Aquatic Toxicology 188 (2017) 26–32

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Dechlorane Plus induces oxidative stress and decreases cyclooxygenase activity in the blue mussel

Pierre-Luc Gagné[a,b], Marlène Fortier[c], Marc Fraser[b,c], Lise Parent[b,d], Cathy Vaillancourt[b,c], Jonathan Verreault[a,b,*]

[a] Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, succursale Centre-ville, Montreal, QC, H3C 3P8, Canada
[b] Center for Interdisciplinary Research on Well-Being, Health, Society and Environment (CINBIOSE), Université du Québec à Montréal, C.P. 8888, Succursale Centre-ville, Montreal, QC, H3C 3P8, Canada
[c] INRS-Institut Armand-Frappier, Université du Québec, 531 boulevard des Prairies, Laval, QC, H7V 1B7, Canada
[d] Département Science et Technologie, Télé-université (TÉLUQ), 5800 rue Saint-Denis, bureau 1105, Montreal, QC, H2S 3L5, Canada

ARTICLE INFO

Keywords:
Dechlorane plus
Flame retardant
Blue mussel
Cyclooxygenase
Lipid peroxidation
DNA damage

ABSTRACT

Dechlorane Plus (DP) is a chlorinated flame retardant used mainly in electrical wire and cable coating, computer connectors, and plastic roofing materials. Concentrations of DP (*syn* and *anti* isomers) are increasingly being reported in aquatic ecosystems worldwide. However, there is exceedingly little information on the exposure-related toxicity of DP in aquatic organisms, especially in bivalves. The objective of this study was to investigate the *in vivo* and *in vitro* effects of DP exposure on histopathology, lipid peroxidation (LPO) levels, cyclooxygenase (COX) activity, phagocytosis capacity and efficiency, and DNA strand breakage in the blue mussel (*Mytilus edulis*) following a 29 days exposure (0.001, 0.01, 0.1 and 1.0 µg DP/L). Blue mussels accumulated DP in muscle and digestive gland in a dose-dependent manner. LPO levels in gills were found to increase by 82% and 67% at the 0.01 and 1.0 µg DP/L doses, respectively, while COX activity in gills decreased by 44% at the 1 µg/L dose. No histopathological lesion was found in gonads following DP exposure. Moreover, no change in hemocyte DNA strand breakage, phagocytosis rate, and viability was observed following DP exposure. Present study showed that toxicity of DP may occur primarily *via* oxidative stress in the blue mussel and potentially other bivalves, and that gills represent the most responsive tissue to this exposure.

1. Introduction

Dechlorane Plus (DP) is a highly chlorinated compound that has been manufactured over the last 40 years as a replacement to Dechlorane (Mirex) (Fang et al., 2014) and is mainly used today as flame retardant (Wang et al., 2016). DP is synthesized by a Diels-Alder reaction of hexachlorocyclopentadiene and cyclooctadiene (Shen et al., 2011). The DP technical mixture contains two stereoisomers (*syn*- and *anti*-DP) in a ratio of approximately 1:3 (Wu et al., 2010). Usages of DP include electrical wire and cable coating, computer connectors, plastic roofing materials, and other polymeric materials (Feo et al., 2012). DP is currently unregulated and listed as a high production-volume chemical in the USA with an annual production or importation volume estimated to 500 t (EPA, 2015). Studies have also reported the presence of DP as an impurity in other chlorinated compound mixtures including the pesticide Chlordane (Shen et al., 2011; Sverko et al., 2010). The environmental persistence, bioaccumulation propensity and potential for toxicity suggest that DP may represent a persistent organic pollutant (POP) candidate (Feo et al., 2012; Sverko et al., 2011).

The global bans of Octa- and Penta-bromodiphenyl ether (BDE) technical mixtures (UN, 2009) have resulted in an increased market demand for alternative flame retardants (Bergman et al., 2012; Chen et al., 2013). DP has been identified as a possible replacement product to the recently phased-out Deca-BDE in North America and Europe (EPA, 2014), and was recently proposed for listing under the Stockholm Convention on POPs (UN, 2015). Due to its high hydrophobicity ($\log K_{ow}$ = 9.3; Hoh et al., 2006), DP has a high tendency to adsorb onto organic materials, and exhibits long half-life in the aquatic environment. DP has been reported in wastewater from Shanghai and water samples from the coastal shore of northern China at concentrations up to 1.4 and 1.8 ng/L, respectively (Xiang et al., 2014; Jia et al., 2011). It has also been determined in sediments of tributaries of the

* Corresponding author at: Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, C.P. 8888, succursale Centre-ville, Montreal, QC, H3C 3P8, Canada.
E-mail address: verreault.jonathan@uqam.ca (J. Verreault).

http://dx.doi.org/10.1016/j.aquatox.2017.04.009
Received 5 December 2016; Received in revised form 12 April 2017; Accepted 14 April 2017
Available online 17 April 2017
0166-445X/

Laurentian Great Lakes (Shen et al., 2011). Also, studies have reported levels of DP in aquatic food chain including marine bivalves, *e.g.*, Asiatic hard clams (*Meretrix meretrix L*) and Manila clams (*Ruditapes philippinarum*) from the Pearl River estuary in China in which elevated DP levels (13–37 ng/g lw) were found (Sun et al., 2015). DP was also determined at lower levels in several marine mollusks from the Northern coast of Spain, *e.g.*, Mediterranean mussels (*Mytilus galloprovincialis*), Manila clams, and cockles (*Cerastoderma edule*) (Villaverde-de-Sáa et al., 2013). A study by Wang et al. (2016) further showed that DP isomers accumulate in a stereoselective manner in the aquatic food web, *i.e.*, f_{anti} (fraction of *anti*-DP to ΣDP) values decreased in species occupying higher trophic levels, suggesting a lower uptake efficiency and higher metabolic capacity for the *anti*-isomer compared to *syn*-isomer. Recent studies have shown that DP is genotoxic to luminous bacteria (*Tetrahymena thermophila*) (Dou et al., 2015), earthworms (*Eisenia fetida*) (Yang et al., 2016) and Mediterranean mussels (Barón et al., 2016). There are, however, limited information available on the bioaccumulation dynamics and toxicity (*i.e.*, reproductive toxicity and immunotoxicity) of DP in bivalves, and there is an urgent need to fulfill these knowledge gaps.

The blue mussel (*Mytilus edulis*) is ubiquitously distributed in the marine environment from Antarctica up to the Arctic regions. Its distribution, reproductive cycle and growth are influenced by an array of environmental factors including temperature, salinity, light, and food supply (Seed, 2009). Their sedentary lifestyle makes blue mussels ideal candidates for contaminant bioaccumulation and toxicity studies. Mussels are well-known to respond to a wide array of contaminants, *e.g.*, municipal wastewater effluent that was shown to induce lipid peroxidation (LPO) in mussel gonads (Gagné et al., 2011a, 2011b). Also, Brooks et al. (2009) have observed DNA damage in mussel hemocytes and microlesions in digestive glands following exposure to polycyclic aromatic hydrocarbons (PAHs).

The first line of defence to xenobiotics in mussels is phagocytosis by the hemocytes as a non-specific immune response. This has been demonstrated in a marine soft-shell clam (*Mya arenaria*) and a freshwater bivalve (*Elliptio complanata*) exposed to municipal wastewater effluent (Blaise et al., 2002). However, there are to our knowledge exceedingly limited information on the immunotoxicity of DP in any aquatic organisms. Nevertheless, bivalves were shown to exhibit inhibition (Pichaud et al., 2008) or stimulation (Croxton et al., 2012) of phagocytosis by hemocytes following exposure to PAHs. Moreover, Lv et al. (2015) observed an immunosuppressive response through formation of reactive oxygen species and apoptosis in peritoneal macrophages of female mice following a 24 h exposure to the PBDE congeners BDE-47 (one major congener in Penta-BDE) and BDE-209 (> 97% of Deca-BDE). Depressed immunity in bivalves may thus lead to susceptible disease vulnerability, *i.e.*, to marine pathogens including *Vibrio anguillarum* as demonstrated in salmon exposed *in vivo* for 40 d to five PBDE congeners (*i.e.*, BDE-47, −99, −100, −153, and −154) (Arkoosh et al., 2010).

DP exposure was also shown to induce a genotoxic response (*i.e.*, DNA strand breakage) in Mediterranean mussels at concentrations far greater than those found in aquatic ecosystems in general (> 1.8 ng/L) (Barón et al., 2016). Interaction with genetic material can also be associated with enhanced oxidative stress (*i.e.*, increased LPO levels) by altering the permeability of cell membrane (Sheehan and McDonagh, 2008). For example, recent study on the molecular properties of the anti-inflammatory, anti-diabetic and anti-hypertensive ethyl acetate-methanol extracts in marine bivalves (*i.e.*, *Villorita cyprinoides* and *Paphia malabarica*) showed significant decrease in cyclooxygenase (COX) activity that was indirectly related to oxidative stress (Joy et al., 2016). Also, Gagné et al. (2011b) showed significant increase in COX activity and LPO levels in gonads of *Elliptio complanata* following exposure to municipal wastewater effluent. Given the key role of COX in the immune system (Shimokawa and Smith, 1992; Gagné et al., 2007) and spawning in invertebrates including bivalves (Matsutani and Nomura, 1987), it is of primary importance to determine the effects of DP on this enzyme.

Due to the ubiquitous environmental presence and potentially growing usage of DP as an alternative flame retardant, and considering the lack of information on the exposure-related effects of DP in aquatic organisms, the objective of the present study was to investigate the accumulation and *in vivo* and *in vitro* effects of DP in the blue mussel at selected doses including environmental concentrations on histopathology, DNA integrity and markers of the immune system, oxidative stress, and reproductive system.

2. Materials and methods

2.1. In vivo exposure

Adult blue mussels (shell length: 62.9 ± 1.7 mm; $n = 72$) were obtained from the mussel husbandry *La moule du large* (Havre-aux-Maisons, QC, Canada). Upon reception, mussels were acclimatized for 14 d in 20 L tanks filled with artificial aerated marine water (AAMW) at a salinity of 31 psu (Instant Ocean, Reef Crystal, Cincinnati, OH, USA) that was changed twice a week. Mussels were kept at 25 ± 2°C under a photoperiod of 12 h light and 12 h dark. Mussels were fed during the acclimatization period three times a week with a commercially available phytoplankton solution containing *Nannochloropsis*, *Tetraselmis suecica*, *Isochrysis* sp. at a concentration of 3.0×10^9 cells/5 mL (Phytoplex; Reef solution, Laval, QC, Canada). After the acclimatization period, mussels were divided into six groups ($n = 12$ per group) in 3 L Pyrex beakers: a control (AAMW only) which was also used to detect potential pathologies, a vehicle control (AAMW with DMSO; 0.002% v/v) and four doses of DP (stock solution: 50 mg/L or mM; Wellington laboratories, Guelph, ON, Canada): 0.001, 0.01, 0.1 and 1.0 µg/L or µM. The selected DP doses represented two environmental concentrations (0.001 and 0.01 µg/L or µM) (wastewater from Shanghai and seawater of coastal shore in Northern China: Xiang et al., 2014; Jia et al., 2011) and 10- and 100-fold greater concentrations. The water in the beakers was renewed and spiked according to exposure groups described above twice a week during the entire exposure period that lasted 29 days. Mussels were not fed during the 29-days exposure period for the following reasons: 1) it has been shown that DP has a high tendency to adsorb onto suspended solids (Fang et al., 2014), and hence the presence of bivalve food (phytoplankton solution) in water would have resulted in varying exposure to mussels, 2) mussels are known to survive without food for 41–154 days during summer and winter, respectively (Gabbott, 2009), and 3) Riisgård et al. (2011) have observed a variation in filtration rates when blue mussels are fed, which also would have resulted in varying DP exposure to mussels. Because the present experimental design aimed to investigate the *in vivo* and *in vitro* effects of accumulated DP concentrations in blue mussel tissues, water was not sampled for DP analysis during the course of the experiment. Regardless, Barón et al. (2016) have investigated the bioavailability of DP in mussels (*Mytilus* sp.) kept in 2 L beakers by exposing them to three concentrations of DP (5.6, 56, and 100 µg L^{-1}). The authors collected three water samples for each treatment immediately after dosing and prior to water change (*i.e.*, after 23 h of exposure), and this during six consecutive days. Barón et al. (2016) reported concentrations (mean ± SEM) of DP in water samples taken immediately after dosing that were as follows: 0.4 ± 0.3, 0.3 ± 0.2, and 0.7 ± 0.5 µg/L for the 5.6, 56, and 100 µg/L doses, respectively. These concentrations thus decreased consistently after 23 h with 77, 79 and 86%, respectively. Therefore, because the environmentally-relevant DP concentrations used in present study (0.001, 0.01, 0.1 and 1.0 µg/L) were between 100 and 5600 times lower compared to those used by Barón et al. (2016), we would not be able to detect the nominal exposure concentrations of DP in water based on current method limits of quantification for DP isomers (Table 1).

During the exposure period, 14 individuals were found dead,