RESEARCH ARTICLES

Comparison of Four Species Sensitivity Distribution Methods to Calculate Predicted No Effect Concentrations for Bisphenol A

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ABSTRACT

Bisphenol A (BPA, CAS RN 80-05-7) is a high production volume chemical used as an intermediate in the production of polycarbonate plastic and epoxy resins. During its manufacture and use, some emissions to surface waters are anticipated. Chronic predicted no effect concentrations (PNECs) for aquatic systems are used to support the assessment of potential risks to aquatic organisms in receiving waters. PNECs for a compound are considered protective of populations, communities, and ecosystems. Traditionally, PNECs are derived by taking the lowest no-observed effect concentration (NOEC) from a set of toxicity studies and dividing by an assessment factor (e.g., 10 to 1000). This traditional approach is appropriate for substances with few data, but may not be necessary for substances with many valid studies. For well-studied substances, statistical approaches (i.e., development of Species Sensitivity Distribution or SSD methods) can be used to calculate a PNEC that makes use of the full distribution of available NOEC values. Bisphenol A has an extensive set of aquatic toxicity studies covering diverse taxa including algae, hydra, rotifers, mollusks, crustaceans (both benthic and pelagic), insects, annelids, fish, and amphibians. The full chronic data set was used to calculate PNEC values using four SSD methods: (1) the Hazard Concentration (HC5) approach developed by The Netherlands National Institute of Public Health and the Environment (RIVM), (2) the U.S. Environmental Protection Agency's water quality criteria procedure, (3) SigmaPlot (Systat 2000) commercial software that calculates percentile values, and (4) a distributional method consistent with that used by Environment Canada. Using these approaches, PNEC values for BPA range from 11 to 71 μg/L. Literature studies suggest that application of

an additional assessment factor is unwarranted if an SSD-based PNEC is based on chronic data. SSD-derived PNEC values and the traditionally derived PNEC value of 1.6 μg/L are then compared to concentrations of BPA that have been measured in North American and European surface waters. Adverse risks to aquatic organisms are not anticipated from measured concentrations of BPA in North American and European surface waters.

Key Words: no observed effect concentration (NOEC), Final Chronic Value (FCV), hazard concentration (HC5), hazard quotient (HQ), PNEC.

INTRODUCTION

Bisphenol A (BPA, also 4,4′-isopropylidine diphenol, CAS Registry No. 80-05-7) is primarily used as an intermediate in the production of polycarbonate plastics and epoxy resins. Lower volume applications include use as additives to polyvinyl chloride (PVC) and other plastics, and other miscellaneous monomeric applications. Small amounts of BPA enter the environment from production and processing facilities, sewage treatment plants, and following disposal of PVC materials. Cousins *et al.* (2002) identified the aqueous compartment as one of the main compartments into which BPA may be found following release into the environment.

To conduct a risk assessment for a chemical entering the environment, estimates of both the hazard (available toxicity data for potentially exposed organisms) and the estimated or measured exposure concentrations are required. For the purposes of this article, the general term Predicted No Effect Concentration (PNEC) was adopted to describe the estimated threshold concentration below which communities of organisms can be chronically exposed without adverse consequences. PNEC values are frequently used to describe the hazard component of ecological risk assessments.

A simple deterministic approach for toxicity assessment relies on single, usually worst case values and is widely used in North America and Europe, particularly if data are limited (USEPA 1984; CEPA 1997; EC 2003). A criticism of this approach for PNEC determination is that an assessment factor (AF) is applied to the most sensitive acute LC50 or chronic NOEC value and the full dataset is not used to characterize potential toxicity. Assessment factors are a precautionary means of addressing perceived uncertainties in a set of toxicity studies. This method therefore produces a single criterion concentration that is assumed to be cautious and protective in terms of adverse environmental effects but which actually may best serve as a screening tool because it provides little information on the likelihood of impact and provides no expression of its uncertainty. In contrast, probabilistic modeling approaches evaluate and compare complete distributions (*i.e.*, the distribution of chemical toxicity to a range of species can be compared to the distribution of exposure concentrations). The result is a distribution of potential outcomes that can be used to examine the probability or likelihood of certain results occurring, versus a single result with deterministic techniques. For any given compound, the distribution of toxicity values for a set of species is often referred to as a species sensitivity distribution (SSD).

It has long been recognized that species of organisms within a community have differing sensitivities to a toxic substance. A distribution of species responses would be

expected to occur rather than a single response value. In addition, SSDs that include a number of studies across a range of species increases the confidence in SSD-derived PNECs. For these reasons, SSDs are increasingly recommended to either complement or replace the use of deterministic criteria in the risk assessment of chemicals in the United States and in Europe (Posthuma *et al.* 2002). For example, the SSD concept has been formally incorporated into ecological risk assessment frameworks at the USEPA (ECOFRAM 1999; http://www.epa.gov/oppefed1/ecorisk/) and within the Organization for Economic Cooperation and Development (OECD 1992). The USEPA has also used the SSD technique to derive water quality criteria for several decades (USEPA 1985).

The assumed goal of a traditionally derived PNEC calculated using the single lowest acute or chronic toxicity value of any taxa and an AF is an attempt to protect all individuals of all species. As Suter (1993) notes, the reproducing population is the smallest ecological unit that can be meaningfully protected. Further, populations require a community of organisms of which the population is a part. In contrast to the traditional AF approach, SSD-derived PNECs reflect the concentration at which a significant risk to the community of species is reached. It is acknowledged that the selection of the appropriate level of protection is a risk management or policy decision, not the decision of the risk assessor. The most common policy-based protection threshold is 5%, that is, effects on more than 5% of species are unacceptable (Postuma *et al.* 2002). Thus, SSD-based PNECs will be derived at the 5% threshold.

The focus of this article is on the hazard definition of BPA in the aquatic environment, in particular the development of PNEC values needed for risk assessment. To give results that are as comparable as possible, the lower-bound 5th percentile concentrations were taken from all SSD approaches, even though some of the SSD models can generate toxicity values of other percentiles (*e.g.*, the 10th percentile). PNEC values for BPA from four SSD methods will be compared and contrasted with each other and with the traditional assessment factor (AF) approach. To demonstrate the use of SSD-based PNEC values, all PNEC values will be compared to measured aquatic BPA concentrations in surface waters in the United States and Europe.

CHRONIC AQUATIC TOXICITY OF BISPHENOL A

Study Quality Assessment

As an initial step, the available chronic ecotoxicity studies were critically reviewed for suitability for use in risk assessment following the criteria and procedures outlined in the European Union (EU) Technical Guidance Document (EC 2003). These procedures are presented and refined in Staples *et al.* (2002). Only studies designated as "valid without restriction" are used in this aquatic hazard assessment. Studies designated as "use with care" or "not valid" are not used. Many of the studies used in this paper were previously evaluated by Staples *et al.* (2002) in a weight-of-evidence analysis of the aquatic toxicity of BPA. Studies that were conducted after the publication of Staples *et al.* (2002) are discussed later. Specific note is made of studies for which there is some controversy as to their acceptability and where additional work

has been performed to obtain more robust data. All valid studies for BPA that are used to calculate PNEC values are presented in Table 1.

Recent Chronic Aquatic Studies with BPA

Fish

As presented in Staples *et al.* (2002), two valid partial life-cycle studies report the effects of BPA on medaka fish. One study measured growth and sexual development over 100 days using adult fish (Metcalfe *et al.* 2001), reporting a NOEC of 120 μg/L. The second study is a 60-day partial life cycle study that measured survival, growth and development with a NOEC of 355 μg/L (Yokota *et al.* 2000). Subsequently, the Japanese government (Japan MOE 2004) reported the results of a multigeneration study with medaka. Although all experimental details of this study are not yet widely available, it is known that the BPA results are part of a series of studies sponsored by the Japanese government that are being used to inform their regulatory management decisions on endocrine modulation-related issues. In this latter study, newly hatched F0 eggs were exposed to BPA until they reached maturity. F0 adults were bred, F1 eggs were hatched, and hatchlings were exposed to BPA until they reached maturity. Endpoints related to survival, growth and development, and reproduction are reported. An overall NOEC of 247 μg/L was reported that is based on F0 survival as the most sensitive endpoint. NOEC for growth, development, and reproduction were similar or less sensitive, and range from 247 to 1179 μg/L. For the PNEC calculations, the NOEC of 247 μg/L based on survival of the F0 generation was taken as the most sensitive endpoint from the longest duration valid medaka study available (Japan MOE 2004).

Caunter and colleagues (1998, 2000) conducted two studies with BPA using fathead minnows. The studies were conducted using flow-through exposures to BPA following standard guidelines and conducted in accordance with Good Laboratory Practices (GLP). An NOEC of 640 μg/L based on survival and growth was determined in a 35-day early life stage test. An NOEC of 16 μg/L based on hatchability of F2 eggs was determined in a multigeneration test (overall 431 days) with fathead minnows. Sohoni *et al.* (2001) reported the results from the F1 exposure through day 164 and includes observations of an apparent effect on spermatogenesis in male fish at concentrations lower than effects reported for mortality, growth, and development, and reproduction endpoints. A third-party pathology review found the gametogenic cell counts reported by Sohoni *et al.* (2001) to be questionable (Experimental Pathology Laboratories, Inc 2005). The study reported by Sohoni *et al.* (2001) was designed to examine effects on growth, reproduction, and hatchability, but was not optimally designed for quantitative examination of gonadal cell types as a supplemental endpoint. In addition, a number of critical inadequacies related to the histological analyses were identified, specifically with respect to the number of fish sampled from each exposure level, methods of tissue collection, procedures used in preparation of slides for histopathological analysis, and the procedures used for quantitative analysis including the identification and number of cells counted in each sample.

To clarify the potential for effects from BPA on spermatogenesis in fathead minnows, a follow-up study sponsored by the Polycarbonate/BPA Global Group was

Table 1. Studies* used for calculation of PNECs for bisphenol A using four different Species Sensitivity Distribution methods. All concentrations in μg/L.

Family	Species	Endpoint: NOEC (LOEC)	Reference
Fam. 1 Salmonids	Rainbow trout *Oncorhynchus mykiss*	28-day, juvenile growth test Growth: 3640 (11,000)	Bayer (1999)
Fam. 2 Other fish family	Medaka fish *Oryzias latipes*	Multi-generation** Mortality: 247 (1179) Growth: 247 (1179) Reproduction: 1179	Japan Ministry of Environment (2004)
Fam. 2 Other fish family	Medaka fish *Oryzias latipes*	60-d** Mortality: 1820 Growth: 355 (1820) Reproduction: 355 (1820)	Yokota *et al.* (2000)
Fam. 2 Other fish family	Medaka fish *Oryzias latipes*	100-d post-hatch** Growth: 120 Reproduction: 120	Metcalfe *et al.* (2001)
Fam. 2 Other fish family	Fathead minnow *Pimephales promelas*	35-d ELS assay Mortality: 640 (1280) Growth: 640 (1280)	Caunter *et al.* (1998)
Fam. 2 Other fish family	Fathead minnow *Pimephales promelas*	Multi-generation Mortality: 640 Growth: 640 Reproduction: 16	Caunter *et al.* (2000)
Fam. 2 Other fish family	Fathead minnow *Pimephales promelas*	164-day partial life cycle Mortality: 160 (640) (males only) Growth: 640 (males and females) Reproduction: 640 (males and females)	Rhodes *et al.* (2007)
Fam. 2 Other fish family	Guppy *Poecilia reticulata*	30-d Mortality: 500 (5000)	Kinnberg and Toft (2003)
Fam. 3 Third phyla in Chordates	African clawed frog *Xenopus laevis*	90-d Mortality: 500 Growth: 500 Sexual differentiation: 500	Pickford *et al.* (2000) Pickford *et al.* (2003)
Fam. 4 Planktonic crustacean	Water flea *Daphnia magna*	21-d Mortality: 3160 Reproduction: 3160	Caspers (1998)
Fam. 5 Benthic crustacean	Amphipod *Hyalella azteca*	Survival, growth, reproduction Mortality: 1000 Growth: 1000 Reproduction: 490 (1000)	Carafella (2005)
Fam. 6 Aquatic insect	Midge *Chironomus riparius*	Growth and development Moulting delay: 100 (1000) Growth: 100 (1000)	Watts *et al.* (2003)
Fam. 7 Family from non-Chordata/ Arthropoda	Rotifers *Brachionus calyciflorus*	Life cycle, intrinsic rate of population increase Population growth: 1800 (3600)	Sayers (2006)

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Table 1. Studies* used for calculation of PNECs for bisphenol A using four different Species Sensitivity Distribution methods. All concentrations in μg/L. *(Continued)*

Family	Species	Endpoint: NOEC (LOEC)	Reference
Fam. 8 Other insect or other phylum	Prosobranch mollusks *Marisa cornuarietis*	84-d adult survival and reproduction; hatchability and 90-d juvenile growth trials (25°C) Mortality: 640 Reproduction: 640 Hatching and juvenile growth: 640	Forbes *et al.* (2007b)
Fam. 8 Other insect or other phylum	Prosobranch mollusks *Marisa cornuarietis*	181-d adult survival and reproduction; hatchability and 90-d juvenile growth trials (25°C) Mortality: 640 Reproduction: 640 Hatchability: 640 Juvenile growth: 25 (640)	Warbritton *et al.* (2007a)
Fam. 8 Other insect or other phylum	Hydra *Hydra vulgaris* Cnidarian	Mortality, growth and development Mortality: 42 (460) Growth: 460	Pascoe *et al.* (2002)
Fam. 8 Other insect or other phylum	Poriferan Sponge *Heteromyenia* sp.	9-d Growth: 1600 (16,000)	Hill *et al.* (2002)
Fam. 9 Algae	Green algae *Selenastrum capricornutum*	4-d EC10 Growth: 1360	Alexander (1988)
Fam. 10 Aquatic macro-phyte	Duckweed *Lemna gibba*	7-d Growth: 7800 (20,000)	Putt (2003)

*All studies used to derive the PNECs have been screened for study quality according to the procedures described in Staples *et al.* (2002). Concentrations presented here are either nominal or mean measured as reported in each study.
**The longest duration medaka study was used for this species (NOEC = 247 μg/L).

conducted (Rhodes 2007). Preliminary work developed and optimized the histopathology techniques (Wolf *et al.* 2004). A range finding test was performed to develop the optimal design for the definitive test, including a power analysis to determine the appropriate number of replicates and appropriate statistical methods (Caunter *et al.* 2006). The definitive study followed the general design of that reported by Sohoni *et al.* (2001), while using a four-fold higher replication per concentration to improve statistical power. Results for survival, growth, and reproduction are similar to those reported by Sohoni *et al.* (2001) with NOEC of 160 μg/L and higher being determined, depending on the specific endpoint. In contrast, the results of the gonadal histopathology analysis were markedly different than reported

by Sohoni *et al.* (2001). Delay in gametogenesis was found at 640 and 160 μg/L, but not at 64 μg/L BPA for males. Thus for fathead minnows, a NOEC of 16 μg/L will be used for the PNEC calculations, based on the F2 hatchability endpoint originally observed in the multigeneration study as reported by Caunter *et al.* (2000).

Two additional fish studies with BPA have been reported, but were considered not valid. Bowmer and Gimeno (2001) measured the effects from BPA exposure on gonadal development of common carp over a 49-day test. Segner *et al.* (2003) reported an EC50 for reproductive success in a life-cycle test with zebrafish. Both of these studies suffer from incomplete description of experimental design and results, and were not considered fully valid for use in deriving an SSD-based PNEC.

Kinnberg and Toft (2003) examined the effects on survival, male reproductive behavior, secondary sex characteristics, sperm count, and gonadosomatic indices in guppy fish. After exposure to BPA for 30 days at concentrations up to 5,000 μg/L, survival was affected at the highest concentration with a NOEC of 500 μg/L. In addition, some effects on testes structure were reported but apparently no other developmental or reproductive effects were found at any exposure concentration. Based on these results a NOEC of 500 μg/L for survival will be used for PNEC calculations.

Amphibians

Kloas and colleagues reported that BPA has an effect on the sex ratio of the African clawed frog, *Xenopus laevis* (Kloas *et al.* 1999; Levy *et al.* 2004). Upon critical review, the studies were not considered valid for deriving a PNEC based on the experimental design, the lack of measured exposure concentrations, and the statistical approaches used in evaluating the results.

In the work of Kloas *et al.* (1999), juvenile frogs were exposed to two different levels of BPA under static renewal conditions. The fact that exposure concentrations were not measured in such systems is problematic, given the ready biodegradability of BPA (West *et al.* 2001). Further, no documentation was provided of the test conditions (temperature, water quality), which may also have an impact on the sex ratio. The study used one replicate test vessel per treatment and therefore it is impossible to determine whether the potential effect is a sampling or statistical artifact.

The statistical analyses used by the authors appear to be inappropriate. Kloas *et al.* (1999) analyzed the statistical significance of the data using the Kruskal-Wallis test combined with the Mann-Whitney test. Independent analysis of the data using the Kruskal-Wallis test failed to show significant differences between treatments. Normally the Mann-Whitney test is used to determine which treated group is statistically different from the control after the Kruskal-Wallis test indicates significant differences between treatments. Because the latter did not reveal such differences, it is unclear how the Mann-Whitney test was applied given that there was only one replicate test vessel. Lastly, the authors compared the sex ratios to a solvent control. However, the more appropriate comparison is to the expected sex ratio (50:50) as long as the controls do not significantly vary from the expected.

Levy *et al.* (2004) attempted to further refine the experimental approaches to assessing changes in sex ratio with *X. laevis*, but the work suffers from many of

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008

461

the same deficiencies of experimental design (although test concentrations were measured) and application of statistical methodologies. Levy *et al.* (2004) did use two replicate test vessels per test concentration; however, the authors then effectively reduced this to a single replicate by pooling the data for the two replicates. In so doing the authors removed any potential for estimation of the experimental error associated with the sex ratio endpoint. The findings of a second experiment described by Levy *et al.* (2004) using three BPA concentrations further highlights the uncertainty in the validity of the results. The effects on sex ratio were only noted at the intermediate dose, indicating a lack of dose response. Furthermore, a histological examination of the gonads failed to indicate intersex conditions at any of the test concentrations, which would be expected if there were significant shifts in sex ratio occurring. Thus, due to methodological deficiencies, the *X. laevis* results reported by Kloas *et al.* (1999) and Levy *et al.* (2004) were not used to calculate SSD-based PNEC values.

Subsequent work by Pickford *et al.* (2000, 2003) with *X. laevis* was conducted in accordance with GLP guidelines. BPA concentrations were carefully maintained using a flow-through system and were analytically verified. At the end of the 90-day test, no skewing of sex ratios was found even at 500 μg/L, the highest BPA concentration tested, and a 50/50 sex ratio was reported for the controls and all test concentrations. The findings of Pickford *et al.* (2000, 2003) were considered valid for use in the PNEC calculations.

Invertebrates

Several chronic aquatic toxicity studies with invertebrates have been recently sponsored by the Polycarbonate/BPA Global Group. Each study was conducted according to standard guidelines in accordance with GLP. Carafella (2006) reports the results of a 42-day study conducted with the amphipod, *Hyalella azteca*. The study reports NOEC for survival and growth endpoints of 1000 μg/L and a NOEC for reproduction of 490 μg/L. Sayers (2006) reports the results of a life cycle test with the rotifer, *Brachionus calyciflorus*. A NOEC of 1800 μg/L was reported, based on the measurement of the intrinsic rate of population increase.

Several other authors have published findings of the effects of BPA on invertebrate species. Pascoe *et al.* (2002) reports the effects of BPA on the survival, growth, and development of the Hydra, *Hydra vulgaris* Cnidarian. The lowest NOEC was 42 μg/L based on survival. In contrast, the work reported by Fukuhori *et al.* (2005) reports inconsistent growth with both enhanced and reduced growth parameters noted during their 35-day test with the *Hydra oligactis* and was not used in the PNEC derivation. Hill *et al.* (2002) reports the results of 9-day growth experiments on poriferan sponges *Heteromyenia* sp. The authors report a NOEC of 1600 μg/L based on growth. Watts *et al.* (2003) examined the effects of BPA on larval molting and mouthpart structure in life-cycle renewal test using the insect *C. riparius*. Time to first molt and mean wet weights of first instar larvae were only affected at 1000 μg/L, but not 100 μg/L. The studies of Pascoe *et al.* (2002), Hill *et al.* (2002), and Watts *et al.* (2003) were scored as valid and will be used for the PNEC calculations.

Aquatic plants

An additional study sponsored by the Polycarbonate/BPA Global Group examined the effects of BPA on the aquatic duckweed plant *Lemna gibba* and was reported by Putt (2003). Duckweed is not very sensitive to BPA with a NOEC of 7800 μg/L based on frond growth.

Mollusks

Recent publications by Oehlmann and colleagues have claimed that BPA strongly stimulates female egg production and increases mortality in the prosobranch snail *Marisa cornuarietis* (Oehlmann *et al.* 2000; Schulte-Oehlmann *et al.* 2001; Oehlmann *et al.* 2006). Closer examination of these publications has identified a number of significant deficiencies in the experimental design, statistical analysis, and interpretation of the results that render them invalid for the PNEC calculations.

Shortcomings in the studies conducted by Oehlmann and colleagues include lack of knowledge of the influence of husbandry conditions on the growth, development, and reproductive performance of *Marisa*, variability of life history traits under laboratory conditions, lack of replication in most of the experiments, and use of inappropriate statistical methods that invalidates the claims of statistical significance (Dietrich *et al.* 2006; Forbes *et al.* 2007 a,b). The influence of husbandry conditions and knowledge of life history traits in control populations of *Marisa* are critical to properly designing toxicity tests with sufficient statistical power to detect the effects of treatments of BPA.

Aufderheide *et al.* (2006), Selck *et al.* (2006), and Forbes *et al.* (2007a,b) reported the results of a series of studies with *Marisa* that were designed to understand the influence of variables such as water quality and food source, while maximizing the ability to discern differences in egg production between controls and treatments. Forbes *et al.* (2007b) used a nested statistical design that enabled the identification of individual snails, which allowed the estimation of inter-snail and inter-vessel variability. No statistically significant effects on survival or reproductive output of adult *Marisa* were observed during a 12-week test using a range of BPA concentrations from 0.1 to 640 μg/L. Likewise, hatching success and juvenile growth were not significantly affected by any BPA treatment.

Statistical analysis showed that reproductive output was the most variable trait (mean within-replicate coefficient of variation was 45%), followed by juvenile growth (CV 28%), percent hatch (CV 18%), and time to egg hatching (CV 6 to 9%). The authors concluded that toxicity tests having sufficient power to detect differences in egg production should, therefore, have sufficient power to detect effects on other endpoints. Power analyses showed that a relatively high degree of replication is needed to detect effects on reproduction of *Marisa*. The authors calculated that an experiment using four treatments of BPA and a control that is capable of detecting a 26% treatment effect on egg production with 80% power would require 6 replicates per treatment and 12 replicates per control. This robust design, therefore, requires 36 tanks and 360 snails.

Warbritton (2007a) conducted a definitive study with *Marisa* that was designed based on the recommendations of Forbes *et al.* (2007b). BPA treatments ranged from 0.1 to 640 μg/L BPA. No treatment related effects on survival, egg production, or

hatching were found. Juvenile growth was marginally, but significantly reduced in females at 640, but not at 25 μg/L. Warbritton (2007b) also conducted a 3-month experiment with one treatment (25 μg/L) at 22°C (all other experiments were conducted at 25°C). This experiment was conducted to ascertain possible differences in fecundity at a lower temperature. No statistically significant effects on egg production related to BPA exposure were reported at 22°C.

Overall, the Forbes *et al.* (2007b) and Warbritton (2007a) studies used experimental designs of sufficient robustness to discern possible reproductive effects caused by exposure to BPA and used appropriate statistical analyses to properly interpret the data. A *Marisa* species NOEC of 25 μg/L was used in the development of SSD-based PNECs.

Summary of the Chronic Aquatic Database for BPA

The U.S. Environmental Protection Agency (USEPA's) approach to developing water quality criteria (USEPA 1985) requires that the toxicity database include, at a minimum, eight unique families of aquatic organisms. The required taxa include two different families of fishes, a non-fish chordate family, planktonic and benthic crustaceans, insects, and other non-chordate or arthropod taxa. In addition, green algae and aquatic plants may be included. The EU technical guidance document (TGD) has tentatively adopted this requirement for diversity of taxa to calculate an SSD-based PNEC (EC 2003).

For BPA, 19 valid chronic aquatic toxicity studies covering 14 different species of organisms were identified in this study (see Table 1). Representing the animal kingdom are four species of fish, one amphibian, two crustaceans, one insect, one mollusk, as well as rotifers, hydra, and sponges. Plants are represented by green algae and an aquatic macrophyte. Overall the dataset covers ten distinct families of aquatic organisms, which meets the requirements of both the USEPA and EU TGD guidance (USEPA 1985; EC 2003).

Each study reports one or more endpoints related to survival, growth and development, and/or reproduction. The lowest NOEC for any endpoint that was determined within each study was identified. If two or more studies for the same species are available, the longest duration study was used to represent the species. This was true for the medaka and fathead minnow species for which partial and full life-cycle studies exist. The observed toxicity of BPA to aquatic organisms ranged from the lowest NOEC value of 16 μg/L from a multi-generation test with fathead minnows to the highest NOEC value of 7800 μg/L for the aquatic macrophyte, duckweed.

DEVELOPMENT OF PNEC VALUES

Four separate SSD methods were examined with BPA, along with the traditional deterministic AF approach. The SSD approaches used were:

1. ETx 2.0 was obtained from The Netherlands National Institute of Public Health and the Environment (RIVM) (RIVM 2004). This program is used to calculate a lower 5th percentile concentration from the distribution of chronic NOEC values

464

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008

(*i.e.*, an HC5 value). The SSD assumes a normal distribution of species sensitivity that is log transformed to perform the calculations. The assumption that the toxicity dataset fits a log-normal distribution is tested using the Anderson-Darling test for normality.

2. A Final Chronic Value (FCV) was calculated using the USEPA method for calculating National Ambient Water Quality Criteria (USEPA 1985, 1995). Using only the four most sensitive toxicity values, the FCV calculation assumes a log triangle distribution of species sensitivity. The FCV is the lower 5th percentile from the SSD.

3. Sigma Plot software (Systat 2000) was used to calculate 5th percentile values. SigmaPlot is a commercially available software package. A log-normal distribution of species sensitivity is assumed.

4. A distribution approach consistent with that used by Environment Canada in their conduct of chemical-specific risk assessments was used (CEPA 1997). All data were simply ranked and Microsoft® Office Excel (2003) software was used to calculate the lower 5th percentile value, assuming a log normal distribution.

RESULTS

Calculation of SSD-Derived PNEC Values

HC5 derived from ETx 2.0 (RIVM 2004)

An HC5 value was calculated for BPA that represents the lower-bound 5th percentile of NOEC values of the distribution of species-specific toxicity results (see Table 2 and Figure 1). The HC5 value calculated for BPA was 18 μg/L.
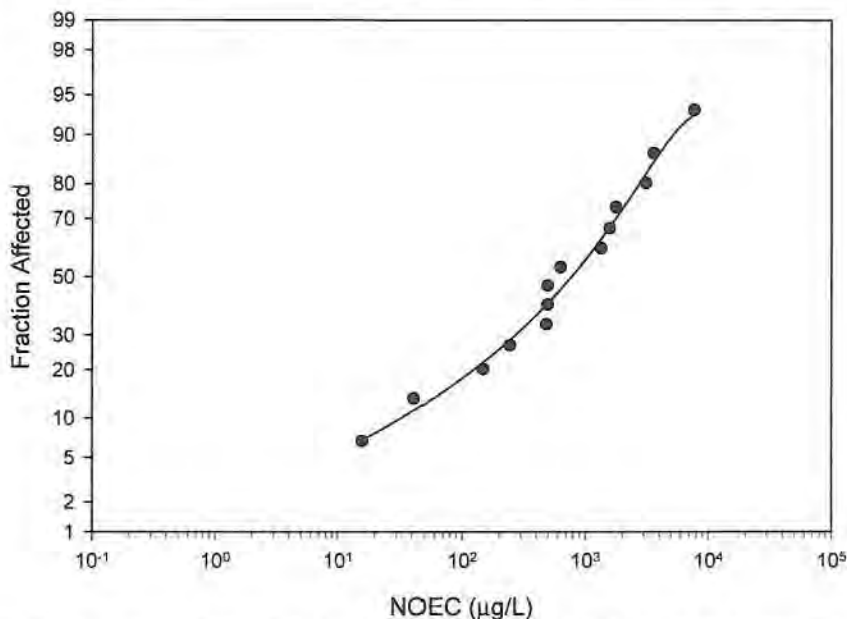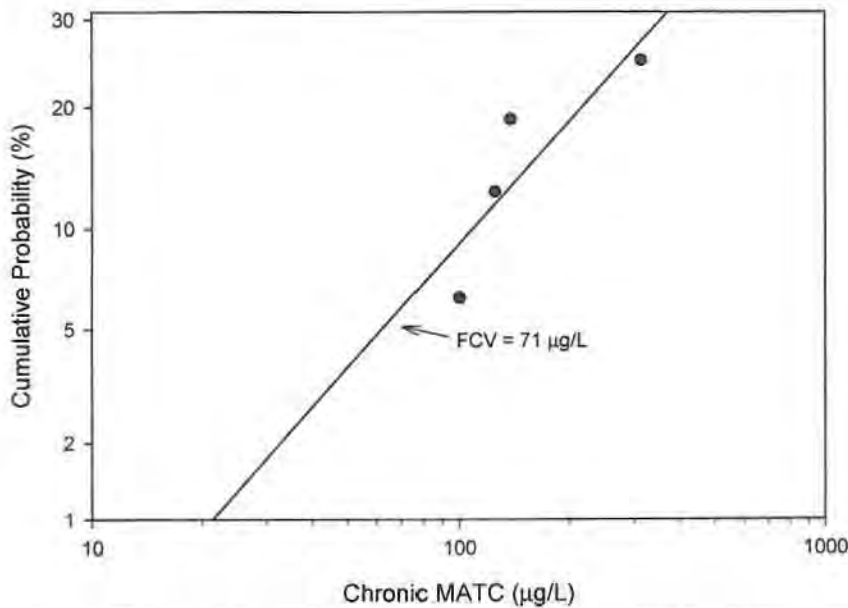

Figure 1. PNEC for bisphenol A calculated using the RIVM ETx software. PNEC = 18 μg/L.

Table 2. PNEC values for BPA derived using either SSD or traditional assessment factor approaches.

| Method	ETx HC5	USEPA FCV	SigmaPlot 5th percentile	Distributional lower 5th percentile	Range of SSD-based PNEC values	Traditional AF approach
PNEC (μg/L)	18	71	11	22	11 to 71	1.6

FCV calculated using USEPA procedures (USEPA 1985)

A Final Chronic Value (FCV) was calculated for BPA using the four most sensitive toxicity results (see Table 2 and Figure 2). FCVs are calculated using the geometric mean of the NOEC and LOEC for each study result yielding maximum-allowed toxicant concentrations (MATCs). The rationale for use of MATCs is that the MATC gives a more likely estimate of the true NOEC, since treatment concentrations are rather arbitrarily chosen (USEPA 1985). In addition, many compounds exhibit threshold levels of toxicity and the MATC may be viewed as the lowest concentration of a chemical that might produce a harmful effect on an organism. If multiple tests of similar duration are available for a species, a species MATC may be calculated. For BPA, an FCV of 71 μg/L was obtained.

Figure 2. PNEC for bisphenol A calculated using the USEPA Final Chronic Value (FCV) approach (only the lowest four toxicity values are used). MATC values are the geometric means of the measured NOECs and LOECs. PNEC = 71 μg/L.

466

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008
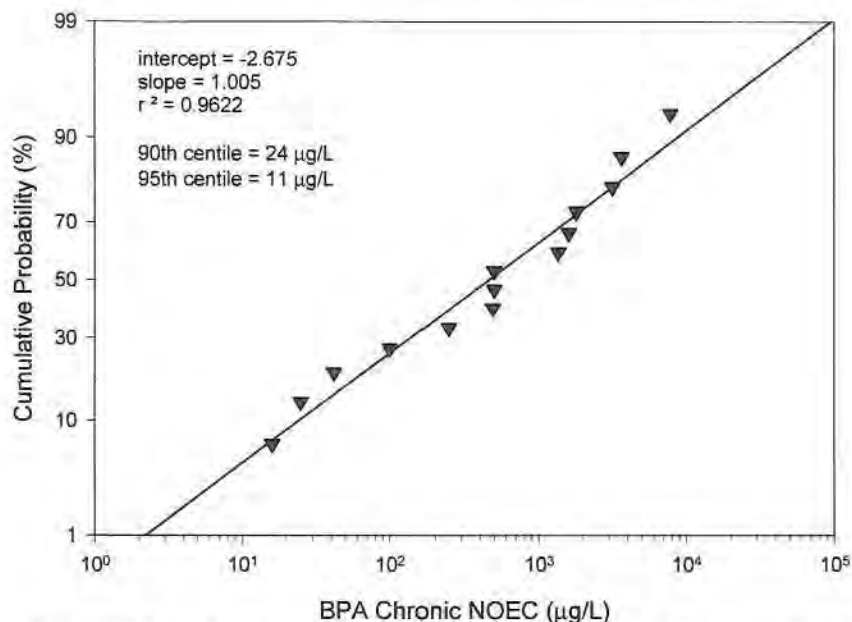
Figure 3. PNEC for bisphenol A calculated using the SigmaPlot (SYSTAT 2000) HC5 approach. PNEC = 11 μg/L.

### Lower-bound 5th percentile value from SigmaPlot (Systat 2000)

SigmaPlot yields lower bound 5th percentile toxicity values (see Table 2 and Figure 3). The SSD derived using SigmaPlot uses species-specific NOEC values, although either LOEC values alone or the MATC values could also be used. For BPA, a lower-bound 5th percentile of 11 μg/L was calculated.

### Lower-bound 5th percentile value from a distribution plot

Using the distributional approach of Environment Canada (CEPA 1997), the lower-bound 5th percentile concentration was calculated. Species NOEC values were used for the SSD plot (see Table 2 and Figure 4). For BPA, a lower-bound 5th percentile concentration of 22 μg/L was determined.

PNEC calculated using the AF approach

The SSD-derived PNEC values can be compared to a PNEC calculated using the traditional AF approach. The species-specific NOEC values were ranked and plotted from low to high concentration (see Table 2 and Figure 5). The lowest NOEC is 16 μg/L from the fathead minnow multigeneration test and this NOEC was divided by an AF of 10. Because three or more NOEC values for different taxa are available (Table 1), the lowest AF of 10 is justified (USEPA 1984; CEPA 1997; EC 2003). The resulting traditionally derived PNEC based on the use of AF of 10 for BPA was 1.6 μg/L.
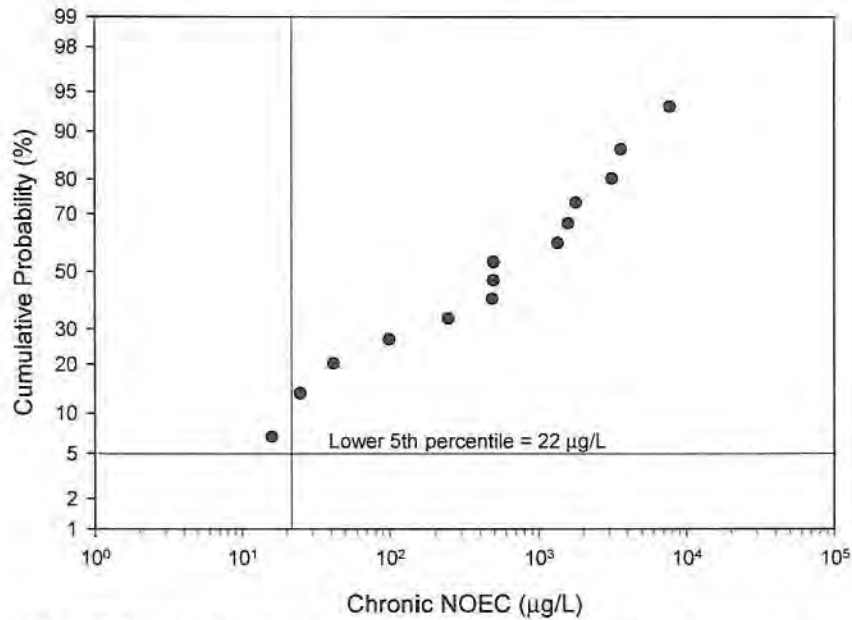
Figure 4. PNEC for bisphenol A calculated using the distributional approach. PNEC = 22 μg/L.
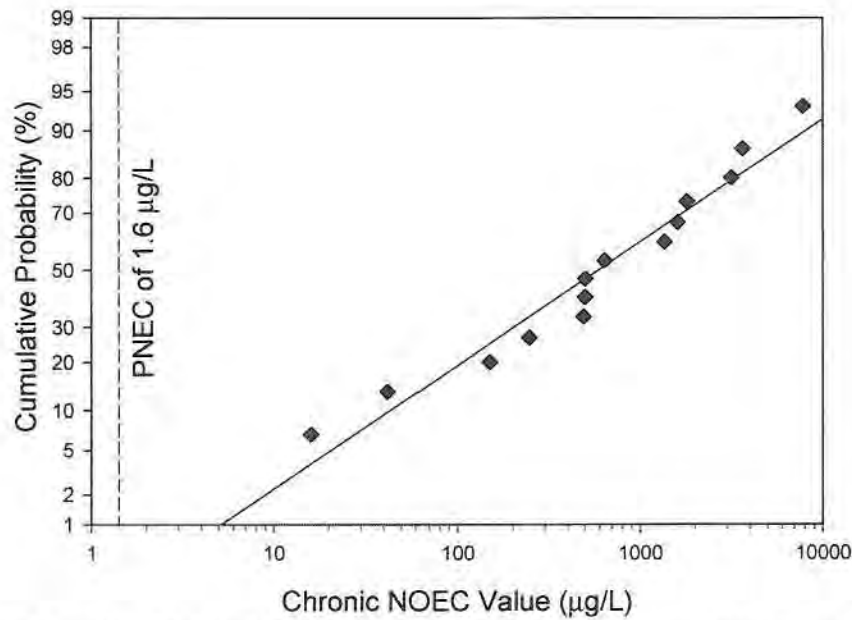
Figure 5. PNEC for bisphenol A calculated using the traditional assessment factor approach (most sensitive species and endpoint divided by an AF = 10). PNEC = 1.6 μg/L.

468

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008

Measured BPA Concentrations in U.S. and European Surface Waters

Concentrations of BPA in surface waters of North America and Europe have been compiled from the scientific literature and government reports (Clark *et al.*, manuscript in preparation). Relevant studies were obtained after identification by comprehensive literature searching. All studies were critically reviewed for validity using the methods presented in Klecka *et al.* (2007). The reviews emphasized the adequacy of sampling strategy and methods, quality assurance data, and analytical methodologies. The number of samples from representative and unique surface water locations that were analyzed for BPA totaled 1068 and 848, for North America and Europe, respectively.

The median and 95th percentile measured BPA concentrations in North American and European surface waters are presented in Table 3. In North America, 80% of the sampling locations had no detectable BPA, whereas in Europe, 49% had no detectable BPA. The difference in percent detections is attributed to the use in the studies conducted by the U.S. Geological Survey of analytical methods developed for the simultaneous analysis of dozens of analytes; a consequence of which often yields less sensitivity (Kolpin *et al.* 2002).

In North America, concentrations of BPA in surface waters have a median of 0.081 μg/L and a 95th percentile of 0.47 μg/L. Most of the U.S. surface water data has been recently collected by the U.S. Geological Survey and has largely focused on areas that are susceptible to contamination (see for example Kolpin *et al.* 2002). In European surface waters, BPA concentrations have a median of 0.010 μg/L and a 95th percentile of 0.35 μg/L.

Comparison of Measured BPA Concentrations to PNEC Values

Using the range of SSD-based PNECs (11 to 71 μg/L) and the concentrations of BPA in North American and European waters, ranges of hazard quotients (HQ) are calculated according to the USEPA (1998) and are presented in Table 3. HQs are the ratio of exposure to effect concentrations. BPA concentrations are most usefully

Table 3. Hazard quotients* and surface water concentrations of BPA in the United States and Europe.

	North America n = 1068 n = 216 (20%)		Europe n = 848 n = 433 (51%)	
No. of samples** No. of detects***				
	BPA concentrations	Hazard quotients	BPA concentrations	Hazard quotients
Median (50th percentile)	0.081 μg/L	0.0011 to 0.0074	0.010 μg/L	0.00014 to 0.00091
95th percentile	0.47 μg/L	0.0066 to 0.043	0.35 μg/L	0.0049 to 0.032

*Hazard quotients calculated as the ratio of the surface water concentration divided by the range of SSD-based PNEC values (11 to 71 μg/L). **Represents only the number of unique sampling locations, eliminating duplicate samples and multiple sampling events at any given location. ***Varying detection limits.

summarised by median or 50th and 95th percentile values as the distributions are skewed by a small number of values at the high end of the concentration range. In North American surface waters, HQ values for BPA range from 0.0011 to 0.0074 using the median concentration of 0.081 μg/L and from 0.0066 to 0.043 using the 95th percentile concentration of 0.47 μg/L. In European surface waters, HQ values range from 0.00014 to 0.00091 using the median concentration and from 0.0049 to 0.032 using the 95th percentile concentration. Based on the lack of overlap in the measured surface water concentrations and the range of calculated PNEC values in this assessment, communities of aquatic organisms in North American and European surface waters are not expected to be adversely impacted by exposure to BPA.

DISCUSSION

PNECs Derived for BPA

The PNEC values that were calculated using four SSD methods are compared to the traditional PNEC calculated using the AF approach as shown in Table 2. The PNECs that were calculated using SSD methods ranged from 11 to 71 μg/L, a range that covers a factor of about seven between the high and low values. Three of the SSD-derived PNECs (calculated using the RIVM HC5, SigmaPlot 5th percentile, and Distributional 5th percentile methods) range from 11 to 22 μg/L, about a factor of two apart. The differences in these PNECs (ranging from 11 to 22 μg/L) are due to slightly differing calculation methodologies. The choice of a specific method would be dependent on the specific regulatory needs of a specific country employing the procedures.

The fourth PNEC of 71 μg/L was calculated using the USEPA FCV method. This latter PNEC is different from the other three primarily due to the use of the species geometric mean of the NOEC and LOEC (*i.e.*, the MATC), instead of strictly using the NOEC values. The rationale for use of MATCs in FCV derivation is that the MATC gives a more likely estimate of the true chronic threshold effect concentration (USEPA 1985). This is due to the fact that many chemical substances exhibit threshold levels, *i.e.*, the lowest concentration of a compound that might produce a harmful effect on an organism. For any threshold material, a continuous exposure or series of exposures below the aquatic threshold concentration should not cause an unacceptable effect on aquatic organisms. As defined by the USEPA, an FCV derived from MATCs is intended to be an estimate of "a threshold of unacceptable effect" to a sensitive aquatic species. In addition, the USEPA has detailed the application of the threshold effect in FCV derivation, noting the following, "if maintained continuously, any concentration above the criterion is expected to cause an unacceptable effect. On the other hand, the concentration of a pollutant can be above the criterion without causing an unacceptable effect if (a) the magnitude and duration of the excursions are appropriately limited and (b) there are compensating periods of time during which the concentration is below the criterion" (USEPA 1985).

In most of the studies identified in Table 1, multiple endpoints were measured. The endpoints may be grouped into the categories of mortality, growth and development, and reproduction. Although the lowest NOEC for any endpoint measured
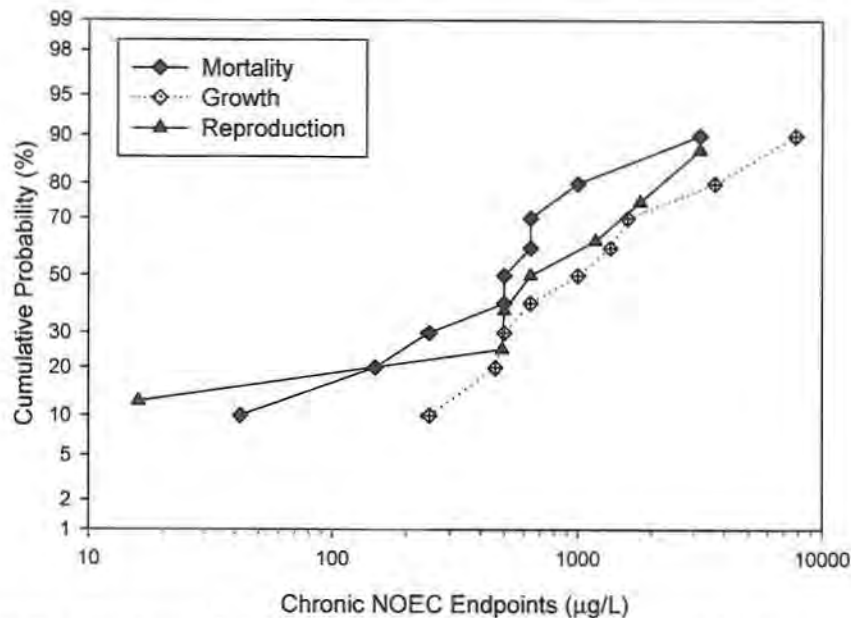
Figure 6. Bisphenol A: NOEC values for mortality, growth, and development and reproduction endpoints. Data from Table 1.

in a study was used as the overall study NOEC to calculate PNEC values regardless of what category it represented, it is useful to compare the NOEC for each category of endpoint. The NOEC for the three types of observed endpoints were ranked from low to high and plotted by concentration (Figure 6). The endpoints at a given rank are not necessarily from the same study. There appear to be few obvious differences between the NOEC for the three general endpoints across studies in the distributions shown in Figure 6. In other words, the distribution of reproduction NOECs do not appear to be more or less sensitive than distributions of growth and development NOECs or mortality NOECs.

To further test this observation, the RIVM ETx 2.0 program was used to calculate individual HC5 values for the distribution of NOECs based on endpoints. The HC5 values for mortality and growth and development NOECs are 62 and 44 μg/L, respectively, as compared to the HC5 of 31 μg/L for reproduction NOECs. The reproduction-based HC5 value is within a factor of 1.7 of the HC5 of 18 μg/L calculated using all data. The HC5 of 31 μg/L based only on reproduction data and the HC5 value of 18 μg/L based on all data are lower than the HC5 values based on either mortality or growth because of the single lowest NOEC of 16 μg/L, which is based on F2 generation hatchability. Absent this value, the HC5 value based on reproduction data is 118 μg/L and the HC5 value based on all data is 30 μg/L. The reason for the changes in the HC5 values is due to the influence on the shape of the species sensitivity distribution from the one NOEC. This type of influence on the HC5 value or any of the PNECs calculated using the other methods is independent of the type of endpoint. Any substantially different NOEC (either high or low) will

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008

471

change the tails of the distributions and affect the PNEC that is calculated (*e.g.*, the HC5).

Key Assumptions about Using the SSD Approach to Calculate PNEC Values

In the SSD approach to determining PNEC values, a concentration at any percentile (here, 5%) is calculated from a distribution of toxicity values. A number of assumptions are inherent in this approach: (1) the SSD is well modeled by the selected distribution; (2) the sensitivity of the species in the laboratory approximates the sensitivity of species in the field; (3) the sample of species is random, or at least representative of a community; and (4) protection of the prescribed percent of species confers an appropriate level of protection on a community.

The assumption that the actual distribution of species sensitivity follows the lognormal distribution (the most common distribution assumed) can be tested with the RIVM ETx 2.0 program. This program uses the Anderson-Darling test for normality of the distribution of effect concentrations. For the BPA dataset, the calculated Anderson-Darling statistic of 0.345 (for n = 14) is well below the critical value of 0.752 at the $p = .05$ level of significance. Thus, the assumption of a normal distribution (log transformed) was confirmed for the BPA dataset. Had this assumption not been supported, other more sophisticated methods of calculating toxicity thresholds are available and may have been needed. For instance, Newman *et al.* (2000) examined toxicity datasets for 30 compounds and found that half did not meet the assumption of normality. The authors proposed the use of bootstrap methods to overcome this problem with good success. Grist *et al.* (2002) extended this type of analysis further proposing a hybrid bootstrap regression method.

When developing a toxicity dataset for a chemical, a standard group of aquatic species (fish, invertebrates, algae) are tested for which validated protocols exist. This base dataset is often supplemented by other less standard species. The combined dataset then may be used to conduct risk assessment. The inherent assumption is that the toxicity dataset with multiple species is representative of a community of species that is to be protected. Buckler *et al.* (2005) stated that although minimum datasets consisting of toxicity values for rainbow trout, bluegill, daphnids, and mysid shrimp provide satisfactory prediction of larger datasets of toxicity values, it is obviously desirable to have high quality data for as many species as possible. In fact, both the USEPA and the European Chemicals Bureau have general requirements (see Table 1) for minimum datasets for deriving a PNEC using SSD approaches, consisting of at least eight major taxa, including fish, non-fish vertebrates, insects, mollusks, crustaceans, annelids, plankton, and algae (USEPA 1985; EC 2003).

It is difficult to assess whether the laboratory-derived toxicity tests are a random sample of species in the field or at least representative of the species in the field. In general, species selected to become standard test organisms are thought to be relatively sensitive, at least to some toxicants. As noted by Versteeg *et al.* (1999), *Daphnia*, fathead minnows, and rainbow trout are often tested and are typically among the most sensitive species in a toxicity dataset. This is partly true for BPA, as the most sensitive species is the fathead minnow. The fact that the most sensitive life stages of these species are exposed in chronic tests further contributes to the conservatism built into a set of laboratory toxicity tests.

Possibly the most important assumption for the use of SSDs to calculate PNEC values is that they confer an appropriate level of protection to a community of species. It is this final point that has garnered the majority of criticisms concerning the SSD approach and extrapolation to protection at the ecosystem level. In examination of this latter assumption, Versteeg *et al.* (1999) compared the application of single-species chronic toxicity data from the open literature (via the SSD approach) with published, high quality chronic model ecosystem data (*e.g.*, microcosms, model ecosystems, and field situations) on the same chemical. If single-species data are indeed useful for extrapolating to the ecosystem level via the SSD approach, there should be substantial overlap in the distribution of single-species and model ecosystem data. Conversely, if single-species/SSD data are of limited use for extrapolating to an ecosystem, there should be limited or no overlap in the distributions. Versteeg *et al.* (1999) compared the SSD output from laboratory studies to ecosystem NOEC values for a variety of substances (n = 11), including organic compounds, metals, pesticides, and surfactants. The authors noted that observed field ecosystem NOEC values corresponded to concentrations expected to exceed the SSD-derived PNEC at the 5th percentile and that the lower 5th percentile values are good predictors of the lower 95% confidence interval on the mean ecosystem NOEC value. Their analysis indicated that a sufficiently large data set of laboratory-generated chronic test data (defined as n > 5 species) may be used to successfully determine concentrations for a compound that are protective of the system that the authors studied.

Additional supporting evidence is available from Van den Brink *et al.* (2006), who analyzed laboratory toxicity data for nine herbicides and compared respective SSD regressions against response data from field mesocosms. These authors noted that the lower confidence limit of the acute 5th percentile (*i.e.*, acute HC5 value) and the median value of the chronic HC5 were protective of adverse effects in aquatic micro/mesocosms even under a long-term exposure regime. In studies using endosulfan, Hose and Van den Brink (2004) examined the utility of non-native laboratory species-based SSDs to be protective of local mesocosm responses and determined that the sensitivities of native organisms were not significantly different from those of non-native species. The authors also found that field mesocosm response to endosulfan was less sensitive than corresponding laboratory species and the laboratory-based acute 5th percentile SSD value was protective of field and mesocosm populations. Finally, Selck *et al.* (2002) compared PNEC values from both deterministic and probabilistic approaches with tributyltin and linear alkylbenzene sulfonate and found both methods yielded PNEC values that were protective of marine mesocosm responses.

The SSD approach represents a technique to reduce uncertainties in our understanding of ecological dynamics following chemical exposure. Evidence from numerous researchers with a wide variety of metal and organic chemicals indicates that low percentile SSD-derived effect concentrations are protective of field systems (Versteeg *et al.* 1999; Selck *et al.* 2002; Hose and Van den Brink 2004; Van den Brink *et al.* 2006).

Risk Management Decisions

At least two risk management decisions accompany the use and application of PNEC values derived using SSDs. The first is the selection of the protection level (*e.g.*,

5th percentile). The lower-bound 5th percentile value has been used throughout this study as it is commonly employed. Other levels of protection could be used as well.

The second risk management issue to be decided is whether the SSD-derived PNEC at the chosen level of protection nevertheless warrants an additional AF to achieve an even greater level of protection. The available data suggest that no additional AF is needed if the PNEC calculated using SSD methods is based on chronic data. Using acute data for a series of pesticides, Brock *et al.* (2006) found that AFs of 2 to 10 may be warranted for acute HC5 values. Similarly, Maltby *et al.* (2005) suggests an AF of 5 be applied to HC5 values that are calculated using only acute studies. However, based on longer term studies, Van den Brink *et al.* (2006) and Versteeg *et al.* (1999) reported that chronic HC5 values with no AF used were protective of long-term exposure in mesocosms. As Forbes and Calow (2002) noted, studies that examine individual-level responses often provide protective estimates of population-level effects, and changes in ecosystem structure. The testing of individual-level responses and changes in species composition rather than on ecosystem processes is unlikely to grossly underestimate effects on ecosystems (Forbes and Calow 2002). Therefore, it does not appear that applying an additional AF to an SSD-derived PNEC based on chronic NOEC values is warranted for BPA.

SSD-Derived PNEC in Other Environmental Compartments

For the purposes of this article, PNECs were calculated for use in risk assessment of BPA in surface waters. Besides aquatic risk assessment, SSDs for any compound may be used to calculate PNECs for use in setting cleanup standards for surface waters, to support discharge permits, or in support of ecological risk assessments for potentially affected sites. With adequate data, PNECs based on SSDs for terrestrial species can also be calculated.

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REFERENCES

Alexander HC, Dill DC, Smith LW, *et al.* 1988. Bisphenol A: Acute aquatic toxicity. Environ Toxicol Chem 7:19–26.

Aufderheide J, Warbritton R, Pounds N, *et al.* 2006. Effects of husbandry parameters on the life-history traits of the apple snail, *Marisa cornuarietis*: Effects of temperature, photoperiod, and population density. Invertebrate Biol 125:9–20

Bayer AG. 1999. Fish, Juvenile Growth Test (Oncorhynchus mykiss) of Bisphenol-A. Study Number: 707 A/98FF. Bayer AG, Leverkusen, Germany

Bowmer T and Gimeno S. 2001. The Effects of Bisphenol-A on the Development of the Male Carp Reproductive Tract When Exposed During Sexual Differentiation—Implications for Test Design and Risk Assessment. Unpublished TNO Report. Netherlands Organization for Applied Scientific Research (TNO), Delft, The Netherlands

Brock TCM, Arts GHP, Maltby L, *et al.* 2006. Aquatic risks of pesticides, ecological protection goals, and common aims in European legislation. Integrated Environ Assess Manage 2:e20–e46

Buckler DR, Mayer FL, Ellersieck, *et al.* 2005. Acute toxicity value extrapolation with fish and invertebrates. Arch Environ Contam Toxicol 49:546–58

Cafarella MA. 2006. Bisphenol A (BPA)—Chronic Toxicity to Amphipods (*Hyalella azteca*) Under Flow-Through Conditions. Report No. 13796.6107. Springborn-Smithers Laboratories, Inc, Wareham, MA, USA

Caspers N. 1998. No estrogenic effects of Bisphenol A in *Daphnia magna* Straus. Bull Environ Contam Toxicol 61:143–8

Caunter JE. 1998. Bisphenol A: Effect on the Embryo-Larval Developmental Stage of the Fathead Minnow (*Pimephales promelas*). Report No. BL6421/B. Brixham Environmental Laboratory, Zeneca, Ltd., Brixham, Devon, UK

Caunter JE. 2000. Bisphenol A: Multigeneration Study with Fathead Minnow (*Pimephales promelas*). Report No. BL6878/B. Brixham Environmental Laboratory, Zeneca, Ltd., Brixham, Devon, UK

Caunter JE. 2006. Bisphenol A: 42-Day Exposure to Mature Adult Fathead Minnows (*Pimephales promelas*)— Phase 2 of the Study "Effects of BPA on Fathead Minnow Gonadal Histology." Brixham Environmental Laboratory, Zeneca, Ltd., Brixham, Devon, UK

CEPA (Canadian Environmental Protection Act). 1997. Environmental Assessments of Priority Substances under the Canadian Environmental Protection Act. Guidance Manual Version 1.0. Report EPS/2/CC/3E. Chemical Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, Ottawa, ON, Canada. March

Clark KE, Klecka GM, Staples CM, *et al.* 2007. Evaluating bisphenol A concentrations in North American and European environments: comparison with predicted environmental fate. Society of Environmental Toxicology and Chemistry Annual Meeting, Milwaukee, WI, USA, P. 172

Cousins IT, Staples CA, Klecka GM, *et al.* 2002. A multimedia assessment of the environmental fate of bisphenol A. Hum Ecol Risk Assess 8:1107–35

Dietrich DR, O'Brien E, Hoffmann S, *et al.* 2006. Effect of bisphenol A in snails. Environ Health Perspect 114:A340–A41

EC (European Commission). 2003. Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances. Part II. ECSC-EC-EAEC. Brussels, Belgium

Experimental Pathology Laboratories, Inc. 2005. Histopathologic Quality Re-Assessment of Spermatogenesis Slides from a Fathead Minnow Study with Bisphenol a Pathology Report. EPL Project Number 685-001. Experimental Pathologies, Laboratories, Inc, Sterling, VA, USA

Forbes VE and Calow P. 2002. Extrapolation in ecological risk assessment: Balancing pragmatism and precaution in chemical controls legislation. BioScience 52:249–57

Forbes VE, Selck H, Palmqvist A, *et al.* 2007a. Does bisphenol A induce superfeminization in *Marisa cornuarietis*? Part I: Intra- and inter-laboratory variability in test endpoints. Ecotoxicol Environ Safety 66:309–18

Forbes VE, Aufderheide J, Warbritton R, *et al.* 2007b. Does bisphenol A induce superfeminization in *Marisa cornuarietis*? Part II: Toxicity test results and requirements for statistical power analyses. Ecotoxicol Environ Safety 66:319–25

Fukuhori N, Kitano M, and Kimura H. 2005. Toxic effects of bisphenol A on sexual and asexual reproduction in *Hydra oligactis*. Archives Environ Contam Toxicol 48:495–500

Grist EPM, Leung KMY, Wheeler JR, *et al.* 2002. Better bootstrap estimation of hazardous concentration thresholds for aquatic assemblages. Environ Toxicol Chem 21:1515–24

Hill M, Stabile C, Steffan LK, *et al.* 2002. Toxic effects of endocrine disruptors on freshwater sponges: Common developmental abnormalities. Environ Pollut 117:295–300

Hose GC and Van den Brink PJ. 2004. Confirming the species-sensitivity distribution concept for endosulfan using laboratory, mesocosm, and field data. Arch Environ Contam Toxicol 47:511–20

Japan MOE (Ministry of Environment). 2004. Test Results of Endocrine Disrupting Effects on the Biological System in Fish 37. Bisphenol A Study Results on Medaka. July 27, 2004. Available at http://www.env.go.jp/chemi/end/kento1601/mat/mat05-2.pdf

Kinnberg K and Toft G. 2003. Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). Ecotoxicol Environ Safety 54:16–24

Klecka GM, Zabik J, Woodburn K, *et al.* 2007. Exposure analysis of C8- and C9-alkylphenol, alkylphenol ethoxylates and their metabolites in surface water systems within the United States. Hum Ecol Risk Assess 13:792–822

Kloas W, Lutz I, and Einspanier R. 1999. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals *in vitro* and *in vivo*. Sci Total Environ 225:59–68

Kolpin DW, Furlong ET, Meyer MT, *et al.* 2002. Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. Environ Sci Technol 36:1202–11

Levy G, Lutz I, Kruger A, *et al.* 2004. Bisphenol A induces feminization in *Xenopus laevis* tadpoles. Environ Res 94:102–11

Maltby L, Blake N, Brock TCM, *et al.* 2005. Insecticide species sensitivity distributions: Importance of test species selection and relevance to aquatic ecosystems. Environ Toxicol Chem 24:379–88

Metcalfe CD, Metcalfe TL, Kiparissis Y, *et al.* 2001. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by *in vitro* assays with Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 20:297–308

Newman MC, Ownby DR, Mezin LCA, *et al.* 2000. Applying species-sensitivity distributions in ecological risk assessment: Assumptions of distribution type and sufficient numbers of species. Environ Toxicol Chem 19:508–15

OECD (Organization for Economic Cooperation Development). 1992. Report of the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment. OECD Environment Monograph 59. OECD/GD (2)169. Paris, France

Oehlmann J, Schulte-Oehlmann U, Tillmann M, *et al.* 2000. Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol. Ecotoxicol 9:383–97

Oehlmann J, Schulte-Oehlmann U, Bachmann J, *et al.* 2006. Bisphenol A induces super-feminization in the ramshorn snail *Marisa cornuarietis* (Gastropoda: Prosobranchia) at environmentally-relevant concentrations. Environ Health Perspect 114(Supplement 1): 127–33

Pascoe D, Carroll K, Karntanut W, *et al.* 2002. Toxicity of 17-ethinylestradiol and bisphenol A to the freshwater cnidarian *Hydra vulgaris*. Arch Environ Contam Toxicol 43:56–63

Pickford DB, Caunter JE, Hethridge MJ, *et al.* 2000. Bisphenol A: Determination of Effects on Larval Growth, Development, and Sexual Differentiation of the African Clawed Frog (*Xenopus laevis*). Report no. AF0072/A. Brixham Environmental Laboratory, Zeneca, Ltd., Brixham, Devon, UK

Pickford DB, Hetheridge MJ, Caunter JE, *et al.* 2003. Assessing chronic toxicity of bisphenol A to larvae of the African clawed frog (*Xenopus laevis*) in a flow-through exposure system. Chemosphere 53:223–35

Posthuma L, Sutter II GW, and Traas TP. 2002. Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, FL, USA

Putt AE. 2003. Bisphenol A—7-Day Toxicity to Duckweed, *Lemna gibba*, Under Static-Renewal Conditions. January 22, 2003. Springborn Smithers Laboratories, Inc, Wareham, MA, USA

Rhodes JE, Wolf JC, and van der Hoeven N. 2007. Bisphenol A: Partial Life-Cycle Toxicity Test with the Fathead Minnow. Pimephales Promelas. Study Number 48972. ABC Laboratories, Inc, Colombia, MO, USA

RIVM (The Netherlands National Institute of Public Health and the Environment). 2004. Bilthoven, The Netherlands

Sayers LE. 2006. Bisphenol A (BPA)—Chronic Toxicity to Rotifers (*Brachionus calyciflorus*) Under Static Conditions. Report No. 13796.6108. Springborn Smithers Laboratories, Inc. Wareham, MA, USA

Schulte-Oehlmann U, Tillmann M, Casey D, *et al.* 2001. Xeno-estrogenic effects of bisphenol A in prosobranchs (Mollusca: Gastropoda: Prosobranchia). Umweltchem Okotox 13:319–33

Segner H, Navas JM, Schaefers C, *et al.* 2003. Potencies of estrogenic compounds in vitro screening assays and in life cycle tests with zebrafish in vivo. Ecotoxicol Environ Safety 54:315–22

Selck H, Riemann B, Christoffersen K, *et al.* 2002. Comparing sensitivity of ecotoxicological effect endpoints between laboratory and field. Ecotoxicol Environ Safety 52:97–112

Selck H, Aufderheide J, Pounds N, *et al.* 2006. Effects of food type, feeding frequency, and temperature on juvenile survival and growth of *Marisa cornuarietis* (Mollusca: Gastropoda). Invertebrate Biol 125:106–16

Sohoni P, Tyler CR, Hurd K, *et al.* 2001. Reproductive effects of long-term exposure to bisphenol A in the fathead minnow (*Pimephales promelas*). Environ Sci Technol 35:2917–25

Staples CA, Woodburn K, Caspers N, *et al.* 2002. A weight of evidence approach to the aquatic hazard assessment of Bisphenol A. Hum Ecol Risk Assess 8:1083–105

Suter GW II. 1993. Ecological Risk Assessment. Lewis Publishers, Chelsea, MI, USA

Systat. 2000. SigmaPlot software. Systat Software, Inc, Richmond, CA, USA

USEPA (US Environmental Protection Agency). 1984. Estimating Concern "Levels" for Concentrations of Chemical Substances in the Environment. Report, Environmental Health Branch, Health and Environmental Review Division, Washington, DC, USA

USEPA. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. PB85-227049. National Technical Information Service, Springfield, VA, USA

USEPA. 1995. Final water quality guidance for the Great Lakes system. Fed Reg 60(56):15366–425

USEPA. 1998. Final Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F. Washington, DC, USA. April

USEPA. 1999. ECOFRAM Materials. Available at http://www.epa.gov/oppefedl/ecorisk/

Van den Brink PJ, Blake N, Brock TCM, *et al.* 2006. Predictive value of species sensitivity distributions for effects of herbicides in freshwater ecosystems. Hum Ecol Risk Assess 12:645–74

Versteeg DJ, Belanger SE, and Carr GJ. 1999. Understanding single-species and model ecosystem sensitivity: Data-based comparison. Environ Toxicol Chem 18:1329–46

Warbritton R, Forbes VF, and van der Hoeven N. 2007a. Bisphenol A: Effects on Fecundity, Hatchability, and Growth of *Marisa cornuarietis* Under Flow-Through Conditions at Twenty-Five Degrees Celsius. Draft report. ABC Laboratories, Inc, Columbia, MO, USA

Warbritton R, Forbes VF, and van der Hoeven N. 2007b. Bisphenol A: Effects on Fecundity of *Marisa cornuarietis* Under Flow-Through Conditions at Twenty-Two Degrees Celsius. Draft report. ABC Laboratories, Inc, Columbia, MO, USA

Watts MM, Pascoe D, and Carroll K. 2003. Exposure to 17α-ethinylestradiol and bisphenol A—effects on larval moulting and mouthpart structure of *Chironomus riparius*. Ecotoxicol Environ Safety 54:207–15

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008

477

West RJ, Goodwin PA, and Klecka GM. 2001. Assessment of the ready biodegradability of bisphenol A. Bull Environ Contam Toxicol 67:106–12

Wolf JC, Dietrich DR, Friederich U, *et al.* 2004. Qualitative and quantitative histomorphologic assessment of fathead minnow *Pimephales promelas* gonads as an endpoint for evaluating endocrine-active compounds: A pilot methodology study. Toxicologic Path 32:600–12

Yokota H, Tsuruda Y, and Maeda M *et al.* 2000. Effect of early life stage in Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 19:1925–30

478

Hum. Ecol. Risk Assess. Vol. 14, No. 3, 2008