Effect of trihalomethanes (chloroform and bromoform) on human haematological count

Asna Lodhi, Imran Hashmi, Habib Nasir and Romana Khan

ABSTRACT

With the increasing concerns about the harmful effects of disinfection products, the process of chlorination is becoming questionable. Bromoform and chloroform are among the most frequently occurring disinfection by-products. Haematological parameters are an important indicator of human well-being which is why the prime objective of the current study was to conduct a dose–response assessment to investigate the effects of trihalomethanes on human haematological count. Blood samples of healthy subjects were exposed to different concentrations (10, 30 and 50 μg/mL) of chloroform and bromoform *in vitro* to analyse how these compounds affected the haematological count with increasing dose concentrations. Headspace gas chromatography analysis was also conducted on samples to assess the difference between measured and spiked values of doses. The results indicated that the damage caused by bromoform was statistically more significant as compared to chloroform. Haemoglobin (HGB) and mean corpuscular haemoglobin concentration levels lowered as they were significantly affected ($p < 0.05$) by bromoform at all administered doses. It also significantly damaged platelet level at doses of 30 ($p < 0.05$) and 50 μg/mL ($p < 0.01$). Conversely, the damage caused by chloroform was statistically less significant ($p > 0.05$).

Key words | bromoform, chloroform, disinfection by-products, haematology, trihalomethanes

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INTRODUCTION

Water consumed by humans must be of a certain quality and should possess no threat to human health (Trujillo *et al.* 2008). One of the major accomplishments in public health is drinking water disinfection which now helps generate large volumes of safe and high quality tap water (Muellner *et al.* 2010). Disinfection of drinking water is crucial to prevent infectious disease outbreaks (Teixido *et al.* 2015). Public water supplies are chemically treated for killing pathogens that may be present in drinking water; subsequently, the risk–benefit balance of drinking water disinfection is therefore considered positive (Colman *et al.* 2011). When chlorine or its compounds are added to water for disinfection they react with dissolved organic matter and result in the formation of disinfection by-products (DBPs) (Nejjari *et al.* 2012). Trihalomethanes (THMs) are a class of disinfection by-products that are present in chlorinated water (Landi *et al.* 2003). There is a strong relation between the amount of organic matter and the formation of trihalomethanes (Takeuchi *et al.* 1991). There are numerous factors that influence the formation of DBPs (Wei *et al.* 2011) and they include dosage of chlorine, contact time, level of pH and concentration of bromide, etc. (Ma *et al.* 2015). Many studies have analysed the presence of DBPs in drinking and recreational waters to be harmful for health because of their adverse effects (Plewa *et al.* 2011).

Among all the trihalomethanes measured, chloroform is the most prevalent one so far (Monarca *et al.* 2004). Bromoform is formed in chlorine disinfected drinking-water when

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chlorine reacts with natural organic matter in the presence of Br^- ion (Richardson *et al.* 2007). Even in untreated water, bromoform has been detected at lower levels. It is the major THM detected during desalination of seawater by chlorination (Kargalioglu *et al.* 2002). The Environmental Protection Agency (EPA) has established a maximum contaminant level of 70 ppb and 5 ppb for chloroform and bromoform, respectively (USEPA 1998).

The presence of chloroform and bromoform has been detected in human blood by various researchers (Riederer *et al.* 2014). The level of these THMs in blood varies greatly and a notable increase in their amount has been observed after human exposure to these compounds through tap water (USEPA 2005). THMs have been widely assessed in water and blood samples in the past few years on the basis of different water use patterns and characteristics of tap water. Studies have revealed that the concentration of individual trihalomethanes varies after consumption of chlorinated water and even after showering with it (Lynberg *et al.* 2001). Several studies have revealed and are still being conducted to examine cytotoxic and genotoxic potential and other adverse health effects of trihalomethanes, especially chloroform and bromoform, on body weights, habits of food consumption, haematology parameters and major organ histopathology. Formation of malignant cells and significant decline in fibrinogen levels and prothrombin time in male mice at high dose has been reported along with changes in many haematology parameters (DeAngelo *et al.* 2007).

The aim of this study was to assess how bromoform and chloroform affect human haematological count. Haematological malignancies were examined at different concentrations of both compounds. Comparative analysis was also done to observe which between these two was the more damaging.

MATERIALS AND METHODS

Test materials and instruments

Standard analytes chloroform and bromoform were purchased from Sigma Aldrich (USA) and Dr. Ehrenstorfer (Germany) respectively, with 99% purity. Methanol was acquired from Merck (Germany), whereas solid-phase micro-extraction (SPME) (75 μm Car-PDMS) fibre was obtained from Supelco (USA). Fully automated instrument Sysmex XP-100 (Singapore) was used for haematology analysis. Gas chromatograph used for analysis was Shimadzu GC-2010 (Japan). Headspace solid-phase micro-extraction (HS-SPME) clear glass vials and EDTA tubes were purchased from Supelco (USA) and Improve Medical (Belgium), respectively.

Blood sample collection

Human whole blood samples were collected from healthy subjects between the age group 20 to 30 years, for gas chromatography (GC) and haematology analysis in EDTA vacutainers through venipuncture. These sterile vacutainer glass tubes contain EDTA, which prevents the blood from clotting. For headspace GC analysis, to avoid headspace loss, vacutainers were filled with blood completely. Immediately after the blood was drawn into the tube it was slightly shaken by hand to carefully dissolve the EDTA. Afterwards, these samples were stored at 4 °C until the time of analysis.

Sample preparation

Prior to cell treatment, the blood samples were removed from the refrigerator and placed at room temperature for 20–30 minutes. Once the blood samples equilibrated with the room temperature, 1 mL aliquots of blood were withdrawn using a clean disposable air tight syringe and transferred into each of the four EDTA vacutainers. Apart from the control, the three vacutainers were spiked with 10, 30 and 50 μg/mL doses of chloroform. These concentrations were selected after extensive literature review. Once the samples were spiked with doses of chloroform and bromoform they were placed in the incubator at 37 °C for 5 hours to allow cell treatment. The same procedure was followed for haematological and HS-SPME analysis.

Haematological analysis

After cell treatment, the samples underwent complete blood count (CBC) test for analysing the effect of target compounds on various haematology parameters. The

equipment used to conduct the test was Sysmex XP-100 Haematology Analyzer. The haematological parameters included in the CBC test were red blood cells (RBCs), white blood cells (WBC), platelets (PLT), haematocrit (Hct), mean corpuscular haemoglobin concentration (MCHC) and haemoglobin (Hgb).

HS-SPME for blood sample analysis

Standard solutions

THM stock solution of 10,000 μg/mL was prepared in methanol following EPA method 551.1. Working standard solutions of 1, 10, 20, 30 40, 50 μg/mL were prepared for chloroform and bromoform to obtain linear calibration curves.

HS-SPME technique

The blood samples were tested using GC to observe the change in concentration of the spiked dose, by means of HS-SPME technique. The extraction method used involved mixing 1 mL of treated blood sample with an equal volume of distilled water in an SPME glass vial using a hot plate magnetic stirrer. Stirring was done at 40 °C for 30 minutes. The SPME fibre was injected through the 1.5 mm thick PTFE/silicone septum of the vial and the sample was allowed to adsorb on the fibre. After 10–15 minutes the SPME fibre was retracted and the sample was injected in GC. After calculating the change in spiked dose concentrations the recovery efficiency (R) was also calculated using USEPA 555.1 method.

$$R = \frac{100\ (A - B)}{C}$$

RESULTS AND DISCUSSION

GC analysis

Linear calibration curves were obtained with a regression coefficient (R^2) of 0.98 and 0.99 for chloroform and bromoform, respectively (Figure 1).

GC results revealed that 16.8–19.9% and 15.1–22.7% change in spiked dose concentration occurred after cell treatment in chloroform and bromoform, respectively, indicating that the cell treatment was successful. This slight change of dose concentration was probably due to headspace loss and volatilization occurring while transferring samples into SPME vials (Blount *et al.* 2006). Moreover, it is quite challenging to measure concentrations of THMs in blood samples and it continues to pose challenges owing to their rapid metabolism and elimination (Aylward *et al.* 2008).

The respective chromatographic peaks for standards and samples of both THMs at a dose of 50 μg/mL can be observed in Figure 2. The figure clearly depicts identifiable chromatographic peaks for chloroform and bromoform at retention time 3.95 and 7.74 minutes, respectively. However, the control showed no significant peaks at these retention times.

Recovery efficiency for HS-SPME was calculated to evaluate the method performance for THMs. According to

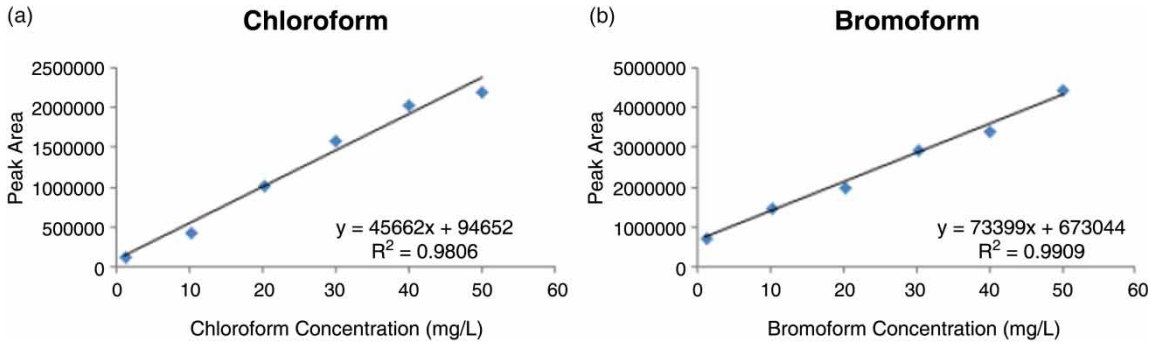

Figure 1 | Calibration curve of concentration against peak response for chloroform (a) and bromoform (b).

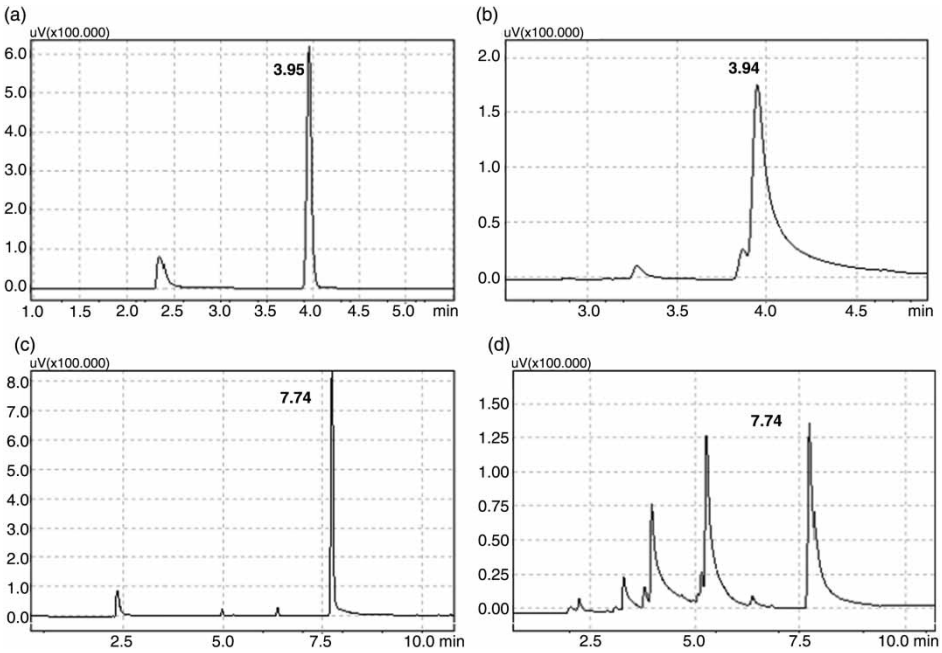

Figure 2 | Chromatographic peaks for standards and samples of chloroform (a) and (b) and bromoform (c) and (d).

USEPA, per cent recoveries must fall in the range of 70 to 120% for THMs. The HS-SPME helped to achieve acceptable recovery efficiency values ranging from 80.1 to 83.2% and 77.29 to 84.9% for chloroform and bromoform, respectively, therefore establishing that HS-SPME technique is reproducible, fast and accurate.

Haematological analysis

The pharmacokinetics of these two trihalomethanes has been well studied through experimentation on laboratory rodents. The uptake and removal of volatile organic compounds (VOC) from the body is not a simple phenomenon but is controlled by a chain of active mechanisms that control the movement of compounds throughout the body and metabolize them into more water-soluble compounds. The work performed on pharmacokinetics of these VOC indicates that repeated and long-term exposures cause bioaccumulation (Meek *et al.* 2002).

The data obtained from the CBC test were statistically analysed using two-way analysis of variance (ANOVA). The dose-dependent changes included that chloroform and bromoform have only minor effects on Hct and WBCs ($p > 0.05$) (Figure 3).

Both haemoglobin (HGB) and MCHC levels were significantly lowered at all administered doses of bromoform ($p < 0.05$) (Figure 4). The effect of DBPs on haemoglobin has previously been reported in the literature (Sen *et al.* 2011). Bromoform is absorbed by the tissues which enhances the chemical activity and so it biotransforms into more reactive compounds. Bromoform is known to metabolize to dibromocarbonyl which is the bromine analogue of phosgene and carbon monoxide. Upon reaction with proteins phosgene is likely to cause either cell damage or cell death (USEPA 2005).

Bromoform significantly lowered PLT level at doses of 30 μg/mL ($p < 0.05$) and 50 μg/mL ($p < 0.01$). RBC count was also lowered significantly by 30 and 50 μg/mL dose of bromoform ($p < 0.05$) whereas chloroform only had a minor effect ($p > 0.05$) (Figure 5).

As mentioned above, bromoform metabolizes to carbon monoxide. This carbon monoxide reacts with haemoglobin in the blood stream and converts it into carboxyhaemoglobin (COHb), which unlike oxyhaemoglobin (O_2HB) prevents haemoglobin from supplying oxygen (O_2) to the body tissues. The cells die because of lack of O_2 and their number begins to decline (Andersen *et al.* 1991). This phenomenon explains the decline in RBCs.

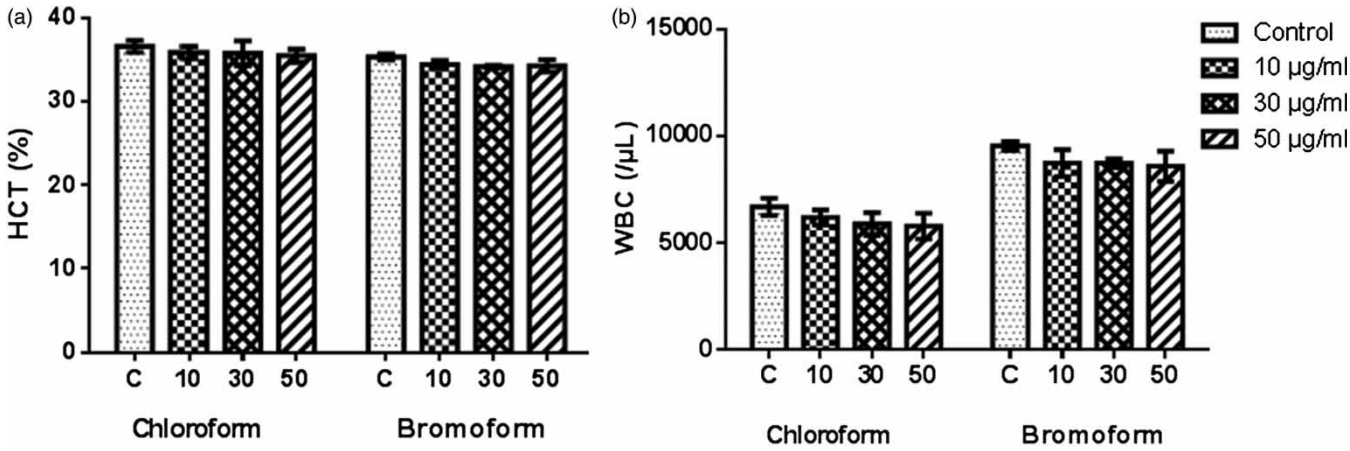

Figure 3 | (a) Change in Hct level and (b) change in WBC level.

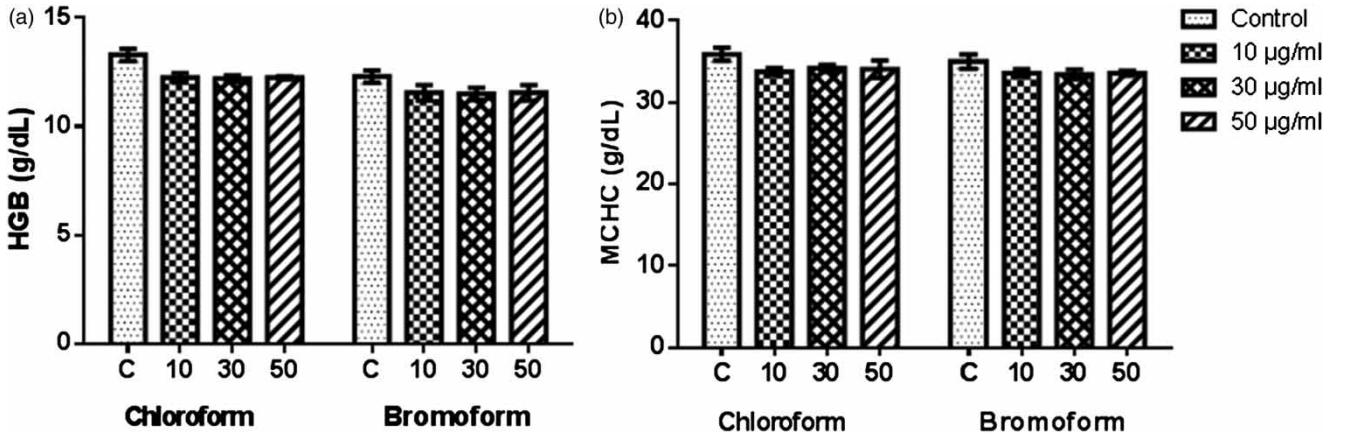

Figure 4 | (a) Change in Hgb level and (b) change in MCHC level.

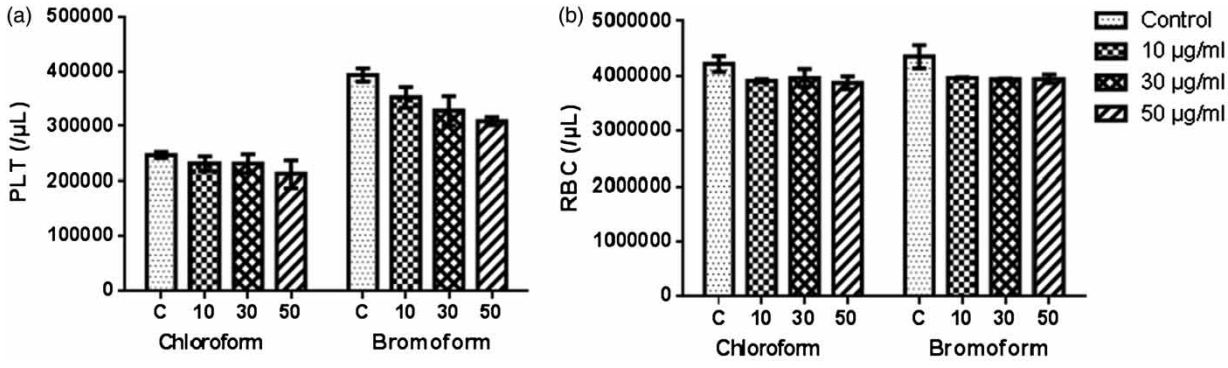

Figure 5 | (a) Change in PLT level and (b) change in RBC level.

Toxicological effects of these compounds are due to the fact that all trihalomethanes are primarily metabolized to either carbon monoxide or carbon dioxide. According to the literature, the toxicity of both the compounds is due to their reactive metabolites. Metabolism of chloroform has been identified and studied in both oxidation and reduction pathways. Chloroform is metabolized to carbon dioxide that is generated via oxidative pathway. Phosgene and

hydrochloric acid are produced during oxidative activation and both are very toxic and cause damage. However, at these observed doses they caused no significant damage to haematological parameters (Borgert *et al.* 2015).

The results showed a strong correlation between concentration of studied DBPs and their effect on haematological parameters. These results are in accordance with the literature (CEPA 2010). Larson *et al.* (1994) treated animals with chloroform in corn oil and severe hepatotoxicity was reported. Ruddick *et al.* (1983) treated female rats with a disinfection by-products dose and the results depicted a decrease in body weight gain, haemoglobin and Hct, and increases in relative liver weight of dams at all dose levels. Hence, this study has depicted that chloroform and bromoform have the potential to change haematological count.

CONCLUSIONS

(1) After cell treatment only minor changes were observed in spiked doses, ranging from 16.8 to 19.9% and 15.1 to 22.7% for chloroform and bromoform, respectively, thereby assuring successful cell treatment.
(2) HS-SPME technique is highly accurate and a rapid technique for quantifying THMs.
(3) Both the target compounds caused minor or significant changes in the haematological count in a dose-dependent way.
(4) Bromoform was statistically more damaging ($p < 0.05$) to haematological parameters than chloroform ($p > 0.05$).

Pertaining to the harmful effects of these compounds, the levels of trihalomethanes must be kept to a minimum in water supplies. In order to do so, either water must be pre-treated before chlorination or alternative methods of water disinfection must be used.

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