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ScienceDirect
Reproductive Toxicology 24 (2007) 225–239
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Review

An ecological assessment of bisphenol-A: Evidence from comparative biology

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Received 7 March 2007; received in revised form 9 May 2007; accepted 11 May 2007
Available online 18 May 2007

Abstract

This review assesses the effects of environmental concentrations of bisphenol-A (BPA) on wildlife. Water concentrations of BPA vary tremendously due to proximity to point and non-point sources, but reported concentrations in stream/river water samples are less than 21 μg/L, and concentrations in landfill leacheate are less than 17.2 mg/L. Extensive evidence indicates that BPA induces feminization during gonadal ontogeny of fishes, reptiles, and birds, but in all cases the amount of BPA necessary to cause such ontogenetic disruption exceeds concentrations in the environment. Extensive evidence also exists that adult exposure to environmental concentrations of BPA is detrimental to spermatogenetic endpoints and stimulates vitellogenin synthesis in model species of fish. Most of the reported effects of BPA on vertebrate wildlife species can be attributed to BPA acting as an estrogen receptor agonist, but mechanisms of disruption in invertebrates are less certain. A comparison of measured BPA environmental concentrations with chronic values suggests that no significant margin of safety exists for the protection of aquatic communities against the toxicity of BPA. Further studies should examine the most vulnerable vertebrate and invertebrate species.

Keywords: Bisphenol-A (BPA); Wildlife; Reproduction; Environmental concentrations; Vitellogenin; Landfill leachate; Spermatogenesis

Contents

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doi:10.1016/j.reprotox.2007.05.008

1. Introduction

Of all the xenobiotics registered for use by the EPA, the monomer bisphenol A (BPA, 2,2-bis-(4-hydroxyphenyl)-propane; CAS Registry No. 80-05-7) has perhaps generated the greatest amount of interest and controversy during the past decade. BPA is one of the key raw materials used to manufacture polycarbonate plastic and epoxy resins; as a result, approximately 1.7 billion pounds of BPA are synthesized and used in the United States per year, leading to BPAs designation as a high production volume chemical [1]. Currently, there is much scientific debate concerning the potential for low doses of BPA to cause adverse reproductive and developmental effects in humans, with some scientific reviews concluding there is little evidence to support this hypothesis (e.g., [2,3]) and other reviews citing overwhelming evidence for low-dose effects in humans (e.g., [4–6]). In this review, we are not attempting to settle this controversy, but will address a different question—are the concentrations of BPA found in the environment potentially harmful to wildlife?

Most studies examining the effects of BPA have been conducted on rats and mice, but the past few years have seen a dramatic increase in research examining BPA exposure on selected invertebrates, fishes, amphibians, reptiles, birds, and wild mammals. The purpose of this review is to examine the influence of BPA on these animals in order to ascertain the ecological impacts of BPA in the environment. Such a review is needed to (1) determine the scope of BPA's effects on various animals and (2) provide an assessment of these effects at ecologically relevant BPA concentrations.

2. BPA in the environment

Human exposure to BPA occurs primarily via hydrolysis of polycarbonate plastics and epoxy resins, resulting in low concentrations of free BPA in food and liquids [7]. This makes dietary consumption the major mode of human exposure [8]. The release of BPA from plastics has been recognized since the early 1990s, when yeast culture media were discovered to contain estrogenic activity neither deriving from the media nor the yeast, but the polycarbonate flasks [9]. Since that recognition, numerous human exposure models have been generated; these models estimate the daily intake of BPA through food consumption to be less than 10 μg/(kg day) [10,11], below the current US EPA oral reference dose (the daily dose deemed without appreciable risk of deleterious effects over a lifetime) of 50 μg/(kg day).

While the source for human exposure to BPA is food and liquid storage containers, BPA is released into the environment through either sewage treatment effluent (via human-ingested BPA being eliminated through sewage; [12]), landfill leachate (via hydrolysis of BPA from plastics; [13]), or natural degradation of polycarbonate plastics. Whereas sewage effluent and landfill leachate are point sources of BPA in the environment, fragments of epoxy resins and polycarbonate plastic debris entering the watershed through runoff are a non-point sources, creating challenges for remediation. BPA can leach from polycarbonate plastic when exposed to heat [14]. Although BPA degrades readily in microbe rich environments [15], its presence as fragments of polycarbonate creates a non-point source for slow leaching as synthetic polymers make up the majority of anthropogenic debris in watersheds and in the marine environment. Studies quantifying marine debris washed ashore consistently report that 60–80% of the volume is plastic [16]. An investigation of pelagic plastics in the eastern region of the North Pacific Ocean approximately 1000 km west of the United States estimated that the weight of plastic floating on the ocean surface outweighs the surface biomass by a ratio of 6–1 [17]. Plastics found washed ashore or floating on the ocean surface are typically positively buoyant, yet most plastic produced, approximately 54%, is negatively buoyant [18], including polycarbonate plastic and epoxy resins. Negatively buoyant plastics have been found in the marine environment worldwide, and represent the majority of marine debris on the seafloor [19–23].

Rivers, lakes, and estuaries are major sinks for BPA. These surface waters accumulate BPA leached from plastic debris and landfill wastes along with BPA-containing sewage and effluent. In aerobic environments such as most rivers, BPA has an environmental half-life of between 4.5 and 4.7 days [24], being degraded primarily by bacteria [25]. However, BPA has lim-

ited biodegradation under anaerobic conditions [26], leading to concerns about BPA accumulation in anaerobic sediments of habitats such as estuaries [27]. Indeed, sediment concentrations are often greater than water concentrations; simultaneous sampling on the Aja River, Japan showed the water concentration to be 0.058 μg/L BPA, whereas sediment concentration was 11 μg/kg [28]. Few studies have quantified BPA concentrations in animal tissues, but concentrations up to 15 μg/kg have been measured in fish in Japan [29].

Water samples vary greatly in their concentration of BPA depending on location and time of sampling. For instance, river water sampling and analyses in Germany have found BPA concentrations between 0.5 and 702 ng/L [30,31], in the United States between non-detectable and 12 μg/L [32], and in Japan up to 19 μg/L [29]. For the majority of rivers sampled to date, concentrations of BPA fall below 0.1 μg/L and very few samples contain BPA concentrations above 1 μg/L. In non-surface water, concentrations vary between non-detectable and 17.2 mg/L [33,34]. Table 1 presents maximum detected BPA concentrations in waters around the world.

Due to such dramatic variation in environmental water concentrations of BPA, the question arises as to what concentration to consider as a typical environmental exposure for aquatic animals. The Bisphenol A Global Industry Group (a coalition of the American Plastics Council, the Association of Plastics Manufacturers in Europe, and the Japan Chemical Industry Association) reports environmental concentrations of BPA as median concentrations of numerous environmental samplings (http://www.bisphenol-a.org). It is our view that a more relevant exposure concentration is the maximum amount detected at a site. This is because acute exposure to "organizational disruptors" such as BPA can have long-term detrimental consequences if exposure happens during critical windows of development. While mean or median concentrations are appropriate values to consider for activational disruptors (substances activating a change in an already formed organ; [137]), organizational disruptors must be considered at the maximum concentration found in the environment as short-term exposure to these concentrations has permanent effects. The Bisphenol A Global Industry Group notes that Kolpin et al. [32] showed that the median detected concentration of BPA in US streams was 140 ng/L. While this is true, the study included a survey of 139 streams, many of which were not in the proximity of a BPA source (sewage effluent or landfill leachate). Thus, the important value produced in the study by Koplin et al. [32] is the maximum—12 μg/L.

We view the maximum measured concentration at a given location as the most relevant BPA exposure concentration because this compound is capable of eliciting reproductive toxicity following short-duration exposure during critical windows of development (organizational effects as discussed below). For instance consider the zebrafish (*Danio rerio*), a model fish species used in aquatic toxicology. Zebrafish embryos that are exposed to 2280 μg/L BPA for only 48 h have elevated brain

Table 1
Maximum environmental concentrations of BPA measured in representative independent assessments

Maximum measured (μg/L)	Sample	Location	Reference
Surface waters			
21	River water	The Netherlands	[114]
19	River water	Japan	[29]
12	Stream water	United States	[32]
0.90	Surface water	Japan	[115]
0.60	Surface water	Austria	[116]
0.494	River water	Italy	[52]
0.41	Surface water	Germany	[30]
0.330	Estuary	The Netherlands	[114]
0.23	River water	Japan	[33]
0.158	Canal water	United States	[117]
0.147	River water	United States	[118]
0.058	River water	Japan	[28]
0.030	Lagoon water	Italy	[119]
0.016	River water	Germany	[31]
0.002	Drinking water	Germany	[31]
Non-surface waters			
17,200	Landfill leachate	Japan	[34]
5,400	Landfill leachate	Japan	[56]
5.1	Treated landfill leachate	Japan	[56]
1.7	Untreated septage	United States	[120]
1.5	Sewage treatment influent	Spain	[121]
0.27	Sewage treatment effluent	Spain	[121]
1.4	Landfill plume	United States	[120]
1.2	Crude wastewater	UK	[122]
0.046	Treated waste water	UK	[122]
0.93	Ground water	Austria	[116]
0.70	Sewage treatment effluent	Germany	[30]
0.49	Sewage treatment effluent	Sweden	[123]

P450aromB, which could result in a feminized brain as P450 aromatase-synthesized estrogen is known to have permanent organizing effects on the developing central nervous system [35]. While this is a high environmental concentration, it illustrates that acute exposures can have significant influence on the developing endocrine system and, thus, the maximum concentration to which an organism is exposed is most relevant to long-term outcome. In summary, studies in fish show us that short-term exposure to BPA can have delayed, sometimes permanent effects on physiology; thus, the maximum concentration in the environment, not the mean or median, should be used to evaluate potential risk.

BPA is rapidly degraded in the environment through both microbial biodegradation and photodegradation and has a low potential to bioaccumulate in animals [36]. Aquatic organisms that are in proximity of point source outputs of BPA are at the greatest risk of harmful effects of BPA. As a result, this review will focus on such aquatic species and the potential for maximum measured environmental concentration of BPA to alter normal structure and function in these species.

3. Effects of BPA on wildlife

Bisphenol A acts as both a teratogen and an endocrine disruptor in various vertebrates. Most teratogenic effects in animals occur at environmentally unrealistic dosages of BPA. For example, exposure concentrations in excess of 4.6 mg/L induce microcephaly, short body length, abnormal gut coiling, and edema in the amphibian *Xenopus laevis* [37], whereas exposure concentrations in excess of 5.7 mg/L cause development of scoliosis, head malformations, and organogenesis suppression [38]. In another amphibian (*Rana nigromaculata*), an environmentally high concentration (200 μg/L) of BPA-induced tail flexure malformations after 45 days exposure [39]. Yolk sac edema and hemorrhage occurred among salmon fry (*Salmo salar*) exposed to 1 mg/L for 6 days [40]. Histological changes were noted in hepatic cell nuclei from salmon exposed to 1 and 0.1 mg BPA/L. While many of these teratogenic malformations are qualitatively similar to those caused by embryonic exposure to 17β-estradiol [41], they may be due to the ability of high concentrations of BPA to suppress thyroid hormone receptor β gene expression [38], rather than activation of the estrogen receptor. Thyroid hormone responsiveness is necessary for proper metamorphic changes in vertebrates.

Unlike most teratogenic endpoints, endocrine-disrupting effects occur at environmentally relevant concentrations (i.e., concentrations found in the environment). The endocrine-disrupting effects of BPA that have been identified in wildlife species include (1) alteration of sex determination from exposure during gonadal organogenesis, (2) alteration of gonadal function from exposure both during and after gonadal organogenesis, and (3) induction of hepatic vitellogenin production following exposure of fully organized individuals. Thus, like many other endocrine-disrupting contaminants [42], BPA is a substance that can act both organizationally to permanently alter organ structure (e.g., the gonad) and activationally to cause a change in normally organized systems (e.g., the gonad and liver).

3.1. Sex determination

Whereas in most vertebrates sex is determined by sex chromosomes containing sex-specific genes, in many reptiles (including all crocodilians, most turtles, and some lizards) gender is determined by the temperature of egg incubation during a critical window of development. Animals with such temperature-dependent sex determination (TSD) are particularly susceptible to environmental perturbations, and thus, genetic sex determination has evolved numerous times in vertebrate ancestry. While there are several environmental perturbations that influence sex ratio in animals with TSD (including habitat change and accelerated environmental temperature change), endocrine-disrupting contaminants have been influencing sex determination in species with TSD only since the industrial revolution. In recent years, animals with TSD have become models of exposure to endocrine-disrupting contaminants due to the obvious sex-altering effects of these compounds [43].

In ovo exposure to BPA in the crocodilian *Caiman latirostris* causes such gender disruption. When Caiman eggs were exposed to 140 ppm (9 mg/egg applied to the egg shell, average egg weighing 65.1 g) BPA during the critical time of gender determination (stage 20 of embryogenesis), all resultant alligator offspring were females regardless of the incubation temperature [44]. Caiman embryos incubated at 30 °C all develop ovaries whereas embryos incubated at 33 °C all develop testes, unless these 33 °C eggs were treated with 140 ppm BPA or 1.4 ppm 17β-estradiol. BPA and 17β-estradiol-treated embryos were all feminized, having apparently normal ovaries.

Embryonic exposure to BPA also induces gonadal ontogenetic changes in some birds. For instance, exposure of eggs to 200 μg/g (200 ppm) BPA in the yolk caused Mullerian duct abnormalities in female quail, whereas the same yolk concentration caused feminization of the left testis in male chicken embryos [45].

Studies using the anuran *X. laevis* are not consistent in their findings of the sex altering effects of BPA (see Fig. 1). The first study of BPA's sex-altering effects on *X. laevis* was conducted by Kloas et al. [46]. In this study, *Xenopus* larvae from stages 38–40 (well before the sex-determining stages of 50–52) through metamorphosis were exposed to 2.28 and 22.8 μg/L of BPA under static-renewal conditions. Frogs ($n = 44$) exposed to 22.8 μg/L BPA had a feminized sex ratio compared to control frogs ($n = 82$). Subsequently, Pickford et al. exposed *Xenopus* larvae from stages 43–45 through metamorphosis to 0.83, 2.1, 9.5, 23.8, 100, and 497 μg/L BPA using a flow-through tank design [47]. While the positive control group of 17β-estradiol-treated frogs did have a feminized sex ratio, none of the BPA-treatment groups ($n = 123$–128 per group) deviated from the expected 50:50 sex ratio. A third study by Levy et al. exposed groups ($n = 40$ per group) of *Xenopus* larvae (stages 42–43 through metamorphosis) to 2.28, 22.8, or 228 μg/L BPA [48]. Similar to the results of Kloas et al., this study found that the 22.8 μg/L group had a feminized sex ratio, but the other treatment groups, the low

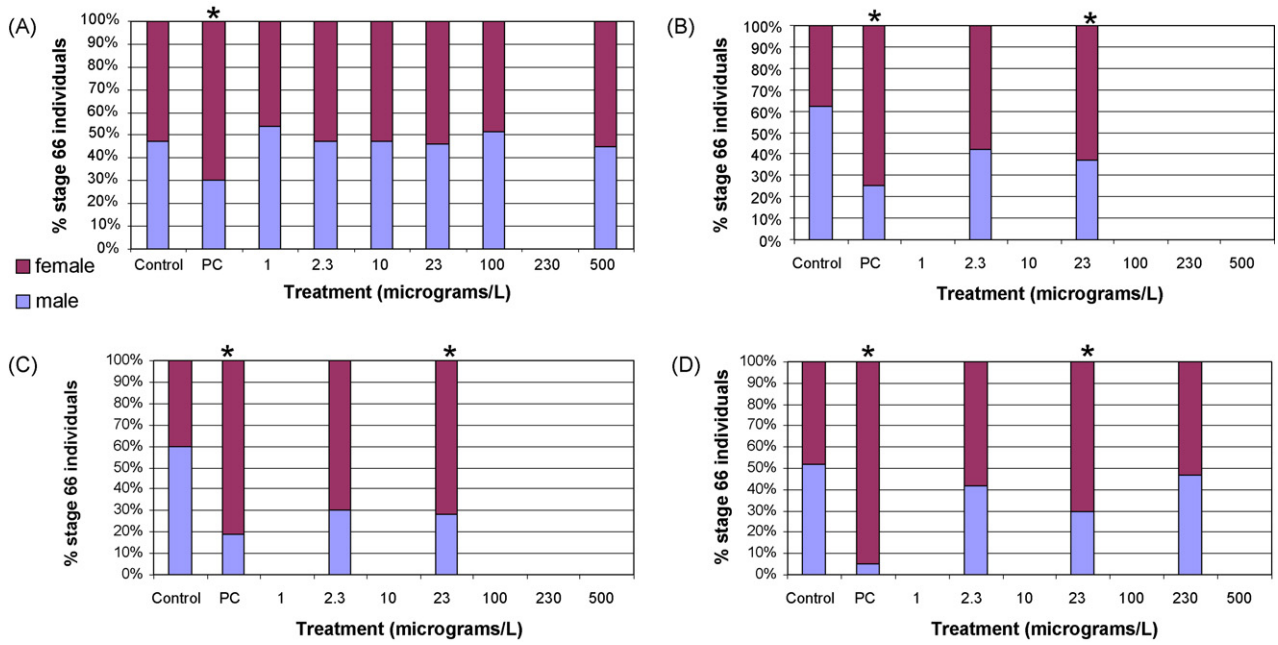

Fig. 1. Sex ratio results from BPA exposure of *Xenopus laevis* tadpoles. The positive control (PC) for each study was 2.7 μg/L 17β-estradiol. * significantly different ratio at $p < 0.05$ as reported in each paper (although different statistical tests were used in the studies, see discussion in text). (A) [47], (B) [46], (C) [48] experiment 1, (D) [48] experiment 2.

and high dose groups, did not. Levy et al. attributed this to the biphasic dose–response relationship seen with many estrogenic compounds [49], where extremely low doses exhibit no effect, low doses induce physiological changes, and higher doses are physiologically compensated for through immune, metabolic, and excretory responses. The BPA-induced sex reversal from male to female in *Xenopus* was deemed a complete reversal, as determined by histology [48].

There are several possible explanations for why the study of Pickford et al. found no gender-altering effects when the studies of Kloas et al. and Levy et al. found feminization. Kloas and Levy used a similar experimental design including <40 animals per treatment group and renewing BPA solutions (using a solvent as a vehicle) three times a week. Pickford et al. delivered BPA in a continuous flow-through design without use of a solvent and included >123 animals per group. Pickford et al. also quantified the concentration of BPA in each group, assuring that concentrations were constant throughout the experiment. Levy et al. determined that with their semi-static exposure, water concentrations of BPA rapidly decline within 48 h, the time period between their treatments. Pickford et al. maintained stable concentrations of BPA whereas Kloas et al. and Levy et al. had fluctuating concentrations. Pickford et al. suggested that stable concentrations of BPA could affect *Xenopus* tadpoles less than fluctuating concentrations, and these fluctuating concentrations are more representative of exposure in the wild. However, this difference in stable verses fluctuating concentrations is not seen for 17β-estradiol, as all three studies used the same 17β-estradiol concentrations and received the same results for this positive control. A second explanation for the discrepancies among the studies is the statistical analyses differences among the studies. Pickford et al. compared sex ratios in all groups with the expected sex ratio of 50M:50F using a goodness of fit test. This differs from the other authors who compared sex ratios of each experimental group to the control group using nonparametric Kruskal–Wallis *H*-test followed by a Mann–Whitney *U*-test. If compared using a goodness of fit test, the results of Kloas et al. and Levy et al. show that there is no difference between control and BPA treatments (raw results estimated from figures in papers). As sex ratio data are binomial (for each experimental group, there is a male number and a female number), the goodness of fit test seems more appropriate [50], thus suggesting lesser differences in the outcomes of the three studies than were originally perceived. Regardless of whether or not the results of Kloas and Levy are statistically significant, these results suggest that BPA exposure has the potential to feminize the developing *Xenopus* gonad.

Sex differentiation can also be altered in zebrafish (*D. rerio*) exposed to BPA during the period of embryonic development associated with gonadal ontogeny. Zebrafish fry fed food with either 17β-estradiol or BPA had abnormal sex ratios, 3.8F:1M for fry fed 1000 mg/kg and 11.5:1 for fry fed 2000 mg/kg [51]. While this is mechanistically informative, it is ecologically irrelevant due to the excessive dose needed to induce gonadal feminization (500 mg BPA/kg exposed fish had a normal sex ratio).

Environmentally relevant levels of BPA can contribute to intersex conditions. For instance, immature barbel (*Barbus* sp.) downstream from a BPA pollution source had a high incidence of intersexuality, whereas intersex barbel were not seen upstream from this polluted tributary [52]. Estimates for the incidence of intersex barbel in this region of the river varied between 30% [52] and 50% [53]. These feminized fish had gonads containing primarily oogonia and previtellogenic oocytes, but also includ-

ing spermatogonia, spermatocytes, and spermatids (however these male germ cells were not enclosed in lobules as normal). River water that the fish were exposed to contained measurable concentrations of various estrogenic contaminants, the highest estrogenic burden by mass coming from BPA (0.302 μg/L). In laboratory experiments, similar feminized testes occurred in male Japanese medaka (*Oryzias latipes*) but only after exposure to 837 μg/L BPA or higher [54]. This suggests that BPA is probably not potent enough in fish to induce the effects reported by Vigano et al. [53] on its own, but it is likely a contributor to the additive effects of estrogenic chemicals in natural waters.

In summary, exposure of cleidoic eggs to BPA concentrations in excess of 100 ppm can alter normal sex-determination pathways in reptiles and birds. However, such high concentrations likely never occur in eggs in the wild, as maternal transportation of BPA into eggs is low [55] and surrounding environmental water concentrations (thus leading to low nesting material concentrations) of BPA would not normally exceed 12 μg/L. Gender determination can also potentially be altered by BPA in anurans and in fishes, but again at concentrations that have only been exceeded in landfill leachates from domestic incombustibles [34,56]. Thus, wildlife populations that are directly exposed to water from landfill effluent should be examined for potential BPA disruptions such as sex ratio skewing and altered gonadal differentiation.

3.2. Gonadal function and secondary sexual characteristics

3.2.1. Vertebrates

Whereas high concentrations of BPA cause abnormal sex determination in some fishes, amphibians, reptiles and birds, lower concentrations cause alterations in gonadal function in a variety of vertebrates (Table 2). For instance, environmentally relevant concentrations of BPA have the potential to alter testicular structure and function in fishes (Fig. 2A). Unlike other vertebrates, teleost fish have distinct populations of maturing sperm in either acinar compartments or tubular lobules [57]. In a normal teleost fish, the testis has a balance of sperm stage populations. However, when male fathead minnows (*Pimephales promelas*) were exposed to 16 μg/L or higher concentrations of BPA, their testes had significantly reduced numbers of mature spermatozoa and significantly increased numbers of immature sperm stages in the seminiferous tubules of their testes [58]. Similarly, sperm count was significantly reduced in male guppies (*Poecilia reticulata*) exposed to 274 μg/L BPA for 21 days although sperm length is unaffected [59]. In Japanese Medaka (*O. latipes*), exposure of the F0 generation to 1.179 mg/L BPA resulted in testicular oocytes in the F1 male offspring [29]. However, BPA is almost never present in river waters at concentrations that are likely to induce these effects *in vivo* [33,58,60]. Future research should determine whether the alterations in sperm maturity noted in fathead minnows and guppies occur in wild fish populations exposed to sewage treatment and landfill effluent where the concentrations of BPA might approach toxicologically relevant levels.

Table 2
Responses of aquatic species to BPA for which chronic values (geometric mean of the NOEC and LOEC) are available

Species	Endpoint	Chronic value (μg/L)	Reference
Physiologic responses			
Rainbow trout	Growth	6300	[124]
Zebrafish	Fertilization rate	5700	[125]
Guppy	Sperm count	387	[59]
Fathead minnow	F2 egg hatchability	101	[126]
Carp	Intersex	32	[127]
Medaka	Ovotestis	22	[128]
Xenopus	Sex ratio	7.3	[46]
Swordtail	Tail length	0.63	[129]
Ramshorn snail	Egg production	0.014	[80]
Vitellogenin induction			
Swordtail		894	[129]
Rainbow trout		84	[130]
Fathead minnow		50	[126]
Xenopus		7.2	[46]

In addition to reducing sperm count and as a result, fertilization success, BPA can also alter the timing of reproduction in wild fish. Lahnsteiner et al. exposed brown trout (*Salmo trutta*) to environmentally relevant concentrations (1.75–5 μg/L) of BPA and examined the influence on semen quality through spawning [61]. Sperm density and motility were reduced at the beginning of spawning, as was motility in the middle of spawning. Sperm were not affected during late spawning, however the effects early and mid spawning resulted in a spawning delay of 4 weeks. As a result of such a delay, larval development would be seasonally delayed to the point where most fish species would have high larval mortality. The sperm count reductions in teleost fish seem similar to those in BPA-induced sperm reductions in laboratory mammals (e.g., [62]), but the mechanism influencing the sperm reductions is likely different as mammalian sperm production is decreased after in utero exposure (organizational disruption) whereas teleost sperm production is decreased in normally organized individuals (activational disruption).

BPA has been shown to adversely affect gonadal function in female fish. Kang et al. found no alteration in fecundity of Japanese medaka exposed to BPA [54] (but male medaka display organizational disruption when exposed to high concentrations of BPA, see above). However, 2.4 μg/L BPA caused female brown trout to delay ovulation 2 weeks, and 5 μg/L BPA eliminated ovulation all together in the trout [61]. These effects on ovulation are consistent with the estrogenic action of BPA, as ovulation in salmonids is suppressed by estradiol [63].

Reptiles also show evidence of BPA-induced gonadal dysfunction. Exposure of Caiman eggs to 1.4 ppm (90 μg/egg) BPA with incubation at a temperature that produces all males, caused hatchlings to have testes with poorly organized seminiferous tubules [44]. Seminiferous tubule organization was also altered in chickens exposed to 20 μg/kg BPA [64]. Disorganized seminiferous tubules are indicative of endocrine disruption, having been seen before in male juvenile alligators from Lake Apopka, Florida, a lake contaminated with numerous xenoestrogens [65].

Embryonic exposure of quail (*Coturnix japonica*) to BPA (67 and 200 μg/g egg) did not result in altered male sexual behavior, testicular weight asymmetry or circulating plasma testosterone concentrations, nor did these concentrations alter

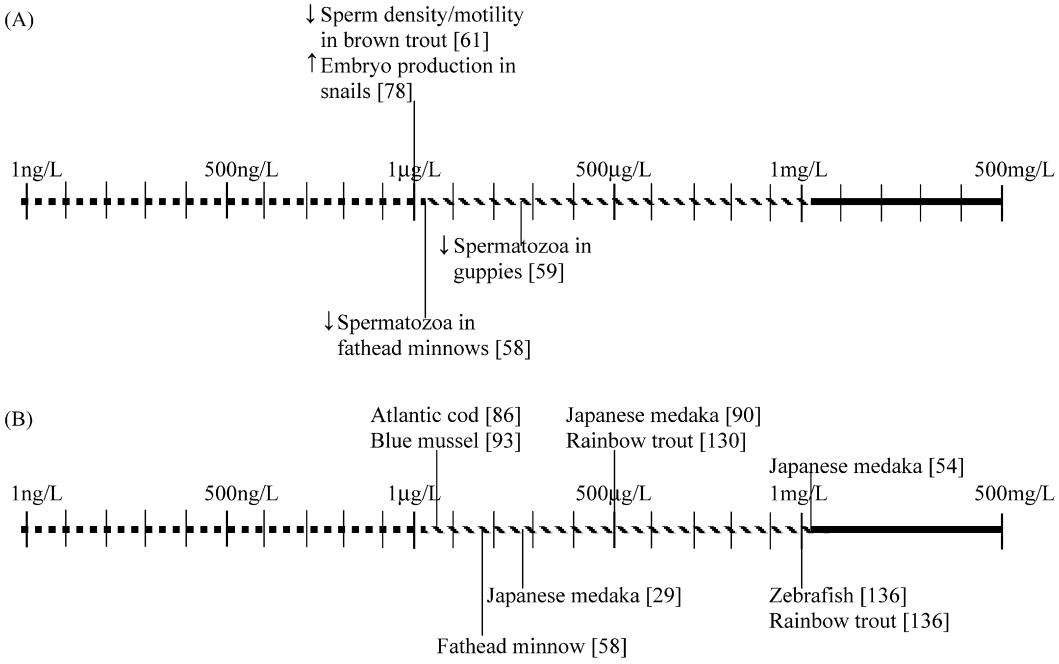

Fig. 2. Water concentrations of BPA that induce (A) activational changes in the gonads and (B) elevated Vtg in various fish and mollusk species. Concentrations are the LOEC reported in each study. Line type indicates concentrations reported for different water sources: dotted = concentrations measured in streams, striped = concentrations measured in landfill leachate, solid = exceeds values measured in environmental sources.

female fecundity [55]. However, these concentrations did cause organizational disruption of male chickens (see above, [45]). In addition, the extremely high dose of 200 mg BPA/week caused activational feminization of male chickens, resulting in testes with small seminiferous tubules and reduced spermatogenesis and a reduction in both testicular weight and comb weight [66]. More environmentally realistic doses of 2 and 20 μg/kg also cause activational disruption in chickens [64]. Males exposed to 2 μg/kg BPA had abnormal primary sexual characteristic (decreased testicular weight) and feminized secondary sexual characteristics (reduced comb and wattle weights).

Very few studies have examined the influence of BPA on wild mammals, but mammalian species appear to vary in susceptibility to the activational effects of BPA. For instance, 250 mg/(kg day) caused increased circulating testosterone concentrations in field voles (*Microtus agrestis*; [67]) but the same dose had no effect on testosterone in polecats (*Mustela putorius*; [68]). Lower doses of BPA (1 mg/(kg day)) cause reductions in circulating testosterone of rats [69]. This variation in activational response is likely due to species-specific ER binding and ER tissue distribution (see Section 4).

3.2.2. Invertebrates

In addition to its effects on vertebrates, BPA has an adverse effect on gonadal function in some invertebrates. For example, exposure of water fleas (*Ceriodaphnia dubia*) to BPA in excess of 1 mg/L (a concentration in excess of those seen in the environment) reduces reproduction [70]. Similarly, Mu et al. report a chronic toxicity threshold of 1.3 mg/L (a concentration close to the lethal dose) for effects on female fecundity in *Daphnia magna* after a 21-day exposure period [71]. In the cnidarian *Hydra vulgaris* the structure and physiology of the polyps was adversely affected at concentrations greater than 42 μg/L over 6 weeks, and a concentration-dependent inhibition of regeneration was seen above 460 μg/L [72]. In the nematode *C. elegans*, Hoshi et al. found a significant increase in the relative percentage of *C. elegans* germ cells when they were exposed to $\geq 10^{-9}$ M bisphenol-A in agar for 6 days, with no effects at 10^{-10} M [73]. Tominaga et al. exposed *C. elegans* to bisphenol-A on agar plates over four generations and found sublethal effects on fourth generation abundance at 1 nM [74].

In the harpacticoid copepod *Tigriopus japonicus,* long-term exposure to 0.1, 1.0 and 10 μg/L BPA caused a significant delay in completion of the naupliar stages (compared to the controls) in the parental generation, and at 0.01 μg/L and above in the F1 generation [75]. The time to sexual maturity was also increased at 1.0 μg/L in the parental generation, and at all concentrations in the F1 generation. The sex ratios of copepods were not significantly different from the controls at any concentration, for either of the generations. There were also no effects on fecundity (as measured by the average number of nauplii per female) at any concentration. The authors concluded that BPA (and the other chemicals tested, alkylphenols and 17β-estradiol) had no extensive effect on reproductive parameters, and would have little impact on the demographic profile of the copepod.

In contrast to the apparent absence of reproductive effects of BPA at environmentally relevant concentrations in most groups of invertebrates, exposure to environmentally relevant BPA concentrations elevated fecundity in three species of prosobranch snails (*Potamopyrgus antipodarum*, *Marisa cornuarietis* and *Nucella lapillus*) [76]. The main effect seems to be an increase in the number of eggs produced. Exposure of ramshorn snail (*M. cornuarietis*) to greater than 1.0 μg/L BPA caused rupture of the oviduct and death [76]. This effect appears to depend

on the morphology of the pallial oviduct, and the observation is so far restricted to this single species. Oehlmann et al. also showed that at the environmentally relevant BPA of 1 mg/L increases the cumulative number of eggs and the cumulative number of egg masses [76]. Similarly, in the parthenogenetically reproducing snail *P. antipodarum,* exposure to sediments containing 30 μg/kg BPA for 2 weeks increased egg production [77], whereas a 3-week exposure to 5 μg/L BPA has the same effect [78]. This pattern of increased embryo production is concentration-dependent, with an inverted U-shaped curve resulting from increasing BPA exposure [78].

The initial studies on *M. cornuareits* have stimulated a great deal of argument and have potential political importance as they indicate that BPA may cause effects at doses that are ecologically relevant and that are lower than those reported to cause effects in all other animals tested. Indeed, the controversy surrounding the validity of these studies led Oehlmann et al. to repeat their own studies, this time including more replication and measurement of the concentrations of BPA. In the first of these repeated studies, the same semi-static exposure system was used with a 180-day duration [79]. The nominal exposure concentrations were 0.05–1.0 μg/L, and the concentrations were checked by analysis following sampling on three occasions. In these studies, Oehlmann et al. was again able to demonstrate a superfeminization syndrome in *M. cornuareits,* and this was observed in all of the treated groups (with the exception of the 0.05 μg/L nominal group). This time, however, the incidence was at a lower level than in the high concentration experiment (although the level of incidence at the concentrations common to both studies, 1 μg/L, was the same). Mortality was not significantly enhanced in any of the BPA groups in comparison to the controls. Egg production was also stimulated as in the previous experiment, although the results over the whole 180-day exposure period showed a significant increase only at the two highest concentrations. The authors observed that the exposure period included the season of the year (October to February) when spawning activity in this population of ramshorn snails increases naturally. It was therefore considered that the effect of BPA might be masked to some degree by the natural increase.This led the research group to conduct further studies, which have been reported recently [80]. This study indicates that both spawning time and temperature influence BPA disruption of snail reproduction. Before and after the spawning season, superfemale responses were observed (NOEC 7.9 ng/L, EC10 13.9 ng/L), which were absent during the spawning season. The adverse effect of BPA was at least partially masked at 27 °C (EC_{10} 998 ng/L) when compared with 20 °C (EC_{10} 14.8 ng/L).

Recently, another study found that 12-week exposure to 0, 0.1, 1.0, 16, 160 or 640 μg/L BPA had no effect on reproduction, egg hatchability, or timing of egg hatching in ramshorn snails [81]. These results do not appear to support those of Oehlmann, although there were significant experimental design differences between the Forbes study and those of Oehlmann. First, snails were exposed to BPA at 25 °C in the Forbes study, a temperature close to the 27 °C that Oehlmann found partially masked the effects of BPA. Second, snail densities differed among the studies. Whereas Forbes et al. had a constant snail density of 0.8 snails/L, Oehlmann et al. had 3.98 snails/L dropping to 0.56 snails/L in the last month of the study (due to mortality). Due to these design differences, results from these experiments are difficult to compare.

3.3. Vitellogenin production

Vitellogenin (Vtg) is the precursor of the egg yolk proteins phosvitin and lipovitelline that are common for all oviparous vertebrates. This phospholipoglycoprotein is produced by the liver in response to estrogen, and Vtg concentrations in the blood increase during oogenesis [82]. Typically males do not synthesize Vtg, but male hepatocytes do produce and release Vtg if exposed to estrogens. Thus, the presence of Vtg in the blood of male oviparous animals has been used extensively as a biomarker for exposure to estrogenic toxicants.

3.3.1. Fish

Numerous studies have shown that male fish captured downstream from sewage treatment plants have elevated serum Vtg concentrations (e.g., [83–85]). These elevations cannot be attributed to BPA because sewage effluent contains numerous estrogenic compounds, including ethinylestradiol (one of the estrogens used in birth control pills) and nonylphenol (a breakdown product of surfactants and detergents). Several fish species have been exposed to only BPA in a controlled laboratory setting and assessed for *in vivo* Vtg concentrations (the fathead minnow *P. promelas*, the Japanese medaka *O. latipes*, the rainbow trout *Oncorhynchus mykiss*, and the zebrafish *D. rerio*). All of these fish species tested in the laboratory show elevated Vtg, but only at concentrations greater than that typically measured in surface waters (Fig. 2B). However, fish exposed to either landfill leachate prior to treatment or untreated leachate can be expected to have elevated Vtg.

Much variation has been noted among fish species in the ability of BPA to elevate serum Vtg (see Fig. 2B). When exposed to 59 μg/L BPA for 3 weeks, male Atlantic cod (*Gadus morhua*) had significantly elevated serum Vtg concentrations, whereas male turbot (*Scophthalmus maximus*) did not [86]. BPA concentrations as high as 22.8 mg/L had no effect on vitellogenic response in bream (*Abramis brama*) hepatocytes [87]. Sensitivity differences in vitellogenin induction by BPA can be due to several factors. First, different sensitivities may be due to species-specific ER binding affinities, BPA uptake rates, metabolism, and elimination rates. For instance, the lower estrogenic sensitivity of zebrafish compared to rainbow trout can be attributed to rapid metabolism of BPA in the zebrafish liver [88]. Second, some of the variation in species sensitivities reported in Fig. 2B could be due to differences in experimental designs. Both concentration and time of exposure influence the vitellogenic response of fish, as the lowest observed effect concentrations (LOECs) needed to elevate circulating Vtg are dependent on duration of exposure. For instance, 8-h exposure of Japaese medaka (*O. latipes*) to 8000 μg/L BPA-induced Vtg mRNA expression [89], whereas 21-day exposure to 3120 μg/L [54], and 28-day exposure to 1000 μg/L [90] elevated circulating Vtg.

3.3.2. Amphibians

Few studies have examined the effect of BPA on amphibians, but high environmental concentrations of BPA have the potential to induce Vtg. Exposure of hepatocytes, isolated from male *X. laevis,* to 22.8 μg/L BPA increased Vtg-mRNA accumulation [46]. In addition to Vtg mRNA accumulation in male *X. laevis*, BPA also induced hepatic Vtg mRNA levels in male *Bombina orientalis* frogs similar to, but 100-fold less potent than, the effect of 17β-estradiol and similar to normal female expression [91]. However, exposure of brown frog (*Rana temporaria*) hepatocytes to BPA (up to 22.8 mg/L) had no effect on vitellogenin production [92].

3.3.3. Invertebrates

Molluscs are commonly used as sentinels of aquatic environments, and they may be sensitive organisms to BPA due both to their increased exposure (stationary, benthic filter feeders). Female freshwater blue mussels (*Mytilus edulis*) exposed to 50 μg/L BPA for 3 weeks have elevated vitellogenin-like protein levels [93]. It is possible that BPA affects other invertebrates independent of reproductive effects, as 1 h exposure to 11.4 μg/L BPA-induced metamorphosis in 50% of the larvae in *Capitella*, an annelid worm [94].

4. Mechanism of action

The estrogenic effects of BPA have been recognized since the early 1900s [95] and these effects are attributed to BPA acting both as an estrogen agonist and to promote the effect of endogenous 17β-estradiol. Support for estrogen agonistic activity comes from numerous studies showing that BPA binds to the estrogen receptor (ER) of vertebrates (reviewed in [96]; see Table 3), but the data from invertebrates is less conclusive. In the ramshorn snail (*M. cornuarietis*), BPA reportedly displaced 17β-estradiol from binding sites within cytosolic tissue extracts; indeed, BPA exhibited greater affinity than the hormone itself [80]. These investigators used this as evidence to claim that molluscs possess an ER that is highly sensitive to activation by BPA. However, several independent studies have shown that the molluscan ER is constitutively active and does not bind 17β-estradiol [97–99]. These reports cast doubt that the binding activity measured in ramshorn snail involved the ER. Nonetheless, ramshorn snails where reported to be exquisitely sensitive to the toxicity of BPA with egg production being enhanced with the EC_{10} (concentration that increased egg production by 10%) of 13.9 ng/L [80].

The action of BPA as an ER agonist in vertebrates is due to the unsubstituted phenolic groups in the chemical structure. Whereas BPA binds to the carp (*Cyprinus carpio*) ER and elevates circulating Vtg in response, related BPA diphenylalkanes such as BPA diglycidyl ether (also a raw material for polymer plastics) have substitutions on their phenol side groups that result in ER antagonistic action [100]. Therefore, conflicting results in wild populations exposed to BPA can be attributed to the interaction of BPA with other BPA-related diphenylalkanes.

Although BPA acts as an ER agonist, gene expression patterns after BPA exposure are often different from those after 17β-estradiol-ER binding [101]. This is in part explained by the fact that BPA binds ERβ with greater affinity than ERα [102]. Indeed, BPA displays pure agonistic action for human ERβ, but both agonistic and antagonistic actions for human ERα [103]. Thus, some of the differing effects and sensitivities of BPA among species can be explained by (1) different ER amino acid structure in various species leading to varying BPA/ER binding affinity and (2) differential ER subtype tissue distribution in various species.

Direct ER binding is the most obvious mechanism through which BPA elicits estrogenic responses. However, as illustrated in Fig. 3, BPA can also act indirectly by promoting the action of endogenous 17β-estradiol. Three indirect mechanisms that increase estrogenicity are: (1) elevating steady state levels of ER, (2) displacing endogenous 17β-estradiol from it's circulating binding protein, and (3) inhibiting the excretion of endogenous 17β-estradiol. First, similar to 17β-estradiol, BPA stimulates the accumulation of ER mRNA [48]. Resulting increased levels of ER protein can enhance responsiveness to both endogenous estrogens and xenoestrogens. A second indirect estrogen-promoting effect of BPA involves sex steroid binding proteins. Normally, sex steroid binding protein regulates the availability of endogenous sex steroids, functioning

Table 3
BPA estrogen receptor binding in various animals

Genus species	Common name	BPA/E2 affinity ratio (BPA IC_{50}/E2 IC_{50})	BPA relative binding affinity for the ER 100 × (IC_{50}E2/IC_{50} BPA)	Comment	Reference
Marisa cornuarietis	Ramshorn snail	33[a]	3.03[a]	High binding affinity compared to aquatic vertebrates	[80]
Cyprinus carpio	Carp	831	0.12		[131]
Oncorhynchus mykiss	Rainbow trout	39	2.56		[132]
O. mykiss	Rainbow trout	485	0.21	ER D, E, F domains only	[133]
Xenopus laevis	African clawed frog	719	0.14		[134]
Anolis carolinensis	Green anole	774	0.13	ER D, E, F domains only	[133]
Gallus gallus	Chicken	2281	0.044	ER D, E, F domains only	[133]
Mus musculus	Mouse	11,481	0.009	ERα D, E, F domains only	[133]
Homo sapiens	Human	12,413	0.008	ERα	[135]
H. sapiens	Human	267	0.37	ERβ	[135]

IC_{50} = inhibitory concentration of BPA needed to displace half of ^3H-E_2 bound to the ER.
[a] Not purified ER, but cytosolic binding.

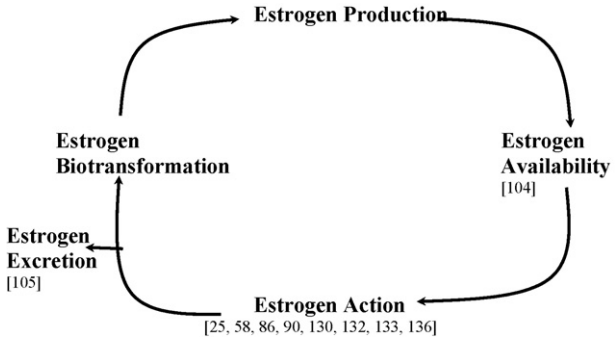

Fig. 3. Example of the multiple mechanisms of action for the endocrine-disrupting effects of BPA in fish. BPA cannot only alter estrogen action by acting as an estrogen agonist, but also can increase the cellular availability of decrease the excretion of endogenous estrogen [136].

as a circulating repository for steroids. The fact that BPA is able to displace ^{3}H-estradiol from rainbow trout sex steroid binding protein [104] suggests that BPA could alter the bioavailability of endogenous estrogens. Third, BPA decreases hepatic metabolism of 17β-estradiol in lake trout (*Salvelinus namaycush*; [105]). Normally, circulating 17β-estradiol is metabolized in the liver to create water-soluble excretory products, thus providing constant excretion of 17β-estradiol. An *in vitro* study of tissues from lake trout showed that 22.8 mg/L BPA reduced 17β-estradiol hepatic metabolism to 24.2% of controls and renal metabolism to 52% of controls [105]. The ability of BPA to upregulate estrogen receptors, displace 17β-estradiol from sex hormone binding globulins, and reduce 17β-estradiol excretion indicates that ER binding alone is not an indicator of BPAs total estrogenicity.

Similarly, other non-estrogenic endpoints are likely involved in BPA toxicity in invertebrates. BPA endocrine-disruption may affect molting of adults by playing a role in shell disease through completely different mechanisms of action. Alkylphenols appear to interfere in the tanning and sclerotization reactions of both insects [106] and crustaceans [107] by interfering with shell hardening by suppressing protein crosslinking, by interfering in the uptake and incorporation of tyrosine, an alkyphelolic amino acid, and its metabolic derivatives during the molting process, leading to shell weakness [107]. The shell weakened by alkylphenols makes it susceptible to microbial invasion resulting in shell disease, which can be fatal to lobsters. Damage to the shell induces affected animals to molt more frequently. In summary, when selecting model species for assessing the effects of BPA, species-specific endpoints should be chosen based on the most susceptible organ or tissue for that species. Varying effects in different phylogenetic groupings are expected to be shown through a conserved mechanism of action.

5. Conclusions and levels of confidence for different outcomes

A comparison of measured BPA environmental concentrations with chronic values (geometric mean of the NOEC and LOEC) suggests that no significant margin of safety exists for the protection of aquatic communities against the toxicity of BPA (Fig. 4). Based upon the species sensitivity distribution depicted in Fig. 4, a concentration of 0.03 μg/L BPA should not be exceeded to ensure protection of 95% of the exposed species against chronic toxicity. However, the upper limit (95th percentile) of BPA concentrations measured in surface waters was estimated to be 8.2 μg/L. Thus, environmental scenarios likely exist where species are being adversely affected by environmental levels of BPA. The adverse effects that have been noted at environmentally relevant (high surface water and landfill leachate) concentrations are activational disruptions of the gonads, and these effects are seen at similar concentrations in lab animals (see Table 4).

5.1. Based on existing evidence, we are confident of the following

> The criterion for an outcome being assessed as achieving this level (we are confident) is that multiple independent studies had been conducted that showed the same or similar outcome.

5.1.1. Environmental concentrations of BPA

Levels of BPA in water and sediment samples have been measured in numerous studies, and environmental concentrations vary based on the source of the sample and time of sampling. For sample source, the highest concentrations are found in landfill leachate and anaerobic sediments. Based on the concentrations that have been measured in aqueous media, we conclude the following: (1) concentrations in stream/river water samples are typically less than 21 μg/L, (2) concentrations in landfill leachate are typically less than 17.2 mg/L, and (3) concentrations exceeding 17.2 mg/L are typically not measured in the environment.

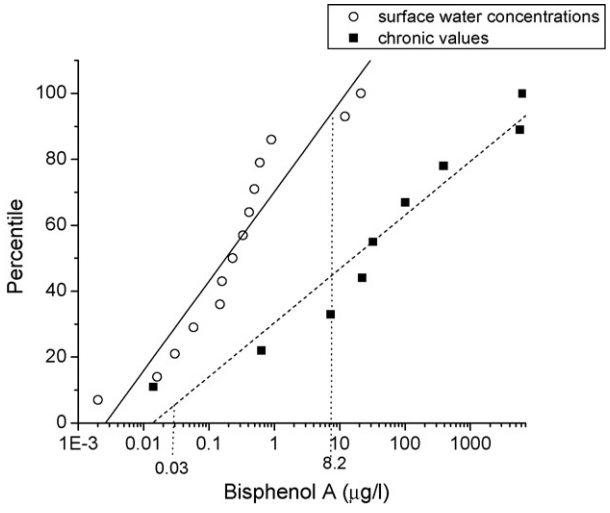

Fig. 4. Hazard assessment of BPA for aquatic species. Closed squares: chronic values, based upon physiologic responses (Table 3), used to estimate the BPA concentration that is protective of 95% of the species for which chronic values were available. Open circles: BPA concentrations found in surface waters (Table 4) used to judge the upper limit (95th percentile) of BPA concentrations found in surface waters. Dotted vertical lines denote these respective values.

Table 4
Comparison of endpoints seen in wildlife with those seen in lab animal (based on Richter et al., this issue), *in vitro* (Wetherill et al., this issue) and human exposure (Vandenbert et al., this issue) studies

BPA	Wildlife studies (water concentrations)	Lab animal studies (dose per day)	In vitro studies (media concentration)	Human studies (measured levels)
10^{-12}		?		
10^{-11}		?		
10^{-10}				
10^{-9}			ERE	90 %
10^{-8}	↓ male reproduction Altered cyclicity	↓ male reproduction Altered cyclicity	↓ male reproduction	population
10^{-7}	↓ male reproduction	↓ male reproduction Altered cyclicity	ERE ↓ male reproduction	
10^{-6}	ERE ↓ male reproduction	↓ male reproduction Altered cyclicity	↓ male reproduction	

▨ = studies have been conducted and effects observed. ▬, reference dose (50 μg/(kg day)). ■ = consistency of response with those seen in wildlife. ERE = activation of transcription after ER binding, ↓ male reproduction = changes in steroidogenesis and/or spermatogenesis.

5.1.2. Organizational effects on the male reproductive system

There is extensive evidence that BPA induces feminization during gonadal ontogeny of fishes, reptiles, and birds. However, in all cases, the concentrations of BPA necessary to cause such ontogenetic disruption exceed concentrations in the environment. Thus, present environmental concentrations are not problematic for sex determination in wildlife species.

5.1.3. Activational effects on the male reproductive system

There is extensive evidence that adult exposure to BPA has a detrimental effect on spermatogenetic endpoints in some species of fish. BPA concentrations found in landfill leachate reduce the number of mature spermatozoa and sperm density in fish. Such spermatogenetic endpoints should be monitored in other wildlife species. There is also extensive evidence that vitellogenin concentrations are elevated in male fish exposed to environmentally relevant levels of BPA. While the physiological ramifications of these Vtg concentrations are unknown, the presence of Vtg is commonly used as an early biomarker of exposure to estrogenic chemicals.

5.1.4. Mechanism of action

Most of the effects of BPA on different vertebrate wildlife species can be attributed to BPA acting as an estrogen receptor agonist. ER binding studies in all vertebrate classes have shown agonistic activity with species-specific receptors that are distinct but similar to the family of estrogen receptors (e.g., [108,109]).

5.2. We consider the following to be likely but requiring confirmation

The criterion for achieving this level is that significant effects have been reported, but the number of independent replications is limited. However, confidence in the findings is increased by the plausibility of the results, based on mechanistic information available from other related studies.

5.2.1. Activational effects on the male and female reproductive systems

A few studies have shown reduced sperm motility, delayed ovulation, and delayed timing of spawning in fish exposed to environmentally relevant concentrations of BPA. Because these effects could be devastating at the level of the population, future studies should further examine the effect of environmental concentrations of BPA on these endpoints.

5.2.2. Organizational effects on the male reproductive system

Published studies are not consistent on whether or not BPA alters gonadal ontogeny in the model amphibian *X. laevis*. This is due to differences in experimental designs and statistical analyses used in the studies. Similarly, published studies are not consistent on whether BPA causes superfeminization in gastropods. This is due to differences in experimental designs and statistical analyses used in the studies. There is some evidence that the superfeminization response may be temperature dependent.

5.2.3. ER-independent mechanisms of action

A few studies have indicated that some of BPA's effects can be attributed to mechanisms of action independent of the ER agonistic action. These mechanisms include upregulating the ER, alteration of estrogen binding proteins, and alteration of biotrasformation and excretion of endogenous estrogens. However, these effects typically have been reported at BPA exposure concentrations that exceed environmental relevance.

5.3. Future research

5.3.1. Unexamined endpoints

Many endocrine-influenced tissues have thus far not been examined for effects of BPA at environmentally relevant exposure levels. Tissues differ in their response to BPA and, while BPA has a conserved mechanism of action in all vertebrates, taxonomic classification should be considered when examining the influence on endocrine-regulated organs. For instance, the prostate appears to be the most sensitive organ to BPA in mice [110], but this is mammalian structure cannot be examined in vertebrates of other classes. In reptiles such as the Caiman, phallus size may be the most sensitive organ, as phallus size in other crocodilians is potently affected by exposure to environmental contaminants [111]. In amphibians, the larynx appears to be very susceptible to endocrine-disrupting contaminants [112], but no study has examined the influence of BPA on this organ. In fish, sperm production thus far seems most sensitive to BPA, as also has been seen in laboratory mammals. However, the effects noted on teleost spermatogenesis have been limited to activational studies; future studies should examine the effects of early life stage exposure on adult spermatogenesis.

5.3.2. Future model wildlife species

As indicated in this review, most of the studies of non-mammalian species have been conducted in a controlled setting; very few studies focus on wild populations. While laboratory studies are necessary to determine mechanisms of disruptions for BPA in various species, studies of wildlife are necessary to determine if indeed populations are at risk from exposure to BPA. Studies of wildlife populations elucidate the influence, or lack of influence, of anthropogenic substances on populations with natural genetic variation and environmental stressors [113]. If we are to have a true picture of the ecological influence of BPA, then thorough studies must be conducted on wildlife populations that are at the greatest risk. As water concentrations of BPA are highest at landfill leachate effluent and sewage treatment effluent sources, populations of aquatic animals at these sources should be examined closely.

Acknowledgements

This review was prepared in conjunction with the Bisphenol A Conference, Chapel Hill, NC, 28–29 November 2006. Funding for the conference and this project was provided by the National Institute of Environmental Health Sciences, the National Institute of Dental and Craniofacial Research, the U.S. Environmental Protection Agency, Commonweal, and Maryville College.

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