table 4.1. The MDR values of all the pesticide mixtures by CA range from 0.75 for Mix-EC10 to 1.31 for Mix-UD5, and its mean value is 0.93. In particular, the MDR values for two UD mixtures of Mix-UD4 and Mix-UD6 are 1.03 and 1.07, respectively, which demonstrate the better predictive potential of the CA model. In contrast, the mean MDR value by IA is 3.3 and in the range of 2.52 for Mix-EC10 to 4.37 for

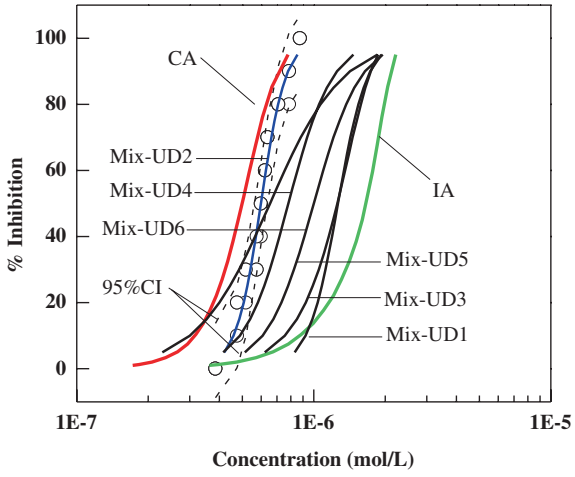

Fig. 4.1 The concentration–response relationships predicted for six pesticide mixtures by the UD method. Experimental data points of Mix-UD2, its fitted concentration–response curve, and the predicted curves by CA and IA as an example. Observed (O); predicted by CA (*red solid line*) and IA (*green solid line*); fitted (*blue solid line* for Mix-UD2 and *black solid line* for other UD mixtures); 95 % confidence intervals (*two dashed lines*)

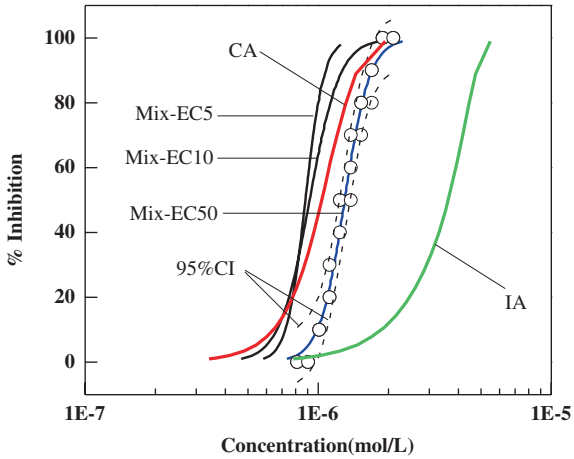

Fig. 4.2 The concentration–response relationships predicted for three pesticide mixtures by the equivalent-effect concentration ratio (EECR) method. Experimental data points of Mix-EC50, its fitted concentration–response curve, and the predicted curves by CA and IA as an example. Observed (O); predicted by CA (*red solid line*) and IA (*green solid line*); fitted (*blue solid line* for Mix-EC50 and *black solid line* for other EECR mixtures); 95 % confidence intervals (*two dashed lines*)

Mix-UD5 and the IA model tends to overestimate the joint toxicity of the OP pesticides in this study.

From the values of p_i in Table 4.1, the mixtures arranged by UD show more extensive concentration compositions than the EECR mixtures. The fitted concentration–effect curves of the EECR mixtures in Fig. 4.2 are somewhat parallel, and the values of the fitted parameters β which demonstrate the slope of the curves are in the same order of magnitude from 15.64 for Mix-EC50 to 25.38 for Mix-EC5, while the β values of the UD mixtures span from one order of magnitude which range from 6.49 for Mix-UD4 to 16.08 for Mix-UD1. The results show that the concentration ratios in the UD mixtures cover all possible concentration compositions of the components compared with the EECR mixtures.

The CA model tends to produce a higher predictive toxicity for the joint toxicity than IA (Backhaus et al. 2000a; Faust et al. 2003; Junghans et al. 2006). In this study, the similar conclusions appear which the CA model results in the bigger predictions for the OP mixture toxicity to *D. magna* than those by IA. However, the predicted toxicity by CA and IA for the pesticide mixtures gives different conditions for different test organisms. For example, the mixture toxicity of OP pesticides to the luminescent bacterium *V. qinghaiensis sp. Nov*-Q67 (Zhang et al. 2008) predicted by IA is higher than those by CA, especially for some mixtures, the IA predictions are almost equal to the CA results. It is difficult to explain from the view of mechanism of action of the pesticides, which might be interpreted mathematically (Drescher and Boedeker 1995; Goldoni and Johansson 2007). The slope of the concentration–effect curves of the mixture components may be

the most important factor to influence the quantitative relationships between the prediction of the CA and IA models. The joint toxicity of the mixtures including fifteen pesticides also provided a case study that the IA predictions are higher than or equal to the CA predictions (Zhang et al. 2010).

From Table 4.1 the predictive concentration–effect curves from CA are closer to the observed curves both of the UD and EECR mixtures even though the CA curves slightly deviate from the 95 % CI curves especially on the section of low effect, while IA tends to overestimate the observed toxicities for the OP mixtures. The predictive accuracy of the CA and IA models is qualitatively explained using the MDR as a criterion. In this study, the MDR values range from 0.75 to 1.31 by CA and from 2.52 to 4.37 by IA. The MDR values between the observed and the predicted by CA do not exceed a factor of 2, which is the maximum MDR value between the effect concentrations predicted by CA and the observed in most studies on the mixture toxicity of pesticides on aquatic organisms (Deneer 2000; Belden et al. 2007). It is demonstrated that CA is able to accurately predict the combined toxicity of OP pesticide mixtures to *D. magna*.

4.3.2 Application of the Mixture Toxicity of OP Pesticides in the Water Quality Criteria

OP pesticides are assumed to have the same target site to the organisms as the acetylcholinesterase enzyme (AChE) inhibitors. From the view of the mechanisms of action, the joint toxicity of OP pesticides to the aquatic organisms could be predicted by the CA model, which is based on the presumption of the chemicals in the mixture share the same or similar mode of action. The shape of the concentration–effect curves of the substances with the same mode of action is assumed to be equal for one organism (DeZwart and Posthuma 2005), which could be extrapolated to other environment organisms. The presumption is considered to be strictly and the maximum deviation of ± 10 % of the slope of the log-logistic distribution for the concentration–effect curves is also acceptable (DeZwart and Posthuma 2005).

An approach is developed to construct the species sensitivity distribution (SSD) to assess the mixtures of chemicals with similar mode of action (Chèvre et al. 2006). The SSD curves constructed by acute toxicity data of the chemicals in the mixture are parallel, i.e., the chemicals with a common slope of the SSD. Then, the relative potential is calculated by 50 % hazardous concentration (HC_{50}) compared to the chemical with most rich data which is designated as the reference substance. The lower confidence interval (95 %) of HC5 (HC5-95 %) of other chemicals in the mixture is calculated by dividing HC5-95 % of the reference substance by the relative potency value on the basis of the hypothesis that the SSD curves by chronic data are parallel to the acute SSD curves.

The triazine herbicides (atrazine, terbuthylazine, simazine, cyanazine, metribuzin, terbutryn) and phenylurea herbicides (linuron, chlortoluron, metoxuron, diuron, isoproturon) (Chèvre et al. 2006) were demonstrated by this approach as

well as six OP pesticides (chlorpyrifos-ethyl, diazinon, dichlorvos, dimethoate, parathion-ethyl, parathion-methyl) (Chèvre et al. 2008). It is considered that these pesticides have similar mode of action, of which the mixture toxicity of the triazine herbicide (Faust et al. 2001) and the phenylurea herbicides (Backhaus et al. 2004b) to *Scenedesmus vacuolatus* was tested and accurately predicted by the CA model. According to the above-mentioned method, the risk quotients of the components can be summed up and be equivalent to the overall risk of the mixture.

The HC5-95 % value derived by this approach is proposed as the water quality criterion. And the approach is considered by the author to be better and a consistent method when deriving the water quality criteria of the chemicals. The HC5-95 % values for triazine herbicides are two to three times higher than the quality standards set under the Water Framework Directive. For the phenylurea herbicides, the HC5-95 % values are equal to or differ only two factors of the standards. However, the results for the OP insecticides have a big difference. The HC5-95 % values for the OP pesticides are ten times bigger than the proposed standards, especially for chlorpyrifos-ethyl, the proposed water quality standard of 0.03 μg/L is about forty times greater than the HC5-95 % of 0.0008 μg/L.

OP pesticides are one kind of pollutants widely distributed in the water bodies in China (Na et al. 2006). The approach of mixture toxicity based on SSD is applied to assess the mixture risk of five OP pesticides (dichlorvos, parathion, methyl parathion, malathion, and dimethoate) in Lake Taihu (Lei et al. 2013). The results demonstrate that the risk quotients of OP pesticide mixtures (RQ_m) were greater than 1, of which the risk of dichlorvos is main distributor to the overall risk. OP pesticide mixtures in Lake Taihu had posed a certain ecological risk for the aquatic organisms. The HC5-95 % values are derived to be 0.0007 μg/L for dichlorvos, 0.0006 μg/L for parathion, 0.001 μg/L for methyl parathion, 0.007 μg/L for malathion, and 0.02 μg/L for dimethoate, which are much higher than the environmental quality standards for surface water set by China (MEP 2002). It is suggested that the mixture toxicity should be taken into account when setting the water quality criteria of OP pesticides.

4.4 Conclusions

The joint toxicities of five OP pesticides to the luminescent bacterium *D. magna* are determined in this study. From the model deviation ratio (MDR) values, the joint toxicity pesticide mixtures designed by the UD method and the EECR method are all close to the predictions by the CA model, particularly for two UDCR mixtures of UD-2 and UD-6, while the IA model tends to underestimate the toxicity of all OP mixtures. The mixture toxicity of OP pesticides can be accurately predicted by CA.

The mixture toxicity based on SSD approach is applied to assess the mixture risk of five OP pesticides in Lake Taihu, and the risk quotients of OP pesticide

mixtures were greater than 1. The HC5-95 % values derived for five OP pesticides are much higher than the environmental quality standards for surface water set by China, which is suggested that the mixture toxicity of OP pesticides should be taken into account when setting the water quality criteria of OP pesticides.

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