Chemosphere 190 (2018) 287–295

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Bisphenol A in artificial soil: Effects on growth, reproduction and immunity in earthworms

I. Verdú [a], D. Trigo [a], J.L. Martínez-Guitarte [b], M. Novo [b,*]

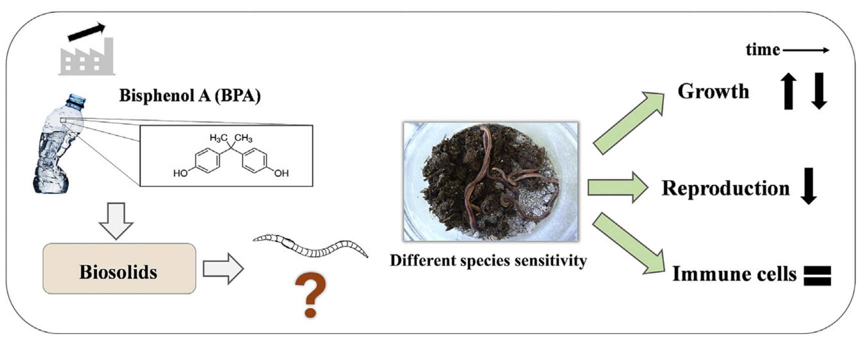
[a] *Soil Zoology Group, Departamento de Zoología y Antropología Física, Facultad de Biología, Complutense University, Jose Antonio Nováis s/n, 28040 Madrid, Spain*

[b] *Environmental Toxicology and Biology Group, Departamento de Física Matemática y de Fluídos, Facultad de Ciencias, UNED, Senda del Rey 9, 28040 Madrid, Spain*

HIGHLIGHTS

- Effect of Bisphenol A was evaluated on life history traits and immunity of earthworms.
- BPA induced an initial decline in growth and the opposite effect afterwards.
- Reproduction was reduced at the highest concentration tested for *E. fetida*.
- Two species showed different sensitivity to BPA.
- Viability of immune cells was not affected by BPA.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 June 2017
Received in revised form
17 September 2017
Accepted 25 September 2017
Available online 27 September 2017

Handling Editor: Jim Lazorchak

Keywords:
Xenobiotics
Ecotoxicological test
Life cycle
Coelomocytes
OECD
BPA

ABSTRACT

The application of biosolids in agricultural fields is increasing annually. They contain not only nutrients but also xenobiotics, such as Bisphenol A (BPA). These compounds are not regulated in the use of biosolids in agriculture, which highlights the need to assess their effects on soil life, of which earthworms are most abundant of the animal representatives. In this study the effect of BPA on life-history parameters, such as mortality, growth and reproduction, and on immunity, is evaluated for *Dendrobaena veneta* and *Eisenia fetida*. Sublethal concentrations were evaluated by a modified OECD artificial soil test. Decline in growth with increasing concentration of BPA was detected during the first two weeks and the opposite effect for the next two, although these differences were only significant at the highest concentration. Reproduction traits were only significantly different for *E. fetida*, for which the number of juveniles decreased at higher concentrations, thus showing different sensitivity in both species. By using a contact test, the potentially harmful effect of direct contact with BPA was shown to be much higher than in soil (resembling natural) conditions. Finally, results indicate that BPA may not affect the immune system of these animals, at least in terms of coelomocyte viability.

1. Introduction

In recent years, industrial and technological development has led to the appearance of new chemical compounds that have a wide range of applications in different sectors. Known as xenobiotics,

* Corresponding author.
E-mail addresses: mnovo@ccia.uned.es, martanovorodriguez@gmail.com (M. Novo).

https://doi.org/10.1016/j.chemosphere.2017.09.122

they are synthetized by the human being and rare or nonexistent in the nature (De Bolster, 1997).

Bisphenol A (BPA) is one of them (Flint et al., 2012; Michalowicz, 2014) and is present in a number of daily products, mainly in plastics (95% of the applications of BPA fall here), particularly in polycarbonate resins (71%) and epoxy resins (29%). Global production has increased from 3.2 million tons in 2003 to 5 million tons in 2010 (Flint et al., 2012; Careghini et al., 2015), thus it has become one of the most widely produced and used chemicals in the world (Michalowicz, 2014). Considered as an endocrine disruptor, BPA is capable of altering the endocrine function by imitating or blocking the endogenous hormones (Schug et al., 2011). The greatest effort has been devoted to studying the possible impacts of BPA in vertebrates, with many studies reporting its estrogenic effect (Flint et al., 2012; Michalowicz, 2014). However, not only do the endocrine and reproductive systems seem to be affected, but also the immune and nervous systems (Michalowicz, 2014). Regarding invertebrates, the studies fall mainly in aquatic environments reporting effects on development and reproduction (Flint et al., 2012). The impact of BPA on the edaphic environment and soil life remains unknown, with only a few studies on isopods (Lemos et al., 2009, 2010) and earthworms (Lee et al., 2005; Babić et al., 2016a, b), even though the presence of BPA in agricultural soils is of concern (Kinney et al., 2008; Oehlmann et al., 2009; Flint et al., 2012; Careghini et al., 2015).

BPA can reach the edaphic medium through sewage sludge or biosolids that are used as organic amendments to fertilize farmland (Kinney et al., 2008; Lemos et al., 2009; Dodgen et al., 2014; Careghini et al., 2015). It is estimated that 6 million tons of biosolids are produced each year in the USA, 60% of which are applied to agricultural fields (Dodgen et al., 2014). In Europe, 37% of the produced biosolids are used for this purpose, representing 2.4 million tons of sludge per year (Kinney et al., 2008). Given the accessibility of this resource, these numbers are tending to increase. Regulation of biosolids application contemplates the control of pathogen levels, nutrients, heavy metals, etc., but xenobiotics such as BPA have been neglected. Nevertheless, there is a recalcitrant BPA fraction in soils (Zhang et al., 2015) due to the high and constant application rates of biosolids.

Earthworms represent 80% of the animal biomass in the soil (Diogéne et al., 1997; Yasmin and D'Souza, 2010). They are considered excellent bioindicators of contamination in the soil, due to the ecosystem services they provide, their low dispersion capacity and their sensitivity to contamination (Bouché, 1992; Paoletti, 1999; Shin et al., 2005; Sánchez-Hernández, 2006; Santadino et al., 2014). Moreover, they are worth studying because of their role in the bioaccumulation and passage of toxins along the food chain, in which they constitute a basic link (Spurgeon et al., 2003; Dodgen et al., 2014). Contaminants found in the soil come into contact with these terrestrial annelids either through the skin that covers the animal, formed by an epidermis and a thin cuticle, or through the ingestion of food (Phipps et al., 1993; Laycock et al., 2016), and the bioaccumulation of BPA in earthworm tissues has been reported before (Markman et al., 2007; Babić et al., 2016a). This work aims to study the effect of BPA on two epigeic species of earthworms *Eisenia fetida* (Savigny, 1826), and *Dendrobaena veneta* (Rosa, 1886). Based on the hypothesis that oligochaetes possess estrogen receptors, and also on previous effects observed in other animals for BPA (obesity, infertility, and/or feminization), an alteration in the growth and reproduction of earthworms at sublethal concentrations can be expected. Moreover, proven effects of BPA on vertebrate immunity suggest that BPA could also affect worms' immune system, so the viability of coelomocytes was analyzed. These cells, which represent part of the earthworm's immune system (Bilej et al., 2010), have been widely used for monitoring pollution at the cellular level in earthworms, due to their ease of extraction, their contact with the external environment through the dorsal pores, and their sensitivity to contaminants (Asensio et al., 2007; Homa et al., 2007; Plytycz et al., 2010; Irizar et al., 2015a). On the other hand, a dose response is expected, as well as lower toxicity through the soil than by direct contact, due to the adsorption of the BPA molecules on the surface of soil colloids. Finally, the two studied species may present different sensitivity to the same toxicant (Newman et al., 2000; Dittbrenner et al., 2011).

Therefore, the specific objectives of this study are:

1. To assess life-history traits, in terms of growth and reproduction of two different species of adult earthworms (*E. fetida* and *D. veneta*) living in soil contaminated with BPA at different sublethal concentrations, that do not kill the entire population in a given time.
2. To explore the effect of different concentrations of BPA on the immune system of said species and to assess the validity of coelomocytes as biomarkers in the case of endocrine disruptor contamination.
3. To study the effect of BPA when individuals are exposed directly to the xenobiotic.
4. To evaluate the sensitivity of different species of earthworms exposed to the same toxicant under the same conditions.

The obtained results will lead to an improvement in the knowledge of the effects of endocrine disruptors such as BPA, in the natural environment, particularly for earthworms, which are essential for the proper functioning of edaphic ecosystems, thus covering a field that has practically no references. This and future evidence will aid forthcoming ecological risk assessment.

2. Materials and methods

2.1. Earthworms

Two species of earthworms, *Eisenia fetida* and *Dendrobaena veneta,* were used. They are epigeic, living close to the surface, feeding on the organic remains present in the most superficial layers of the soil, and have short reproductive cycles when compared with other species of earthworms. The population of *E. fetida* was provided by Dr. Domínguez (University of Vigo, Spain), whereas *D. veneta*, was acquired from Decathlon. Adults (clitellated) with weights between 250 mg and 600 mg for *E. fetida,* and between 300 mg and 900 mg for *D. veneta,* were used for the tests. All earthworms were kept in culture chambers in the dark under controlled temperature conditions of 21 ± 0.5 °C and food (untreated horse manure).

There are numerous studies on the cryptic speciation of earthworms, which often present genetic differences without showing morphological differences (King et al., 2008; Novo et al., 2009, 2010; James et al., 2010; Buckley et al., 2011; Cunha et al., 2014). To ensure that the populations of earthworms used were genetically homogeneous, randomly selected individuals from the original cultures were genotyped by amplifying a fragment of subunit 1 of the cytochrome oxidase (COI) gene. Genetic divergences could imply different responsiveness to toxicants and therefore different sensitivity (Dallinger and Hockner, 2013; Voua Otomo et al., 2013), which represents one of the problems of toxicological studies, since some of the species used as models may be a set of cryptic species. DNA was extracted from 25 mg of epidermal tissue from the caudal part (easily regenerated) of 11 individuals of each species. A DNeasy Blood and Tissue Kit from QIAGEN was used following the manufacturer's instructions. A 485 bp fragment of COI gene was