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Rapid and complete dehalogenation of halonitromethanes in simulated gastrointestinal tract and its influence on toxicity

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HIGHLIGHTS

- HNMs were rapidly dehalogenated in human gastrointestinal tract.
- Digestive juice played major roles in the dehalogenation process of HNMs.
- Dehalogenation process reduced hepatotoxicity of HNMs.
- HNMs changed the microbial community in gastrointestinal tract.

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ABSTRACT

Halonitromethanes (HNMs) as one typical class of nitrogenous disinfection byproducts in drinking water and wastewater are receiving attentions due to their high toxicity. This study applied a simulator of the human gastrointestinal tract to determine the dehalogenation processes of trichloronitromethane, bromonitromethane and bromochloronitromethane for the first time. Influence of digestion process of HNMs on gut microbiota and hepatotoxicity was further analyzed. Results showed that the three HNMs were rapidly and completely dehalogenated in the gastrointestinal tract, especially in the stomach (2 h retention Time) and small intestine (4 h retention Time). Mucin, cysteine, pancreatin and bile salts in the digestive juice played major roles in the dehalogenation process. HNMs and their dehalogenation products in the resulting fluids of stomach induced the highest toxicity followed by those in intestine and colon, exhibiting dose-dependent effects. Although most HNMs were degraded in the stomach and small intestine, residual HNMs entered into colon changed the microbial community. Abundance of several genera, such as *Bacteroides, Lachnospiraceae_unassigned* and *Lactobacillus* had high correlation with exposure concentration of HNMs. This study sheds new light on dehalogenation and toxic processes of HNMs by oral exposure, which provides basic data for their human health risk assessment.

1. Introduction

Halonitromethanes (HNMs) are a type of nitrogenous disinfection byproducts (N-DBPs) that have been widely detected in drinking water and wastewater (Hu et al. 2010a, 2010b; Krasner et al., 2006). HNMs are receiving attention due to their higher cytotoxicity, genotoxicity and developmental toxicity than currently regulated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs) (Liviac et al., 2009; Richardson et al., 2007). Nine HNMs have been identified, including mono-, di- and trihalogenated species. Among these HNMs, trichloronitromethane (TCNM) is the most frequently occurring one in drinking water, followed by dichloronitromethane (DCNM), chloronitromethane (CNM), bromonitromethane (BNM) and bromochloronitromethane (BCNM) (Richardson et al., 2007). Meanwhile, toxicity of TCNM has been widely studied by *in vitro* experiment. For example, TCNM can induce high levels of DNA strand breaks in human lymphoblastoid TK6 cells (Liviac et al., 2009) and intracellular reactive oxygen

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species (ROS) in human epithelial cells (Pesonen et al. 2014, 2015). For other HNMs, their genotoxicity and mutagenicity were also reported (Kundu et al. 2004a, 2004b; Plewa et al., 2004). The bromo-HNMs were found to have higher toxicity than chloro-HNMs (Yin et al., 2017).

Dehalogenation readily occurs for HNMs in the environment. Previous studies have shown that TCNM can be dehalogenated to nitromethane (NM) by microorganisms in soil (Qin et al., 2016; Yates et al., 2015). The dehalogenation process of HNMs can change their toxicity. For example, DCNM and CNM are found to have more cytotoxicity than TCNM (Liviac et al., 2009). Thus, dehalogenation process of HNMs in the animal body should be completely characterized to better understand their toxic effects and health risk. The primary exposure route for HNMs in drinking water is via oral consumption. Complex conditions in the gastrointestinal tract including food, digestive enzymes and enormous numbers and diversity of microbes might induce dehalogenation of HNMs (Gill et al., 2006; Kau et al., 2011). However, little knowledge is available on which section of gastrointestinal tract and which components in digestive juice play major roles in the dehalogenation process. Moreover, toxicity of DBPs is associated with halogeno-groups, and brominated THMs (Criquet et al., 2012), HAAs (Zhang et al., 2010) and haloacetamides (HAcAms) (Plewa et al., 2008) were found to have higher genotoxic and carcinogenic effects than chlorinated counterparts. Thus, it is also important to determine the influence of substituent groups on the dehalogenation process of HNMs.

Animal experiments can effectively determine the distribution and excretion of xenobiotics in body. However, it is very difficult to identify the roles of different parts of gastrointestinal tract (such as stomach, small intestine and colon) on bioaccessibility and metabolism of xenobiotics. The *in vitro* simulated gastrointestinal system provides an alternative tool for these studies. Among current *in vitro* systems, the simulator of the human intestinal microbial ecosystem (SHIME) can simulate physiological conditions in human stomach, small intestine and colon and take into account the roles of gut microbiota (Van den Abbeele et al., 2013; Venema and van den Abbeele, 2013). Presently, the SHIME has been widely used to investigate the bioaccessibility and metabolism of xenobiotics, such as aromatic hydrocarbon and polybrominated diphenylethers in the gastrointestinal tract (TR Van de Wiele et al., 2004a,b; Yu et al., 2009).

The objective of this study was to determine the different dehalogenation processes of three HNMs with different halogen-groups (TCNM, BCNM and BNM) in different part of gastrointestinal tract by an *in vitro* SHIME system. Roles of culture medium, digestive enzymes and gut microbiota on the dehalogenation process were systematically characterized. The impact of gastrointestinal digestion on HNMs toxicity was analyzed by human HepG2 cell, since liver is the main organ of xenobiotic metabolism. This study sheds new lights on the metabolism and toxicity of HNMs by oral exposure, which are critical for the health risk assessment of HNMs.

2. Materials and methods

2.1. Chemicals

TCNM (99.9%) and BNM (90.1%) were obtained from Supelco (USA). CNM (93.1%), DCNM (98.2%) and BCNM (93.3%) were supplied by Cansyn Chem. Corp. (Canada). NM (99.0%) and 1,2-dibromopropane (99.0%, as the internal standard) were purchased from Sigma-Aldrich (USA). Methyl *tert*-butyl ether (MTBE) was supplied by Tedia (USA). Dimethyl sulfoxide (DMSO) and anhydrous sodium sulfate (Na_2SO_4) were purchased from Nanjing Chemical Reagent Corporation (China). All solvents and salts were of analytical grade or better. The ultrapure water was produced using a Millipore S.A.S. SMART water purification system (France) in the laboratory.

2.2. SHIME

The SHIME consists five double-jacketed vessels, which simulate the stomach, small intestine, ascending colon, transverse colon and descending colon, respectively. Basic operation conditions are described in the literature (Van den Abbeele et al., 2013) and shown in Figure S1. The three colon vessels of SHIME were inoculated with microbial community from faecal sample of healthy volunteer. The faecal sample was collected as previously described by Van de Wiele et al. (van de Wiele et al., 2007; Wiele et al., 2010) Since three SHIME runs were carried out for TCNM, BCNM and BNM exposure, respectively, to enable comparison of the results among the three runs, the three SHIME reactors were inoculated with bacteria from the same feacal sample of a healthy adult volunteer (aged 30 years) with no history of antibiotic treatment 6 months prior to the faecal sample collection for this study. The fresh fecal sample (30 g) was homogenized in sterile physiological salt solution (pH 7.0, 150 mL) and centrifuged at 1200 × g for 10 min to remove particulate matter. Supernatant (30 mL) was introduced into the three colon vessels (Yu et al., 2016). Then, the SHIME was fed with culture medium for 3 weeks, which enabled the microbial community to adapt to nutritional and physicochemical conditions in the different colon vessels (T Van de Wiele et al., 2004a,b).

During exposure experiment, the SHIME was sequentially exposed to culture medium with 0.1% DMSO as vehicle (CK) and culture medium with 0.1% DMSO + HNMs solution at 1, 60 and 120 μg/L. The 1 μg/L was chosen according to the detected concentrations (μg/L level) of HNMs in drinking water (Alwis et al., 2008). The exposure duration was set to one week for each condition according to the previous studies (Ouethrani et al., 2013; Yu et al., 2016). At the end of each exposure duration, 60 mL sample was taken from five vessels, respectively. Then the samples were centrifuged at 10400 × g for 10 min. The separated supernatants and pellets were flash frozen with liquid nitrogen, and stored at −80 °C for further analyses.

2.3. Analysis of factors associated with dehalogenation

Influence of components of culture medium and pancreatic juice on HNMs dehalogenation was determined. The components of the stomach culture medium were tested separately in three different incubations, including mucin (4 g/L), cysteine (0.5 g/L) and others (arabinogalactan 1 g/L, pectin 2 g/L, xylan 1 g/L, starch 4 g/L, glucose 0.4 g/L, yeast extract 3 g/L and peptone 1 g/L). The components of the pancreatic juice added into the small intestine were tested separately in two different incubations, including pancreatin (0.9 g/L) and bile salts (6.0 g/L). 60 μg/L TCNM, BNM and BCNM were incubated in the above five groups according to the operation conditions of simulated stomach and small intestine. Then 60 mL mixed solution was selected and centrifuged at 10400 × g for 10 min. The supernatants were flash frozen and stored at −80 °C for further analyses.

Influence of pH on HNMs dehalogenation was also analyzed. The pH value and reaction time were set according to the SHIME system. The 60 μg/L TCNM, BNM and BCNM solutions were firstly adjusted pH to 2 and held for 2 h at 37 °C. Then the solutions were adjusted pH to 7 and held for 4 h at 37 °C. 60 mL of solution was obtained for each pH value, and stored at −80 °C for further analyses.