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Variation in the toxicity of sediment-associated substituted phenylamine antioxidants to an epibenthic (*Hyalella azteca*) and endobenthic (*Tubifex tubifex*) invertebrate

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HIGHLIGHTS

- 28-d LC50s *H. azteca* exposed to sediment-associated SPAs ranged 22 – > 403 μg/g dw.
- 28-d EC50s *T. tubifex* exposed to sediment-associated SPAs ranged 3.6–15 μg/g dw.
- Variation in toxicity between SPAs corresponded with Koc.
- Variation in toxicity between species due to variation in pathway of exposure.

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ABSTRACT

Substituted phenylamine antioxidants (SPAs) are produced in relatively high volumes and used in a range of applications (e.g., rubber, polyurethane); however, little is known about their toxicity to aquatic biota. Therefore, current study examined the effects of chronic exposure (28 d) to four sediment-associated SPAs on epibenthic (*Hyalella azteca*) and endobenthic (*Tubifex tubifex*) organisms. In addition, acute (96-h), water-only exposures were conducted with *H. azteca*. Mortality, growth and biomass production were assessed in juvenile *H. azteca* exposed to diphenylamine (DPA), *N*-phenyl-1-napthylamine (PNA), *N*-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (DPPDA), or 4,4'-methylene-bis[*N*-*sec*-butylaniline] (MBA). Mortality of adult *T. tubifex* and reproduction were assessed following exposure to the four SPAs. The 96-h LC50s for juvenile *H. azteca* were 1443, 109, 250, and >22 μg/L and 28-d LC50s were 22, 99, 135, and >403 μg/g dry weight (dw) for DPA, PNA, DPPDA, and MBA, respectively. Reproductive endpoints for *T. tubifex* (EC50s for production of juveniles > 500 μm: 15, 9, 4, 3.6 μg/g dw, for DPA, PNA, DPPDA, and MBA, respectively) were an order of magnitude more sensitive than endpoints for juvenile *H. azteca* and mortality of adult worms. The variation in toxicity across the four SPAs was likely related to the bioavailability of the sediment-associated chemicals, which was determined by the chemical properties of the SPAs (e.g., solubility in water, Koc). The variation in the sensitivity between the two species was likely due to differences in the magnitude of exposure, which is a function of the life histories of the epibenthic amphipod and the endobenthic worm. The data generated from this study will support effect characterization for ecological risk assessment.

1. Introduction

There are a large number of chemicals used in commerce for which there is little data on their potential fate and effects in the environment. For this reason, several initiatives have been adopted to prioritize these chemicals for hazard assessment. For example, in 2006, the Government of Canada introduced the Chemicals

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Management Plan, to prioritize and evaluate the hazard of approximately 23,000 chemicals, used in commerce in the previous two decades, to human health and the environment by 2020 Government of Canada (2014). Similar programs to assess chemicals in commerce have been initiated in the United States and Europe (USEPA, 2015; ECHA, 2016). It is important to note that these lists of chemicals do not include pharmaceuticals and pesticides, which are extensively assessed under different federal programs (e.g., Health Canada (2009), USFDA (2015), European Commission (2016)). A class of chemicals that is present on these lists for prioritization and hazard assessment is substituted phenylamine antioxidants (SPAs) (Table 1).

SPAs are used as a primary antioxidant in a variety of commercial products, e.g., rubber, polyurethane, and engine lubricants, for the purpose of extending the lifespan of the products (WHO, 1998; OECD, 2004; ECHA, 2008). SPAs prevent the formation of free radicals produced by heat, stress, exposure to oxygen and/or ozone, or UV radiation, which contribute to the degradation of polymers (Maier and Calafut, 1998). There is a dearth of data on the potential effects of SPAs on aquatic ecosystems. The majority of research has focused on the acute toxicity of SPAs due to exposure via water (Sikka et al., 1981; Tonogai et al., 1982; Geiger et al., 1990; Drzyzga et al., 1995; Murin et al., 1997). However, SPAs will likely partition into the sediment when they enter an aquatic system due to their relatively high organic carbon-water partition coefficient (Koc) and low solubility in water (Table 1). Hence, research is needed to investigate the effect of sediment-associated SPAs on benthic organisms. Therefore, the objective of our study was to examine the effect of sediment-associated SPAs on two species of benthic invertebrates that would be exposed via different pathways; *Tubifex tubifex* (oligochaete) are endobenthic (burrow into sediment) while *Hyalella azteca* (amphipod) are epibenthic (live at sediment-water interface). The magnitude and pathway of exposure to sediment-associated contaminants will vary between these two species, as *T. tubifex* live exclusively in sediment and are primarily exposed to contaminants bound to the sediment and within the pore water, while *H. azteca* live at the sediment-water interface and are primarily exposed to contaminants in the overlying water. These two species are also representative of the different pathways of exposure by which most aquatic invertebrates would be exposed to sediment-associated SPAs. As the primary route of exposure for *H. azteca* is via water, acute, water-only exposures with SPAs were also conducted with this species. Four SPAs were chosen for this study: diphenylamine (DPA), *N*-phenyl-1-napthylamine (PNA), *N*-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (DPPDA), and 4,4'-methylene-bis[*N-sec*-butylaniline] (MBA) (Table 1). The four SPAs chosen are representative of the range of physicochemical properties (e.g., log K_{OW}, solubility in water) that are found in this class of chemicals. The data generated from this study will provide information to assist in assessing the risk that SPAs may pose to aquatic ecosystems.

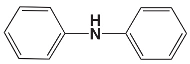
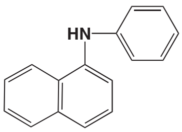
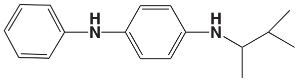
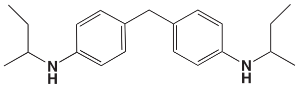
2. Methods

2.1. Sediment

The sediment used in this study was a mixture of two reference sediments used by Environment and Climate Change Canada (ECCC) for invertebrate culturing and toxicity testing. The sediments were collected from two sites in Lake Erie in Ontario, Canada (Long Point marsh, 42.583683N, 80.443726W and Long Point Bay, 42.58472N 80.21806W). The physicochemical properties of the sediments are presented in the Supplementary Information (SI) (Table S1). The sediments were mixed to produce sediment with an organic carbon content of ~2%, which is representative of a large range of sediments across Canada. Long Point marsh and bay sediments were sieved through a 250-μm sieve before being mixed in a 2:3 ratio by volume. Tables S2 and S3 contain the physicochemical properties of the mixed sediment and the results of monitoring for potential contaminants. Physicochemical properties of sediment were determined by standard methods developed by ECCC's National Laboratory for Environmental Testing (NLET). Sediment was stored at ~4 °C before being used in the study (stored 2–10 months).

Due to their relatively low solubility in water, solid SPAs (DPA: purity > 99%, Sigma Aldrich; PNA: purity > 98.0%, TCI; DPPDA: purity > 98.0%, TCI; MBA: purity ≥ 99%, CHEMOS Gmbh) were dissolved in acetone to produce stock solutions of different concentrations for each individual SPA for spiking of sediment. Sediment was spiked with the same volume of stock solution to produce the different treatments used in each test and the volume of stock solution used for spiking represented <1% of the volume of sediment. However, the use of a solvent required the addition of a solvent control in each test. Solvent control treatments were

Table 1
Physicochemical properties of substitute phenylamine antioxidants diphenylamine (DPA), *N*-Phenyl-1-naphthylamine (PNA), *N*-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (DPPDA), and 4,4′-Methylene-bis(*N-sec*-butylaniline) (MBA). Physiochemical properties were modeled using USEPA EPI Suite.

Name	CAS #	Chemical structure	log K_{OW}	log K_{OC}	Solubility in water (25 °C) (mg/L)	Henry's law constant (Pa·· m^3/mol)
Diphenylamine (DPA)	122-39-4		3.3	2.9	63	0.106
N-phenyl-1-napthylamine (PNA)	90-30-2		4.5	4.5	9.0	0.010
N-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (DPPDA)	793-24-8		4.7	4.4	2.8	0.003
4,4'-methylene-bis[*N-sec*-butylaniline] (MBA)	5285-60-9		6.1	4.9	0.07	0.0002