Subchronic toxicity evaluation of potassium bromate in Fischer 344 rats

Darol E. Dodd\textsuperscript{a,*}, Debra K. Layko\textsuperscript{a}, Katherine E. Cantwell\textsuperscript{a}, Gabrielle A. Willson\textsuperscript{b}, Russell S. Thomas\textsuperscript{a}

\textsuperscript{a} The Hamner Institutes for Health Sciences, Six Davis Drive, P.O. Box 12137, Research Triangle Park, NC 27709, USA
\textsuperscript{b} Experimental Pathology Laboratories, Inc., P.O. Box 12766, Research Triangle Park, NC 27709, USA

\textbf{A R T I C L E   I N F O}

Article history:
Received 18 August 2013
Received in revised form
7 October 2013
Accepted 9 October 2013
Available online 19 October 2013

Keywords:
Potassium bromate
F344 rats
Hyaline droplets

\textbf{A B S T R A C T}

Male F344 rats were exposed to potassium bromate (KBrO\textsubscript{3}) in drinking water at concentrations of 0, 5, 20, 100, 200, or 400 mg/L for 2 or 13 weeks. Endpoints evaluated included clinical observations, body weights, serum chemistry, gross pathology, organ weights, and select tissue histopathology (kidney, lung, liver, thyroid, and tunica vaginalis). Weekly body weight and water consumption means were similar between KBrO\textsubscript{3} and control groups throughout the study. Increases in kidney weights were observed in rats of the 400 mg/L group following 2- or 13-weeks exposure. Hyaline droplets were observed in renal tubules of rats of the 200 and 400 mg/L groups following 2 weeks exposure and in rats of the 400 mg/L group at 13 weeks. There were no KBrO\textsubscript{3}-related microscopic findings in the lung, liver, thyroid, and tunica vaginalis at the 2- and 13-week time points. A no observed effect level of 100 mg/L KBrO\textsubscript{3} (8.1 mg/kg/day) was selected based on the absence of microscopic alterations in the kidney.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Potassium bromate (KBrO\textsubscript{3}) is an oxidizing agent used in a variety of applications and products, such as a food additive in baked goods, a neutralizer in hair wave preparations, an industrial cleaning agent, or in skin bleaching compounds (HSDB, 2009). Bromate is a disinfection byproduct that is formed during the ozonation of waters containing bromide ion. Laboratory studies indicated that the degree of bromate formation depends on ozone concentration, pH, and contact time (Haag and Hoigne, 1983). Potential routes of exposure of KBrO\textsubscript{3} are ingestion, inhalation, and dermal contact. The U.S. Environmental Protection Agency derived an oral reference dose (RfD) for bromate of 0.004 mg/kg/day (U.S. EPA, 2001). For occupational exposure, the American Industrial Hygiene Association’s Workplace Environmental Exposure Level (WEEL) guideline for potassium bromate is 0.1 mg/m\textsuperscript{3} (8-h time-weighted average) (AIHA, 2013).

There are numerous in vitro and in vivo toxicity studies with KBrO\textsubscript{3}, many of which have been summarized by the U.S. EPA in a toxicology review of bromate (U.S. EPA, 2001). The major health concern is carcinogenicity due to results of two-year toxicity studies using laboratory animals. Specifically, tumors of the kidneys, thyroids, and tunica vaginalis were identified as KBrO\textsubscript{3} exposure-related in the F344 rat studies. For example, Kurokawa et al. (1983) observed high incidences of renal cell tumors in male and female F344 rats and of peritoneal mesotheliomas in male rats when KBrO\textsubscript{3} was administered in the drinking water at concentrations of 250 or 500 ppm (250 or 500 mg/L) for 110 weeks. These results were confirmed in subsequent two-year drinking water studies using F344 rats...
at similar or lower concentrations of KBrO₃ (Kurokawa et al., 1986; DeAngelo et al., 1998). Tumors of the thyroid were also reported (Kurokawa et al., 1986; DeAngelo et al., 1998) with a carcinogenic effect level as low as 20 mg/L KBrO₃ (DeAngelo et al., 1998) and an incidence time as early as 26 weeks in male F344 rats administered 100 or 200 mg/L KBrO₃ (Wolf et al., 1998). The current study selected the kidneys, thyroids, and tunic vaginalis as target tissues for more detailed investigation of dose-related and temporal effects of KBrO₃ exposure.

When KBrO₃ was identified as a suspect carcinogen, a number of scientific investigations ensued to ascertain its mechanism or mode of action for producing tumors. Since KBrO₃ is an oxidizing agent, a relationship between KBrO₃, oxygen radicals, and DNA injury was hypothesized. Kasai et al. (1987) observed a significant increase of 8-hydroxydeoxyguanosine (8-OHdG) in kidney DNA following a single gavage dose of 400 mg/kg KBrO₃ in F344 rats, and though oxygen radicals, lipid peroxidation, protective roles of antioxidants (glutathione and cysteine) and related oxidative stress mechanisms have been implicated with tumor formation (Kurokawa et al., 1990; Sai et al., 1992; Umemura et al., 1995), an unequivocal link between these mechanisms and KBrO₃ carcinogenicity has not been established (U.S. EPA, 2001). However, the kinetics of 8-OHdG formation in kidney DNA and associated elevation of renal cell proliferation may partially explain sex differences associated with KBrO₃-induced renal tumors (Umemura et al., 1998, 2004). In male F344 rats, kidney 8-OHdG levels elevated rapidly following one week of KBrO₃ exposure (500 mg/L) and the increase in 8-OHdG persisted throughout the 13-week study (Umemura et al., 1998). Increased renal cell proliferation coincided with the increases in 8-OHdG. But in female F344 rats, kidney 8-OHdG levels did not increase until three weeks of KBrO₃ exposure (500 mg/L), and renal cell proliferation was observed only at the end of the 13-week study (Umemura et al., 1998). Changes in renal cell proliferation in male and female rats were dose-dependent (Umemura et al., 2004). Results of in vitro or in vivo genotoxicity studies indicate KBrO₃ is mutagenic in bacteria (Ishidate et al., 1984), induces chromosomal aberrations in Chinese hamster fibroblasts (Ishidate et al., 1984), and increases the incidence of chromosome aberrations in rat bone marrow cells following gavage or intraperitoneal administration (Fujie et al., 1988). Genetically altered mouse models (TG.AC hemizygous or p53 haploinsufficient mice) were used in subchronic dermal and drinking water studies to further evaluate development of carcinogenicity following sodium bromate exposure (NTP, 2007). No increases in tumors were observed in these studies. Since sodium bromate and KBrO₃ are related structurally, similar results would likely have occurred with KBrO₃. Since a clear mechanism for bromate carcinogenicity remains unidentified, EPA used a low-dose linear default approach for cancer risk estimation (U.S. EPA, 2001).

The EPA concluded clear evidence of carcinogenic activity of KBrO₃ based on increased incidences of renal cell tumors, thyroid follicular tumors, and mesotheliomas (tunica vaginalis) in male F344 rats (only males were tested) administered drinking water concentrations of 0 (control), 20, 100, 200, or 400 mg/L KBrO₃ for a lifetime (100 weeks exposure). Due to a dose-related and statistically significant increase in the incidence of mesotheliomas in male F344 rats at all KBrO₃ doses tested (DeAngelo et al., 1998), a no observed adverse effect level (NOAEL) was not established in this two-year bioassay. In our study, the objective was to focus on the toxicity of previously identified target tissues (kidney, thyroid, and tests) associated with oral KBrO₃ exposure and to characterize both dose response and time course relationships. The liver and lung were also selected for evaluation to compare potential effects of KBrO₃ exposure in non-target tissues with those of target tissues. It was anticipated that a more thorough characterization of target organ effects with dose and exposure time would provide more definitive results to support the selection of a NOAEL and to improve human risk assessment of KBrO₃. Five concentrations of KBrO₃ and two time points were selected to assess toxicity in male F344 rats. Male rats appear to be more responsive to KBrO₃ exposure than female rats (Umemura and Kurokawa, 2006), and the tunica vaginalis is a target tissue of carcinogenicity. One of the five KBrO₃ concentrations selected in the current study was below the lowest concentration used in EPA’s 2-year bioassay study of KBrO₃. Endpoints evaluated included clinical observations, body weights, water consumption, target organ weights, serum clinical chemistry, gross pathology, and target organ histopathology.

2. Materials and methods

Test Materials. Potassium bromate (CAS No. 7758-01-2) was obtained from Sigma–Aldrich, Milwaukee, WI, USA (ACS reagent grade, Product No. 309087). A certificate of analysis from Sigma–Aldrich indicated a purity of ≥99.8%. A purity check performed by The Hamner Institutes Analytical Chemistry Group confirmed a purity of ≥99.8%.

2.1. Study design, animals, and animal husbandry

One hundred and thirty-two 4–5 week old male Fischer 344 rats (rat F344/NCr) from Charles River Laboratories (Kingston, NY) were used in this study. The selection of the F344 strain was based on the findings of the NTP bioassay that showed increased incidences of tumors of the kidney, thyroid, and mesothelium when KBrO₃ was administered in drinking water at 20–400 mg/L (DeAngelo et al., 1998). To keep the number of animals on study at a minimum, only one sex of F344 rats was used. Since male rats appear to be more responsive to KBrO₃ exposure than female rats (Umemura and Kurokawa, 2006), and the tunica vaginalis was a target tissue of carcinogenicity, male rats were chosen for the current study. Treatment group sizes were 10 with an additional two to three rats assigned to the control group per time point to be used to assure a group size of at least 10 for evaluation of biological endpoints. Upon arrival, rats were acclimated to housing and animal room environment for 12–14 days. Rats were weighed and randomized using Provantis8 (Provantis™, Conshohocken, PA) to ensure mean body weight in each treatment group was approximately the same. Animals were ear-tagged and housed one per cage in shoebox style cages separated by treatment group. Alpha-dri cellulose bedding (Shepard Specialty Papers, Kalamazoo, MI) was used. Animals had access to reverse osmosis (RO) water (Hydro Systems, Durham, NC).
with or without KBrO3 and NIH-07 certified pellet feed (Zeigler Brothers, Gardners, PA) ad libitum. The animal room was kept within the standard temperature and relative humidity parameters (64–79°F and 30–70% relative humidity) and standard light cycle (07:00–19:00 h).

The Hamner Institutes for Health Sciences is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Currently acceptable practices of good animal husbandry were followed per National Research Council’s Guide for the Care and Use of Laboratory Animals and were in compliance with all appropriate parts of the Animal Welfare Act. In addition, the study design and protocol were approved by The Hamner Institutes’ Institutional Animal Care and Use Committee (IACUC) prior to the initiation of study.

2.2. Preparation and administration of KBrO3

Target KBrO3 drinking water concentrations were 0, 5, 20, 100, 200, and 400 mg/L. Selection of water concentrations was based on the concentrations used in EPA’s 2-year drinking water study in F344 rats (DeAngelo et al., 1998) of 0, 20, 100, 200, and 400 mg/L, but extended lower (5 mg/L) to assure a NOAEL. A NOAEL was not observed in the EPA study (DeAngelo et al., 1998), and KBrO3 drinking water concentrations of 600 mg/L significantly decreased body weight gain in male F344 rats (Onclera, unpublished data cited in Kurokawa et al., 1990).

A KBrO3 pre-mix was prepared by adding a weighed amount of the test material into 0.5 L of either RO or distilled water. The test material was dissolved through vigorous shaking before adding the pre-mix to the desired volume of drinking water. RO water was used initially (study weeks 1 through 6), but beginning on study week 7, KBrO3 pre-mix and drinking water concentrations were prepared in distilled water (Barnstead Nanopure, Thermo Fisher Scientific, Asheville, NC) to improve the signal-to-noise ratio for quantitating bromate ion. Batches of KBrO3 pre-mix and drinking water concentrations were prepared approximately weekly. On the day of preparation, triplicate aliquots from each batch were diluted with water and submitted for analysis. Analytical calibration curves (0.3–10.0 mg/L) were prepared on the same day as sample analysis by spiking water with a freshly prepared stock solution of KBrO3 in water. Samples of prepared drinking water batches were diluted to fit the concentration range of the analytical calibration curve and analyzed for bromate using an API-3000 triple quadrupole mass spectrometer (Danaher, Washington, DC, USA) with a PE-200 Perkin-Elmer high performance liquid chromatography (HPLC) system (Perkin-Elmer, Waltham, MA, USA). Chromatographic separation was conducted on a Restek C18 column (Allure C18, 50 mm × 2.1 mm, 5 µm) (Restek®, Bellefonte, PA). Aqueous mobile phase used for analyses was 0.1% formic acid (Sigma–Aldrich, MO) and the organic phase was mass spectrometry grade methanol (Sigma–Aldrich, MO). Sample separation and analysis were conducted using the following gradient conditions in negative mode: (1) 5% organic for 0.5 min; (2) linear gradient ramp to 70% organic over 3.5 min; (3) maintain 70% organic for 0.5 min; (4) linear gradient ramp to 5% organic over 1.0 min; and (5) maintain 5% organic for 0.5 min prior to the next injection. Total analysis time was 5.5 min per sample. Injection sizes of 10 µL with a column temperature of 35°C and a constant HPLC flow rate of 200 µL/min was introduced into the mass spectrometer in splitless mode. Chromatograms were manually integrated and dosing solution concentrations were calculated through the use of the generated calibration curves and dilution factors. The mass used to quantify the bromate ion was 127.

KBrO3 drinking water concentration stability was conducted at two concentrations, 5 mg/L and 400 mg/L, under two different environmental conditions. Samples of drinking water were stored at either room temperature or 4°C. Subsamples of the fractions were analyzed and quantified through comparison to a freshly prepared calibration curve (described above). Sample analysis was conducted every few days for a total of five analyses in three weeks. Following completion of the stability studies, it was observed that the concentration of KBrO3 in drinking water was not impacted by any of the different storage conditions, with final concentrations ±10% of the original concentration.

The mean ± standard deviation concentration of the prepared water batches were 5.0 ± 0.3, 19.6 ± 0.6, 98.6 ± 5.7, 201 ± 11, and 401 ± 28 mg/L KBrO3 for target concentrations of 5, 20, 100, 200, and 400 mg/L, respectively.

2.3. Mortality checks, clinical observations, body weights, water consumption, and administered dose

Animals were checked daily for clinical signs of toxicity, morbidity or death. Body weights and detailed clinical observations were measured twice during the first week and then weekly, including immediately prior to scheduled necropsy. Water consumption was measured weekly. Detailed clinical observations, body weights, and water consumption were recorded in Provantis®. The administered dose of KBrO3 was calculated using the mean (relative to body weight) water consumption data and the mean analytical concentration of KBrO3 in the administered drinking water. Means of water consumption and analytical drinking water concentration per dose group were combined, and the results were expressed as mg KBrO3 per kg body weight per day.

2.4. Necropsy, serum clinical chemistry, organ weights, and tissue histopathology

Animal necropsies occurred in the morning of the day following scheduled time points. Animals were weighed and anesthetized with a lethal intraperitoneal injection of Euthasol containing sodium pentobarbital. A cardiac puncture was performed to collect blood samples, and the animal was then exsanguinated via transection of the abdominal aorta. The left and right kidney, liver, and thyroid were removed, weighed, and results were recorded in Provantis®. Blood samples were collected into serum separator tubes (gel barrier), centrifuged and separated into two aliquots per animal. One aliquot was used for analysis of the following clinical chemistry analytes: aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin (total), blood urea nitrogen (BUN), creatinine, and lactate dehydrogenase (LDH). An Olympus AU600 (Olympus America, Center Valley, PA) chemistry analyzer was used for analysis of serum
Table 1 – Absolute and relative (to body weight) organ weights of male F344 rats following 2 weeks of daily exposure of KBrO₃ by drinking water.

<table>
<thead>
<tr>
<th>Organ</th>
<th>KBrO₃ (mg/L)</th>
<th>0</th>
<th>5</th>
<th>20</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>g</td>
<td>7.41 ± 0.89</td>
<td>7.87 ± 0.89</td>
<td>7.41 ± 0.83</td>
<td>7.04 ± 0.69</td>
<td>7.36 ± 0.93</td>
<td>7.84 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>4.33 ± 0.27</td>
<td>4.46 ± 0.23</td>
<td>4.35 ± 0.17</td>
<td>4.23 ± 0.12</td>
<td>4.33 ± 0.26</td>
<td>4.41 ± 0.18</td>
</tr>
<tr>
<td>Kidney-left</td>
<td>g</td>
<td>0.69 ± 0.08</td>
<td>0.71 ± 0.06</td>
<td>0.70 ± 0.07</td>
<td>0.68 ± 0.05</td>
<td>0.71 ± 0.06</td>
<td>0.79 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td>0.41 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Kidney-right</td>
<td>g</td>
<td>0.69 ± 0.08</td>
<td>0.70 ± 0.06</td>
<td>0.68 ± 0.07</td>
<td>0.68 ± 0.05</td>
<td>0.70 ± 0.07</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>Thyroid</td>
<td>g</td>
<td>0.011 ± 0.004</td>
<td>0.011 ± 0.003</td>
<td>0.012 ± 0.002</td>
<td>0.011 ± 0.002</td>
<td>0.011 ± 0.002</td>
<td>0.013 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0.006 ± 0.002</td>
<td>0.006 ± 0.002</td>
<td>0.007 ± 0.001</td>
<td>0.007 ± 0.002</td>
<td>0.007 ± 0.002</td>
<td>0.007 ± 0.001</td>
</tr>
</tbody>
</table>

* p < 0.01 Compared to control.
** p < 0.001 Compared to control.

a g, Grams.
b % = [Organ weight/terminal body weight] × 100.
c Mean ± standard deviation for n = 10 except for the 0 mg/L group (n = 12 for liver and kidneys; n = 11 for thyroid) and for the 20 mg/L group (n = 9 for thyroid).

samples. Reagent sets for the serum analytes and quality control (QC) materials, including standards were obtained from Carolina Liquid Chemistries (Winston Salem, NC). The AU600 operator manual and laboratory standard operating procedures (SOP) were followed for conducting analyses. The second serum aliquot was used for the measurement of total triiodothyronine (T₃), total thyroxine (T₄), and thyroid-stimulating hormone (TSH) via radioimmunoassay (RIA). Instructions supplied with the RIA kits were followed. A Packard Gamma Counter was used for measuring radioactivity.

For kidney histopathology, a longitudinal slice from the left kidney and a cross-sectional slice from the right kidney were taken. For lung tissue, the trachea was cannulated below the larynx and the left lung infused under approximately 30 cm of hydrostatic pressure with 10% neutral buffered formalin (NBF). When filled, a suture around the left bronchus was pulled tightly to tie off the left lobe. The left lobe with trachea attached was removed and placed in NBF. Liver slices from 3 of 4 lobes (median, right, and left) were taken. The left thyroid and left tunica vaginalis were also removed for histopathology. Kidney, liver, thyroid, and tunica vaginalis slices were placed in cassettes and immersed in NBF for approximately 48 h. The tissue cassettes and left lung were then transferred to a cup containing 70% ethanol followed by paraffin embedding. Embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin for microscopic examination by a board-certified pathologist. Histomorphologic findings were graded from one to five where 1 = minimal, 2 = mild, 3 = moderate, 4 = moderately severe, and 5 = severe. The liver lobes were not evaluated independently to assess interlobe variability, but were evaluated together to characterize an overall hepatic effect of KBrO₃ exposure.

2.5. Statistical analysis

Body weight, water consumption, and organ weight data were analyzed using the statistical tests provided by the Provantis software system (NT2000 versions 8.2.0.1 or 8.2.0.6, Instem, Coshocton, PA). A one-way analysis of variance (ANOVA) was used. If significant, the ANOVA was followed by a Dunnett’s test to compare KBrO₃ concentration groups with the control group. Serum chemistry data were analyzed using JMP 9.0.0 software (SAS Institute, Inc., Cary, NC). A goodness of fit test (Shapiro–Wilk, p < 0.01) and homogeneity of variances test (Levene’s, p < 0.05) were conducted. If pre-test assumptions were met, an ANOVA was used and, if significant (p < 0.05), KBrO₃-exposed groups were compared to the control group using Dunnett’s test. For data sets of non-normal distributions or unequal variances, a Welch ANOVA followed by Steel’s test was used. A result of p < 0.05 was considered significant.

3. Results

Clinical Observations, Body Weights, Water Consumption, and Administered Dose. There were no KBrO₃ exposure-related clinical observations throughout the study. Weekly body weight and water consumption means were similar between all KBrO₃ exposure and control groups throughout the study, except for a statistically significant increase (approximately 20%) in mean water consumption during study week 13 in rats of the 400 mg/L group (data not shown). The administered doses calculated from drinking water consumption (relative to body weight) and analytical drinking water concentration at the conclusion of the 13-week study were 0.4, 1.6, 8.1, 16.5 and 34.9 mg KBrO₃/kg body weight/day for drinking water concentrations of 5, 20, 100, 200, and 400 mg/L KBrO₃, respectively. Administered doses at the conclusion of the 2-time point were 23–37% higher than the 13-week administered dose calculations, due to much higher relative (to body weight) water consumption values for younger rats.

3.1. Organ weights

Statistically significant increases were observed in mean absolute and relative kidney weights of the 400 mg/L group compared to means of the control animals following 2 weeks (Table 1) or 13 weeks exposure (Table 2). The increases were
observed in both left and right kidneys and ranged from 10 to 15% higher than control values. Mean liver and thyroid weights for all KBrO₃ groups were similar to control means at both time points (Tables 1 and 2).

### 3.2. Clinical chemistry and histopathology

Following 2 weeks exposure, mean serum ALP concentration was mildly increased in rats of the 400 mg/L group only (427 U/L) compared to the mean ALP concentration of control animals (384 U/L). There were no additional statistically significant differences between KBrO₃ and control groups for the other serum analytes measured. At 13 weeks, a statistically significant decrease in mean TSH was observed in rats of lower KBrO₃ groups only (20 and 100 mg/L) compared to the control mean TSH value. There were no additional statistically significant differences between KBrO₃ and control groups for the other serum analytes measured.

There were no KBrO₃ exposure-related gross observations during animal necropsy at the 2- and 13-week time points. Following two weeks exposure, hyaline droplets were observed in the kidneys of rats of the 200 and 400 mg/L groups (Table 3). The hyaline droplets were observed within the cytoplasm of tubule epithelial cells and within the lumens of the tubules and were eosinophilic in color. The severity of hyaline droplets in affected rats was minimal. Following 13 weeks of KBrO₃ exposure, renal hyaline droplets were observed in rats of the 400 mg/L group only and the severity changed from minimal to mild (Table 3 and Fig. 1). The incidence of nephropathy was similar between KBrO₃ and control groups (Table 3), though the group incidence was greater following 13 weeks exposure (80–100%) compared to 2 weeks exposure (10–40%). Nephropathy was characterized by a focal tubular change of basophilia with thickened basement membranes. The severity of nephropathy increased slightly in rats of the 400 mg/L group compared to the control animals (Table 3). There were no KBrO₃ exposure-related microscopic findings in the lung, liver, thyroid, and tunica vaginalis at the 2- and 13-week time points.

### 4. Discussion

In previous KBrO₃ drinking water carcinogenic studies utilizing F344 rats, the kidney, thyroid, and tunica vaginalis were identified as critical target organs following chronic exposure (DeAngelo et al., 1998; Kurokawa et al., 1983, 1986). However, exposure of KBrO₃ in drinking water for much shorter durations produces effects. For example, eosinophilic bodies in the renal tubules of male F344 rats were observed following 4 weeks of exposure of 600 mg/L KBrO₃ (Onodera et al., 1985; Kurokawa et al., 1990). A dose-dependent increase in the numbers of eosinophilic droplets within the proximal tubule epithelium was observed in male F344 rats exposed to 20, 100, 200, or 400 mg/L KBrO₃ for 12 weeks (Wolf et al., 1998). Kurokawa et al. (1987a) reported similar lesions in proximal renal tubules of male F344 rats following 13 weeks exposure of 500 mg/L KBrO₃. An increase in cell proliferation in the proximal convoluted tubules and a concomitant accumulation of α₂-globulin were observed in male F344 rats following 1, 2, 3, or 13 weeks exposure of 500 mg/L KBrO₃ (Umemura et al., 1998). Results in the current study agree very well with results of these subchronic studies and demonstrate a continuum of KBrO₃ effects in male rats at low drinking water concentrations (200–400 mg/L) and short durations of exposure (2 and 13 weeks).

Following 2 weeks exposure, water consumption and body weights were similar between control and KBrO₃ groups, but serum ALP was mildly increased in rats of the 400 mg/L group. Kidney weights were also mildly increased in rats of the 400 mg/L group (Table 1), and hyaline droplets were observed in renal tubules of rats exposed to ≥200 mg/L (Table 3). Increases in relative kidney weights, proximal convoluted tubule cell proliferation, and accumulation of α₂-globulin droplets in cells of the renal cortex were observed in male F344 rats as early as one week of exposure at 500 mg/L KBrO₃ (Umemura et al., 1998).

When the exposure duration increased from 2 to 13 weeks, weekly body weight and water consumption values of KBrO₃ exposed rats were similar to control values, except for an
Table 3 – Selected histopathologic changes in the kidneys of male F344 rats following daily exposure of KBrO3 by drinking water.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Finding</th>
<th>KBrO3 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>Hyaline droplets, renal tubules</td>
<td>–</td>
</tr>
<tr>
<td>13 Weeks</td>
<td>Hyaline droplets, renal tubules</td>
<td>–</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>Nephropathy</td>
<td>2/12 (0.2)</td>
</tr>
<tr>
<td>13 Weeks</td>
<td>Nephropathy</td>
<td>12/13 (0.9)</td>
</tr>
</tbody>
</table>

a Indicates no finding.

b Incidence-number with finding/number examined (mean severity).

increase in water consumption at 13 weeks in the 400 mg/L group. Increases in water consumption in KBrO3 treated rats had been observed in previous studies (DeAngelo et al., 1998; Kurokawa et al., 1986). Serum ALP concentration in rats of the 400 mg/L group returned to normal value at 13 weeks, indicating that the observed increase at 2 weeks was transient and the source of the increase was unknown. A decrease in serum TSH at 13 weeks was not dose-related, and changes in serum T3 or T4 were absent. Wolf et al. (1998) measured serum T3 and T4 in male F344 rats following 12 weeks of KBrO3 exposure (20–400 mg/L in drinking water). Serum T3 levels were decreased in an exposure-dependent, but not dose-dependent manner, while T4 concentrations in KBrO3 exposed rats were similar to the control value (Wolf et al., 1998). In the current study, means of thyroid and liver weights of KBrO3 exposure groups were similar to control values, but kidney weights continued to be increased at 13 weeks (Tables 1 and 2). However, the magnitude of the observed increases in kidney weights between 2 and 13 weeks exposure (approximately 13–15%) did not change.

Microscopically, of the target tissues (kidneys, thyroid, and tunica vaginalis) and non-target tissues (liver and lung) evaluated, only the kidneys had alterations (Table 3). Specifically, hyaline droplets were observed in the cytoplasm of renal tubule epithelial cells and within the lumens of the tubules. The droplets were eosinophilic in color. This observation was consistent with results in previous drinking water studies with KBrO3 at similar doses in male F344 rats (Onodera et al., 1985; Kurokawa et al., 1987a; Umemura et al., 1993; Wolf et al., 1998; DeAngelo et al., 1998). A number of KBrO3 investigations in rats were conducted to determine the relationship, if any, of kidney hyaline droplets/eosinophilic bodies with that of renal carcinogenicity (Kurokawa et al., 1987b; Kurata et al., 1992; Umemura et al., 1998, 2004). A general consensus is that the eosinophilic bodies occur as a result of oxidative damage from KBrO3. Although α2u-globulin has been associated with the hyaline droplets in male rats, it does not appear that α2u-globulin accumulation is related to renal tumor development, since both male and female rats develop renal tumors. Umemura and Kurokawa (2006) observed an increase in renal cell proliferation in both male and female rats with male rats showing greater sensitivity at lower KBrO3 doses, likely due to the accumulation of α2u-globulin in males. Further, the observed nephropathy in the current study (Table 3) did not appear to be KBrO3 related. Results by both Kurokawa et al. (1987a) and Wolf et al. (1998) concluded that KBrO3 did not enhance the development of nephropathy in rats and that the mechanism of renal tumor development by KBrO3 was not related to nephropathy.

There was excellent agreement in calculated administered doses between the current study and previous studies. Calculated KBrO3 doses in the current 13-week study were 0.4, 1.6, 8.1, 16.5, and 34.9 mg/kg/day for KBrO3 drinking water concentrations of 5, 20, 100, 200, and 400 mg/L, respectively. DeAngelo et al. (1998) reported KBrO3 doses of 1.5, 7.9, 16.9, and 37.5 mg/kg/day in male F344 rats for KBrO3 drinking water.

![Fig. 1](https://example.com/fig1.png) – (a) Kidney from male rat administered 400 mg/L KBrO3 for 13 weeks showing eosinophilic hyaline droplets (arrows) in the cytoplasm of renal tubule epithelial cells and within tubule lumens and (b) 0 mg/L (control) male rat kidney; H&E; 64×.
concentrations of 20, 100, 200, and 400 mg/L, respectively. Kurokawa et al. (1986) reported KBrO₃ doses of 0.9, 1.7, 3.3, 7.3, 16.0, and 43.4 mg/kg/day in male F344 rats for KBrO₃ drinking water concentrations of 15, 30, 60, 125, 250, and 500 mg/L, respectively. Following oral administration of KBrO₃ solutions in rats, bromate was rapidly absorbed from the digestive tract and partly excreted in the urine unchanged within 2 h of dosing (Fujii et al., 1984). No bromate was detected in rat blood or tissues 24 h after administration and the urinary excretion rate was proportional to dose when bromate was detected in the urine (KBrO₃ doses ≥5 mg/kg).

In conclusion, male F344 rats administered KBrO₃ by drinking water at concentrations up to 400 mg/L for 13 weeks had exposure-related effects; namely increased weights and microscopic alterations in the kidney. These observations were congruent with results of previous KBrO₃ drinking water studies conducted in male F344 rats for the last 30 years. Much of the focus of those studies was related to KBrO₃ carcinogeticity, including dose-dependency, time-dependency, and mechanism of action. A clear understanding between the induction of hyaline droplets and tumor development in the kidney has not been established for KBrO₃. In the current study, a NOEL of 100 mg/L KBrO₃ (8.1 mg/kg/day) was selected based on the absence of microscopic kidney alterations at the 2- and 13-week time points. The selection of this NOEL is in agreement with previous studies where renal hyaline droplets/eosinophilic bodies, renal cell proliferation, α2µ-globulin accumulation, and renal oxidative stress (lipid peroxidation or DNA oxidation) were statistically significantly increased at ≥125 mg/L KBrO₃. Tumor development in the male rat kidney has also been observed at ≥125 mg/L KBrO₃.

**Conflicts of interest statement**

The authors declare that there are no conflicts of interest.

**Acknowledgements**

Carol Bobbitt, Kathy Bragg, Nigel Edgerton, Kay Roberts, Mark Sochaski, and other members of The Hamner Institutes’ laboratory animal care, necropsy, clinical chemistry, and analytical chemistry groups; EPL’s histology staff for technical support; and AnTech Diagnostics GLP (Morrisville, NC) for serum thyroid analysis. The study was funded by the American Chemistry Council’s Long-Range Research Initiative.

**REFERENCES**


