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Assessing the genotoxicity of two commonly occurring byproducts of water disinfection: Chloral hydrate and bromal hydrate

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ABSTRACT

Water disinfection treatments result in the formation of disinfection byproducts (DBPs) that have been linked to adverse human health outcomes including higher incidence of bladder and colorectal cancer. However, data about the genotoxicity of DBPs is limited to only a small fraction of compounds. Chloral hydrate (CH) and bromal hydrate (BH) are two trihaloacetaldehydes commonly detected in disinfected waters, but little is known about their genotoxicity, especially BH.

We investigated the genotoxicity of CH and BH using a test battery that includes three *in vitro* genotoxicity assays.

We conducted the Ames test using *Salmonella* bacterial strains TA97a, TA98, TA100 and TA102, and the alkaline comet assay and the micronucleus test both using Chinese hamster ovary cells. We carried out the tests in the absence and presence of the metabolic fraction S9 mix.

CH did not exhibit statistically significant genotoxic effects in any of the three assays. In contrast, BH exhibited mutagenic activity in the *Salmonella* strain TA100 and induced statistically significant DNA lesions in CHO cells as appeared in the comet assay. The genotoxic potential of BH in both assays decreased in the presence of the metabolic fraction S9 mix. BH did not induce chromosomal damage in CHO cells.

Our results show that BH exhibited genotoxic activity by causing mutations and primary DNA damage while CH did not induce genotoxic effects. Our findings highlight concerns about the higher genotoxicity of brominated DBPs in comparison to their chlorinated analogues.

1. Introduction

One of the most significant public health advances of the twentieth century was the adoption of drinking water disinfection in many countries [1]. This practice has sharply reduced the incidence of infectious diseases such as cholera, typhoid, and dysentery [2,3]. After this dramatic success, disinfection practices have been introduced into swimming pools and other recreational water venues to ensure the elimination of pathogenic microorganisms and the prevention of waterborne disease outbreaks [4]. However, disinfection treatments result in the undesirable formation of chemical contaminants known as disinfection byproducts (DBPs), in consequence to reactions taking place between disinfectants and organic matter present in water [5,6]. Exposure to DBPs in humans can take place through ingestion of drinking water or inhalation and dermal absorption during showering or swimming [7–10]. Many studies have suggested associations between exposure to DBPs and adverse health effects. Increased incidence of asthma [11], bladder cancer [12,13], and colorectal cancer [14] have been reported. Adverse pregnancy outcomes such as spontaneous abortions [15], stillbirth [16], and fetal growth restriction [17] have also been noted. To date, more than six hundred DBPs including trihalomethanes, haloacids, halonitriles, haloaldeydes, haloketones, halonitromethanes, haloamines, haloamides, haloalcohols, and halobenzoquinones have been identified in disinfected waters [9,18–23]. Several laboratory-controlled studies have been conducted to evaluate potential toxicities of DBPs providing evidence about cytotoxic, genotoxic, carcinogenic and teratogenic potentials [20,24–28]. However, the toxicological data are limited to only a small fraction of identified DBPs. In consequence, many DBPs that have been detected in disinfected waters remain

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with unknown toxicological profiles. Chloral hydrate and bromal hydrate, the hydrated forms of trichloroacetaldehyde and tribromoacetaldehyde respectively, belong to the chemical class of haloacetaldehydes. This class of DBPs has been reported to be one of the most abundant DBP classes by weight [19,25,29,30]. Occurrence studies have shown that the predominant trihaloacetaldehyde in chlorinated waters is chloral hydrate, while bromal hydrate is the predominant trihaloacetaldehyde in chlorinated waters containing high levels of bromide [19,31]. In a recent study, BH was detected as one of the degradation byproducts of benzophenone-3, a UV filter commonly used in sunscreens, in chlorinated swimming pools filled with seawater [32].

Toxicokinetic studies have shown that CH is rapidly absorbed after oral administration, and enters the liver where it undergoes extensive metabolism in rodents [33,34] and in humans [35,36]. Studies of the potential carcinogenicity of CH in mice have demonstrated that it is able to induce hepatocellular adenomas and carcinomas, and exposure to CH has been associated with increases in malignant lymphoma and adenoma of the pituitary gland [37–39]. However, there was still no persuasive evidence to connect chloral hydrate exposure and the development of cancers in humans [40]. CH was also found to induce significant aneugenic effects in mice [41]. Furthermore, micronuclei were produced in germ cells of male mice treated intraperitoneally with CH [42]. CH was also reported to be able to lead to chromosomal loss in mouse spermatids [43] and in human lymphocytes [44]. Nevertheless, most of the investigations incorporated only one or two *in vitro* assays [25,45] and results from genotoxicity assessment of CH remain inconclusive. Concerning BH, although little is known about its toxicity, the U.S. Environmental Protection Agency (EPA) included this compound to the list of priority DBPs to be monitored in a nationwide occurrence study [46] due to anticipations of potential toxicity based on alarming structure-activity relationships [47]. To address this scarcity of data, we analyzed the genotoxicity of CH and BH using a battery of three genotoxicity assays, namely the Ames test, the comet assay, and the micronucleus assay. The use of a test battery is critical since no single genotoxicity test is capable of detecting all genotoxic mechanisms [48]. We performed the three assays in the absence and presence of the metabolic activation fraction S9 mix to assess the effects of metabolic reactions on the toxicity of the two compounds.

2. Methods

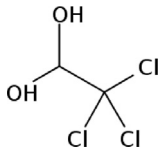
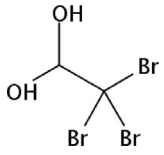
2.1. Chemicals

The identifiers and structures of CH and BH are shown in Table 1. CH (crystallized, ≥98%) was obtained from Sigma-Aldrich (China). BH was prepared by adding tribromoacetaldehyde (bromal, Sigma-Aldrich, UK, 97% purity), to ultrapure water and then recrystallizing the product from a small volume of water. Ultrapure water was produced from a Millipore water system (resistivity = 18.2 MΩ.cm). Before toxicological analyses, stock solutions were prepared in dimethyl sulfoxide (DMSO, Chromasolv plus, ≥99.7%) obtained from Sigma-Aldrich (UK) and were immediately stored in amber glass vials at −80 °C.

2.2. Metabolic activation mixture (S9 mix)

The metabolic activation mixture was a 9000 g centrifuged supernatant of a liver homogenate (S9). It was prepared from male OFA rats (Charles River Laboratories, France). Five days before sacrifice, the rats were treated with a single injection of Aroclor 1254 (500 mg/kg body weight). The final protein concentration of the S9 mix was 26 mg/mL as determined by the method of Lowry et al. [49]. In the Salmonella mutagenicity assay, the composition of the metabolic mixture (S9 mix) included 4% S9, 10 mM glucose-6-phospahate (G6P) and 8 mM nicotinamide adenine dinucleotide phosphate (NADP) (De Méo et al., 1996). In the comet and micronucleus assays, the S9 mix contained 10% S9, 5 mM G6P, 4 mM NADP, 33 mM KCL and 8 mM $MgCl_2$ diluted in 0.15 M saline phosphate buffer [50].

Table 1
Names, CAS numbers, and chemical structures of CH and BH.

Name	CAS Number	Structure
Chloral Hydrate 2,2,2-Trichloro-1,1-ethanediol Trichloroacetaldehyde hydrate Bromal Hydrate	302–17-0	
2,2,2-Tribromo-1,1-ethanediol Tribromoacetaldehyde hydrate	507–42-6	

2.3. Cell cultures

The comet and the micronucleus assays were performed using Chinese Hamster Ovary cells (CHO-K1, ATCC). Cells were grown in McCoy's 5A medium (Sigma-Aldrich, St Quentin Fallavier, France) supplemented with 10% fetal calf serum, 1 mM glutamine and penicillin-streptomycin (100 U/mL and 10 μg/mL), and incubated in a humidified atmosphere containing 5% carbon dioxide (CO_2).

2.4. Salmonella/microsome mutagenicity assay

The Salmonella typhimurium tester strains TA97a, TA98, TA100 and TA102 used in the Ames test were supplied by Prof. B.N. Ames (Berkeley, CA, USA). These strains were used to detect different types of mutations in agreement with the recommendations of Maron and Ames [51]. The strain TA100 is able to detect base-pair substitution mutations. The strains TA97a and TA98 are able to detect frameshift mutations. The strain TA102 detects cross-linking mutagens [52]. The strains were stored at −80 °C and regularly checked for genetic markers. The mutagenicity assay was carried out according to Maron and Ames [51], with a modified version of the liquid-incubation technique [53]. Salmonella strains were grown in Oxoid Nutrient Broth N° 2 with ampicillin (25 μg/mL) for 12 h at 37 °C with gentle shaking. After the incubation period, various volumes of solutions of the test substances (four test doses per compound), not exceeding 10 μL (0.5%, v/v) to avoid toxicity, were added to 0.1 mL of the overnight culture and 0.1 mL of PBS or S9 Mix. The mixtures were incubated either for 60 min in the dark. Then, 2 mL of melted top agar containing 0.045 mM histidine and biotin were added, and the mixtures were poured onto Vogel–Bonner (VB) minimal plates. For each series of experiments, negative controls with 5 or 10 μL of DMSO were included to determine the number of spontaneous revertants/plate. Positive controls were also included to ensure the performance of the tester strains: 0.002 μg/plate IRC191 for TA97a, 0.002 μg/plate 2,4,7-trinitrofluorenone for TA98, 5 μg/plate NaN3 for TA100, 0.002 μg/plate mitomycin C for TA 102. After a 48-h incubation period, revertants were counted with an automatic counter (Scan 1200, Interscience, Saint Nom La Bretèche, France).