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Short communication

Evaluation of the effect of brominated flame retardants on hemoglobin oxidation and hemolysis in human erythrocytes

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ABSTRACT

Brominated flame retardants (BFRs) are widely used in many everyday products. Numerous studies have shown that BFRs can be released into the environment. Environmental pollution with these compounds raises concerns about their potentially adverse health effects. The aim of this study was to evaluate the effect of tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), 2,4-dibromophenol (2,4-DBP), 2,4,6- tribromophenol (2,4,6-TBP) and pentabromophenol (PBP) on hemolysis induction and hemoglobin oxidation in human erythrocytes. The erythrocytes were incubated with selected BFRs in a wide concentrations ranging from 0.01 to 100 μg/ml for 24 h, 48 h and 72 h. All compounds studied, exhibited hemolytic potential and induced methemoglobin formation. Hemolytic and oxidative potential of BFRs increased along with the increasing concentrations of the compounds studied and elongation of the incubation time. Our study showed that both the number of aromatic rings and the number of bromine atoms in the molecule of the compounds examined influence hemoglobin oxidation and damage to the cellular membrane. Furthermore, we may conclude that 2,4-DBP is potentially most toxic compound because it causes statistically significant changes at the lowest concentration, while the highest toxicity at the highest concentrations was noted for TBBPA.

1. Introduction

Flame retardants (FRs) are synthetic compounds used to reduce flammability or burning rate of polymeric materials. Production and usage of these chemicals is still growing, which is associated with introduction of numerous legislative changes aimed to raise fire safety (Guerra et al., 2011). Brominated flame retardants (BFRs) constitute up to 25% of the total market of FRs (Guerra et al., 2011). In this group, more than 70 different chemicals can be identified, which is due to no restrictions within the backbone of the structure of these substances (BSEF, 2017; Guerra et al., 2011). BFRs cover many substances including polybrominated diphenyl ethers (PDBEs), polybrominated biphenyls (PBBs), hexacyclobromododecane (HBCDD), tetrabromobisphenol A (TBBPA) and others (Guerra et al., 2011). Annual production of TBBPA has been estimated to be higher than 220,000 tones and refers mainly to the United States, Israel and Japan (Covaci et al., 2009). TBBPA production accounts for about 60% of the market of all BFRs (Covaci et al., 2009). BFRs are used in the production of epoxy resins and polycarbonate plastics. Epoxy-resins are mainly used in the production of printed circuit boards and electronic components, and thus BFRs are used in electrical engineering, electronics, construction and telecommunication. Those substances are also found in many other everyday products such as furniture, carpets or textiles (Bjermo et al., 2017).

The widespread use of these compounds contributes to the exposure of the environment and humans to their action (de Wit, 2002; Abdallah, 2016). It is worth noting that human exposure to these substances is mainly associated with food consumption, while inhalation of indoor dust is considered to be the second

Abbreviations: BB, bromobenzene; BFRs, brominated flame retardants; BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; 2,4-DBP, 2,4-dibromophenol; DMSO, dimethyl sulfoxide; FRs, flame retardants; Hb, hemoglobin; HBCD, hexacyclobromododecane; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; PBDCs, polybrominated diphenyl ethers; PBP, 2,3,4,5,6-pentabromophenol; TBBPA, tetrabromobisphenol A; TBBPS, tetrabromobisphenol S; 2,4,6-TBP, 2,4,6-tribromophenol.

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major route to BFRs exposure (especially for the youngest individuals) (EFSA, 2011; Harrad et al., 2010; Rasmussen et al., 2017).

Numerous BFRs like PBDCs and TBBPA have been determined in human biological matrices such as blood plasma, adipose tissue and milk of mothers (Mazdai et al., 2003; Abdallah and Harrad, 2011; Cariou et al., 2008; Johnson-Restrepo et al., 2008). Leonetti et al. (2016) found a prevalence of 2,4,6-TBP in the placenta tissue at an average concentration of 15.4 ng/g fat, which indicate that exposure exists to this compound from the fetal stage. Kim and Oh (2014) investigated the presence of TBBPA in maternal and infant serum. The mean concentration of this compound was 10.7 ng/g of fat for mothers while 72.5 ng/g of fat for newborns. Abdallah et al. (2008) estimated the exposure of people living in Britain to TBBPA at the concentration of 1.3 ng/kg of body weigh per day in adults and 3.3 ng/kg of body weigh per day in children.

An increasing number of studies show that the BFRs may have adverse effects on living organisms. A large number of data concerns toxicity of PBDEs in humans but limited results are available for both TBBPA and HBCDD (Guerra et al., 2011). Similarly, there is lack of data concerning the effect of other BFRs examined such as 2,4-DBP, 2,4,6-TBP, PBP and TBBPS on human organism. The majority of toxicological studies concerning BFRs relate to disturbances in the functioning of thyroxine, disorders in reproduction and neurotoxicity (Choi et al., 2011; Park et al., 2014; Liu et al., 2016). Anisuzzaman and Whalen, 2016 assessed the effect of TBBPA at different concentrations (0.05–5 mM) on the ability of the immune cells to secrete IL-1. Both an increase and a decrease in IL-1 secretion, depending on concentration and incubation time, were observed.

Chen et al. (2017) conducted a study on the effects of PBP on mink lung epithelial cell line (Mv1Lu cells). They found that it contributes to TGF-β signal modulation. Lee et al. (2016) found that 2,4,6-TBP may interfere with the normal functioning of thyroid hormones. Moreover, Lim et al. (2008) suggested that PBDEs and PBBs accumulate in adipose tissue, and thus may contribute to the development of diabetes and metabolic syndrome.

Due to high exposure of human population to the above mentioned compounds and their well-documented presence in the circulation (Kim and Oh, 2014), we have decided to determine the effect of these chemical substances on human erythrocytes. The erythrocytes are the most plentiful cells of circulatory system, which show their major role in the body. In addition to their basic function, which is the transport of oxygen and carbon dioxide, the erythrocytes can be involved in the transport of xenobiotics. That is why, the erythrocytes are potentially exposed to various toxicants entering human organism (Stasiuk et al., 2009). Furthermore, red blood cells can transfer various xenobiotics to other organs and tissues of the body. Inouye et al. (1979) conducted a study in which they found that TBBPA induces changes in the permeability of human erythrocyte membrane. Szymańska et al. (2001) revealed that TBBPA conjugated to radioactive C-14 carbon accumulated with a 10-fold higher concentration in the erythrocytes than in plasma.

The mechanism of the impact of BFRs on blood cells including red blood cells has been poorly investigated, therefore, we aimed to determine the effect of 2,4-DBP, 2,4,6-TBP, PCP, TBBPA and TBBPS (Fig. 1) on hemolysis induction and hemoglobin oxidation in human erythrocytes. BFRs used in this study were chosen in regard to their significant market share among all FRs. Moreover, TBBPA is the most widely used BFRs worldwide. In respect to the data showing potential toxicity of TBBPA, some its analogs such as TBBPS are introduced into the market. Similarly to TBBPS other BFRs like 2,4-DBP, 2,4,6-TBP and PBP are widely used in the manufacture but their toxic effect has been very poorly evaluated.

2. Materials and methods

2.1. Chemicals

Dibromophenol (98%, 2,4-dibromophenol), tetrabromobisphenol A (99%, 2,2-bis(3,5-dibromo-4-hydroxyphenyl)propane), pentabromophenol (98%, 2,3,4,5,6-pentabromophenol) were purchased from LGC Standards, Germany. Tribromophenol (pure ≤ 100%, 2,4,6-tribromophenol) was purchased from Sigma-Aldrich, USA. Tetrabromobisphenol S (98.8%) was synthetized in the Institute of Industrial Organic Chemistry in Warsaw, Poland. All other chemicals were bought in POCH, Poland. The compounds studied were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the sample was 0.4%. We have compared changes in the parameters studied for the samples consisting of the erythrocytes with Ringer buffer and the samples containing the erythrocytes with Ringer buffer and DMSO. As the result, we have not observed any statistically significant differences between the tested samples, which showed that DMSO concentration used in our experiments was not toxic for red blood cells.

2.2. Isolation of red blood cells

Human erythrocytes were obtained from leukocyte buffy-coat separated from blood taken from blood donors from the Regional Blood Center in Lodz. The leucocyte-buffy coat were centrifuged (600*g*, 10 min, 4 °C) to separate erythrocytes from plasma, platelets and leucocytes. Isolated red blood cells were washed three times with Ringer buffer (125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose, 32 mM HEPES, 25 mM Tris, pH 7.4) by centrifugation (600*g*, 10 min, 4 °C). In order to determine hematocrit, the erythrocytes were centrifuged for 3 min in a microhematocrit centrifuge. The cells were diluted to 5% hematocrit (about 630 mln cells/ml) using a Ringer solution and incubated with BFRs (0.01–100 μg/ml) at 37 °C for 24 h, 48 h or 72 h in total darkness. The control samples were incubated with 0.4% DMSO. Due to the long incubation times, we added antibiotics, i.e. streptomycin and penicillin (0.2%) to the cells suspension. Additionally we prepared a control samples with 0.2% streptomycin/penicillin to exclude negative effect of antibiotics on the erythrocytes. The use of human blood (leucocyte buffy-coat) in the investigation of the effect of flame retardants on human erythrocytes was approved by Bioethics Committee for Scientific Investigation, University of Łódź (agreement no. 7/KBBN-UŁ/II/2015).

2.3. Hemolysis

The measurement of the amount of hemoglobin released from the erythrocytes was considered as the parameter of cell viability (Drabkin, 1946). After the incubation, the samples were centrifuged (600*g*, 10 min, 4 °C). Next, the supernatant from samples was collected and assayed for hemoglobin content by measuring the absorbance of the sample at a wavelength of 542 nm (Specord 250 plus, Analytik Jena AG, Germany). Samples with total hemolysis (100%) were prepared by adding deionized water to control samples.

The percent of hemolysis was calculated using the following formula:

$$H[\%] = \frac{A_s}{A_{100\%}} \times 100\%$$

where:

H [%] – the percentage of erythrocyte hemolysis.

A_s – absorbance of Hb in the supernatant at 542 nm.