Ecotoxicology and Environmental Safety 163 (2018) 417–426

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Single and combined effects of selected haloacetonitriles in a human-derived hepatoma line

Guanghua Lu[a,b,*], Donghong Qin[b], Yonghua Wang[b], Jianchao Liu[b], Wei Chen[b]

[a] Water Conservancy Project & Civil Engineering College, Tibet Agriculture & Animal Husbandry University, Linzhi 860000, China
[b] Key Laboratory for Integrated Regulation and Resources Development on Shallow Lakes of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, China

ARTICLE INFO

Keywords:
Haloacetonitriles
Cytotoxicity
DNA damage
ROS
Combination toxicity
CA and IA models

ABSTRACT

Haloacetonitriles (HANs) are nitrogenous disinfection byproducts (N-DBPs) detected in drinking water that have high toxicity and are a high risk to human health. The cytotoxicity and genotoxicity as well as the oxidative stress of five HANs, namely chloroacetonitrile (CAN), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), bromoacetonitrile (BAN), and dibromoacetonitrile (DBAN) on a hepatoma cell line (HepG2) were determined by single, binary or ternary exposure. The median effective concentrations, based on cell viability, ranged from 0.8360 mg/L for BAN to 256.9 mg/L for DCAN, with a cytotoxicity order of BAN > DBAN > CAN > TCAN > DCAN. The lowest observed effective concentrations regarding DNA damage were 0.01 mg/L for CAN and DCAN, 0.1 mg/L for DBAN and TCAN, and 1 mg/L for BAN. The DNA damage induced by CAN, DCAN and TCAN was repaired to about 80% in 30 min, and when induced by BAN and DBAN, it was repaired completely in 60 min. The intracellular reactive oxygen species (ROS) levels were significantly increased by the five HANs, and bromo-acetonitrile produced a stronger oxidative stress than chloro-acetonitrile. Co-exposure of DCAN, TCAN and DBAN significantly inhibited cell viability, induced DNA damage and facilitated ROS generation in HepG2 cells. However, the interactive effects were inconsistent for the different endpoints, which seemed to be antagonism for cell viability but synergy for ROS generation.

1. Introduction

Disinfection by-products (DBPs) have received attention since they were identified in drinking water in the early 1970s (Bellar et al., 1974; Grünwald et al., 2002). Chlorine, chloramines, ozone, and chlorine dioxide are commonly used as disinfectants in drinking water to decrease the outbreak of waterborne illnesses, but these chemicals may react with some organic matter (such as amino acids, proteinaceous compounds, microbial products, pyrimidine and purine bases, and antibiotic chloramphenicol and its analogues) to form DBPs, which are harmful to human health (Chu et al., 2012; Jia et al., 2016; Zhang et al., 2017a; Zhang et al., 2017b). Until now, more than 600 DBPs including carbonaceous DBPs (C-DBPs) and nitrogenous DBPs (N-DBPs), have been identified (Plewa et al., 2008). These DBPs are generally present at sub-μg/L or low- to mid-μg/L levels in drinking water (Richardson et al., 2007). A US study in 2000–2002 encompassed over 70 emerging DBPs, and among the analyzed N-DBPs were haloacetonitriles (HANs), halonitromethanes (HNMs) and haloacetamides (Bond et al., 2011). C-DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs) have been studied comprehensively, while N-DBPs represent a new emerging concern in drinking water, due to their higher toxicity compared to C-DBPs (Yang et al., 2014; Yu et al., 2015).

HANs, including chloroacetonitrile (CAN), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), bromoacetonitrile (BAN), dibromoacetonitrile (DBAN) and bromochloroacetonitrile (BCAN), are the main N-DBPs that typically occur at a higher concentration in water disinfected by chlorine, chloramine, or chlorine dioxide compared to some N-DBPs. In order to reduce the generation of THMs and HAAs, many water plants decreased the use of chlorine disinfectants and increased chloramine disinfection, and thus, the formation of HANs increased. HANs levels in drinking water by chloramine disinfection ranged from non-detectable (< 0.5 μg/L) to 41.0 μg/L, with a maximum of 14 μg/L according to the U.S. EPA ICR database, and more brominated HANs formed with higher bromide levels (Richardson et al., 2007). The same tendency was observed in Israeli drinking water with high bromide (Richardson et al., 2003). Another US survey in 2006–2007 showed that the median value of the sum of DCAN, BCAN, DBAN and TCAN (collectively HAN_4) was slightly higher at 4.0 μg/L,

* Corresponding author at: Water Conservancy Project & Civil Engineering College, Tibet Agriculture & Animal Husbandry University, Linzhi 860000, China.
E-mail address: ghlu@hhu.edu.cn (G. Lu).

https://doi.org/10.1016/j.ecoenv.2018.07.104
Received 28 March 2018; Received in revised form 2 July 2018; Accepted 25 July 2018
Available online 31 July 2018
0147-6513/

while HAN_4 levels reached 36 μg/L in Australia (Bond et al., 2011). Furthermore, HANs were also detected in Canada, Scotland and South Korea, and CAN, DCAN, TCAN, BCAN and DBAN were the most commonly measured species (Goslan et al., 2009; Richardson et al., 2007; Shin et al., 2013; Williams et al., 1997; Yang et al., 2010). HANs concentrations ranged from < 0.2 μg/L to 12.2 μg/L in 155 water samples from Guangzhou, Foshan and Zhuhai in China (Gan et al., 2013). Another study using the drinking water from 70 waterworks from 31 cities in China showed that the total concentration of HANs was as high as 39.2 μg/L (Ding et al., 2013). As HANs continue to be detected, there is increasing concern regarding current drinking water sanitation practices and their potential health risks.

HANs possess greater genotoxic potency than C-DBPs as well as other N-DBPs and produce DNA strand breaks in human lymphoblastic cells and Hela S3 cells (Lin et al., 1986; Muller-Pillet et al., 2000; Zhang et al., 2017c). DCAN induced acute toxicity in zebrafish, including developmental toxicity to the embryos, heart function alterations, neuronal function disturbances and DNA damage, and it easily accumulated in adult zebrafish (Lin et al., 2016). Another study indicated that DBAN resulted in a significant generation of reactive oxygen species (ROS), lipid peroxidation, the accumulation of oxidized proteins and the inhibition of proteasomal activity in C6 cells (Ahmed et al., 2008). A report from health advisories from the US EPA showed that the oral reference dose is 8 μg/kg/d, and the non-cancer dose for a lifetime is 6 μg/kg/d (Tan et al., 2017). The World Health Organization (WHO) guideline for DCAN is 20 μg/L, and for DBAN it is 70 μg/L (WHO, 2011). However, there is no guideline data for HANs in drinking water in China, and adequate toxicology data are very important for establishing drinking water sanitation standards.

In drinking water, HANs exist in the form of a mixture, instead of a single substance. However, no information on their combined effects has been reported to date. In the present study, we conducted HepG2/CCK-8/SCGE to determine the median effective concentration (EC_{50}) or the lowest observed effective concentration (LOEC) regarding the cytotoxicity, genotoxicity and ROS formation of five HANs. Co-exposure of DCAN, TCAN and DBAN was conducted to determine their combined effects, and the concentration addition (CA) and independent action (IA) models were used to evaluate the toxic interaction of the mixtures on cell viability. Moreover, we detected the damaged DNA repair ability by repair kinetics experiments. The results from this study provide important information for the risk assessment of coexistent HANs in drinking water.

2. Materials and methods

2.1. Test materials

Five HANs, including CAN, DCAN, TCAN, BAN and DBAN, were purchased from J&K Scientific (China). The CAS number, purity and physicochemical parameters of these compounds are presented in the Supporting information (SI) Table S1, which were provided by the manufacture and ecological structure activity relationships (ECOSAR). The low melting agarose (LMPA, 98% of purity) and normal melting agarose (NMPA, 98% of purity) were from the Nanjing Jiancheng Bioengineering Institute (China). Trypan blue (97% of purity) was obtained from Nanjing Zhongdong Reagent (China). Dimethyl sulfoxide (DMSO, 99% of purity) was purchased from Nanjing Ronghua Reagent (China). The fluorescent stain Gel Red (98% of purity) was purchased from KeyGEN Biotech (China).

2.2. Cell culture

HepG2 cells were provided by KeyGEN Biotech (China). The cells were grown in Dulbecco's modified Eagles medium (DMEM) containing 10% foetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2. Stock solutions of the HANs, at approximately 100 mg/mL, were prepared in DMSO. The stock solutions were diluted with DMEM to achieve final concentrations before exposure. Before treatment with HANs, the cells were rinsed with PBS, trypsinized, and were then transferred on to plates. DMSO (0.05%, v/v) was used as the solvent control.

2.3. Cell viability assay

The cells were transferred to 96-well plates at a density of 1×10^4 cells/well. After 24 h of growth, the cells were exposed to HANs for 24 h, and the solvent control (containing 0.05% of DMSO, cells and medium) and blank control (containing medium only) were set. A preliminary experiment was completed following 24 h of exposure in order to determine the concentration ranges of different HANs inhibiting cell viability. According to the results of the preliminary experiment, the exposure concentrations were set to 5–200 mg/L for CAN, 1–500 mg/L for DCAN and TCAN, and 0.1–10 mg/L for BAN and DBAN in the cell viability assay. Three independent trials were performed, and each trial was replicated six times. The cell viability of HepG2 was determined by the cell counting kit-8 (CCK-8, KeyGEN, Nanjing, China). Ten microlitres of the CCK-8 solution was added to each well of the 96-well plate, and it was incubated for 2 h at 37 °C with 5% CO_2. Then, the optical density (OD) of cells were measured at 450 nm with a microplate reader (Synergy H4, BioTek). Cell viability was calculated by the following formula: Cell viability = (OD of HANs - OD of blank control)/(OD of solvent control - OD of blank control) × 100%.

2.4. SCGE assay and DNA damage repair test

For the single cell gel electrophoresis (SCGE) assay, 1×10^6 cells were added to 6-well plates in 2 mL of DMEM and were incubated overnight. Then, the cells were pretreated with different concentrations of HANs (0.001–10 mg/L for CAN, 0.01–10 mg/L for DCAN, 0.1–20 mg/L for TCAN, 0.001–5 mg/L for BAN and DBAN) for 4 h. For each treatment group, the acute cytotoxicity was measured using the trypan blue vital dye exclusion assay, and only the concentrations at which the cell viability exceeded 75% were applied for the SCGE assay. In SCGE assay and DNA damage repair test, three independent trials were performed, and each trial was replicated two or three times.

Approximately 100 μL of the cell suspension in 0.5% LMPA was spread over microscope slides, which were pre-coated with 1% NMPA. Then, the cells were lysed in 4 °C lysing solution (146.1 g/L NaCl, 37.22 g/L EDTA-Na_2, 8.0 g/L NaOH, 1% TritonX-100%, and 10% DMSO) for 1.5 h and were placed in the 4 °C unwinding buffer solution (12 g/L NaOH) for 20 min. After that, the slides were electrophoresed in alkaline buffer (12 g/L NaOH, 0.3722 g/L EDTA-Na_2, pH = 13) for 20 min at 25 V and 300 mA. Then, the slides were treated with 48.5 g/L Tris buffer (pH = 7.5) for 12 min, stained with Gel Red (20 mg/L) and analyzed using a fluorescence microscope (Zeiss Axioskop 40, Shanghai). For SCGE analysis, 100 cells were randomly selected from two replicated slides per treatment. The entire process was executed in the dark, and the experiments were repeated three times independently. A software program (CASP) was used to measure the Olive tail moment (OTM) to evaluate DNA damage.

A repair kinetics assay was carried out to determine the DNA damage repair capacity over time (0, 30, 60, 90, 120, 180 and 240 min, as well as 24 h). We selected one concentration of each HAN, which significantly induced DNA damage (the highest or the second highest effective concentrations), to evaluate the DNA damage repair capacity. After the treatment, the cells were incubated in DMEM in an incubator at 37 °C with a humidified atmosphere of 5% CO_2 until its specific repair time. Then, the cell samples followed the same steps as the SCGE assay. The DNA repair capacity was calculated using the average OTM value and the following formula: Percent DNA repair capacity = [(DNA damage immediately after treatment-DNA damage at the time of repair)/(DNA damage immediately after treatment)] × 100%.