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# Drinking water toxicity study of the environmental contaminant——Bromate

Liu Dongmei [a, *], Wang Zhiwei [a], Zhu Qi [b], Cui Fuyi [a, **], Shan Yujuan [c], Liu Xiaodong [d]

[a] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, PR China
[b] Key Laboratory of Chemical Engineering Process & Technology for High-efficiency Conversion, School of Chemistry and Material Sciences, Heilongjiang University, Harbin 150080, PR China
[c] School of Food Science and Engineering, Harbin Institute of Technology, Harbin 150090, PR China
[d] Shenzhen Water (Group) Co., Ltd., Shenzhen 518031, PR China

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ABSTRACT

Bromate is a byproduct of water disinfection that is produced when waters contain bromide treated with ozone. To investigate the level of the toxicity of bromate and find the most sensitive indicators in a short time, a series of toxicological assessments were conducted including the acute toxicity, cumulative toxicity, genetic toxicity and subacute toxicity of bromate (using Potassium Bromate to represent bromate). The $LD_{50}$ of orally administered Potassium Bromate was 215 mg/kg in Wistar rats and 464 mg/kg in ICR mice. The cumulative toxicity of Potassium Bromate was not obvious. The Ames test, mouse bone marrow cell micronucleus test and mouse sperm abnormality test did not indicate mutagenicity. The results of the subacute study did not exhibit significant differences in most of the parameters, except the white blood cell count, which was significantly decreased in male rats. In addition, Potassium Bromate influenced the albumin, creatinine, total cholesterol, triglycerides and glucose levels in male rats to various extents. A thorough analysis of the above tests clearly demonstrates that bromate has toxicity, not obvious cumulative toxicity and the white blood cell count can be used as an indicator to reflect the toxicity of bromate and investigate bromate's toxic mechanism.



## 1. Introduction

Bromate ($BrO_3^-$) has been extensively used as an oxidizer in food processes, such as flour milling, beer malting and cheese making (Dupuis, 1997). Because it induced renal cell tumors in F344 rats after oral administration for 104 weeks (Kurokawa et al., 1986), bromate is limited as a food additive (IARC, 1999). Nevertheless, it has also been used as a neutralizer in cold-wave hair lotions and the dyeing of textiles via sulfur dyes (Khan et al., 2004). Furthermore, bromate is one of the most prevalent water disinfection byproducts from the ozonation of drinking water (Cavanaugh et al., 1992). The U.S. Environmental Protection Agency (EPA) Stage 1 Disinfectants and Disinfection Byproducts Rule, which was signed in 1998, set a maximum bromate level of 10 μg/L (Butler et al., 2005). In the United Kingdom, The Water Supply (Water Quality) Regulations 2000 specified a maximum bromate level in drinking water of 10 μg/L. Furthermore, the Standards for Drinking Water Quality issued in 2006 in China also adopted this level.

Ozonation is used as an alternative method to hypochlorite disinfection because it avoids the formation of halogenated byproducts and improves the taste of the water. However, bromate is a common byproduct from the oxidation of bromide in surface water containing high amounts of it, especially in regions with saltwater intrusion. Additionally, bromate is not easily removed once formed with ozone oxidation. Although granular activated carbon (GAC) filtration has been reported to be effective under certain circumstance, conventional water treatment processes are not designed to remove bromate in compliance with the new regulatory standards. Many pilots and full-scale trials are currently under laboratory evaluation and in the development stages. Therefore, a potential hazard risk is associated with the exposure of humans to bromate (Weinberg et al., 2003), and the target level (no more than 10 μg/L) complicates the use of ozone as a disinfectant

* Corresponding author. State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, 73 Huanghe Road, Nangang District, Harbin, Heilongjiang Province 150090, PR China.
** Corresponding author.
*E-mail addresses:* mei18@hit.edu.cn (L. Dongmei), hit_cuifuyi@hotmail.com (C. Fuyi).

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for bromide-containing waters. So it is necessary to evaluate the toxicity of bromate.

Bromate is classified as a Group 2B or "possible human" carcinogen by the International Agency for Research on Cancer (IARC) because of tumor induction in rats and mice and lack of evidence of carcinogenicity to human (IARC, 1999). However, species differ in their sensitivity to bromate. For example, the $LD_{50}$ ranges from 160 to 495 mg/kg in rats, mice and hamsters in the oral acute toxicity test (Kurokawa et al., 1990). The results of the Ames test, gene mutation assay in Escherichia Coli, chromosome aberration and micronucleus tests of various reports in the literature are also inconsistent (Li, 2006). Furthermore, the target tissues also depend on the animal species. Lesions in the kidney, peritoneal mesothelium and thyroid gland of male F344 rats, in the kidney of female F344 rats, the kidney of male Syrian golden hamsters, the kidney and small intestine of male $B6C3F_1$ mice, the liver of male $CDF_1$ mice (Crosby et al., 2000) and the intestine of male Wistar rats have been reported (Ahmad et al., 2012). However some researchers have suggested that bromate might be inactivated by antioxidants in the gastrointestinal tract, and the relationship between the blood and toxic dose response was not linear at low doses (Fawell and Walke, 2006). As discussed above, more comprehensive research on the toxicity of bromate is essential.

The toxicity of bromate has been studied in the long-term tests, but the sensitive indicators for bromate toxicity in the short-term tests were rarely investigated. The short-term tests have the advantages of reducing anything unnecessary, shortening the experiment period and improving the efficiency. In this study, we report our thorough evaluation and toxicological assessment of bromate based on acute toxicity test, cumulative toxicity test, Ames test, bone marrow cell micronucleus test, sperm aberration test and 30-day animal oral toxicity study (including body weight, vital organ weight, food intake, hematology and blood biochemistry).

## 2. Materials and methods

### 2.1. Animals

Equal numbers of male and female ICR mice and Wistar rats were purchased from the experimental animal center of Heilongjiang University of Chinese Medicine, Heilongjiang, China (laboratory animal reproduction license # SCXK (H) 2008-004). They were housed in the experimental animal center (laboratory animal use permit # SYXK (H) 2006-010). The temperature was controlled at 21-23 °C, and the relative humidity was controlled at 55-58%. The feed was provided by the Beijing Branch of Australian Cooperative Feed Limited Company (license # SCXK (J) 2005-0007).

### 2.2. Acute toxicity test

The acute toxicity test was performed to determine the $LD_{50}$ using Horn's method. Forty Wistar rats (20 male and 20 female) and 40 ICR mice (20 male and 20 female) were obtained as described previously with body weights ranging from 180 to 220 g and 18 to 22 g, respectively. The animals were randomly divided into five groups with four males and four females in each group. Potassium Bromate ($KBrO_3$, using Potassium Bromate to represent bromate) was dissolved in distilled water and administered by oral gavage at doses of 46.4, 100, 215, 464 and 1000 mg/kg bw to Wistar rats, and 100, 215, 464, 1000 and 2150 mg/kg bw to ICR mice. The test article was given a single intragastric administration in a volume of 1% bw for Wistar rats and 2% bw for ICR mice. They were monitored daily for 14 days for toxicological signs and mortality.

### 2.3. Cumulative toxicity test

To investigate the cumulative toxicity of $KBrO_3$, a dose-escalation approach was used based on the acute $LD_{50}$ of $KBrO_3$ in mice. In the dose-escalation approach, 4 days were selected as a period, the initial infected dose was 1/10 $LD_{50}$ and the infected dose increased by 1.5 times every period. The equal numbers of male and female ICR mice were randomly divided into two groups, the experimental group and negative control group. Distilled water was used as a negative control and was administered at a volume of 2% bw via oral gavage. In the experimental group, $KBrO_3$ was administered by oral gavage in sequentially increased doses.

The experiment was terminated either when 50% of the mice had died or the cumulative dose was equal to 5.0 times of the $LD_{50}$ (20 days).

### 2.4. Ames test

The Ames test is the most often used traditional method to test the genotoxicity of a compound. The mutagenic potency of $KBrO_3$ was tested with two tester *Salmonella strains* (TA98 and TA100) that were provided by the Ames lab at the University of California. Both of the strains were biologically identified prior to the experiment, and they all conformed to the test requirements. Polychlorobiphenyl (PBC)-induced rat liver (S9 mixture) was used as the metabolic activator in vitro. The tested bacteria were exposed to $KBrO_3$ at four concentrations (10, 30, 200 and 600 μg/plate), with and without the addition of S9 mixture. The solvent (distilled water), positive (Dexon 50 μg/plate, 2-AF 10 μg/plate) and negative controls were run simultaneously with the test. The plates were cultured at 37 °C for 48 h. Each concentration was tested with three parallel plates.

### 2.5. Micronucleus test of mice bone marrow erythrocyte

Fifty ICR mice with body weights between 25 and 30 g were housed in a clean chamber for 5 days to adapt to the environment and then randomly divided into five groups with 5 male and 5 female mice per group. Distilled water was used as the negative control, and Cyclophamide (40 mg/kg bw) was used as the positive control. $KBrO_3$ was dissolved in distilled water at doses of 46.4, 92.8 and 232 mg/kg bw for mice. Distilled water, Cyclophamide and $KBrO_3$ were administered by oral gavage in a volume of 2% bw at 24 h intervals. The mice were killed 6 h after the fifth administration, and the bone marrows cells were then flushed out. Smear slides were prepared and were double-blind coded under a fluorescence microscope. 1000 polychromatic erythrocytes (PCE) were observed to record the numbers of PCE with micronuclei for each mouse, and the frequency of micronucleated PCE were calculated.

### 2.6. Sperm abnormality test of mice

Sperm is usually used to theoretically evaluate the effects of chemicals on genetic materials. In this experiment, 25 male ICR mice with body weights ranging from 25 to 30 g were randomly divided into five groups with 5 mice in each group. The mice were administered a negative control (distilled water), a positive control (Cyclophosphamide, 40 mg/kg bw) and $KBrO_3$ at doses of 46.4, 92.8 and 232 mg/kg bw, respectively, by oral gavage daily for 5 days. Thirty-five days after the first administration, the mice were killed and both sides of the epididymides were obtained. Smear slides were then prepared with 2% eosin as the stain. 1000 sperm from each mouse were assessed, and the sperm abnormalities were calculated.