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A Weight of Evidence Approach to the Aquatic Hazard Assessment of Bisphenol A

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ABSTRACT

Bisphenol A (BPA; 4,4-isopropylidene diphenol) is a chemical intermediate used primarily in the production of epoxy resins and polycarbonate products. BPA has been identified in surface waters and, hence, has been the subject of considerable research into its potential effects on aquatic organisms. Available literature on the aquatic toxicity of BPA was reviewed for quality against European Union TGD and Organization of Economic Cooperation and Development GLP principles. From this review, studies of suitable quality covering numerous ecologically relevant endpoints were identified to evaluate the survival, growth, and reproductive success of aquatic organisms exposed to BPA. Those studies yielded approximately 70 no observed effect concentrations (ranging from 16 to 3640 μg/L) and lowest observed effect concentrations (160 to 11,000 μg/L) that were considered in this weight of evidence assessment. Across all data, adverse effects on survival, growth, and reproduction occurred only at concentrations of 160 μg/L and above. Secondary biochemical (*e.g.*, vitellogenin induction) and morphological (*e.g.*, gonad histology) data provide insight into mechanisms of action, but do not correlate with apical endpoints related to survival, growth, and reproduction. Comparing the weight of the evidence of the aquatic toxicity data that showed chronic effects at 160 μg/L and higher with typical surface water concentrations in the range of 0.001 to 0.10 μg/L, BPA is unlikely to cause adverse effects on aquatic populations or ecosystems.

Key Words: ecotoxicity, multi-generation test, ecologically relevant, reproductive success.

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INTRODUCTION

Bisphenol A (BPA, 4,4'-isopropylidine diphenol, CAS Registry No. 80-05-7) is a commercially important chemical with an estimated worldwide production of 2.5 million tonnes in 2001; the structure of BPA is shown in Figure 1 (Staples *et al.* 1998). Approximately 90% of all BPA is used as an intermediate in the production of polycarbonate plastics and epoxy resins, such as impact-resistant eyeglass lenses, food and beverage containers, helmets, and compact discs. Lesser amounts are used to produce fire retardants or as an antioxidant and stabilizer in the production of 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.

The physical-chemical properties and fate characteristics of BPA control its distribution and ultimate fate in the environment. BPA is a moderately water-soluble compound at ambient temperatures (300 mg/L) that dissociates under alkaline conditions (pK_a = 9.6 to 11.3) (Staples *et al.* 1998). BPA has been found to be readily biodegradable, achieving 81 to 93% mineralization to carbon dioxide in 28 days. These studies were conducted in compliance with Guideline 301F of the Organization of Economic Cooperation and Development (OECD) (West *et al.* 2001). Subsequent work has shown that BPA is rapidly degraded in surface waters taken from diverse geographies in the United States and Europe. Following lag phases of 2 to 4 days, BPA in natural river water was biodegraded with measured half-lives of 0.5 to 3 days (Klečka *et al.* 2001). In summary, BPA's physical, chemical, and environmental fate characteristics influence the nature of its presence in aquatic systems, and need to be considered when conducting or evaluating ecotoxicity studies.

BPA has been the subject of considerable aquatic toxicity testing in recent years. Because the aquatic environment receives direct discharges of BPA from production, processing, and sewage treatment plant effluents, most research on toxicity to wildlife has focused on aquatic organisms. The focus of this paper is therefore on potential hazards to aquatic species. However, since aquatic toxicity data can be used to estimate potential toxicity in sediments and soil (EC 1996), a thorough review of aquatic toxicity data supports the assessment of ecological risks to wildlife inhabiting aquatic and terrestrial environments. Studies investigating the toxicity of BPA to aquatic organisms are presented and discussed in detail below. In brief, conventional acute tests (ASTM 1980; USEPA 1982; ECETTC 1996) were followed by longer-term testing using conventional early life stage (OECD 1992) or life cycle test methods (USEPA 1986; OECD 1996). More recently, a number of studies have used new methodologies, novel test species, and nontraditional endpoints to exam-

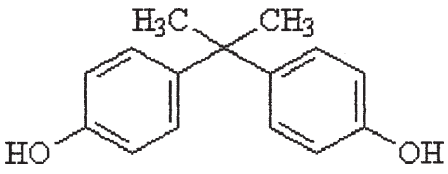

Figure 1. Molecular structure of bisphenol A.

ine the ecotoxicity of BPA, some of which have generated data that are at odds with those obtained with conventional and well accepted procedures. For such nonconventional studies, the ecological relevance and the scientific validity of the studies must be thoroughly examined prior to the use of such data in a risk assessment process. For example, large-scale field studies conducted on pesticide products have demonstrated that hazard assessments based on laboratory data generated with conventional species and testing procedures are protective of ecosystem structure and functional attributes (Giesy *et al.* 1999; Giddings *et al.* 2000).

A recent multistakeholder workshop sponsored by various U.S. regulatory agencies, industry, and advocacy groups addressed screening methods for detecting the potential effects of endocrine active substances in wildlife (Ankley *et al.* 1998). The workshop reviewed screening assays and endpoints for detection of endocrine active substances, including the induction of the egg protein vitellogenin (VTG), fluctuation of plasma steroid levels, receptor binding assays, alteration of external sexual characteristics, alteration of sexual differentiation, changes in organ weights, and maturation of male and female gametes. However, screening methods and endpoints are not intended to be directly predictive of the ecologically relevant endpoints of survival, growth and development, and reproductive success. As noted in the workshop proceedings, screening tests and their endpoints are not predictive of subsequent effects at the population or community levels, or dose-response relationships (Ankley *et al.* 1998).

Compounds such as BPA that have been identified as weakly estrogenic substances (Sohoni *et al.* 2001) have been used frequently in exploratory research to study various aspects of estrogenicity, such as interaction with the estrogen receptor in liver cells (Celius *et al.* 1999) or the induction of serum vitellogenin (Lindholst *et al.* 2000). Such findings serve as biomarkers of exposure of the cells or organisms to estrogenically active substances (Arcand-Hoy and Benson 1998). Other studies have focused on tissue-level endpoints such as alterations of gonad cell structure (Haubruge *et al.* 2000), changes in the relative proportion of cell types in spermatogenesis (Sohoni *et al.* 2001), and the observation of ovarian cells within male gonadal tissue (Metcalfe *et al.* 2001). Although these various endpoints can provide relevant information confirming exposure to endocrine active substances and may elucidate potential mechanisms of action, they are not substitutes for conventional endpoints that predict effects on aquatic populations or communities. Additional research directly comparing supplemental mechanistic endpoints with population and community-level effects will better elucidate this issue.

There are two objectives to this article. The first objective is to critically review the available literature on the aquatic toxicity of BPA. To meet this objective, the recent draft of the European Union (E.U.) risk assessment for BPA (UKEA 2001) was used as a starting point, as it includes a compilation of considerable relevant literature and nonpublished information. Extensive literature searches were also performed and identified additional studies. The second objective of this article is to conduct an aquatic hazard assessment for BPA using a weight-of-evidence approach. Consequently, emphasis was placed on work using the ecologically relevant endpoints of survival, growth and development, and reproductive success. We also included in this review an analysis of recent investigations that examined secondary biochemical and morphological endpoints, because such information may provide further un-

derstanding of potential mechanisms of toxicity and estrogenic activity. However, these secondary endpoints are not included in our weight of evidence approach to hazard assessment because such tests and endpoints are not predictive of population or ecosystem-level effects (Ankley *et al.* 1998).

As an initial step, all studies were critically reviewed for suitability for use in risk assessment following the criteria and procedures outlined in the E.U. Technical Guidance Document (EC 1996). Specific criteria for deciding on the quality and usefulness of a study included:

1. Thorough description of the experimental design, including exposure regime and replication,

2. Analytical confirmation of test concentrations;

3. Description of ecologically relevant endpoints and all supplemental secondary biochemical and morphological information collected;

4. Use of test procedures that are based, at least generally, on internationally accepted procedures and practices. Newly developed test procedures must be able to be repeated, and meet all other required criteria.

5. Clear linkage of reported findings with the exact experimental design, and,

6. Sufficient reporting of results, including system performance, toxicity results, and statistical methods employed to ascertain how the data support the conclusions that are drawn.

Consideration was also given to whether the studies were conducted under E.U., U.S., and/or OECD principles of Good Laboratory Practices (GLP). Compliance with GLP provides some assurance that the data reflect the conduct and findings of the study; that the raw data will be retained for additional analyses, if necessary; that data are not mistakenly changed or omitted, and that the study can be reconstructed from the raw data (OECD 1982). That is not to say good research cannot be conducted in the absence of GLP, only that certain documentation procedures are in place to track the execution and reporting of an experiment.

If all or most (four or more) of the guidance criteria appeared to have been met, the study was designated as "valid", or generally consistent with current practices with E.U. risk assessments (UKEA 2001). Accordingly, when some (<4), but not all, of the criteria were fulfilled, the studies were designated as "use with care." Experiments with insufficient information to allow proper evaluation or with other obvious flaws, received a designation of "not valid" and were deemed unsuitable for hazard assessment. An example of an obvious flaw is a long-term study of a readily biodegradable test substance conducted using a static test system where test chemical concentrations are not measured. All studies designated as "valid" and "use with care" were deemed acceptable for use in this aquatic hazard assessment. Other studies deemed to be "not valid" are presented and the reasons for excluding them from the hazard assessment are discussed in the relevant sections below.

EFFECTS ASSESSMENT

Survival — Acute and Chronic

In order for wildlife populations to remain viable in the presence of an exogenous substance such as BPA, individual organisms must be able to survive both short- and long-term exposures to BPA. Test results evaluating survival, growth, and reproductive fitness, following exposure to BPA, are summarized in Table 1. Lethal effects from aqueous exposure to BPA have been investigated in experiments with exposure periods ranging from 48 h to more than 400 days. The starting age of test organisms ranged from newly hatched to adults. Short-term tests that were considered acceptable (*i.e.*, both "valid" and "use with care" studies) followed standard protocols such as OECD method 203, ASTM methods for fish and aquatic invertebrates (ASTM 1980; ECETTC 1996), and U.S. Environmental Protection Agency (USEPA) methods for algae (USEPA 1982). Longer-term tests that were deemed to be acceptable also followed OECD methods (Table 1).

Survival of aquatic organisms following exposure to BPA has been examined with at least four species of fish and three species of invertebrates in acute toxicity studies that were deemed "valid" or "use with care" (Table 1). All nine results, expressed as acute 48- to 96-h LC_{50} values, ranged from 4600 to 17,930 μg/L in fish and from 960 to 10,000 μg/L with invertebrate species.

The effects of chronic exposures to BPA on the survival of aquatic organisms has been examined in longer-term studies ranging from 14 to more than 400 days with at least four species of fish, one species of amphibian, and one species of invertebrate. Of the 10 studies examined, eight were deemed "valid" or "use with care", while two were judged as not valid (Table 1). Study duration had little effect on survival of aquatic organisms exposed to BPA. For most species, no mortality was observed at the highest concentrations tested, which ranged from 100 to 3160 μg/L for vertebrates and invertebrates. In summary, BPA has apparent minimal effect on the survival of aquatic organisms at elevated dose levels.

Growth and Development

In order for populations to be able to reproduce, individual organisms must be able to grow and develop normally. A variety of endpoints can be monitored to assess the effects of a chemical on growth and development, including length and weight measurements, examination for structural deformities or other sublethal effects, weight of organs (relative to whole body weight), and development of secondary sexual characteristics that enable or aid reproduction. Effects on algae are generally based on measures of population growth. Results of studies that examined growth and development of fish, invertebrates, and algae are summarized in Table 1.

Chronic effects of BPA on growth and development of aquatic vertebrates have been reported for six species of fish and one amphibian; the exposure duration ranged from 28 to 431 days. Eight studies were deemed "valid" or "use with care." The lowest concentrations of BPA that have been reported to cause chronic effects on growth of fish range from 1820 to 11,000 μg/L. No observed effect concentrations (NOECs) for fish and amphibians fell within the range of 120 μg/L to 3640 μg/L, although in several studies these were the highest concentrations tested.

Table 1. Acute and chronic effects based on survival, growth, reproduction in aquatic organisms exposed to bisphenol A.

Species	Endpoint	Result (μg/L)*	Comment	Status	Reference
Rainbow trout *Oncorhynchus mykiss*	28-d, growth:	NOEC = 3640 LOEC = 11,000	juvenile growth test OECD proposed guideline, measured concentrations, GLP	Valid	Bayer AG (1999b)
Atlantic silverside (marine) *Menidia menidia*	96-h, survival:	96-h LC_{50} = 9400	ASTM method, flow-through, measured concentrations, non-GLP	Valid	Alexander (1988)
Zebrafish *Branchydanio retio*	14-d, survival:	NOEC = 3200 LOEC = 10,150	OECD 204, measured concentrations, GLP	Valid	Bayer AG (1999a)
Zebrafish *B. rerio*	120-d, fertilization rate: Mortality, egg production, hatchability, growth, time to onset of reproduction:	NOEC=750 LOEC = 1500 (EC_{10} = 390) NOEC = 1500	lifecycle test, renewal, measured concentrations, F_0 to F_1 to F_2, full details not available	Use with care	Fraunhofer (2000)
Medaka fish *Oryzias latipes*	96-h, survival:	96-h LC_{50} = 13,000	OECD 203, measured concentrations, non-GLP	Valid	Yokota (2000)
Medaka fish *O. latipes*	60-d, survival: 60-d, growth: 60-d, hatchability: 60-d, time to hatch: 60-d, embryo survival: 60-d, sex ratio:	NOEC = 1820 NOEC=355, LOEC=1820 NOEC = 1820 NOEC = 1820 NOEC = 1820 NOEC=355, LOEC=1820	modified OECD 210 ELS test, measured concentrations, renewal until hatch, then flow-through, non-GLP	Valid	Yokota (2000)
Medaka Fish *O. latipes*	100-d post-hatch, growth: 100-d post hatch, sex ratio:	NOEC = 120 NOEC = 120	renewal, measured concentrations, non-GLP	Valid	Metcalfe (2001)
Medaka Fish *O. latipes*	14-d, no. of eggs: 14-d, no. of hatched eggs:	NOEC/LOEC = 684/2280 NOEC/LOEC = 684/2280	renewal, males exposed for 2 weeks, bred with unexposed females in clean water, nominal-only	Valid	Shioda (2000)
Medaka Fish *O. latipes*	72-h survival 72-h survival	LC_{50} = 7500 adults LC_{50} = 5100 embryos	general procedures described, renewal, nominal-only	Use with care	Tabata (2001)
Medaka Fish *O. latipes*	100-d survival 100-d sex ratio:	NOEC = 100 NOEC = 100	general procedures described, no statistics, nominal-only	Not valid	Tabata (2000)
Fathead minnow *Pimephales promelas*	96-h survival:	96-h LC_{50} = 4600 to 4700	ASTM methods, static and flow-through tests, measured concentrations, non-GLP	Valid	Alexander (1988)
Fathead minnow *P. promelas*	32-d post hatch survival: 32-d post hatch, growth:	NOEC = 640 NOEC = 640	OECD 210 ELS assay, measured concentrations, GLP	Valid	Caunter (1999)
Fathead minnow *P. promelas*	F_0 71-d, growth: F_0 egg production:	NOEC=640, LOEC=1280 NOEC=640, LOEC=1280	multi-generation study, F_0 breeding adults exposed, F_1 eggs hatch, grow	Valid	Caunter (2000)

Species	Endpoint	Result (μg/L)*	Comment	Status	Reference
	F_1 egg hatchability: F_1 60-d, survival: F_1 30 to 431-d, growth: F_1 egg production: F_2 egg hatchability: F_2 160 to 260-d, survival: F_2 130 to 260-d, growth:	NOEC = 160, LOEC=640 NOEC = 640 NOEC = 640 NOEC = 160, LOEC=640 NOEC = 16, LOEC=160 NOEC = 640 NOEC = 640	to adults, F_1 adults bred, F_2 eggs hatch, survive, grow, total exposure 431 days, measured concentrations, based on USEPA lifecycle method USEPA 540/9-86-137, July 1986, GLP		
Carp *Cyprinus carpio*	28-day, survival: 28-d growth:	NOEC = 740 NOEC = 740	28-day, modified juvenile growth test OECD 215, non-GLP, abstract only	Use with care	Bowmer (1999)
Guppy *Poecilia reticulata*	21-d sperm counts: 21-d gonad size: 21-d sperm length:	LOEC = 274 NOEC = 549 NOEC = 549	non-standard protocol, renewal, no solvent control, nominal only, non-GLP	Use with care	Harbruge (2000)
Swordtail fish *Xiphophorus helleri*	96-h survival:	96-h LC_{50} = 17,930	non-standard method, renewal, nominal only, non-GLP	Use with care	Kwak (2001)
Swordtail fish *X. helleri*	60-d tail length, growth:	NOEC = 0.2, LOEC=2.0	non-standard method, static test, nominal only, non-GLP	Not valid	Kwak (2001)
African clawed frog *Xenopus laevis*	90-d mortality: 90-d development/growth: 90-d sex ratio:	NOEC = 500 NOEC = 500 NOEC = 500	larval development, hatch to maturity, flow through, concentrations measured, GLP	Valid	Pickford (2000)
African clawed frog *X. laevis*	12-week survival: 12-week development/growth: 12-week sex ratio:	NOEC = 23 NOEC = 23 NOEC = 2.3, LOEC = 23	larval development, hatch to maturity, renewal, nominal-only, method development paper, non-GLP	Not valid	Kloas (1999)
Water flea *Daphnia magna*	48-immobilization:	48-h EC_{50} = 10,000	ASTM method, concentrations measured, static, non-GLP	Valid	Alexander (1988)
Water flea *D. magna*	21-d, survival: 21-d molting success, growth: 21-d reproduction:	NOEC = 3160 NOEC = 3160 NOEC = 3160	OECD 202, renewal, concentrations measured GLP	Valid	Caspers (1998)
Mysid shrimp (marine) *Mysidopsis bahia*	96-h, survival:	96-h LC_{50} = 1100	ASTM method, concentrations measured, flow through, non-GLP	Valid	Alexander (1988)
Copepod (marine) *Acartia tonsa*	5-d larval growth:	EC_{10} = 100	No report available, cited in EU RA (Feb. 01), experimental details unknown, examined metamorphosis through different life stages, results not reported, non-GLP	Not valid	Anderson (2000)
Copepod (marine)	48-h survival:	LC_{50} = 3400 to 5000	methods generally described, paper's	Use with	Kusk (1999)

Table 1. (continued)

Species	Endpoint	Result (μg/L)*	Comment	Status	Reference
A. tonsa			intent was to compare aqueous marine test media	care	
Copepod (marine) *A. tonsa*	72-h immobilization	EC_{50} = 960	acute ISO method (no. not reported), details not reported	Not valid	Anderson (1999)
Copepod (marine) *A. tonsa*	12-d stimulated egg production:	Effect levels based on feeding with BPA-sorbed algae were unstated	renewal test, dosing with sorbed BPA on algae, feed concentrations not reported	Not valid	Anderson (1999)
Chironomid *Chironomus riparius*	Life-cycle test, emergence, egg production, numbers, sex ratio:	Effect levels based on sediment concentrations were unstated	Incorporated aqueous BPA in 15% organic matter artificial sediment, concentrations not reported or estimated	Not valid	Watts (2001)
Prosobranch snails *Marisa cornuarietis* *Nucella lapillus*	3 to 12 months, stimulated egg production, female and male gonadal malformations:	LOEC = 1, reports both feminization and viralization in females (*Marisa*), feminization of females & males (*Nucella*)	method development studies, no replicates, nominal concentrations, egg production, sex organ malformations	Not valid	Oehlmann (2000)
Green algae *Selenastrum capricornutum*	96-h, cell count, reproduction: cell volume, growth:	96-h EC_{50} = 2730 96-h EC_{10} = 1360-1680 96-h EC_{50} = 3100 96-h EC_{10} = 1360-1680	USEPA methods, static, measured concentrations, non-GLP	Valid	Alexander (1988)
Diatom (marine) *Skeletonema costatum*	96-h, cell count, reproduction: chlorophyll a, growth:	96-h EC_{50} = 1000 96-h EC_{10} = 400-690 96-h EC_{50} = 1800 EC_{10} = 400-690	USEPA methods, static, measured concentrations, non-GLP	Valid	Alexander (1988)

* When a NOEC is presented but no LOEC is presented, the NOEC occurred at the highest concentration tested.

Exposure duration to BPA appears to have few consistent effects on growth in fish. In a 33-day early life stage test with fathead minnows (*Pimphales promelas*), Caunter *et al.* (1999) noted adverse effects on growth only at 640 μg/L. In a two-generation study, also with fathead minnows, Caunter *et al.* (2000) noted that growth was reduced after 71 days in F_0 adults at 640 μg/L, but had recovered to control levels by exposure day 164. No effects on growth were observed at 640 μg/L at any time between days 30 and 431 in F_1 offspring or between day 30 and 260 days in F_2 offspring. Gonad weights in F_0 males and females were reduced in a consistent and dose-related manner only at 640 μg/L.

In recent work with swordtail fish (*Xiphophorus helleri*), Kwak *et al.* (2001) have suggested that tail length in male fish correlates with reproductive success. Reduced tail length was reported for fish exposed to 2.0 μg/L BPA (Kwak *et al.* 2001). However, this test is considered not acceptable as exposures were conducted under static conditions for 60 days and BPA concentrations were not measured. Static tests are inadequate to study readily biodegradable compounds like BPA.

Effects on growth have also been examined with two species of aquatic invertebrates, a freshwater algae, and a marine diatom. With the water flea, *Daphnia magna*, Caspers (1998) reported no effects on molting at 3160 μg/L, the highest concentration tested. Growth effects were reported by Anderson (2000) with the marine copepod *Acartia tonsa* (EC_{10} = 100 μg/L); however, there were few details available concerning the experimental procedures or the results, which would allow proper evaluation of the conclusions. The study (Anderson 2000) was therefore considered not valid for use in hazard assessment. Growth effects, as characterized by chlorophyll a concentration and cell volume over 96 hours of exposure, were reported for freshwater and marine green algae (Alexander *et al.* 1988). With the freshwater green algae, *Selenastrum capricornutum*, a range of EC_{10} values of 1360 to 1680 μg/L was reported, while in the marine diatom, *Skeletonema costatum*, growth effects were reported with a range of EC_{10} values of 400 to 690 μg/L.

Thus, the majority (7 of 12) of the acceptable studies (both "valid" and "use with care") had no effect on growth at the highest levels tested. Exposure to BPA does not reduce growth of vertebrates, invertebrates, and algae in a consistent and dose-related manner at concentrations below about 400 μg/L.

Reproductive Success

In order for wildlife communities and ecosystems to remain viable, populations of individual species that make up those communities and ecosystems must successfully reproduce. Measures of reproductive success include production of eggs, fertilization success, time to hatch and hatchability of eggs, survival of embryos, differentiation of sexes, sperm counts, sperm motility, and sperm length. Such chronic effects of BPA exposure have been examined in at least four fish, one amphibian, five invertebrates, and two algal species.

Six of the seven studies examining reproductive effects in fish were deemed acceptable ("valid" or "use with care"). A life-cycle test was conducted with zebrafish (*Danio rerio*) exposed to BPA for 155 days at nominal concentrations ranging from 94 to 1500 μg/L (Fraunhofer Institute 2000). F_1 generation eggs collected from breeding F_0 adults exposed to BPA were raised to maturity (120 days). Newly

hatched F_2 offspring were raised (exposed to BPA) through their early life stages (30 days). The most sensitive reproductive endpoint, fertilization rate, was reportedly reduced at 1500 μg/L, but not at 750 μg/L. Shioda and Wakabayashi (2000), in a 14-day exposure study with medaka fish (*Oryzias latipes*), noted that egg production and hatching were reduced at 2280 μg/L, but not at 684 μg/L. In a longer exposure, a 60-d test with medaka fish, Yokota *et al.* (2000) observed no adverse effects of BPA on time-to-hatch, overall hatchability, or embryo survival at 1820 μg/L. However, sex ratio was significantly skewed ($P<0.001$) to females at 1820 μg/L, but not at 355 μg/L. No skewing of sex ratio was observed by Metcalfe *et al.* (2001) in a 100-day test with medaka fish exposed to BPA at 120 μg/L. In a 431-d, two-generation test with fathead minnows, Caunter *et al.* (2000) observed that egg production was reduced in F_0 breeding pairs exposed to 1280 μg/L and further reduced in F_1 breeding pairs exposed to BPA at 640 μg/L; hatchability of F_1 and F_2 eggs was reduced at 640 and 160 μg/L, respectively. In the same study, the gonadosomatic index, which relates gonad weight to the total body weight of the individual, was significantly reduced at 640 μg/L, but not at 160 μg/L BPA (Caunter *et al.* 2000). In breeding pairs of guppies (*Poecilia reticulata*), neither gonad size nor sperm length were reduced after 21 days of BPA exposure at 549 μg/L; however, sperm counts were reduced following 21 days exposure to 274 μg/L (Haubruge *et al.* 2000). The authors attributed this reduction to blockage of the sperm production process through *in vivo* interference with normal Sertoli-cell function, rather than any changes or alteration in spermatogenesis (Haubruge *et al.* 2000). In brief, in acceptable studies examining fish reproduction endpoints following exposure to BPA, reduced egg production and fertilization rate, and altered sex ratios and gonadosomatic indices were observed at 640 μg/L and higher (NOECs of 120 to 160 μg/L), while hatchability was reduced at 160 μg/L (NOEC 16 μg/L).

Two independent studies on BPA have been conducted with the amphibian *X. laevis*. Kloas *et al.* (1999) exposed larvae to BPA for 12 weeks, measuring survival, growth, and sexual differentiation. Test concentrations of 0, 2.3, and 23 μg/L (nominal) were used in the static renewal test (three times weekly), but were never analytically verified. Because the objective of the test was method development, the test was not conducted according to the principles of Good Laboratory Practices (GLP). Further, control and documentation of temperature and other system conditions were never reported. In contrast, subsequent work by Pickford *et al.* (2000) with *X. laevis* was conducted under GLP for 90 days with BPA concentrations carefully maintained using a flow-through system and analytical verification of test concentrations. Concentrations used by Pickford *et al.* (2000) were 0, 1.0, 2.3, 10, 23, 100, and 500 μg/L and average recoveries of nominal concentrations ranged from 78 to 96%. Kloas *et al.* (1999) reported a significant skewing of sex ratio at the highest concentration of 23 μg/L (60% male in control versus ~40% male at 23 μg/L). Temperature variability may directly influence gender ratio in amphibians, as is known to occur in reptiles (Crews *et al.* 1989), and consistent thermostatic control was not attempted in the work of Kloas *et al.* (1999). However, in the second test (Pickford *et al.* 2000), conducted under GLP and carefully maintained test concentrations and incubation conditions, no skewing of sex ratios was found, even at 500 μg/L, the highest BPA concentration tested (approximately 50% males in controls and all test concentrations). Kloas *et al.* (1999) did not address why the sex ratio of

the control animals was not 50% males and females, but temperature variability could have been a factor, if amphibians are as sensitive as reptiles (Crews *et al.* 1989). Because of the absence of documentation of test system condition and maintenance, test concentrations and the skewed of control sex ratio (60% males), the first *X. laevis* test (Kloas *et al.* 1999) is not considered acceptable for use in aquatic hazard assessment. The second test (Pickford *et al.* 2000), conducted under GLP, with documented test system performance and the anticipated control sex ratio of 50%, is considered valid and hence should be used to assess effects on survival, growth, and sexual differentiation in amphibians represented by *X. laevis* exposed to BPA.

In planktonic organisms chronically exposed to BPA, Caspers (1998) reported no adverse effects on reproduction in the parthenogenic *D. magna* after 21 days at 3160 μg/L. Anderson *et al.* (1999) fed BPA-sorbed algae to the marine copepod *A. tonsa* for 12 days and examined reproductive parameters. The authors noted that egg production of the exposed copepods was stimulated above that of the controls. This stimulation reverted to normal within one day following movement of the exposed organisms to clean saltwater and therefore was not considered an adverse effect on the reproduction of *A. tonsa*. However, this study (Anderson *et al.* 1999) is not acceptable for risk assessment, since the exact dose of BPA ingested by the copepods is unknown, the bioavailability of the ingested algae-bound BPA was not considered, and the likely biotransformation of BPA within the copepods was not addressed.

Watts *et al.* (2001) conducted a full life cycle test with BPA-sorbed artificial sediment and the freshwater midge, *Chironomus riparius*. Midge larva were exposed to an unreported range of sediment concentrations until the adults emerged after 16 to 18 days incubation at 16°C. Time to emergence, sex ratio, and number of adults, egg production, and egg viability were measured. The artificial sediment had a 15% organic matter content and the authors were unable to confirm the BPA concentrations in the sediment. Based on BPA's octanol/water partition coefficient (K_{ow}) of ~2500 (Staples *et al.* 1998), the compound is expected to sorb substantially to organic sediments. In addition, BPA is readily biodegradable and dissipates rapidly when exposed to river water-containing sediment (Klečka *et al.* 2001; West *et al.* 2001). As Watts *et al.* (2001) did not measure BPA sediment residues or pore water concentrations, results pertaining to the reproductive success of the midge in this study are not meaningful. The work of Watts *et al.* (2001) therefore is not considered acceptable to support an aquatic hazard assessment on BPA.

Few studies have examined effects on mollusks exposed to BPA. A series of studies by Oehlmann *et al.* (2000) with two prosobranch snail species, the freshwater ramshorn snail (*Marisa cornuarietis*) and the marine dogwhelk (*Nucella lapillus*), examined the effect of BPA exposure on both spawning and malformations of female and male sex organs. The purpose of the research was to examine the utility of these species to study xenoestrogens, including BPA as a model compound. Three experiments were conducted. For all experiments, exposure was conducted using static-renewal (24- to 48-h exposure periods) of test media in single systems, *i.e.*, no replicate solutions were included, nor was analytical confirmation of BPA concentrations performed. The first experiment exposed adult *Marisa* to 1 to 100 μg/L BPA for five months. At monthly intervals, 30 snails were taken for examination of female anatomy and daily counts of egg masses and total number of eggs were recorded. The authors reported stimulation of egg masses and egg production

(roughly 20% at 1 μg/L to 300% at 5, 25, and 100 μg/L) greater than controls and observations of malformed female sexual organs at all concentrations of BPA. No adverse effects on male gonads were reported. Mortality rates of about 15% across all concentrations were reported (control 3.8%), and the authors postulated that mortality was biased towards females. Although not statistically significant, sex ratio appeared to be shifted slightly towards greater number of males in all treatment groups; however, the basis for this type of effect was related to the potential for greater female mortality and not a feminization of males. Further, histopathological analyses of female and male gonad tissue showed that neither spermatogenesis nor oogenesis was altered at any dose level. In a second experiment with *Marisa*, offspring from the first test were raised for one year at BPA test concentrations of 1 or 100 μg/L (Oehlmann *et al.* 2000). Increases in egg masses, egg production, and mortality were reported at both concentrations; however, no effects on male gonads were reported. Enlarged or even ruptured female sex organs and glands associated with egg production were reported at all concentrations. Analysis of female gonad tissue reportedly showed significant increase in imposex intensity ($P<0.001$) at 100 μg/L. Imposex intensity is a scale based on the vas deferens sequence index, which scores the apparent degree to which male structures have formed within female gonad tissue, and indicates a masculization or virilization effect. The imposition of the vas deferens tissue disrupts the structure of the oviduct and function, disrupting breeding activity (Matthiessen and Gibbs 1998). This increase in virilization within the female gonad tissue is in contrast to the feminization process with *Marisa*, indicated by stimulated egg production and female sex organ enlargement. In addition, these data contradict the results reported with other invertebrates that had no estrogenic response with BPA (Caspers 1998) and studies with fish that reported feminizing effects on male fish upon exposure to BPA, resulting in reduced egg production and hatchability at 160 to 640 μg/L (Caunter *et al.* 2000) and skewed sex ratios toward females (Yokota *et al.* 2000) at 1820 μg/L. In a third experiment with mollusks, adult *Nucella* were exposed to 1, 25, and 100 μg/L BPA for a 3-month period (Oehlmann *et al.* 2000). Enlargement of the female organs and increases in egg production were reported at all test concentrations. In addition, the number of males with sperm stored in their seminal vesicles was reduced at all doses and penis and prostate gland lengths were reduced at all doses ($P<0.05$). If penis length correlates with the ability of the male and female to mate, the reductions in size were apparently not biologically significant.

In summary, the effects of BPA on the mollusks, *Marisa* and *Nucella* snails, are difficult to ascertain. With *M. cornuarietis*, the experiments showed no feminization of males, but did show a statistically significant increase in the feminization of females at all concentrations. However, in contrast to the feminization of the *Marisa* females was other evidence of masculinization of females (imposex) at 100 μg/L. The experiment with *Nucella* reported feminization effects in both females (stimulated egg production) and males (reduced penis and organ lengths). Other difficulties with these studies are that the *Marisa* population originated from a breeding colony at a zoo. It is not known how wild populations of this Central and South American species would react to aqueous exposures of BPA. Due to flaws in the experimental designs that were used (*e.g.*, no replicates and no analytical confirmation of test concentrations), with the endpoints examined (*e.g.*, increased female sex

organ weights that has not been experimentally observed previously by the authors), and by the reported feminization and masculization results, this study is considered to be not valid for use in the aquatic hazard assessment on BPA. Considerable additional research on the life history of prosobranch snails using wild populations is needed before these mollusk species can be successfully used as a hazard assessment tool.

In summary, reproductive success in aquatic organisms has been assessed for BPA using acceptable tests with fish, amphibians, invertebrates, and algae. For fish species, exposure studies examining reproduction have been carried out for 14 to 100 days, covering the early life stages of development (time for eggs to hatch, numbers of eggs hatched, embryo survival, and sex ratio), for multiple generations measuring reduced egg production, hatchability, gonad weights, and effects on breeding adults, including egg production and hatch, gonad weight, and sperm condition. The lowest-observed effect concentrations (LOECs) observed with BPA and these endpoints ranged from 160 to 2280 μg/L. No-observed effect concentrations (NOECs) for fish chronically exposed to BPA ranged from 16 to 1820 μg/L. For invertebrates, no adverse effects were reported at the highest concentration tested, 3160 μg/L. In green algae, adverse effects on growth began at concentrations above the range of 400 to 1680 μg/L.

Secondary Biochemical and Morphological Data

Several studies have examined the interaction of BPA with the estrogen receptor in fish, or included such endpoints in studies that examined more traditional endpoints of survival, growth, and reproduction. Additionally, various endpoints have been studied that examined tissue-level alterations following exposure to BPA. Together, these endpoints provide supplemental information on the effects of exposure to BPA, but are themselves not suitable for defining hazard in aquatic organisms. The collected secondary biochemical and morphological data for aquatic organisms exposed to BPA are shown in Table 2.

Various studies have investigated the stimulation of the egg protein, vitellogenin, in the liver, generally by quantifying vitellogenin levels in serum. The induction of vitellogenin has been reported in male amphibians (*X. laevis)* following exposure to 23 μg/L BPA for 1.5 days (Kloas *et al.* 1999), at 2000 μg/L after 3 days in swordtail fish (Kwak *et al.* 2001), at 100 μg/L after 12 days in rainbow trout (*Oncorhynchus mykiss*) (Lindholst *et al.* 2000), and at 160 μg/L in F_0 males and F_1 and F_2 males and females during a two-generation test with fathead minnows (Caunter *et al.* 2000). Collectively, these data indicate that BPA has the potential to induce vitellogenin production in vertebrate species. However, the induction of vitellogenin does not generally correlate well with conventional reproductive endpoints. For instance, vitellogenin induction by *X. laevis* was reported at 23 μg/L after 1.5 days exposure (Kloas *et al.* 1999), but no effects on sex ratio were reported at 500 μg/L in a repeat test (Pickford *et al.* 2000). Reproductive effects were reported at 640 μg/L and higher for all endpoints except hatchability (effects at 160 μg/L), while vitellogenin was reported at all BPA exposures of 160 μg/L and higher (Caunter *et al.* 2000).

Studies examining tissue-level effects following BPA exposure include a 72-h test with swordtail fish (*X. helleri*) that reported testis cell damage and apoptosis occur-

Table 2. Secondary biochemical and morphological parameters.

Species	Endpoint	Result (µg/L)	Comment	Reference
Rainbow trout *Oncorhynchus mykiss*	12-day VTG induction	NOEC = 70, LOEC = 100	non-standard protocol, flow-through, measured concentrations, non-GLP	Lindholst (2000)
Swordtail fish *Xiphophorus helleri*	72-h VTG expression 72-h testis cell damage, apoptosis	NOEC = 400, LOEC = 2000 NOEC = 2000, LOEC = 10,000	non-standard methods, renewal, nominal only, non-GLP	(Kwak (2001)
Carp *Cyprinus carpio*	28-d oviduct formation in testis 56-d oviduct formation in testis	NOEC = 60, LOEC = 230 NOEC = 17, LOEC = 60	28-day, modified juvenile growth test OECD 215, non-GLP, abstract only	Bowmer (1999)
Medaka fish *Oryzias latipes*	60-d, testis-ova (male) 60-d, ovarian abnormalities (female)	NOEC = 355, LOEC = 1820 NOEC = 1820	modified OECD 210 ELS test, renewal until hatch, then flow-through, non-GLP	Yokota (2000)
Medaka Fish *O. latipes*	100-d post-hatch, testis/ova (male): 100-d post-hatch, oogenesis advanced (females): Testicular fibrosis:	reported in 2/25 male fish at 10 µg/L, but 0/31, 0/28, and 0/24 at three highest levels NOEC = 100, LOEC = 200 NOEC = 10, LOEC = 50	renewal, measured concentrations, non-GLP	Metcalfe (2001)
Medaka Fish *O. latipes*	100-d testis-ova (male) 35-d induction of female-specific protein (fsp) in males: 14-d induction of fsp in males:	NOEC = 100 LOEC = 10 LOEC = 100	general procedures described, sex ratio skewed to more males, contradicts feminization (increased fsp in males), only 1 in 16 males had T/O, no statistics, nominal-only	Tabata (2001)
Fathead minnow *Pimephales promelas*	F_0 males, VTG induction F_1 males and females, VTG induction	NOEC = 16, LOEC = 160 NOEC = 16, LOEC = 160	multi-generation study, F_0 breeding adults exposed, F_1 eggs hatch, grow to adults, F_1 adults bred, F_2 eggs hatch, survive, grow, total exposure 431 days, measured concentrations, based on USEPA lifecycle method USEPA 540/9-86-137, July 1986, GLP	Caunter (2000)
African clawed frog *Xenopus. laevis*	36-hour, VTG induction	NOEC = 2.3, LOEC = 23	larval development, hatch to maturity, renewal, nominal-only, method development paper, non-GLP	Kloas (1999)

ring at 10,000 μg/L (Kwak *et al.* 2001) and a 28-day test with carp (*Cyprinus carpio*) that reported the formation of partial oviducts in testis tissue at 230 μg/L (Bowmer and Borst 1999). Other studies have reported finding indications of ova tissue forming in testis (testis-ova). Yokota *et al.* (2000) reported the formation of testis-ova in medaka fish (*Oryzias latipes*) following 60 days of exposure at 1820 μg-BPA/L, although no testis-ova were observed at doses of 355 μg/L or less. In contrast, in a 100-day exposure, Metcalfe *et al.* (2001) observed testis-ova in 2 of 25 medaka fish at 10 μg/L, but no testis-ova were reported in tissue from fish exposed at 50, 100, and 200 μg/L. Thus, the finding of testis-ova in only 2 of more than 100 fish and only at the lowest concentration suggests that the testis-ova Were not formed in medaka fish in a dose-related fashion attributable to BPA exposure. In summary, testis-ova formation in medaka fish was reported only at a concentration of 10 μg/L, but not at 50 to 200 μg/L by Metcalfe *et al.* (2001) and at concentrations 100-fold higher by Yokota *et al.* (2000). Therefore, it is unlikely that BPA was responsible for the formation of testis-ova observed by Metcalfe *et al.* (2001) at 10 μg/L. While Metcalfe *et al.* (2001) also reported observations of morphological changes in testis structure at 50 to 200 μg/L, no impacts on sex ratio were observed at any test concentration.

The relative proportion of cell types in stages of oogenesis and spermatogenesis have been examined in fish chronically exposed to BPA. In a 60-day study with medaka fish, no abnormalities of the gonads in female fish were reported at the highest concentration tested, 1820 μg/L (Yokota *et al.* 2000). In a 100-day study, also with medaka fish, oogenesis was reported to be advanced after exposure to 200 μg/L (Metcalfe *et al.* 2001). However, this was a qualitative observation and was not deemed statistically significant. In a two-generation study with fathead minnows, gonadal cell structure and the relative proportion of cell types encountered during spermatogenesis were assessed (Caunter *et al.* 2000; Sohoni *et al.* 2001). In males, none of the testes examined contained oocytes, and no other unusual features were observed. In females, no effects on oocyte development after 164 days of exposure were observed at any test concentration. A significant retardation of spermatogenesis occurred in the highest two treatments in F_0 males (640 and 1280 μg/L) and in the highest treatment in F_1 males (1280 μg/L), which follows the observations of overt toxicity with the conventional endpoints of survival, growth and reproduction at the higher concentrations (640 to 1280 μg/L). Potential effects at lower concentrations of BPA did not correlate well with adverse effects to the organism. Considerable additional research is required on the standardization of methods to analyze and interpret histological alterations (cell structure and gametogenesis) in fish. In addition, research into the natural variability of spermatogenesis in fathead minnows is needed to better place these secondary findings in perspective. Such work will help us to understand the utility and applicability of these secondary morphological data in interpreting ecologically relevant reproductive endpoints.

HAZARD ASSESSMENT

Approximately 60 NOECs and LOECs covering numerous ecologically relevant endpoints are available to evaluate the survival, growth, and reproductive success of aquatic organisms following exposure to BPA. These chronic endpoints have been

derived from more than 23 acceptable ("valid" or "use with care") studies conducted with 10 different species of aquatic organisms representing fish (six species), amphibians (one species), invertebrates (one species) and aquatic plants (two species). As illustrated in Figure 2, all but one of the approximately 60 LOECs and NOECs across all endpoints fall between 160 and 11,000 μg/L BPA. The single exception represents the two-generation NOEC derived for hatchability of the F2 generation of fathead minnows (16 μg/L). Because this NOEC value for BPA of 16 μg/L is based on a multigeneration fish test (Caunter *et al.* 2000) using a relevant and well-studied fish species, it can be considered a worst case estimate of levels that may cause ecologically relevant effects on aquatic organisms.

The abundant acute and chronic toxicity data from laboratory studies with BPA can be used to support an ecological risk assessment for the aquatic environment. There are numerous procedures to extrapolate effect data from the laboratory to the field (Chapman *et al.* 1998). The extrapolation procedures attempt to address uncertainties between laboratory and field systems. An approach commonly employed by ecological risk assessors is to divide a lower-bound toxicity value by an application or safety factor (EC 1996). In the case of a recent risk assessment conducted by the UK Environment Agency (2001) for the E.U. Existing Chemicals Program, an assessment factor of 10 was applied to the lowest NOEC of 16 μg/L, yielding a predicted no-effect concentration (PNEC) for BPA of 1.6 μg/L. However, according to Chapman *et al.* (1998), the use of application factors was originally intended to be attenuated by greater amounts of toxicity data and not be rigidly applied.

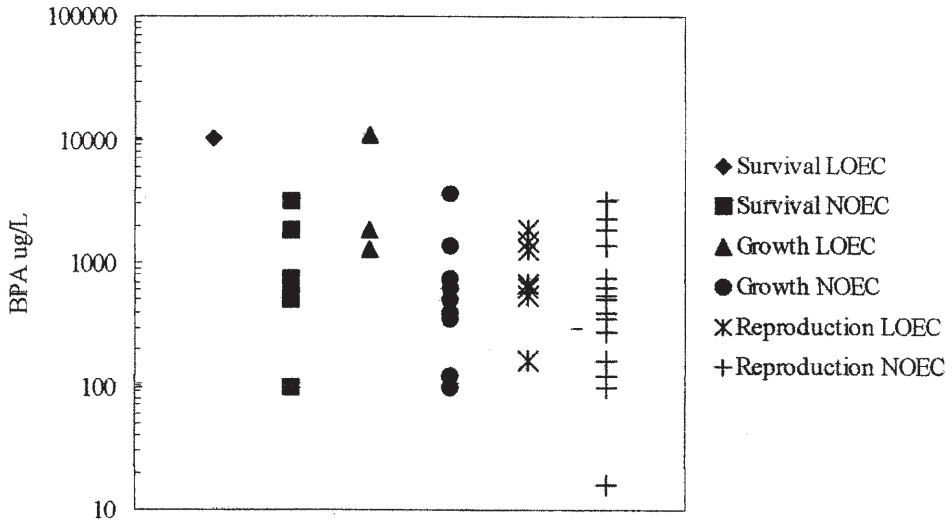

Figure 2. Examination of lowest observed effect concentrations (LOECs) and no observed effect concentrations (NOECs) for aquatic organisms chronically exposed to BPA. Results only for valid ("valid" and "use with care") studies are shown here. Endpoints include survival, growth (body length and body or organ weight) and measures of reproductive success (egg production, time to hatch, hatching success, embryo survival, sexual differentiation, and condition of sperm).

Another method used to address uncertainty is the measurement of one or more endpoints numerous times, then assigning the greatest weight to results that are confirmed by a combined evaluation of the procedures and approaches used, *i.e.*, weight of evidence (Chapman *et al.* 1998). Applying these principles in the context of BPA, we looked at the concentrations at which numerous NOEC and LOEC values clustered. As shown in Figure 2, approximately equal numbers of NOEC and LOEC values exist for the endpoints of survival, growth, and reproduction. All but one value clusters between 160 to 11,000 μg/L BPA. Thus, the weight of evidence from the abundant toxicity dataset for BPA suggests that aquatic effects on mortality, growth, and reproduction are most likely to occur between the concentrations of 160 to 11,000 μg/L, based on repeated measurements of these endpoints.

Statistical extrapolation procedures can also be used where there is a large database of ecotoxicity data to estimate no-effect concentrations that are protective of aquatic populations and ecosystems (USEPA 1995; van Leeuwens and Hermens 1995). While prominently used by the USEPA (1995) to develop national ambient water quality criteria, considerable debate exists in the E.U. as to the utility of such approaches. Recently, a workshop sponsored by the European Chemicals Bureau (2001) proposed that at least 8, if not 10 or 15, different species from different taxonomic families should be tested using chronic assays to develop statistically based no-effect concentrations. Sufficient chronic data (≥ 8 species, a single endpoint and a NOEC for each species) do not yet exist for BPA to employ statistical methods, however, the addition of only a few additional studies should enable the calculation of a statistically based no-effect value that is considered protective of aquatic populations and ecosystems. While standardized and validated tests may not yet exist for some of these additional studies, sound test procedures that have previously been used are available. However, it is important to note that standard, validated test procedures do not exist for many of the proposed test species that would be required.

Although the existing chronic toxicity dataset for BPA is not as extensive as the database discussed recently by the European Chemicals Bureau (2001), Staples *et al.* (2001) have used the available data to calculate a refined PNEC for BPA using the HC5 approach previously described by van Leeuwen and Hermens (1995). Based on chronic endpoints of growth and reproduction, the refined PNECs for BPA ranged from 145 μg/L to 177 μg/L. Further sensitivity analysis revealed that the addition of hypothetical "new" and lower NOECs to the database does not appreciably alter the PNEC, suggesting that the existing database is sufficient for deriving values that will be protective of the aquatic environment. For instance, the exclusion of algal data from the dataset and only using fish and invertebrate reproduction data with the addition of one "hypothetical" new NOEC at a 1 μg/L, gives a refined PNEC of 94 μg/L BPA.

Aquatic hazard assessment of endocrine active substances should include the examination of relevant endpoints under the control of the endocrine system. As established by the multigeneration study with fathead minnows, BPA is a weakly estrogenic substance (Caunter *et al.* 2000; Sohoni *et al.* 2001). Test guidelines that generate data useful for aquatic hazard assessment, which in turn will be the basis of ecological risk assessment, provide for the assessment of ecologically relevant endpoints, including development, growth, reproduction, and survival. It is these

ecologically relevant endpoints that are appropriate to assess the effects of endocrine-active substances (Campbell and Hutchinson 1998). The sustainability of an ecosystem is based on the competition between individuals of the populations that make up the ecosystem. Competition or natural selection affords the greatest opportunity to individuals that are best able to survive, grow and develop, and reproduce. Therefore, it should be the goal of the aquatic hazard assessment process for endocrine active substances to assess the most critical factors, particularly reproductive performance (Campbell and Hutchinson 1998).

Reproductive performance is central to evaluating the effects of exposure to endocrine-active xenobiotic substances (Campbell and Hutchinson 1998). Secondary behavioral and morphological effects support the interpretation of mechanisms by which the effects on individual reproductive performance occur. Individual reproductive performance (*e.g.*, behavior, growth, and development of sexual organs) directly impacts a population's reproductive fitness as measured by such effects as reduced fecundity or hatchability. Reduction in population abundance or elimination of populations can damage an ecosystem's structure and function. As part of this overall aquatic hazard assessment for BPA, data are now available that evaluate the reproductive fitness of fish populations. In a multigeneration, 431-day study with fathead minnows (Caunter *et al.* 2000; Sohoni *et al.* 2001), effects were observed on various reproductive endpoints at concentrations of 160 μg/L and higher. No adverse effects were noted on reproductive success endpoints at chronic BPA concentrations of 16 μg/L or less. These data are supported by numerous studies described above with other species and test procedures that have also addressed reproduction, survival, and growth. Thus, aquatic hazard data are available for BPA that are suitable to assess potential risks to populations and ecosystems. Use of the weight-of-evidence approach for hazard assessment shows that effects on survival, growth, and reproduction are expected to occur at concentrations between 160 and 11,000 μg/L BPA and not below this range. The use of the van Leeuwens and Hermens (1995) method of calculating a refined PNEC with the available data suggests that a refined PNEC for BPA of approximately 100 μg/L is protective of populations and ecosystems. Typical ambient concentrations of BPA in surface waters from around Europe (UKEA 2001) are in the range of 1 to 100 ng/L (0.001 to 0.10 μg/L). A recent study of 85 U.S. sites directly downstream of intense urbanization, livestock production, and sewage outfalls showed that BPA concentrations at 75% of all sites were less than the method quantitation limit of 0.09 μg/L (Kolpin *et al.* 2002). Thus, adverse impacts on aquatic organisms, populations, or ecosystems are unlikely due to the lack of overlap between routine exposure to BPA and chronic endpoints relating to survival, growth, or reproduction.

DISCUSSION

The issue of endocrine disruption in wildlife species has generated an immense amount of scientific, political, and public interest over the last several years (Colborn *et al.* 1993; Tattersfield *et al.* 1997; and Kendall *et al.* 1998). Endocrine-disrupting chemicals can, as a consequence of their molecular structure, bind to hormone receptors and may mimic or antagonize the action of the natural hormonal ligand. Naturally occurring endocrine-disrupting chemicals, or phytoestrogens, found in

soy, alfalfa, clover, and other vegetables may exhibit endocrine activity by directly or indirectly interfering with metabolic activity in a variety of wildlife species (Findley *et al.* 1973; Verdeal *et al.* 1980; Maclatchy and van der Kraak 1995). Pelissero *et al.* (1991) and Latonnelle *et al.* (2000) have examined phytoestrogen activity in rainbow trout and Siberian sturgeon (*Acipenser baeri*) and have found these compounds to be far more potent disruptors of endocrine activity than most xenobiotic chemicals. Previous studies have shown that endocrine-disrupting chemicals released via sewage outfalls may have an adverse impact on aspects of sexual development with some resident fish species. These findings were initially attributed to industrial chemicals with a low level of endocrine activity, but now are largely believed to be due to presence of human hormones in wastewater (Desbrow *et al.* 1998).

It has been demonstrated in laboratory studies that BPA is a weak estrogen, with a potency of 3×10^{-4}, relative to 17β-estradiol (Metcalfe *et al.* 2001). The presence of aquatic residues of BPA in industrial wastewater raises the question of whether environmental concentrations of BPA may adversely impact fish and wildlife populations. The environmental toxicology of BPA has been studied extensively in multiple species, and at several trophic levels of ecosystems. The collective weight of evidence from the abundant toxicity dataset for BPA suggests that aquatic effects on mortality, growth, and reproduction are not expected below test concentrations of 160 μg/L.

Adverse impact should be judged on the basis of environmentally relevant endpoints that affect populations and communities of organisms, such as survival, growth, and reproductive success. Matthiessen (2000) examined the laboratory and field data associated with endocrine-disrupting chemicals and their effect on wildlife species. He reports that although endocrine disruption has indeed been observed as a mechanism of toxicity in some invertebrate and vertebrate wildlife populations, few population-level effects can be demonstrated. For example, while fish populations in the United Kingdom are exposed to endocrine active compounds in wastewater effluents, there is no current evidence of major population declines (EDMAR 1997). This lack of impact has been attributed to the resilience of populations and ecosystems to perturbing influences and the sexual plasticity and redundancy of fish populations (Mathiessen 2000). Similarly, Dawson (2000) has examined endocrine disruption in avian wildlife and found that while in vitro data indicate xenobiotic chemicals can mimic gonadal steroid actions, in vivo evidence of endocrine disruption due to such chemical exposure is lacking and concluded that there is no evidence that xenobiotic chemicals are able to overwhelm endogenous homeostatic endocrine control (Dawson 2000). While endocrine disruption has been cited as a possible cause for eggshell thinning and supernormal clutches in some avian species, Dawson (2000) found little data supporting this conclusion.

A number of new parameters and endpoints to address mechanisms of action are now being added to traditional chronic ecotoxicity studies. Many of these endpoints have been suggested by research programs or regulatory initiatives (*e.g.*, EDSTAC, EMSG) that are being developed to assess endocrine disruption potentials. The endpoints frequently include vitellogenin levels in male fish, blood hormone levels, and histology of reproductive tissues. While these additional parameters provide additional insight into mode of action, mechanism, and biomarkers of exposure, for example, most are still in the "development" stage, *i.e.*, correlation with conventional endpoints and relevance to the environment are still uncertain. Few positive

control studies have been conducted to assess the population- and ecosystem-level applicability of these parameters. For example, vitellogenin appears to be a useful biomarker for exposure, but it is unclear if induction of vitellogenin in male fish leads to adverse effects at the population or community level (Mathiessen 2000). Additional work is needed to validate these supplemental endpoints and establish the relevance to effects on populations and communities.

It is important to note that many of these new supplemental endpoints require sophisticated techniques and significant technical expertise. Often, only a limited number of laboratories with specialized expertise can reliably perform some of these procedures. Other studies are being conducted using novel test organisms that are not generally available to the scientific community, nor has their relevance to the environment been established. Because of the sophistication and expertise required, as scientists explore the utility of these methods and report on their findings, potentially conflicting reports can be found in the literature. This will add to the confusion and create problems for the regulatory community. Hence, we strongly recommend that risk assessment and risk management of chemicals be based on the weight of evidence from studies of acceptable quality that measure conventional ecotoxicological endpoints related to survival, growth, and reproduction, which are generally accepted as relevant to assessing the impact of chemicals at the population/community level.

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