Ecotoxicology and Environmental Safety 104 (2014) 194–201

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Neurotoxic evaluation of two organobromine model compounds and natural AOBr-containing surface water samples by a *Caenorhabditis elegans* test

Jingjuan Ju [a,b,1], Thora Lieke [b,1], Nadine Saul [b], Yuepu Pu [a], Lihong Yin [a], Cindy Kochan [c], Anke Putschew [c], Nora Baberschke [b], Christian E.W. Steinberg [b,*]

[a] Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, China
[b] Department of Biology, Freshwater and Stress Ecology, Humboldt-Universität zu Berlin, Späthstr. 80/81, 12437 Berlin, Germany
[c] Chair of Water Quality Control, Technische Universität Berlin, Straße des 17, Juni 135, 10623 Berlin, Germany

ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form
7 March 2014
Accepted 11 March 2014
Available online 13 April 2014

Keywords:
Cyanobacteria
Autonomic function
Sensory function
Organobromine compounds
AOBr
Surface water samples

ABSTRACT

Brominated organic compounds are known as disinfection byproducts. Very recently, however, even natural brominated organic compounds (analyzed as adsorbable organic bromine; AOBr) have been found in decaying freshwater cyanobacteria blooms. Among the identified compounds was dibromoacetic acid (DBAA), which has proven to be neurotoxic at rather high concentrations in mammalian assays. Currently it is open how single compounds as well as complex mixtures impact organisms at environmentally realistic concentrations. Furthermore, it is also unclear how natural organic matter, mainly humic substances (HS), which are present in all freshwater systems, modulates the toxic impact of AOBr. Therefore, two AOBr compounds (DBAA and tetrabromobisphenol-A; TBBP-A) and AOBr-containing water samples were tested using a *Caenorhabditis elegans* neurotoxicity assay that measured autonomic and sensory functions. TBBP-A had an impact on three response variables of *C. elegans* and can be classified neurotoxic. In contrast to our expectations, DBAA led to neurostimulation of two autonomic functions, but had a temporary impact on the defecation interval.

All surface water samples contained measurable amounts of AOBr. Exposure of *C. elegans* to concentrated water samples – one in particular – increased three of the four locomotion traits and left defecation activity and both sensory variables unchanged. This stimulation might be due to unidentified compounds in the samples or to a hormetic effect of the AOBr compounds. Thermotactic behavior was characterized by a temporary preference for the colder environment, indicating a temporary mild neurotoxicity. Overall, the set of relative simple phenotypic tests used in the current study revealed a meaningful neurotoxic or neurostimulative profile in response to chemical compounds or natural samples. Furthermore, it shows that the resulting response to natural AOBr compounds at environmentally realistic concentrations was not necessarily adverse, but instead, that the mixtures of natural AOBr were neurostimulatory.

1. Introduction

It is well understood and documented that the proliferation of cyanobacterial blooms is a significant hazard to public health worldwide, since these blooms produce a variety of toxins, which are dominated by various microcystins (MCs) (Dittmann and Wiegand, 2006; Paerl and Otten, 2013). However, freshwater contamination by cyanobacterial metabolites is actually very complex, since even natural brominated organic compounds have been identified recently. In two Berlin surface waters, for instance, organobromine compounds, which were measured as adsorbable organic bromine (AOBr) were found at levels up to 35 $\mu g\,L^{-1}$ (Hütteroth et al., 2007a), which exceeds common toxin concentrations (Welker et al., 2003; Wiedner et al., 2008). The occurrence of AOBr was associated with the decay of the cyanobacteria (Hütteroth et al., 2007a). Dibromoacetic acid (DBAA), bromochloro acetic acid (BCAA), and 4-bromo-3-nitrobiphenyl have been detected as single compounds in the AOBr pool (Hütteroth, 2006). These compounds have been shown to be toxic. For instance, Andrews et al. (2004) reported that DBAA and BCAA were toxic to the development of mouse embryos. Additionally, Melnick et al. (2007) found toxic effects of DBAA in the livers and testes of rats and mice, when they used concentrations that far

* Corresponding author. Fax: +49 30 636 9446.
E-mail address: christian_ew_steinberg@web.de (C.E.W. Steinberg).
[1] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.ecoenv.2014.03.009
0147-6513/

exceed field concentrations. Even neurotoxicity, genotoxicity, and immunotoxicity have been observed in rodent models (Gao et al., 2008; Moser et al., 2004; Zhang et al., 2010) if concentrations well above those observed in the environment were applied.

The cocktail of these direct and indirect cyanobacterial metabolites should potentiate the toxic challenge of exposed organisms, although the mode of toxic action may differ. It is well understood that organisms living under conditions close to their environmental tolerance limits appear to be generally the most vulnerable to additional challenges (Heugens et al., 2001). Therefore, we expected that exposure to mixtures of AOBr compounds from surface waters would clearly exceed the potential neurotoxicity of the single compounds tested. This was tested by using the recently developed *Caenorhabditis elegans* Maupas assay, which is a suitable invertebrate alternative to vertebrate assays for neurotoxic evaluations (Ju et al., in revision). The nematode *C. elegans* has been a model animal for toxicological evaluations (Popham and Webster, 1979) and ecotoxicological (reviewed by Traunspurger et al. (1997) and Leung et al. (2008)) and neurotoxicological studies (Anderson et al., 2004; Helmcke et al., 2010; Rajini et al., 2008).

In this study, samples from eutrophicated surface water that contained AOBr compounds in Berlin were tested for neurotoxicity. The autonomic and sensory functions of *C. elegans*, including chemotactic and thermotactic behavior, were used in order to achieve a comprehensive understanding of the neurotoxic risk of the exposed samples. To get an idea of the potential effective compounds in the complex water samples, we tested two individual organobromine compounds, DBAA and tetrabromobisphenol-A (TBBP-A). The latter is the most widely used brominated flame retardant and is, therefore, ubiquitously distributed (Covaci et al., 2009; Zhang et al., 2009). Additionally, a derivative of TBBP-A was recently identified as being neurotoxin (Qu et al., 2011). In conclusion, if individual AOBr compounds lead to neurotoxic symptoms, we hypothesize that a mixture of these compounds will increase the adverse effects additively or synergistically.

2. Material and methods

2.1. Strains

The wild-type *C. elegans* strain N2 and the *Escherichia coli* strain OP50 were originally obtained from the Caenorhabditis Genetics Center (CGC; USA). L4 larvae were used as the starting exposure stage. Nematodes were maintained on nematode growth medium (NGM) plates seeded with OP50 at 20 °C (Brenner, 1974). All quantitative measurements were carried out using a digital microscope (Keyence VHX 600D, Osaka, Japan) or a stereo microscope (Nikon, Tokyo, Japan).

2.2. Surface water samples

During late summer and early autumn in 2010, three samples of approximately 10 L were collected from Lake Stößen See (52° 30′ 34″N 13° 12′ 34″ E), a bay of the Havel river in the western part of the city of Berlin, Germany. Samples were filtered (0.45 μm, cellulose nitrate membrane filters; Sartorius, Göttingen) and analyzed for AOBr and microcystins. Subsamples were deep-frozen and stored for bioassays. For the bioassays, the samples were lyophilized (ABiTEP, Berlin, Germany) to a remaining volume of about 1 L and the AOBr content of each sample was analyzed again. The samples were added to the bacteria and the NGM agar in a calculated volume in order to reach the final AOBr concentrations, which were, due to a higher robustness of the tests, 6–8 times higher than what was originally in the surface water (Table 1).

2.3. AOBr analysis

The determination AOBr was done as described by Oleksy-Frenzel et al. (2000). A 100 mL water sample was acidified to a pH of 2 with HNO_3 (conc.). Dissolved AOBr was enriched on activated carbon and combusted. The reaction gases were collected in 5 mL ultrapure water containing 4.5 μM Na_2S. The solution was injected into an ion chromatographic system for bromide analysis.

2.4. Analysis of cyanotoxins

2.4.1. Solid phase extraction of microcystins

Solid phase extraction (SPE) was used to concentrate dissolved MCs from the water samples prior to HPLC (DIN, 2007). Then, 500 mL water was passed through Lichrolute RP 18 (300 mg, 3 cc; Merck Millipore) SPE cartridges that were conditioned with 4 mL 5 percent methanol and eluted with 6.5 mL methanol acidified with 0.1 percent formic acid (v/v). Organic solvent was removed by a gentle nitrogen stream. Extracts were dissolved in 0.5 mL ultrapure water.

2.4.2. Solid phase extraction of cylindrospermopsin

For enrichment of dissolved cylindrospermopsin, 500 mL water was passed through Supelclean ENVI-Carb (250 mg, 3 cc; Supelco) SPE cartridges that were conditioned with 7 mL H_2O and backflow-eluted with 6.5 mL methanol acidified with 0.1 percent formic acid (v/v). Organic solvent was removed by a gentle nitrogen stream. Extracts were dissolved in 0.5 mL ultrapure water.

2.4.3. LC/MS-MS analysis of cyanotoxins

The separation of cyanotoxins in extracts was achieved using a Zorbrax Eclipse XDB C18-UPLC column at 40 °C with a flow rate of 0.4 mL min^{-1} (Accela pump; Thermo Fisher Scientific), and injection volume of 10 μL (CTC-PAL; CTA Analytics AG). For LC gradient elution, both water (A) and methanol (B) contained 0.006 percent acetic acid (v/v) and 5 mM $HCOONH_4$. The gradient for cyanotoxin separation was 0–2 percent B, 0–1.5 min; 2–50 percent B, 1.5–5 min; 50–70 percent B, 5–8 min; 70 percent B, 8–13 min; and 0 percent B for re-equilibration. The compounds were detected by positive ESI-MS/MS (TSQ Vantage; Thermo Fisher Scientific) in the multiple reaction monitoring modes using argon as the collision gas (1.5 mTorr). The vaporizer temperature was 300 °C, the transfer capillary was set to 270 °C, the spray voltage was 3 kV, and the sheath gas and auxiliary gas pressures were 35 and 15 (arbitrary units), respectively. The recorded transitions, determined by infusion of standards, were m/z 416.1→194.2 and 176.1 (cylindrospermopsin, collision energy (CE) 39 eV), m/z 519.8→135.0 and 239.1 (Microcystin-RR, CE 55 eV), m/z 995.6→135.0 and 861.0 (Microcystin-LR, CE 57 eV), and 1045.5→135 and 174.0 (Microcystin-YR, CE 66 eV). Due to a lack of internal standards, the compounds were analyzed by standard addition of 0, 50, 100 and 150 ng/L, with addition prior to SPE. The limit of quantification was 3 ng L^{-1} for cylindrospermopsin and 10 ng L^{-1} for each microcystin variant.

2.5. Preparation of plates and exposure conditions

TBBP-A and DBAA were dissolved in DMSO and then added to the NGM agar and the OP50 bacteria at a final concentration of 50 μM. The final concentration of the DMSO was 0.3 percent [v/v]. The preparation for surface water samples were described as above. All neurotoxic tests were performed after exposing nematodes for 24 and 72 h. At least three replicates and 8–30 nematodes per replicate were used for the assays with chemicals and two replicates for the assays with the water samples.

2.6. Neurotoxic tests

The test protocol followed the recently developed adaptation of vertebrate neurotoxic evaluations to *C. elegans* (Ju et al., in revision). All neurotoxins that

Table 1
Sampling dates and contents of AOBr and cyanotoxins.

Lake Stößen, see samples	AOBr in original water, μg L^{-1}	AOBr after lyophilization, μg L^{-1}	Microcystin variants, ng L^{-1}	Cylindro-spermopsin, ng L^{-1}
August 23, 2010	17.77	147.50	nd	27.3
September 14, 2010	16.21	105.36	nd	9.0
October 12, 2010	11.24	94.00	nd	6.9

nd = Not detectable.