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Impact of common environmental chemicals bisphenol A and bisphenol S on the physiology of *Lumbriculus variegatus*

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ABSTRACT

Bisphenol A (BPA) is a component of polycarbonate plastics and a near ubiquitous environmental endocrine disrupting chemical. Bisphenol S (BPS), a substitute of BPA, is also hormonally active. This study examines the effects of aqueous exposure to BPA and BPS on the freshwater annelids *Lumbriculus variegatus*, a keystone species in shallow water ecosystems. Both BPA and BPS, at both low dose (10^{-9} M) and high dose (10^{-6} M), retarded the initial phase of body regrowth after cutting/fragmentation, which is the main mode of reproduction of L. *variegatus*. Both acute and five day exposure to BPA and BPS increased pulse rate of the dorsal blood vessel. For all the measured endpoints, the effects of BPA and BPS were nearly indistinguishable. These results indicate that BPA and BPS have similar and significant effects on the physiology of L. *variegatus*. These findings have implication for the potential impact of these bisphenols on invertebrates in the ecosystem.

1. Introduction

Bisphenol A (BPA) is a synthetic chemical commonly used in the manufacturing of polycarbonate plastics and epoxy resins, and is present in a wide range of consumer and industrial products. BPA is a high-production volume chemical, with global annual production of 3.9 million metric in 2006 and 5 million metric tons in 2010 (Careghini et al., 2015). Because of the pervasive use of BPA in modern manufacturing, human exposure to BPA is widespread (Vandenberg et al., 2010). Human exposure routes to BPA include dietary, dermal, air, and dust (Vandenberg et al., 2007; Welshons et al., 2006). BPA is an estrogenic endocrine disrupting chemical (EDC). Numerous epidemiological and animal studies have linked BPA to diseases such as cancer, diabetes, obesity, and various disorders in the reproductive, neuronal, immune, and cardiovascular systems (Diamanti-Kandarakis et al., 2009; Melzer et al., 2010; Zoeller et al., 2012).

Due to the public's growing concern about the potential adverse health effects of BPA, manufacturers are switching away from BPA-based consumer plastics and have introduced various “BPA-free” alternatives. These plastics are made from BPA substitutes, such as Bisphenol S (BPS). Although its usage in manufacturing is a relatively recent event, BPS has already been detected in the environment. BPS was reported in most river water samples in Japan, China, Korea and India with median concentration in the low ng/L or low µg/L range (Yamazaki et al., 2015). Wide human exposure to BPS has been reported in various populations including that of the US (Liao et al., 2012), indicating common presence of BPS in the environment. Recent research has suggested that BPS shares a similar potency and behavior with BPA in estrogenic, antiestrogenic, androgenic, and antiandrogenic ways (Rochester and Bolden, 2015). We have recently shown that BPS has arrhythmogenic effects on rodent cardiac hearts and myocytes that are indistinguishable from those of BPA (Gao et al., 2015).

In addition to their negative health effects, environmental EDCs can have major impact on wildlife and ecosystems (Hutchinson et al., 2000; Tyler et al., 1998). The presence of BPA in the environment is near ubiquitous. Major sources of BPA entering the environment include release during manufacturing and transportation, sewage effluent, landfill leachate, domestic waste combustion, and degradation of polycarbonate plastics in the environment (Barral et al., 2000; Kalmykova et al., 2013; Masoner et al., 2014; Meesters and Schroder, 2002; Wintgens et al., 2003). Bisphenols are found at relatively high concentrations in rivers, lakes and estuaries throughout Asia, Europe, and North America, especially in those near urban areas (Corrales et al., 2015). BPA and other estrogenic EDCs have a range of impact on wildlife species, including alteration of sex determination during development, alteration of gonadal function and secondary sexual characteristics and reproductive behaviors, neurobehavioral alterations, stimulating vitellogenin production, alterations of gene expressions,

Abbreviations: ANOVA, one-way analysis of variance; BPA, bisphenol A; BPS, bisphenol S; EDC, endocrine disrupting chemical; ER, estrogen receptor; DBV, dorsal blood vessel

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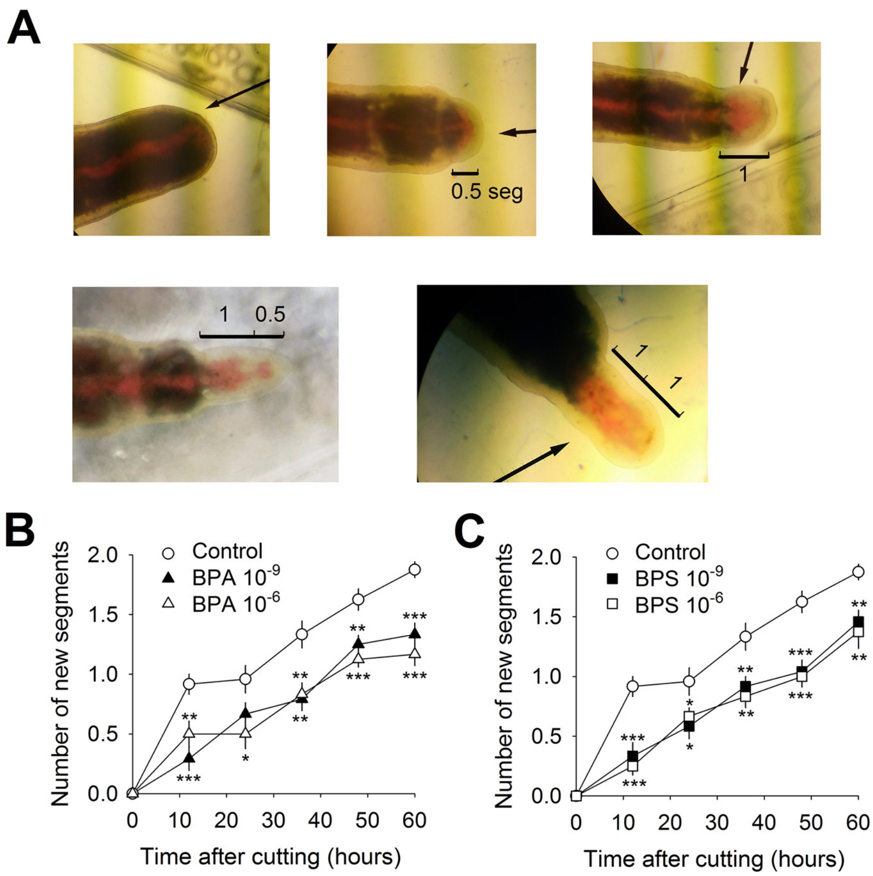

Fig. 1. BPA and BPS affected L. *variegatus* regeneration following cutting. (A), representative images of L. *variegatus* with zero, 0.5, 1, 1.5 and 2 of regrown segments (seg). A half segment was not a precise quantification and was defined as any regrowth that was obviously less than a full segment. Arrows on the images were an indicator built into the ocular lens and do not have a specific meaning. (B), average number of regrown segments at different time points following cutting in vehicle control, 10^{-9} M BPA and 10^{-6} M BPA treatment groups. (C), average number of regrown segments at different time points following cutting in vehicle control, 10^{-9} M BPS and 10^{-6} M BPS treatment groups. N = 12 for all data points. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$ vs control. Data are expressed as average ± SEM.

and increasing genetic mutations (Bhandari et al., 2015; Crain et al., 2007; Flint et al., 2012; Oehlmann et al., 2009).

In this study, we examined the impact of aqueous exposure to BPA and BPS on the freshwater oligochaete *Lumbriculus variegatus,* commonly referred to as the blackworm. L. *variegatus* lives in the benthic zone at the edges of ponds, lakes and marshes, and is found throughout North America and Europe. In nature, the head of L. *variegatus* feeds in the sediments and debris, while the posterior extends to the water for respiration. Thus, the oligochaetes are exposed both to the sediments and to the surface water. They are a prey for a variety of animals, from birds to fish to amphibians, and are also responsible for aiding in decomposition and sediment content. As such, they are a keystone species in the freshwater ecosystem. L. *variegatus* can be easily cultured and handled, has transparent body and easily identifiable anatomical structures. For these reasons, L. *variegatus* is a common model for bioaccumulation and chemical toxicity tests as well as for classroom teaching (Kukkonen and Landrum, 1994; Lesiuk and Drewes, 1999; Phipps et al., 1993; Schubauerberigan et al., 1993). In the present study, we evaluated the effects of BPA and BPS on two key physiological endpoints of L. *variegatus*, segmental regeneration and dorsal blood vessel (DBV) pulsing rate.

2. Materials and methods

2.1. Animals

It is typically advised that L. *variegatus* be cultured in fresh water such as bottled spring water. To minimize background xenoestrogen contamination, an artificial, BPA- and BPS-free “fresh water” was reconstituted by adding trace amount of salts to BPA-and BPS-free distilled water (18 MΩ; < 6 parts per billion total oxidizable organics; produced using a Mill-Q A10 system, Millipore Sigma, Burlington, MA). The formulation was based on the analyses of several bottled spring waters, including Evian Natural Spring Water, Ice Mountain bottle water, and Poland Spring bottle water (posted on the respective company website), as well as analysis of groundwater from a spring in the Sierra Nevada Mountains (http://www.waterencyclopedia.com/En-Ge/Fresh-Water-Natural-Composition-of.html). It contained (in mM) $CaCl_2$ 0.2, $NaHCO_3$ 0.25, KCl 0.02, and $MgSO_4$ 0.12 (pH = 6.6 to 6.7).

L. *variegatus* worms were purchased from Monfort Aquarium and Pets store (Cincinnati, OH). They were transferred from the water that they had been purchased in, rinsed multiple times with the BPA-free artificial fresh water, and maintained in the artificial fresh water in glass containers at room temperature (24 °C) for three days, until the beginning of the experiments. The worms were fed fish food flakes every 3 days; containers were cleaned and water replaced following each feeding.

2.2. Reagents and solutions

Bisphenol A, CAS 80-05-7, was from TCI America, lot 111,909 (ground by Battelle), and was provided by the Division of the National Toxicology Program at the National Institute of Environmental Health Sciences, National Institute of Health. Bisphenol S (4,4′-Sulfonyldiphenol, CAS 80-09-1) was from Sigma-Aldrich. BPA and BPS were dissolved in DMSO as stock solutions (10^{-3} M), and were stored in −20 °C. Experimental solutions were prepared fresh using glass containers. All experimental apparatuses were free of polycarbonate plastic. Solutions were prepared using BPA-free water. HPLC-grade DMSO and other chemicals were all from Sigma-Aldrich (St Louis, MO). All treatment solutions were prepared from the stock solutions and in the artificial fresh water. The control group solution contained vehicle (DMSO, 10^{-6} M).