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Comparative toxicity of chloro- and bromo-nitromethanes in mice based on a metabolomic method

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HIGHLIGHTS

- Halonitromethanes (HNMs) exposure increased oxidative DNA damage in mouse liver.
- Amino acid, carbohydrate and lipid metabolisms were disturbed by HNMs exposure.
- Bromo-HNMs are more toxic than chloro-HNMs.
- Induction of oxidative stress is one of the toxicity mechanisms of HNMs.

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ABSTRACT

Halonitromethanes (HNMs) as one typical class of nitrogenous disinfection byproducts have been widely found in drinking water. *In vitro* test found HNMs could induce higher cytotoxicity and genotoxicity than trihalomethanes and haloacetic acids. However, data on toxic effect from *in vivo* experiment is limited. In this study, bromonitromethane (BNM), bromochloronitromethane (BCNM) and trichloronitromethane (TCNM) were chosen as target HNMs, and exposed to mice for 30 d. Hepatic toxicity and serum metabolic profiles were determined to reveal toxic effects and mechanisms of the three HNMs. Results showed the three HNMs significantly decreased relative liver weight, indicating liver is one of the target organs. Further, the three HNMs exposure damaged hepatic antioxidant defense system, and increased oxidative DNA damage. Nuclear magnetic resonance based metabolomics analysis found amino acid metabolism and carbohydrate metabolism were disturbed by HNMs exposure. Some metabolites in these metabolisms are related to oxidative stress and damage. Combined with above results, BNM had the highest toxicity, followed by BCNM and TCNM, indicating bromo-HNMs had higher toxicity than chloro-HNMs. Induction of oxidative stress is one of the toxicity mechanisms of HNMs. This study firstly provides the insight into *in vivo* toxicity of HNMs and their underlying mechanisms based on metabolomics methods, which is very useful for their health risk assessment in drinking water.

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1. Introduction

Disinfection based on chlorine is widely applied in drinking water supply to protect public health (Sedlak and Gunten, 2011). However, disinfection also generates toxic disinfection byproducts (DBPs), and over 600 DBPs have been identified in drinking water. Adverse effects of some DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs) have been characterized (Shi et al., 2009; Linge et al., 2013). In recent years, in order to reduce human health risk of DBPs, some other disinfection processes based on the usage of chloramine, chlorine dioxide and ozone are developed to replace chlorine. Unfortunately, these disinfection approaches generate more nitrogenous disinfection byproducts (N-DBPs), which have received more and more attentions due to their high toxicity (Richardson et al., 2007). Halonitromethanes (HNMs), as one typical class of N-DBPs, are widely detected in drinking water (0.1–3.0 μg L^{-1}) and effluents of wastewater treatment plant (0.9–1.5 μg L^{-1}) (Plewa et al., 2004; Song et al., 2010). In a U.S. nationwide survey at drinking water treatment plants, the HNMs have been listed in 50 high priority monitored DBPs (Hu et al., 2010).

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Although the concentration of HNMs in drinking water is low, recent researches based on mammalian cell culture found that HNMs could induce 1–2 orders of magnitude higher cytotoxicities and genotoxicities than currently regulated DBPs, such as THMs and HAAs (Plewa et al., 2004; Muellner et al., 2007). However, data on HNMs toxicity from *in vivo* animal experiments on is limited. Only few reports on trichloronitromethane (TCNM) are available, which showed that TCNM could react with biological thiols in mouse liver tissue (Sparks et al., 1997). Thus, toxic effects on other HNMs in animals should be further characterized. Besides, toxicities of DBPs are associated with halogeno-groups. For examples, brominated THMs (Pegram et al., 1997), HAAs (Zhang et al., 2010) and haloacetamides (HAcAms) (Plewa et al., 2008) were found to have higher genotoxic and carcinogenic effects than chlorinated counterparts. For HNMs, their cytotoxicity and genotoxicity in cell lines showed the same conclusion that brominated forms of HNMs are more toxic than their chlorinated analogues (Plewa et al., 2004; Liviac et al., 2009). However, these results were from the *in vitro* tests. It is necessary to identify whether the differences could be found in *in vivo* animals, and determine the underlying mechanism(s).

Metabolomics is a powerful approach for gaining a comprehensive understanding of biological mechanisms, including toxicity (Lindon et al., 1999; Jones et al., 2008). Recently, the nuclear magnetic resonance (NMR) - based metabolomics approach has been used to reveal the toxicities of N-DBPs, such as monohaloacetamides and trichloroacetamide, and found the altered metabolites are associated with lipid, xenobiotics, amino acid and energy metabolisms. Moreover, the bromo-acetamide induced higher changes in metabolic profiles than chloro-acetamide (Deng et al., 2014; Zhang et al., 2013). However, there is no report that apply metabolomics approach to characterize toxic effects of HNMs, and compare the differences of chloro- and bromo-HNMs toxicities.

Objective of this study is to evaluate and compare *in vivo* toxicities of HNMs, and determine their underlying mechanisms. Since acute toxicity of HNMs is limited, oral LD_{50} values of nine HNMs was firstly predicted based on their molecular structure. Then three HNMs were chosen and exposed to mice. Hepatic toxicity and serum metabolic profiles were determined to reveal toxic effects and mechanisms of the three HNMs. Results of this study could provide the insights to the toxic effects and risk assessment of HNMs in drinking water.

2. Materials and methods

2.1. Acute toxicity prediction of HNMs

Nine HNMs were studied in this study, which include TCNM, bromonitromethane (BNM), bromochloronitromethane (BCNM), chloronitromethane (CNM), dichloronitromethane (DCNM), dibromonitromethane (DBNM), tribromonitromethane (TBNM), bromochloronitromethane (BCNM), dibromochloronitromethane (DBCNM) and bromodichloronitromethane (BDCNM). Their oral LD_{50} values were estimated by toxicity estimation software tool (T.E.S.T. version 4.2.1) (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test), which is provided by US EPA. The T.E.S.T. is a program that estimates toxicity of chemical based on its molecular structure. The predicted LD_{50} value for each chemical is shown as the mean value of three individual prediction methods, including hierarchical clustering, flexible discriminant analysis and nearest neighbor methods.

2.2. Mouse administration

Three-week-old male mice (ICR, body weight 18–22 g) provided by Qinglongshan Animal Breeding Center (Nanjing, China) were housed in stainless-steel cages under the controlled conditions: 25 ± 3 °C, 50 ± 5% humidity, 12 h/12 h light dark cycle. Based on the predicted LD_{50} values of HNMs, TCNM, BNM and BCNM were chosen as target chemicals for further analyses. After acclimated for one week, a total of 104 mice were randomly divided into 13 groups (8 mice for each group). Control group (CK) was given distilled water with 0.1% dimethylsulfoxide (DMSO). The treated groups were exposed to three HNMs (TCNM, BNM and BCNM) solution with 0.1% DMSO at four concentrations, meaning four groups per chemical. The TCNM (purity, >99.9%), BNM (purity, >90.1%) and BCNM (purity, >93.3%) were purchased from Supelco (USA). Exposure water with HNMs was prepared every day, and mice had free access to HNMs solution or distilled water. The concentrations of target HNMs in exposure water were measured every day to identify the actual exposure concentration of HNMs. Daily water intake was recorded based on a group water consumption. After 30 d exposure, blood was collected and centrifuged for 10 min at 3000 rpm to get serum samples. Liver of each mouse was weighted and stored at −80 °C until further processes. All experiments were performed according to NIH Guide for the Care and Use of Laboratory Animals.

2.3. Determination of HNMs in water

Analytical method of HNMs in exposure water was based on EPA method 551.1 with some modifications (Chen et al., 2016). Briefly, water sample (50 mL) containing HNMs was adjusted to pH 4.5–5.5 with phosphate buffer. Then, the sample was saturated by addition of 4 g Na_2SO_4 and extracted with 4 mL methyl *tert*-butyl ether (MTBE). HNMs extracted was analyzed by an Agilent 7890A gas chromatograph equipped with an Agilent DB-1 capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 mm film thickness) and an electron-capture detector. Initial oven temperature of gas chromatograph was set at 35 °C (3 min), the temperature was increased at 35 °C min^{-1} to 140 °C (1 min) and then 10 °C min^{-1} to 180 °C (2 min). Injector and detector temperatures were set at 170 °C and 280 °C, respectively. Carrier and make-up gases were helium at 1 mL min^{-1} and nitrogen at 60 mL min^{-1}, respectively.

2.4. Hepatic histopathology test

Parts of liver were dissected and fixed in 10% formalin solution for 48 h. After dehydration in a graded alcohol series, the hepatic tissues were embedded in paraffin wax and sectioned at 5 μm thickness using a rotary microtome. Sections were stained with hematoxylin-eosin, dehydrated in a graded series of alcohol solutions, cleared in xylol, and mounted in neutral gum. The slides were examined by an optical microscopy (Zeiss, Germany).

2.5. Hepatic oxidative stress analysis

As the key biomarkers of hepatic oxidative stress and damage, activities of catalase (CAT), glutathione peroxidase (GSH-Px) and level of lipid peroxidation product malondialdehyde (MDA) were measured by commercial kits (Jiancheng, China) (Liu et al., 2014). Part of liver in phosphate buffer saline was homogenized by ultrasonication. The supernatants after centrifugation were used for various measurements. Protein contents were determined using the modified Lowry method (Lowry et al., 1951). CAT and GSH-Px activates, and MDA levels were normalized by protein content. Additionally, level of 8-hydroxy-2-deoxyguanosine (8-OHdG) in

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