Toxicity and Carcinogenicity of Potassium Bromate—A New Renal Carcinogen

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Potassium bromate ($KBrO_3$) is an oxidizing agent that has been used as a food additive, mainly in the bread-making process. Although adverse effects are not evident in animals fed bread-based diets made from flour treated with $KBrO_3$, the agent is carcinogenic in rats and nephrotoxic in both man and experimental animals when given orally. It has been demonstrated that $KBrO_3$ induces renal cell tumors, mesotheliomas of the peritoneum, and follicular cell tumors of the thyroid. In addition, experiments aimed at elucidating the mode of carcinogenic action have revealed that $KBrO_3$ is a complete carcinogen, possessing both initiating and promoting activities for rat renal tumorigenesis. However, the potential seems to be weak in mice and hamsters. In contrast to its weak mutagenic activity in microbial assays, $KBrO_3$ showed relatively strong potential inducing chromosome aberrations both *in vitro* and *in vivo*. Glutathione and cysteine degrade $KBrO_3$ *in vitro*; in turn, the $KBrO_3$ has inhibitory effects on inducing lipid peroxidation in the rat kidney. Active oxygen radicals generated from $KBrO_3$ were implicated in its toxic and carcinogenic effects, especially because $KBrO_3$ produced 8-hydroxydeoxyguanosine in the rat kidney. A wide range of data from applications of various analytical methods are now available for risk assessment purposes.

Introduction

In the mid-1970s, the close correlation between mutagenicity and carcinogenicity of many chemicals became striking to those engaged in studies on carcinogenesis. Accordingly, a cooperative program was designed to evaluate the predictability of mutagenicity tests for carcinogenicity. This program commenced in 1974 under the auspices of the Ministry of Health and Welfare of Japan (*1,2*). To date, 85 bioassays using rats and/or mice have been conducted on 51 chemicals, including medical drugs, pesticides, and food additives.

$KBrO_3$ was selected as one of the chemicals for carcinogenicity testing because of its positive mutagenicity and widespread use as a food additive. It is used mainly in the maturation process of flour because of its oxidizing properties. In 1978, long-term bioassays of $KBrO_3$ were started by using rats and mice; as a result, this oxidizing agent was found to be carcinogenic in rats after 2 years of oral administration (*3*).

The present review covers the various toxicological studies that have mainly been conducted in our laboratory (*4–19*), with the aim of elucidating the mode of

action and mechanisms of $KBrO_3$ carcinogenicity. The International Agency for Research on Cancer (IARC) recently evaluated all of the data on $KBrO_3$ and concluded, "There is sufficient evidence for the carcinogenicity of $KBrO_3$ in experimental animals. No data were available on the carcinogenicity of $KBrO_3$ to humans" (*20*). $KBrO_3$ has been also classified as a compound belonging to the group 2B, a possible human carcinogen (*21*).

Chemistry and Use

Since this paper is primarily intended as a review of biological data on $KBrO_3$, we encourage readers to refer to the International Agency for Research on Cancer (IARC) Monograph for further details on the subjects covered in this chapter (*20*).

Chemistry and Production

Potassium bromate ($KBrO_3$, CAS No. 7758-01-2, molecular weight 167.01; density, 3.27) exists as white crystals, crystalline powder, or granules. It is highly soluble in water (7.5 g/100 mL at 25°C; 49.8 g/100 mL at 100°C), slightly soluble in ethanol, and almost insoluble in acetone; it is very stable when dissolved in water at room temperature. $KBrO_3$ decomposes at temperatures above 370°C (melting point: 350°C), with the evolution of oxygen and toxic fumes containing potassium

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oxide and BrK; it reacts vigorously as a strong oxidizing agent with organic materials.

KBrO$_3$ can be produced by passing bromine through a solution of potassium hydroxide. However, the compound is manufactured mainly by large-scale industrial electrolytic processes.

Occupational exposure to KBrO$_3$ occurs mainly in production plants during packaging processes in which areas in excess of 100 mg/m^3 require the use of a dust respirator.

Use

KBrO$_3$ has been used primarily as a maturing agent for flour and as a dough conditioner in the bread-making process for over 50 years (22–24), and this application is now used worldwide. Food additive-grade KBrO$_3$ is specified to contain KBrO$_3$ at levels of 99.0 to 101.0% after drying. In Japan, allowable limits are specified to be no more than 4 ppm for arsenic and heavy metals and 10 ppm for lead. The levels of contaminants in KBrO$_3$ used in our studies were within an acceptable range (6).

The Joint Food and Agricultural Organization (FAO)/World Health Organization Expert Committee on Food Additives (JECFA) has temporarily recommended a maximum level of 75 ppm of KBrO$_3$ for treating flour, provided that baking products prepared from such treated flour contain negligible residues of KBrO$_3$ (25). In Japan, the level has been set at 30 ppm under the same conditions as for JECFA (26). The effects of KBrO$_3$ on various nutritional values of the flour are reported to be negligible (22).

In the past, especially in Japan, KBrO$_3$ was used to improve the quality of fish-paste products (Kamaboko) at concentrations less than 270 mg/kg. However, this application of KBrO$_3$ is no longer allowed (26).

As nonfood usage, KBrO$_3$ has been introduced as an oxidizing agent, a primary standard, and a brominating agent in analytical chemistry. Its oxidizing property has further been used in home permanent-wave neutralizing compounds at concentrations of between 5 to 25% at pH 4 to 9, together with sodium bromate, sodium perborate, or hydrogen peroxide (27).

Analysis

KBrO$_3$ can be determined by iodometric titration methods, photometric ion chromatography (28–31), and by high-performance liquid chromatography (HPLC) (32). In Great Britain levels of KBrO$_3$ in bread assessed by the iodometric titration method were negligible when the dough was treated with KBrO$_3$ < 50 ppm (33,34). Furthermore, bromate could not be detected by ion chromatography in bread treated with < 50 ppm of KBrO$_3$ (28). Recently it was reported that the detection limit of a newly developed HPLC method, based on the formation of triiodide ion by the reduction of bromate and iodide (0.05 ppm) (32), is much lower than that detected by ion chromatography (1 ppm) (29). However,

bromate was not detected in 10 samples of commercial bread in Japan by this very sensitive approach (32).

Toxicity

Acute Toxicity in Experimental Animals

Groups of five males and five females each of F344 rats, B6C3F$_1$ mice, and Syrian golden hamsters were given a single intragastric (IG) administration of KBrO$_3$ and observed for 7 days (9). In all species given high doses (900–700 mg/kg of body weight), two-thirds of the animals died within 3 hr of the treatment; the remaining one-third survived up to 48 hr. Major toxic signs and symptoms observed were animals lying in a prone position, suppression of locomotor movement, ataxic gait, tachypnea, hypothermia, diarrhea, lacrimation, and piloerection. At autopsy the major findings were strong hyperemia of the glandular stomach mucosa and congestion of the lung. Microscopically, epithelial dilatation and desquamation of the distal convoluted tubules were noted in rats as early as 1 hr after KBrO$_3$ administration. Necrosis and degenerative changes of the proximal tubular epithelium were observed after 3 hr, and regenerative changes of the tubular epithelium occurred within 48 hr, becoming more extensive after 2 weeks. In mice and hamsters, however, these histological changes were observed later and to a lesser degree. No glomerular lesions were found in any of the species examined.

LD$_{50}$ values calculated by the Probit method are shown in Table 1. Although LD$_{50}$ values were higher in females than in males in all species examined, there were no marked species differences. The fact that the values were distributed in the range of around 300 to 500 mg/kg of body weight in all three species implies that KBrO$_3$ should be classified as a very toxic chemical. Furthermore, in Wistar rats the LD$_{50}$ values for both sexes were reportedly to be approximately 160 to 180 mg/kg body weight (Kawachi, personal communication).

An increase in the levels of cholesterin and phospholipids was observed in the brain and kidney in mice after a single IG administration of KBrO$_3$ (35).

Acute Toxicity in Humans

Case reports on KBrO$_3$ poisoning in humans are not uncommon because of the widespread use of the compound in home permanent-waving kits. However, there seem to be different geographical trends in occurrence. In Western countries most poison cases are by acciden-

Table 1. LD$_{50}$ values in three species.

Species	Strain	LD$_{50}$, mg/kg body weight	
		Male	Female
Rat	F344	400(348–460)[a]	495(446–549)
Mouse	B6C3F$_1$	280(250–314)	355(311–405)
Hamster	Syrian golden	388(318–473)	460(400–529)

[a] Numbers in parentheses are 95% confidence limits.

tal ingestion, mainly among children; in Japan, $KBrO_3$ is more often ingested for attempted suicide by young women, especially hairdressers (Table 2). The lethal dose of $KBrO_3$ in man has variously been estimated at 5 to 50 mg/kg of body weight (*36*) or 200 to 500 mg/kg of body weight (*37*). In reported cases the actual amount ingested ranged from 12 to 50 g, and 9 out of 24 adults died 3 to 5 days after ingestion (*35,37*).

In the acute phase of poisoning, vomiting and diarrhea with abdominal pain are the main symptoms. Subsequent features include oliguria, anuria, deafness, vertigo, hypotension, depression of the central nervous system, and thrombocytopenia. Clinically, acute renal failure is evidenced by the impairment of various renal functions and an associated development of hemolytic uremic syndrome (*36*). On biopsy of the kidney atrophy, necrosis, degeneration, and regeneration of the proximal tubular epithelium have often been reported. In the later stages, however, sclerosis of the glomeruli and interstitial fibrosis become evident; cardiotoxicity and hepatotoxicity have also been reported (*37*). Although bromate is converted to bromide *in vivo*, it is clear that observed effects in different organs can be attributed to bromate ions themselves, because only very low serum bromide levels were evident in the patients investigated.

The curious relationship between nephrotoxicity and ototoxicity induced by aminoglycoside antibiotics (streptomycin, kanamycin, neomycin) and diuretics (ethacrynic acid and furosemide) is well known. Since $KBrO_3$ has been added to the list of substances that selectively attack these two organs in man (*39–41*), some experiments were conducted to determine the cause.

Ototoxicity of $KBrO_3$ and $NaBrO_3$ was studied in guinea pigs after IP injection at a daily dose of 10 to 20 mg/kg body weight for 10 to 20 days (*41*). Histologically, degeneration of the cochlear sensory cells, particularly of the outer hair cells of the inner ear was observed. At the same time, nephrotoxic effects of $KBrO_3$ and $NaBrO_3$ were also confirmed in guinea pigs. The fact that the kidney and the inner ear are both highly efficient systems for transport of water and electrolytes might explain the coincidental occurrence of nephro- and ototoxicity by these chemicals (*40*).

Subacute Toxicity

Administration of KBrO₃ in Drinking Water of Mice. Groups of 10 male and 10 female $B6C3F_1$ mice were given $KBrO_3$ at doses of 4000, 2000, 1000, 500 and 250 ppm for 10 weeks (*15*). Doses > 2000 ppm were not palatable. No treatment-associated deaths of animals given < 1000 ppm or particular histopathological changes attributed to $KBrO_3$ administration were observed.

Table 2. Case reports of $KBrO_3$ poisoning.[a]

Case no.	Age, years	Sex	Occupation	Circumstances	Dosage	Renal failure	Deafness	Onset of deafness	Blood pressure	Prognosis
1	1.5	M		Accident		+				Died
2	2.5	M		Accident	½ cup	+			96/54	
3	3	M		Accident		+				
4	2.5	F		Accident	⅓ cup	+				
5	3	F		Accident	70 g	−				
6	14	F		Accident		+				
7	3	M		Accident	4 g	+			85/45	
8	4	M		Accident		−				
9	2	M		Accident	½ cup	−				
10	1.8	M		Accident		+				Died
11	Adult	F		Accident		+	+			
12	Adult	F		Accident		+	+			Died
13	Unknown			Suicide			+			
14	18	F	Hairdresser	Suicide	2 g	−	−		88/50	
15	21	F		Suicide		+	+			Died
16	39	F	Hairdresser	Suicide	15 g	+	+	6 hr	60/30	
17	21	F		Suicide		+	+			Died
18	23	F			10 g	+	+			Died
19	29	F	Hairdresser	Suicide	25 g	+	+	16 hr	120/70	Died
20	47	F	Hairdresser		25 g	+	+			Died
21	19	F	Hairdresser	Suicide	25 g	+	−		60/40	
22	18	F	Student	Suicide	40 g	+	+	4 hr	96/50	Died
23	29	F	Hairdresser	Suicide		+	+	6 hr	88/60	Died
24	34	M			30 g	+	+			
25	25	F	Hairdresser	Suicide	15 g	+	+		130/20	
26	19	F	Hairdresser	Suicide	15 g	+	−		150/70	
27	30	F	Hairdresser	Suicide	25 g	+	+	8 hr	88/70	Died
28	18	M	Student	Suicide	6 g	+	+	4 hr	130/60	
29	30	F	Housewife	Suicide	15 g	+	+	12 hr		
30	22	F	Hairdresser	Suicide	10 g	+	+	12 hr	90/70	
31	6	M			0.5 g	+	+		90/60	

[a] Modified from Matsumoto (*39*).

Study of Rats Administered KBrO₃ in Drinking Water. Groups of 10 male and 10 female F344 rats were administered KBrO₃ at doses of 10000, 5000, 2500, 1250, 600, 300, and 150 ppm for 13 weeks (Onclera, unpublished data). Doses ≥ 2500 ppm were not palatable. All animals given > 1250 ppm died within 7 weeks, whereas all animals given ≤ 600 ppm survived for 13 weeks. A significant inhibition of body weight increase was observed in males given 1250 or 600 ppm. Significantly elevated levels of GOT, GPT, LDH, ALP, BUN, serum-Na and Ch-E were noted in rats of both sexes given 600 ppm. Serum-K levels were also significantly decreased. Many various-sized droplets stained strongly with eosin were observed in the cytoplasm of the proximal tubular epithelium in treated males. Extensive regenerative changes were seen in the renal tubules.

For analyzing the droplets observed in the renal tubules, groups of five male F344 rats were given 600 ppm KBrO₃ orally for 12 weeks (10). In renal tubules, various-sized droplets were found as early as 4 weeks after the treatment began (Fig. 1). The incidence of the droplets decreased to control levels 4 weeks after terminating the treatment. These droplets were positive for Azan, negative for PAS, and partially positive for hemoglobin staining; they were also observed in control rats, though to a far lesser degree. As observed by electron microscopy, the droplets demonstrated high electron density and were surrounded by a limiting membrane layer (Fig. 2). The origin of these droplets seemed to be the lysosomes, and they seemed to result from material being reabsorbed. From the morphological characteristics, it was concluded that these droplets were eosinophilic bodies rather than hyaline droplets (42,43). Recently, droplets showing similar characteristics were observed in the kidneys of rats given decalin, 2,2,4-trimethylpentane, or unleaded gasoline. In these cases, however, they were classified as hyaline droplets (44,46). α₂ᵤ-Globulin, which is specific for the kidneys of male rats, seems to be involved in the appearance of the hyaline droplets. In preliminary studies the gen-
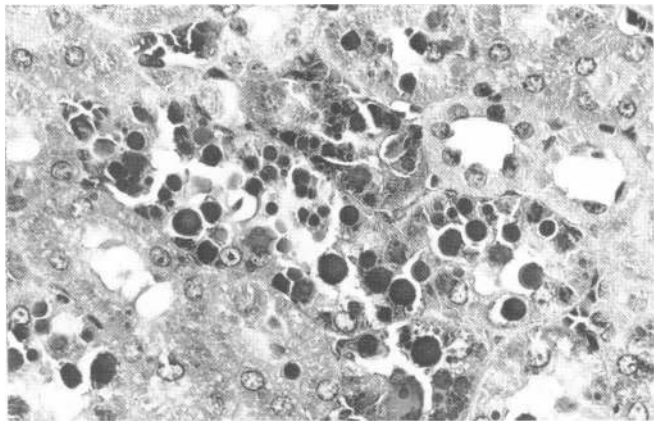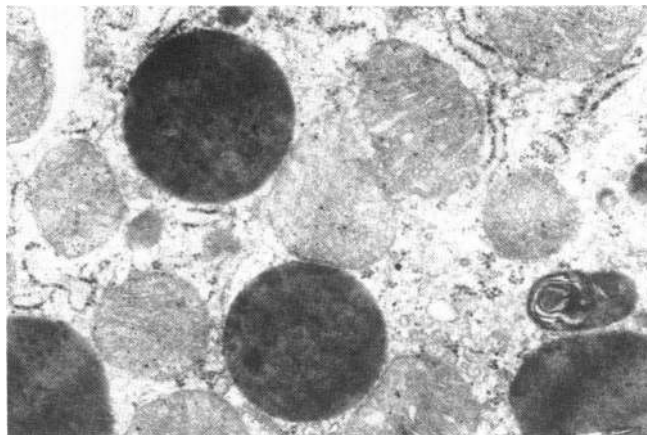

FIGURE 2. By electron microscopy, various-sized electron-dense droplets are observed surrounded by a limiting membrane layer.

eration of eosinophilic bodies in rats treated with KBrO₃ was inhibited by castration, ovariectomy, or treatment with cysteine (47,48). Numerous lipofuscin pigments were also observed in the proximal tubular epithelium of treated rats.

Administration of KBrO₃-Treated Flour or Bread. Japanese researchers have concentrated on the effects of administration of KBrO₃ dissolved in water, whereas researchers in Great Britain fed animals KBrO₃-treated flour itself, or bread made from flour treated with KBrO₃ (22). Eighteen rats, three dogs, and three monkeys were fed a diet containing 84% flour treated with KBrO₃ at a level of about 75 ppm for a period of 4, 12, and 8 weeks, respectively. No adverse effects were observed in any of the species. Bread made from flour treated with 200 ppm KBrO₃ was fed to 12 rats and 2 dogs for 16 days, and flour treated with 200 ppm KBrO₃ was given to rats for 10 weeks, again without any ill effects. Similarly no clinical symptoms were apparent in three dogs fed diets containing flour treated with 70 ppm KBrO₃ for 6 weeks. Four dogs administered bread made from flour containing 200 ppm KBrO₃ for 17 months also showed no adverse effects attributable to the diet.

Multigeneration Studies

Bread made from flour treated with 14 or 100 ppm of KBrO₃ was fed to groups of 6 male and 20 female rats over three generations; the entire experiment lasted 10 months (22). The health, behavior, weight gain, and reproductive performance remained normal throughout. There were no histological abnormalities and analyses of the brain and the liver showed no accumulation of bromine.

Mice and rats (numbers were not specified) were fed flour treated with 15 ppm KBrO₃ over eight and five generations, respectively. In both studies, no effects were observed on weight gain, reproductive performance, or survival.

FIGURE 1. Droplets strongly stained by eosin in the cytoplasm of proximal renal tubules of rats given KBrO₃ for 4 weeks.

Carcinogenicity Including Chronic Toxicity

Studies in the United Kingdom

Long-term toxicity and carcinogenicity studies in rats and mice were conducted by feeding animals with bread treated with $KBrO_3$ (49,50). The levels of $KBrO_3$ chosen for the treatment of flour were 50 and 75 ppm because it was determined that $KBrO_3$ was quantitatively converted to bromide during the dough-mixing process with the $KBrO_3$ at concentrations below 75 ppm (34). Thus, the purpose of the studies was to ascertain the safety of bread made from $KBrO_3$-treated flour, in which bromate levels were presumed to be negligible. The bread made from $KBrO_3$-treated flour was crumbed and dried for incorporation in the diet at a 79% concentration.

Groups of 90 males and 90 females of Wistar-derived Porton strain rats and mice of the Theillers original strain (900) were fed diets made from bread treated with 75 ppm (high-dose group), 50 ppm (low-dose group), or 0 ppm (control group) of $KBrO_3$ for 104 and 80 weeks, respectively.

Rat Study. No differences were noted in appearance or behavior between test and control rats (49). Cumulative mortality rates at week 104 were 20.0%, 38.3%, and 26.7% in males; and 30.0%, 51.7%, and 51.7% in females in the high-dose, low-dose, and control groups, respectively. No intergroup differences were found regarding food intake in either sex. No dose-related changes in the absolute or relative organ weights were apparent. Histopathological data are shown in Table 3. Although not pointed out in the original report, it is noteworthy that the occurrence of periarteritis in the

pancreas of male rats was significantly increased in a dose-related manner. Also the various aging pathology of the adrenals was significantly increased in the female high-dose group. No dose-dependent variation in the incidences of any tumors was apparent. Dose-related reduction in blood glucose levels were observed in treated rats of both sexes at week 104. There was no retention or accumulation of significant amounts of covalently bound bromine in the adipose tissue of treated rats.

Mouse Study. General appearance and behavior were good in both test and control groups (50). Mortality rates at week 80 were 61.8, 65.1, and 65.1% in males; and 56.8, 48.4, and 56.8% in females for the high-dose, low-dose, and control groups, respectively. There were no significant differences in the mean body weights or food intake among groups. Normalized (weighted mean) bromine intakes derived from $KBrO_3$ were 2.64 and 1.76 mg/kg/day in males and 2.99 and 2.03, mg/kg/day in females for the high- and low-dose groups, respectively.

Significant dose-related reduction in the absolute weights of the heart and the pituitary was found in treated males. Absolute thyroid weights were significantly higher in the high-dose males. Anemia was prevalent in male high- and low-dose groups at 3 months. However, no histopathological differences attributable to the treatment were found between test and control males. Small amounts of bromine were detected in the adipose tissues, i.e., at a level of 1 ppm in males of the high- and low-dose groups and at a level of 2 ppm in females of the low-dose group. Ginocchio et al. concluded that there was no evidence that flour treatment with $KBrO_3$ affected the incidence of neoplastic and non-neoplastic lesions in the mouse study (50).

Other Studies on Rats and Mice. Groups of 90

Table 3. Results of histopathological examination in rats given diets based on bread made from flour treated with $KBrO_3$.[a]

| | KBrO₃ dose, ppm | | | | | |
| | Males | | | Females | | |
Pathology	0 (88)[b]	50 (90)	75 (88)	0 (89)	50 (89)	75 (88)
Nonneoplastic lesions						
Pancreas						
Pancreatitis	4	0	2	2	2	3
Periarteritis	2	9[*]	12[†]	9	15	10
Giant islet	0	2	0	2	1	0
Adrenals						
Various aging pathology	23	35	35	47	42	71[‡]
Testes						
Periarteritis	8	8	8			
Uterus						
Cystic endometrial hypertrophy or cystic dilatation				17	8	6
Neoplastic lesions						
Tumors						
Benign	33	42	33	43	60	50
Malignant	4	6	4	6	3	3
Total tumors	37	48	37	49	63[†]	53

[a] Modified from Fisher et al. (49).
[b] Numbers in parentheses are numbers of rats examined.
[*] $p < 0.05$.
[†] $p < 0.01$.
[‡] $p < 0.001$.

males and 90 females rats and mice were fed bread made from two kinds of flour, namely, *a*) flour treated with 50 ppm KBrO₃, 30 ppm ascorbic acid, and 50 ppm benzoyl peroxide; and *b*) flour treated with 50 ppm KBrO₃, 30 ppm ascorbic acid, 50 ppm benzoyl peroxide, and 15 ppm chlorine dioxide (*49,50*). Although these findings were not emphasized in the literature, two significant findings were noted in rats given the latter diet. The incidence of periarteritis in the pancreas in males (13.3%, 12/90) was significantly increased over controls (2.3%, 2/88); and the rates were markedly elevated for the ocurrence of various aging pathology in the adrenals at 51.1% (46/90) and 75.0% (66/88), in males and females, respectively.

Studies in Japan

After learning about the United Kingdom's results on long-term studies in which animals were fed a bread basal diet prepared from KBrO₃-treated flour, we became interested in the possible carcinogenicity of the chemical. Hence, KBrO₃ was given to animals by oral administration as a drinking water supplement at high concentrations, the highest doses being the maximum tolerated doses. Detailed protocols for the experiments have been described (*3–6,8–10,13–15*).

Carcinogenicity Tests in Rats (3,4,6,9,15). Groups of 53 males and 53 females of F344 rats received KBrO₃ for 110 weeks at concentrations of 500 and 250 ppm in the drinking water. However, for males treated at the 500 ppm level, the dose was reduced to 400 ppm at week 60, because exposure to 500 ppm caused too great an inhibition of growth.

Dead or moribund rats were found earlier among males given 500 ppm than in other groups. Mean survival time in males given 500 ppm (88.1 weeks) was significantly shorter than that in controls (104.5 weeks). In females, the survival rates of treated and control groups were very similar. Daily intakes of KBrO₃ (mg/kg body weight) were 27.7 and 12.5 in males and 25.5 and 12.5 in females in the high- and low-dose groups, respectively.

We suspected the kidney to be the target organ dur-

ing the observational period by preliminary histological examination. Therefore, 10 to 15 step-serial sections were examined from each kidney. This procedure is not routine for pathological assessment in carcinogenicity studies. The results were high incidences of renal cell tumors (RCTs) in dosed males and females. Data for separated and combined incidences of renal adenocarcinomas and adenomas are summarized in Table 4. Significant increases were evident for both sexes, as compared to the control group values. The incidences of RCT on the basis of routine microscopic examinations, in which one slide per kidney was checked, also showed significant differences from those of controls (i.e., 56, 30, and 2% in males; and 40, 10, and 0% in females given 500, 250, and 0 ppm KBrO₃, respectively, $p < 0.01$, except in females given 250 ppm), as shown in our first report (*3*). Although lesions were found much earlier in high-dose males than they were in the other groups, RCTs did not appear to be the main cause of death in the experiment. Dysplastic foci (DF), which are preneoplastic lesions for RCT, were observed in almost all of the treated rats of both sexes. Other tumors found in the kidney were two transitional cell papillomas, two transitional cell carcinomas, and one angiosarcoma in treated rats and one liposarcoma in a control rat.

Tumors of the peritoneum, all diagnosed as mesotheliomas, also occurred at a significantly higher incidence in male rats given 250 or 500 ppm than in the controls (Table 5). On the other hand, no mesotheliomas were observed in either treated or control female rats. The mesotheliomas usually resulted in implantation onto the surfaces of various abdominal organs, with massive hemorrhagic ascites causing severe anemia leading to early death. Findings of nonneoplastic lesions of the kidney are described in the section "Pathological Lesions of the Kidney after KBrO₃ Administration."

Significantly decreased values were found in GPT, the albumin/globulin (A/G) ratio, serum K, and Ch-E in females that were treated with 500 ppm KBrO₃. Also, we noted that BUN levels were slightly increased in treated rats. There were no significant differences in the red blood cell (RBC) counts.

We concluded from the above findings that clear evi-

Table 4. Incidences of renal cell tumors (RCT) and dysplastic foci (DF) in male and female F344 rats in the carcinogenicity test of KBrO₃.

Group	Effective number of rats[a]	Mean induction time ± SD, week	Earliest RCT found, week	Number of rats (%) bearing			
				RCT	Adeno-carcinomas	Adenomas	DF
Male							
500 ppm	52	88.9 ± 18.9	14	46(88)[*]	44(85)[*]	5(10)[+]	40(77)[*]
250 ppm	53	103.7 ± 9.1	77	32(60)[*]	24(45)[*]	10(19)[+]	32(60)[*]
0 ppm	53	111.0 ± 0	111	3(6)	3(6)	0(0)	6(11)
Female							
500 ppm	49	107.9 ± 5.6	85	39(80)[*]	36(69)[*]	9(17)[+]	9(17)[+]
250 ppm	50	107.6 ± 5.8	89	28(56)[*]	21(40)[*]	8(15)[+]	13(25)[+]
0 ppm	47	—	—	0(0)	0(0)	0(0)	0(0)

[a] Males and females surviving longer than 14 and 85 weeks, respectively, when the earliest RCTs were found.
[*] $p < 0.001$.
[+] $p < 0.01$.

Table 5. Incidences of mesotheliomas in male F344 rats in the carcinogenicity test.

Group	Effective number of rats[a]	Mean induction time ± SD, week	Mesothelioma Earliest found, week	Mesothelioma Number of tumor-bearing rats (%)
500 ppm	46	91.0 ± 10.7	73	28(59)[*]
250 ppm	52	95.0 ± 14.4	72	17(33)[+]
0 ppm	53	98.8 ± 12.1	80	6(11)

[a] Males surviving longer than 72 weeks when the earliest mesothelioma was found.
[*] $p < 0.001$.
[+] $p < 0.05$.

dence exists that when $KBrO_3$ is given orally in drinking water, it is carcinogenic in rats of both sexes.

Dose-Response Studies in the Rat (14). Based on the results of the carcinogenicity test in rats, studies at low doses were conducted to further characterize the dose-response relationship (14). Groups of 20 to 24 male F344 rats were given $KBrO_3$ orally at concentrations of 500, 250, 125, 60, 30, 15, and 0 ppm for 104 weeks.

As in the previous carcinogenicity test, the mean survival time of the animals given 500 ppm (82.8 weeks) was significantly shorter than that of controls (103.1 weeks). However, at doses of 250 ppm or lower, the survival rates were comparable in treated and control groups and inhibition of body weight gain was not apparent.

Renal adenocarcinomas developed in 3 of the 20 rats given 500 ppm (Table 6), and the incidences of renal adenomas and RCTs were significantly elevated in rats receiving concentrations of 500, 250, and 125 ppm. A sigmoidal curve was obtained when the incidences of RCTs were plotted against the dose of $KBrO_3$ (Fig. 3). Furthermore, significant dose-related increases in the incidences of DF were also noted in all groups given doses higher than 30 ppm.

Follicular adenomas and adenocarcinomas of the thyroid were found in the groups treated with 500, 250, and 60 ppm (Table 7); the combined incidences of benign and malignant follicular lesions were significantly in-

creased in rats of the 500 ppm dose group. Mesotheliomas of the peritoneum were also observed in treated rats at doses higher than 30 ppm; again, the incidence in animals receiving 500 ppm was significant.

Although the RCT rates in high-dose males were somewhat lower than those in the previous carcinogenicity study, they were nevertheless significantly increased in a dose-related manner. The yield of RCTs in the 125-ppm group was significantly higher than that of controls, indicating that oral doses higher than 60 ppm may induce RCTs after long-term treatment. The incidence of mesotheliomas of the peritoneum was significantly increased only at a dose of 500 ppm in this study, in contrast to the significantly higher rates at both 500 ppm (59%) and 250 ppm (33%) in the former test. On the other hand, this experiment demonstrated elevated combined incidences of follicular adenocarcinomas and adenomas of the thyroid that were not observed in the previous study (6). Although the incidence in rats given 250 ppm (15%, 3/20) was not significantly higher than controls, the observed dose-related increase seems to suggest that the thyroid follicular cells are also the target in $KBrO_3$ carcinogenesis. The slight differences in results between the two studies are presumably because of variation in animal lots and the numbers of rats used. The virtually safe dose (VSD) values calculated for RCTs and DF on the basis of this experiment are discussed in the section "General Discussion and Summary."

Relationship between the Duration of Treatment and the Incidence of RCTs in Rats (17). Subsequently, an experiment was designed to ascertain the minimum induction time, minimum treatment period, and total dosage of $KBrO_3$ required for the development of RCTs (17). The dose of $KBrO_3$ chosen for this study was 500 ppm in the drinking water because significant incidences of RCTs had already been observed at this dose level. The experimental protocol using 232 male F344 rats is illustrated in Figure 4.

CONTINUED-TREATMENT PROTOCOL (COMPARISON BETWEEN GROUPS 1 TO 5 AND GROUPS 6 TO 10). Table 8 shows the results of the histopathologic diagnosis of

Table 6. Incidences and average numbers of renal cell tumors (RCT) and dysplastic foci (DF) in male F344 rats in the dose-response study.

Group (dose, ppm)	Effective number of rats	Number of rats (%) bearing Adenocarcinoma	Number of rats (%) bearing Adenoma	Number of rats (%) bearing RCT	Mean number of RCT/cm² of kidney ± SD	Number of rats (%) with DF	Mean number of DF/cm² of kidney ± SD
500	20	3(15)	6(30)[*]	9(45)[+]	0.25 ± 0.35[‡]	19(95)[+]	1.44 ± 1.10[‡]
250	20	0	5(25)[*]	5(25)[*]	0.05 ± 0.08[δ]	19(95)[+]	0.83 ± 0.47[‡]
125	24	0	5(21)[*]	5(21)[*]	0.04 ± 0.08[δ]	12(50)[+]	0.16 ± 0.20[‡]
60	24	0	1(4)	1(4)	0.01 ± 0.04	6(25)[*]	0.07 ± 0.12[‡]
30	20	0	0	0	0	5(25)[*]	0.05 ± 0.08[δ]
15[a]	19	0	0	0	0	1(5)	0.01 ± 0.04
0	19	0	0	0	0	0	0

[a] One kidney lipoma was found in this group.
[*] $p < 0.05$.
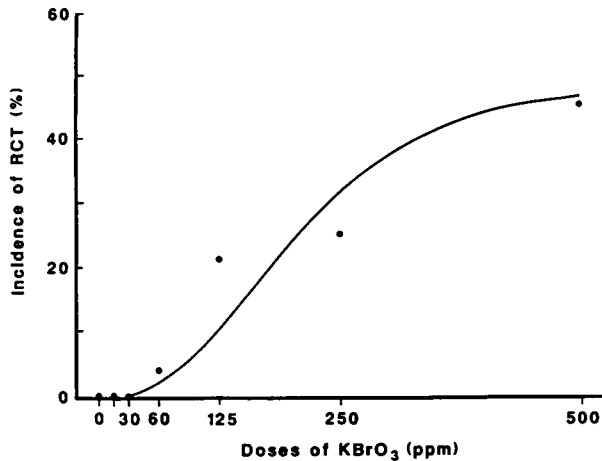[+] $p < 0.001$.
[‡] $p < 0.001$.
[δ] $p < 0.05$.

FIGURE 3. Dose-response curve for incidences of renal cell tumors in male F344 rats.

tumors observed at relatively high incidence. DF and renal adenomas were found as early as 26 weeks in the administration period (group 7). The incidences of DF and adenomas in rats treated with $KBrO_3$ for 52 weeks (group 9) were significantly higher than those in controls (group 4). When $KBrO_3$ was given continuously for 104 weeks, renal adenocarcinomas and adenomas developed in 3 and 6 of 20 rats, respectively (group 10).

A few follicular adenomas of the thyroid were observed in rats given $KBrO_3$ (groups 7 to 9). The combined incidences of follicular adenomas and adenocarcinomas of the thyroid were significantly increased in rats treated continuously for 104 weeks (group 10). A significant increase in the rate of peritoneal mesotheliomas was also found in the group continuously treated for 104 weeks (group 10). Two mesotheliomas were observed in rats exposed to $KBrO_3$ for only 39 weeks.

LIMITED DURATION PROTOCOL (COMPARISON BETWEEN GROUPS 5 OR 10 AND GROUPS 11 TO 14). As shown in Table 8, RCTs were observed in rats of all groups receiving $KBrO_3$ for limited durations (groups

11 to 14), the incidences all being significantly higher than in controls and approximately equal to or slightly higher than in rats given $KBrO_3$ continuously for 104 weeks (group 10), probably because of the longer survival times in the former groups. On the other hand, the mean numbers of DF or RCTs were increased in relation to the length of exposure. The combined incidences of follicular adenomas and adenocarcinomas of the thyroid were significantly higher in rats in which treatment was discontinued at 26 or 52 weeks (groups 12 and 14) when compared to control values (group 10). Significant increases in the yield of peritoneal mesotheliomas were also observed in all limited duration groups.

The limited duration study thus revealed that the yields of preneoplastic and neoplastic lesions remained high, and therefore the effects of $KBrO_3$ were not reversible. Furthermore, only 13 weeks of exposure was necessary to produce increases in the incidences of RCTs and mesotheliomas. Nonneoplastic changes in the kidney were not evident in limited duration groups, demonstrating that toxic lesions, in contrast, do not persist.

The mean total intake of $KBrO_3$ in rats given a dose of 125 ppm for 104 weeks (5.3 g/kg) in the dose-response study was close to that of rats receiving a dose of 500 ppm for only 13 weeks (4.2–4.3 g/kg) in this study. However, the eventual incidence of RCTs was approximately 2-fold higher in the latter than in the former group (50% vs. 21%). This phenomenon was also noted in mice treated with 2-acetylaminofluorene (51,52). In this study much higher incidences of both liver and bladder tumors were observed in animals dosed for 9 or 12 months and sacrificed at 24 months than in groups receiving equivalent total doses spread over 18 or 24 months. Thus, higher doses of $KBrO_3$ given for a shorter period of time appear more effective for producing a high yield of tumors than a lower dose given for a longer period.

It was concluded that the minimum induction time for the development of renal adenomas was between 13 and 26 weeks, and the minimum treatment period and

Table 7. Incidences of thyroid tumors and mesotheliomas in male F344 rats in the dose-response study.

Categories	Number (%) at doses of $KBrO_3$, ppm						
	500	250	125	60	30	15	0
Number of rats at commencement	20	20	24	24	20	20	20
Effective number of rats	20	20	24	24	20	20	19
Site and tumor type							
Thyroid, number examined[a]	19	20	24	24	20	19	16
Follicular adenoma	5(26)	2(10)	0(0)	1(4)	0(0)	0(0)	0(0)
Follicular adenocarcinoma	2(11)	1(5)	0(0)	0(0)	0(0)	0(0)	0(0)
Follicular adenoma and adenocarcinoma combined	7(37)*	3(15)	0(0)	1(4)	0(0)	0(0)	0(0)
C-cell adenoma	1(5)	1(5)	0(0)	3(13)	5(25)	1(5)	2(13)
C-cell adenocarcinoma	0(0)	0(0)	1(4)	0(0)	0(0)	0(0)	1(6)
C-cell adenoma and adenocarcinoma combined	1(5)	1(5)	1(4)	3(13)	5(25)	1(5)	3(19)
Peritoneum, mesothelioma	15(75)†	3(15)	2(8)	4(17)	3(15)	0(0)	0(0)

[a] Number of rats for which thyroids were histologically examined.
* $p < 0.05$.
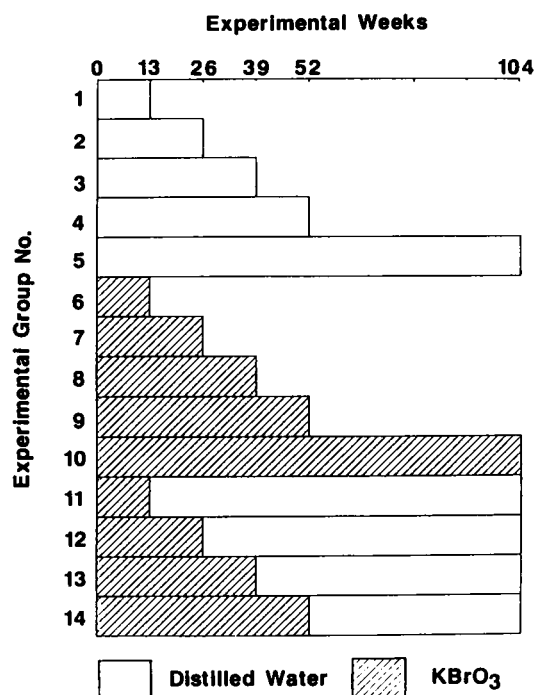† $p < 0.001$.

Experimental Weeks

FIGURE 4. Experimental protocol for the continued and limited duration protocol showing the duration of administration of DW and KBrO₃ solution.

total dose for the induction of renal adenomas and adenocarcinomas were less than 13 weeks and less than 4 g/kg body weight, respectively, when the rats were maintained thereafter on distilled water (DW) for 2 years. However, it is probable that the values for the true minimum treatment period and total dose will be smaller than 13 weeks and 4 g/kg body weight if experiments involving shorter exposure to higher doses of KBrO₃ were performed.

Long-Term Observation of Rats after a Single IG Administration. A total of 81 male F344 rats were given a single IG administration of KBrO₃ at doses of 600, 300, or 0 mg/kg and were observed for 87 weeks (*35*). In the group administered 600 mg/kg, a large renal tumor developed that was diagnosed as an adenocarcinoma. Three renal adenomas were also found in the same group. The final incidences of RCTs were 13.6% (4/41), 0% (0/20), and 0% (0/20), respectively, in groups given 600, 300, and 0 mg/kg. Considering the very low spontaneous rate of RCT development in rats, it appears probable that KBrO₃ exerted the initiation activity for induction of the lesions, despite the fact that the incidence was not statistically significant.

Long-Term Oral Administration in Mice. A total of 50 female B6C3F₁ mice were given KBrO₃ at doses of 1000 or 500 ppm in the drinking water for 78 weeks, and then tap water for 26 weeks until sacrifice at week 104 (*15*). Although body weight gain was markedly inhibited in the 1000-ppm group, the survival was comparable among groups. Daily intakes of KBrO₃ were 119.8 and 56.5 mg/kg body weight/day in the animals given 1000 and 500 ppm, respectively. Although relatively high incidences of lung, liver, and lymph node tumors were observed in the 1000-ppm group, the incidences of these tumors were not significantly different from those of the controls.

A further study was conducted to ascertain the effects of 750 ppm KBrO₃ administered orally for 88 weeks to groups of 27 male mice in B6C3F₁, BDF₁, and CDF₁ strains (*35*). This study also resulted in no statistically significant differences in growth rate or survival time between the treated and control groups. The intake of KBrO₃ was in the range of 60 to 90 mg/kg body weight/ day for all three strains.

As shown in Table 9, one renal adenocarcinoma was found in a B6C3F₁ mouse treated with KBrO₃. Also, a total of four renal adenomas developed in treated mice, and DF were found in more treated mice than controls of both B6C3F₁ and BDF₁ strains. There is a potential for KBrO₃ to also induce RTC in mice. This is attributed to the fact that *a*) the spontaneous occurrence of RCT in mice is very low [for example, in B6C3F₁ mice the incidences are reported to be 0.1% (3/2543) in males and 0.08% (2/2522) in females (*53*)], and *b*) the observed RCTs were morphologically quite similar to those induced by KBrO₃ in rats. Furthermore, significant increases in the occurrence of adenomas of the small intestine in CDF₁ mice and of adenomas of the liver in B6C3F₁ were observed.

Long-Term Oral Administration in Hamsters. Groups of 20 male Syrian golden hamsters were given a KBrO₃ supplement in their drinking water at concentrations of 2000, 500, 250, and 125 ppm for 89 weeks (*11*).

No apparent differences were noted in the survival times. The mean final body weights of animals treated with 2000 ppm KBrO₃ were significantly reduced, and the mean absolute and relative kidney weights in animals given 2000 or 250 ppm KBrO₃ were significantly higher than controls. Renal adenomas developed in 1, 2, and 4 hamsters in groups given 250, 500, and 2000 ppm, respectively, for a total incidence of 7 in 75 treated animals (9.3%). RCTs were not observed in controls, and the structural and cellular morphologic characteristics of RCT, as well as DF found in the exposed hamsters, were quite similar to those induced in rats. Because the spontaneous development of RCT in hamsters is known to be extremely low (*55*), it is highly likely that the observed lesions, although of low incidence, were induced by KBrO₃.

Subcutaneous Injection into Newborn Mice and Rats. KBrO₃ was given at doses of 200, 100, 50, 25, and 12.5 mg/kg body weight to newborn ICR mice and at doses of 100, 50, 25, and 12.5 mg/kg body weight to newborn F344 rats, either as single (24 hr after birth) or 4 weekly SC injections until weanling (*16*). All the surviving mice and rats were killed at weeks 78 and 82, respectively.

Table 8. Histopathologic diagnosis of tumors observed at relatively high incidence in male F344 rats given 500 ppm KBrO₃ in the drinking water for various periods.

Experimental group number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Treatment with KBrO₃ (week)	0	0	0	0	0	13	26	39	52	104	13	26	39	52
Treatment with DW (week)	13	26	39	52	104	0	0	0	0	0	91	78	65	52
Number of rats at start	8	8	8	8	20	20	20	20	26	20	20	20	20	14
Number of rats effective	8	8	8	8	19	20	20	20	26	20	20	19	19	14
Site and number of tumors (%)														
Kidney														
Dysplastic foci (DF)	0	0	0	0	0	0	1(5)	6(30)	16(62)*	19(95)‡	13(65)‡	17(89)‡	19(100)‡	14(100)‡
Adenoma	0	0	0	0	0	0	2(10)	3(15)	15(58)*	6(30)†	10(50)‡	9(47)‡	13(68)‡	8(57)‡
Adenocarcinoma	0	0	0	0	0	0	0	0	0	3(15)	2(10)	1(5)	6(32)§	3(21)
RCT	0	0	0	0	0	0	2(10)	3(15)	15(58)*	9(45)‡	10(50)‡	9(47)‡	14(74)‡	9(64)‡
Mean number of DF/rat	0	0	0	0	0	0	0.1	0.4	1.8	7.3	1.2	3.7	7.1	11.5
Mean number of renal cell tumors/rat	0	0	0	0	0	0	0.05	0.10	0.81	1.25	0.55	0.65	1.26	0.93
Thyroid														
Follicular adenoma	0	0	0	0	0	0	1(5)	1(5)	2(8)	5(40)‡	2(10)	3(16)	0	3(21)
Follicular adenocarcinoma	0	0	0	0	0	0	0	0	0	2(10)	0	3(16)	1(5)	2(14)
Follicular adenoma and adenocarcinoma combined	0	0	0	0	0	0	1(5)	1(5)	2(8)	7(35)§	2(10)	6(32)§	1(5)	5(36)§
C-cell adenoma	0	0	0	0	2(11)	0	0	0	0	1(5)	0	0	1(5)	1(7)
C-cell adenocarcinoma	0	0	0	0	1(5)	0	0	0	0	0	1(5)	0	0	0
C-cell adenoma and adenocarcinoma combined	0	0	0	0	3(16)	0	0	0	0	1(5)	1(5)	0	1(5)	1(5)
Peritoneum														
Mesothelioma	0	0	0	0	0	0	0	2(10)	4(15)	15(75)‡	6(30)†	8(42)§	7(37)§	5(36)§

*$p < 0.01$ (compared to group 4).
†$p < 0.02$.
‡$p < 0.001$.
§$p < 0.01$.

Table 9. Results of histopathologic diagnosis of lesions in three strains of male mice given 750 ppm KBrO₃ orally.

Strain	B6C3F₁		BDF₁		CDF₁	
Treatment	KBrO₃	DW	KBrO₃	DW	KBrO₃	DW
Number of mice at start	27	15	27	15	27	15
Number of mice effective	26	15	27	15	27	15
Site and type of tumors						
Kidney						
Adenocarcinoma	1	0	0	0	0	0
Adenoma	2	0	1	0	1	0
RCT	3	0	1	0	1	0
Dysplastic foci	2	1	4	1	0	0
Small intestine						
Adenocarcinoma	1	0	0	0	0	0
Adenoma	1	1	0	1	14*	1
Liver						
Adenocarcinoma	6	3	1	2	0	2
Adenoma	7†	0	3	1	0	1
Hemangioma	1	0	0	0	0	0

*$p < 0.01$.
†$p < 0.05$.

Histologically, no nonneoplastic or neoplastic lesions of the skin were observed at the injection sites in either species. While no RCTs were observed, the numbers of animals bearing DF were high in both treated and control groups, with the frequency of lesions being in the range of 1.5 to 6.0 (mean 3.4) per mouse. Only a few tumorous lesions of the kidney were observed in the rats. Therefore, it was concluded that KBrO₃ does not exert any potent carcinogenic action for local or distant organs when administered SC to newborn mice and rats for 4 weeks at doses up to 200 (rats) or 100 (mice) mg/kg body weight.

Pathological Lesions of the Kidney after KBrO₃ Administration

Neoplastic and Preneoplastic Changes

Renal Cell Tumors. Recent studies on the histogenesis of renal adenocarcinomas induced by chemical carcinogens in rats have revealed that adenomas gradually progress to adenocarcinomas and that hyperplastic tubular epithelium constitutes the preneoplastic lesion (*6*). However, there is still some controversy as to the best nomenclature for differential diagnosis of renal adenomas and adenocarcinomas in experimental animals and man. Therefore, the incidences of adenocarcinomas and adenomas were combined as RCTs for evaluation of the carcinogenic and promoting activities of KBrO₃ in all of our studies. In this chapter, however, the definition and histopathologic features of renal adenomas and adenocarcinomas induced by KBrO₃ are separately described.

Macroscopically, some tumors were observed as round yellowish-white or grayish-white projections of the renal cortex, clearly distinguishable from the surrounding tissues. However, most tumors grossly appeared as small yellowish-white nodules on cut surfaces or were only detectable by microscopic examination after long-term treatment with KBrO₃. The majority of lesions closely resembled one another in their histologic appearance.

Adenomas appeared as oval, solitary nodules consisting of closely packed polygonal cells and were located in the cortical area. They were well circumscribed with thin, fibrous capsules or pseudocapsules of compressed neighboring tissues. Although most tumors demonstrated a solid growth pattern, some presented as cystic structures with papillary projections protruding into the lumen. The tumor cells were shown to have clear cytoplasm (clear cell), eosinophilic granular cytoplasm (granular cell), or homogeneously basophilic cytoplasm (dark cell). There was no nuclear pleomorphism and mitotic figures were rare (Figs. 5–7).
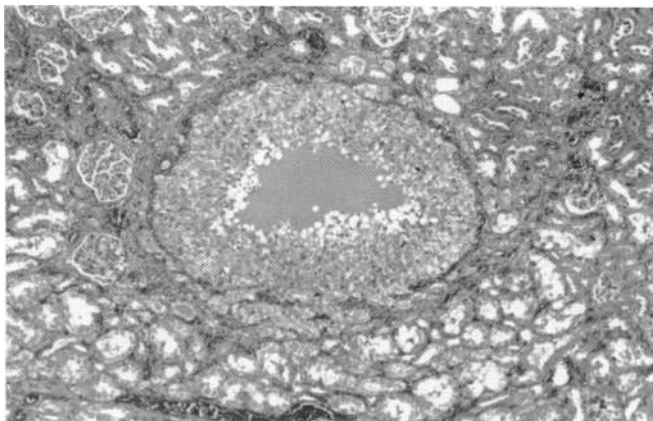
FIGURE 5. Solid adenoma filled with eosinophilic material.
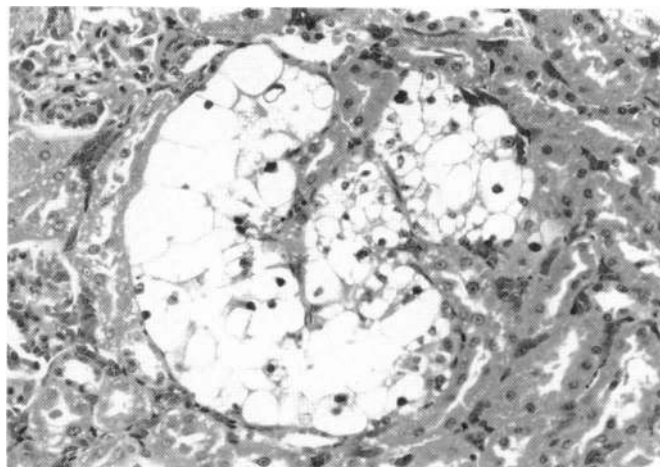
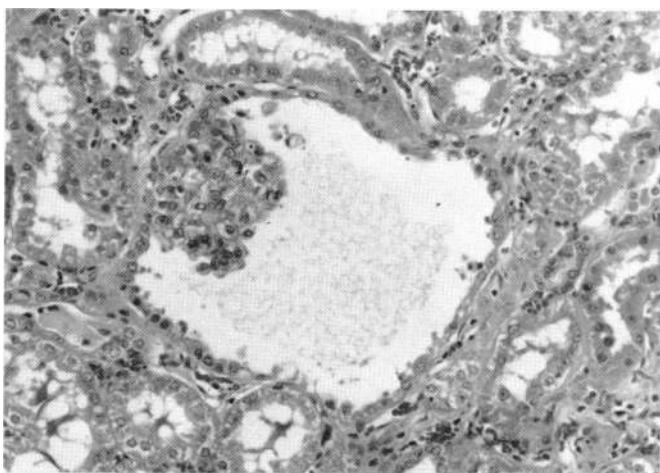FIGURE 6. Solid adenoma composed of clear, polygonal cells.

FIGURE 7. Cystic adenoma with papillary projection protruding into the lumen.

Adenocarcinomas were usually irregularly contoured as if two or more small nodules became aggregated. These tumors were mostly localized in the cortical areas, although occasionally they exhibited a deep downward growth from the cortex into the medulla. Most exhibited a solid growth pattern, but in some cases, they showed trabecular, tubular, or papillary patterns (Figs. 8 and 9). In some large adenocarcinomas, extensive areas of necrosis and hemorrhage were observed. The malignant tumor cells—like those in adenomas—were also polygonal in shape and demonstrated clear, granular, or dark cytoplasm. Mitotic figures were occasionally seen but nuclear pleomorphism was not apparent (Fig. 10). Apparent infiltrative growth was observed in cases of grossly large tumors. A lung metastasis was found in one case in the rat carcinogenicity test.

Essentially, the histologic features of RCTs found in KBrO₃-treated male hamsters and male mice were quite similar to those observed in KBrO₃-treated rats.
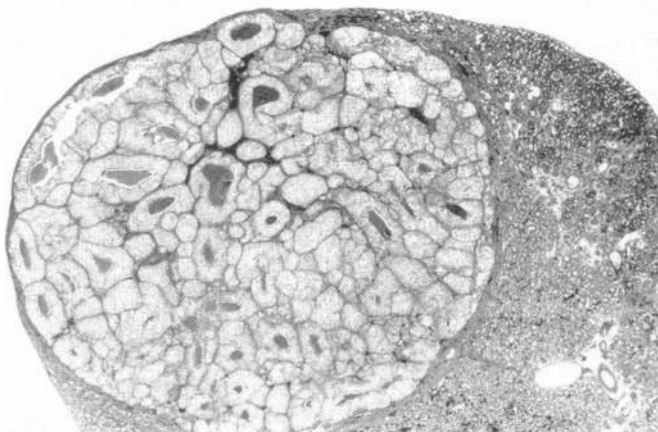
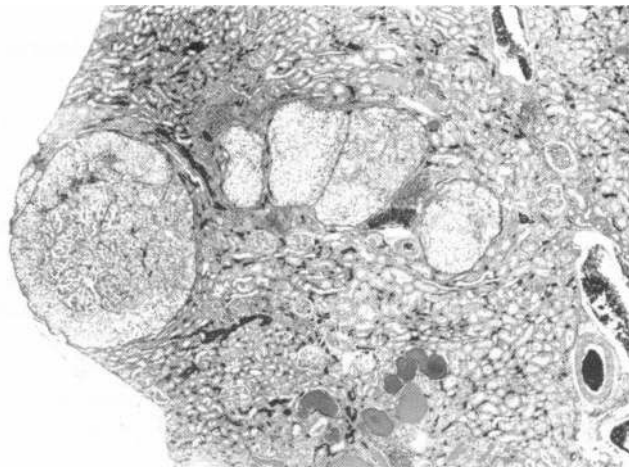FIGURE 8. Large adenocarcinoma demonstrating tubular structures.

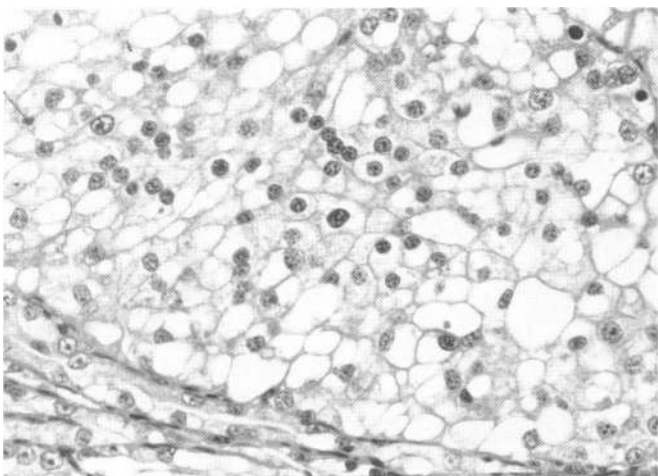FIGURE 9. An adenocarcinoma composed of multiple small nodules extending into the medulla.

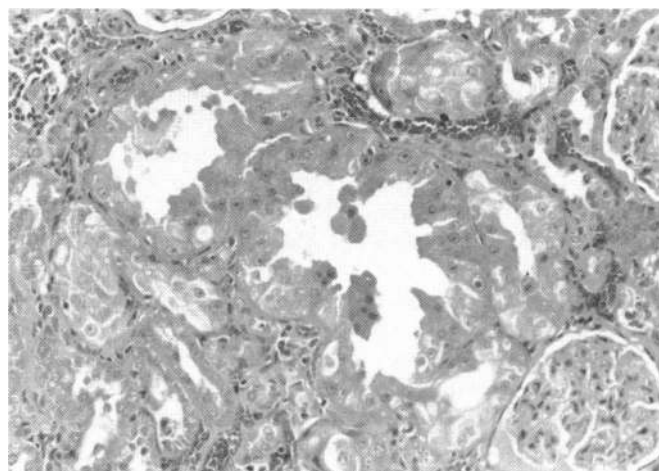FIGURE 10. Adenocarcinoma consisting of granular cells; nuclear pleomorphism is not evident.

FIGURE 11. Dilated tubular lesion demonstrating hyperplastic epithelium.

Dysplastic Foci. Focal tubular lesions that showed hyperplasia of the tubular lining epithelium, which often resulted in narrowing of the tubular lumina, were diagnosed as dysplastic foci (DF) throughout our studies (Fig. 11). Dilated tubules with multilayered and/or enlarged epithelial cells with a papillary growth pattern were also classified as DF (Fig. 12).

DF in our study seem to correspond to putative preneoplastic lesions described by others as atypical cell foci, pathologically changed tubules, focal areas of dysplastic tubular epithelium, small nodules, or dysplasia (8).

Nonneoplastic Changes

Renal tubules in $KBrO_3$-treated rats demonstrated various degenerative, necrotic, and regenerative changes. Numerous eosinophilic bodies were observed in the cytoplasm of proximal renal tubules in rats treated continuously with $KBrO_3$ for 13 to 104 weeks. However, these changes were not evident in rats of the limited duration experiment, indicating that they are reversible. Hyaline casts in the tubular lumen, hyaline droplets, and brown pigments in the tubular epithelium were also commonly observed. Although these lesions were also found in control rats, they were more extensive in both degree and distribution in treated rats, especially in males. Vascular changes, which were observed in the rat study after feeding $KBrO_3$-treated bread (49), were not evident in any organs.

The transitional epithelium of the renal pelvis showed thickening, papillary hyperplasia, and growth, especially in treated males. Calcium deposits in the renal pelvis were also marked in rats showing the hyperplastic changes.

These nonneoplastic changes occurred to a lesser degree in hamsters and mice given $KBrO_3$ than in rats.
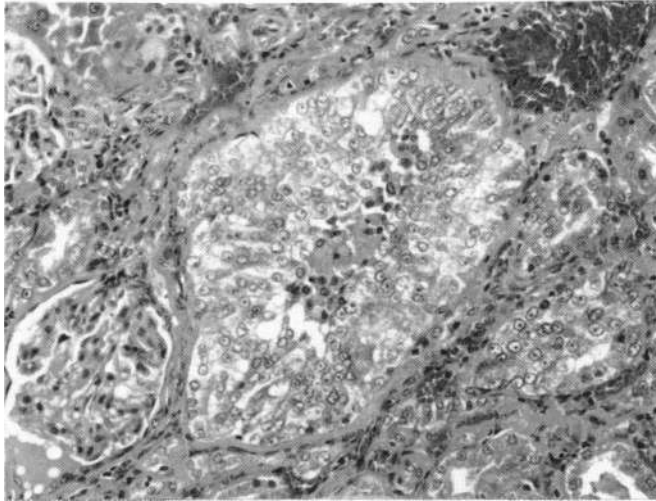
FIGURE 12. Focal lesion demonstrating multilayering of the tubular epithelium.

Initiation and Promotion

Assay for Promoting Potential for Kidney, Liver, and Urinary Bladder Tumorigenesis in the Rat

Although the carcinogenicity of $KBrO_3$ in rats was definitely established by several experiments, it was thought necessary to test the promoting effects of this compound to better understand the mechanisms underlying its carcinogenic action and organ specificity (5). N-Ethyl-N-hydroxyethylnitrosamine (EHEN) was used as an initiator for this purpose because this carcinogen is known as a potent initiator useful for determining the promotion potential of exogenous chemicals on kidney and liver tumorigenesis (56–58).

A total of 128 male F344 rats were given EHEN orally for 2 weeks and then 500 ppm $KBrO_3$ orally for the following 24 weeks. The kidney and liver were cut into

6 to 8 serial slices. The numbers of microscopic neoplastic lesions were counted, and the entire areas of the sections were measured with a semiautomatic image analyzer (TAS-plus, Leitz Wetzlar, West Germany). There were significantly increased incidences of DF and average numbers of both DF and RCT/cm^2 in groups given $KBrO_3$ after initiation as compared to values for animals treated with EHEN alone; therefore these findings clearly demonstrated enhancing activity of $KBrO_3$ on kidney lesion development (Table 10). No hyperplastic or neoplastic changes were observed in the renal pelvis or urinary bladder in any of the groups, and no significant differences in the incidences and average numbers of hyperplastic foci, neoplastic nodules, and hepatocellular carcinomas were found.

Recently, a very effective rapid bioassay system for rat hepatocarcinogenesis was developed, based on the two-stage carcinogenesis concept (59). In this model rats were given a single dose (200 mg/kg) of diethylnitrosamine IP as an initiator and then fed $KBrO_3$ in the diet (4000 ppm) for 6 weeks. A two-thirds partial hepatectomy was performed at week 3. As in the long-term experiment, $KBrO_3$ showed no enhancing effect on hepatocarcinogenesis.

Dose-Response Studies on the Promoting Potential for Renal Tumorigenesis in the Rat

Dose-response studies using a total of 180 male F344 rats were undertaken to clarify whether a threshold level of $KBrO_3$ treatment exists for promotion of renal tumorigenesis (8). The promoting effect of KBr was also tested since $KBrO_3$ is easily degraded to KBr during the baking process. Experimental protocols were similar to those used in the previous promotion study (5).

The mean numbers of DF/cm^2 were found to be significantly increased in a dose-related manner in rats treated with > 30 ppm $KBrO_3$ (Table 11). A parabolic curve was obtained by exponential regression analysis when the numbers of DF/cm^2 were plotted against the

Table 10. Incidences and average numbers of dysplastic foci (DF) and renal cell tumors (RCT) in male F344 rats treated with EHEN and $KBrO_3$.

Experimental group number	Treatment, ppm	Effective number of rats	Number of rats with DF(%)	Average number of DF/cm^2	Number of rats with RCT (%)	Average number of RCT/cm^2
1	DW	19	2(11)	0.02 ± 0.05	0(0)	0.00
2	EHEN (1000) + DW	22	17(77)	0.39 ± 0.37	9(41)	0.08 ± 0.12
3	EHEN (500) + DW	23	15(65)	0.20 ± 0.20	4(17)	0.03 ± 0.06
4	EHEN (1000) + $KBrO_3$ (500)	19	19(100)	1.11 ± 0.45[a]	9(47)	0.11 ± 0.14
5	EHEN (500) + $KBrO_3$ (500)	20	19(95)[b]	0.98 ± 0.38[c]	10(50)	0.13 ± 0.16[d]
6	$KBrO_3$ (500)	20	7(35)	0.07 ± 0.10	0(0)	0.00

[a] $p < 0.01$ (compared with group 2).
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.05$ (compared with group 3).

Table 11. Incidences and average numbers of dysplastic foci (DF) and renal cell tumors (RCT) in male F344 rats treated with $KBrO_3$ or KBr at various doses after initiation with EHEN at a dose of 500 ppm orally.

Experimental group number	Treatment, ppm	Effective number of rats	Number of rats with DF (%)	Average number of $DF/cm^2 \pm SD$	Number of rats with RCT (%)
1	DW + DW	15	0(0)	0.00	0(0)
2	DW + $KBrO_3$ (500)	15	0(0)	0.00	0(0)
3	DW + KBr(1750)	14	0(0)	0.00	0(0)
4	EHEN + DW	15	12(80)	0.29 ± 0.16	3(20)
5	EHEN + $KBrO_3$(15)	14	11(79)	0.39 ± 0.19	4(27)
6	EHEN + $KBrO_3$(30)	15	14(93)	0.49 ± 0.30^b	4(27)
7	EHEN + $KBrO_3$(60)	15	14(93)	0.61 ± 0.29^a	3(20)
8	EHEN + $KBrO_3$(125)	15	15(100)	0.79 ± 0.33^a	6(40)
9	EHEN + $KBrO_3$(250)	15	15(100)	1.22 ± 0.56^a	5(33)
10	EHEN + $KBrO_3$(500)	15	15(100)	1.25 ± 0.76^a	8(53)
11	EHEN + KBr(350)	15	8(53)	0.15 ± 0.16	3(20)
12	EHEN + KBr(1750)	15	8(53)	0.16 ± 0.20	5(33)

$^a p < 0.01$.
$^b p < 0.05$ (from group 4).
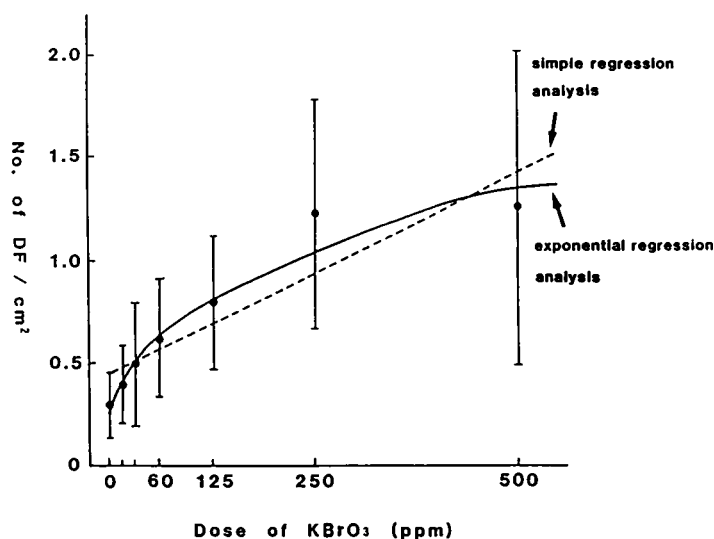
FIGURE 13.   Dose-response curve for DF/cm^2 in rats treated with $KBrO_3$ after EHEN initiation.

dose of $KBrO_3$ (Fig. 13). No promoting effect was observed with KBr.

Thus, the threshold level of $KBrO_3$ in the drinking water for promotion of renal tumorigenesis seems to lie between 15 and 30 ppm. In view of the lack of dose-dependent increases in the mean size and areas of RCT per kidney and the fact that lesion morphology and growth rate appeared unaffected, it is probable that $KBrO_3$ exerted its promoting effect by directly altering expression of other characters in the initiated cell population.

Assay of Two-Stage and Complete Carcinogenesis in Mouse Skin

Since $KBrO_3$ has been used as a neutralizer in permanent wave preparations that have contact with the skin (27), studies on the promoting or complete carcinogenic potential of direct application of $KBrO_3$ to this tissue were considered of interest (7).

In the experiment to determine promoting activity, groups of 20 female Sencar mice received a single topical application of DMBA (20 nmole) followed by treatment with $KBrO_3$ (40 mg/mL), 12-O-tetradecanoylphorbol-13-acetate (TPA) (10 µg/mL), or the acetone solvent alone for 51 weeks. To test for complete carcinogenic activity, groups of 20 mice were also given $KBrO_3$ (40 mg/mL) without prior initiation. Histopathological examination did not reveal any epidermal hyperplasias, squamous cell papillomas, or squamous cell carcinomas in mice treated with $KBrO_3$ from either the promotion or complete carcinogenesis studies. In complete contrast, a strong promoting action was evident in positive control mice administered TPA.

Two-Stage Whole Body Carcinogenesis in the Rat

Methylnitrosourea (MNU) has been shown to be useful as an initiator to detect the promoting potential of

chemicals for tumors of the nervous, hematopoietic, and GI tract systems; and thyroid, liver, and urinary bladder (*60*). The MNU two-stage carcinogenesis model was applied to ascertain whether $KBrO_3$ may act as a promoter in organs other than the kidney (Kurokawa, unpublished data). Groups of 20 male F344 rats were given 4 IP injections of MNU at doses of 40, 20, and 10 mg/kg body wt. for 2 weeks, and then given 500 ppm $KBrO_3$ orally in the drinking water for 24 weeks. Although relatively high incidences of mesotheliomas, leukemias, and tumors of the tongue, forestomach, small intestine, and lung were observed, no significant promoting effects of $KBrO_3$ on development of these tumors were evident.

Assay of Two-Stage Forestomach Carcinogenesis in Mice

Groups of 12 male C57BL mice received a single IG administration of dimethylbenzanthracene (DMBA, 25 or 50 mg/kg) as the initiation step and were then administered 500 ppm $KBrO_3$ orally for 26 weeks (Kurokawa, unpublished data). No increases in the incidences of either papillomas or hyperplasias in the forestomach epithelium of mice were observed, $KBrO_3$-treated as opposed to control mice. No positive controls were used in this study.

Assay of Two-Stage Esophageal Carcinogenesis in the Rat

Groups of 15 male F344 rats were given dibutylnitrosamine (DBN) at a dose of 500 ppm orally for 4 weeks as the initiation step and then administered 500 ppm $KBrO_3$ orally for 32 weeks (Kurokawa, unpublished data). The incidences of neoplastic lesions of the esophagus and other GI tract organs in rats given $KBrO_3$ after initiation were not significantly increased as compared to control values.

Mutagenicity

Microbial Assays

In Japan microbial testing of $KBrO_3$ was conducted simultaneously in several laboratories under the cooperative program on short-term assays (*2*). Using *Salmonella typhimurium* TA100, $KBrO_3$ was found to be weakly positive at a concentration of 3 mg/plate after metabolic activation. However, the compound proved negative in TA98, TA1535, TA1537, TA1538, *E. coli* WP2try⁻ and *E. coli* WP2try⁻his⁻ with or without metabolic activation (*2,61,62*). Similarly, in the USA no mutagenic activity could be demonstrated for $KBrO_3$ in *Salmonella typhimurium* (strains not specified) or *Sarcina cerevisiae* (Litton Bionetics, personal communication).

Very recently, microbial tests were reconducted in our laboratory (Kurokawa, unpublished data). Weak

mutagenic activities were again demonstrated in TA100 at doses of 2 to 4 mg/plate with or without metabolic activation. $KBrO_3$ was also mutagenic in TA102 and TA104, strains which are sensitive to chemicals that generate active oxygen radicals (*63*) in the presence of metabolic activation (Fig. 14). KBr was negative in both TA98 and TA100.

Microbial assays of $NaBrO_3$ and silver bromate ($AgBrO_3$) were also conducted in strains TA 97, 98, 100, and 102 in order to ascertain the general mutagenic potential of bromate (BrO_3^-) compounds. Both $NaBrO_3$ and $AgBrO_3$ proved negative in all strains tested at the maximum levels of 5 mg/plate and 25 µg/plate, respectively (Kurokawa, unpublished data). The very low solubility of $AgBrO_3$ might be the reason for negative data.

The results of Rec assays on $KBrO_3$ using *Bacillus subtilis* (17Arec⁺, 45Trec⁺) were also negative with or without metabolic activation (*2*).

Chromosome Aberration Tests

The rates of chromosome aberrations in Chinese hamster lung (CHL) cells treated with $KBrO_3$ were found to be significantly higher than in controls at dose ranges of 0.0625 to 0.25 mg/mL, in a dose-dependent manner, without metabolic activation (*2,64–66*) (Table 12). Mainly chromatid type breaks and exchanges were induced. The value D_{20}, representing the dose at which aberrations were detected in 20% of metaphase cells, was calculated to be 0.071 mg/mL. Therefore, the clastogenic activity of $KBrO_3$ was considered to be relatively strong (*66*).

Chromatid breaks were also induced in cultured Chinese hamster DON-6 cells by the addition of 5×10^{-4} M (0.0835 mg/mL) $KBrO_3$ (*67*). *In vivo* clastogenic activity of $KBrO_3$ was further examined in bone marrow cells of male Long-Evans rats administered $KBrO_3$ by IP and oral routes. The incidences of aberrant metaphase cells were significantly increased, reaching a maximum of 10.5% at 18 hr (IP, 250.5 mg/kg body weight) and of 10.8% at 18 hr (oral, 334.0 mg/kg body weight). On the contrary, an IP injection of heat treated $KBrO_3$ (190°C or 230°C for 20 min) at a dose of 167 mg/kg body weight had no effect on numbers of aberrant cells (*68*).

KBr was positive in the chromosome aberration test at doses greater than 4 mg/mL, inducing chromatid gaps, breaks, and exchanges (*62,66*). However the D20 of KBr (3.7 mg/mL) was much higher than that of $KBrO_3$.

Micronucleus Tests

Micronucleated polychromatic erythrocytes were induced in male ddY mice in a dose-dependent manner when $KBrO_3$ was administered at doses higher than 25 and 100 mg/kg body weight by IP and oral routes, respectively (*69*). Among 47 compounds tested, including 39 synthetic and natural food additives, only $KBrO_3$ was positive by both IP and oral application. In contrast,
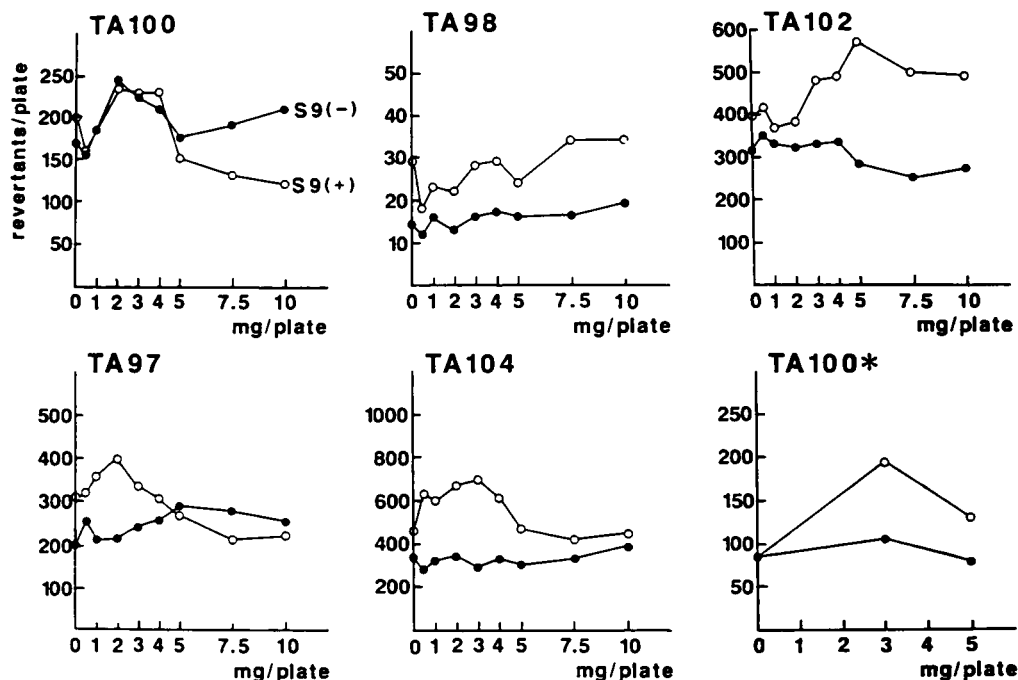
FIGURE 14.   Microbial assays of KBrO₃ using five strains of *Salmonella typhimurium*. *Previous test in 1976; others are data in 1987.

Table 12. Chromosome aberration test on KBrO₃ and KBr.

	Treatment time, hr	Concentration, mg/mL	Cells with structural chromosomal aberrations, %	Judge
KBrO₃	24	0	4	
	24	0.0625	10	+
	24	0.125	27	+
	24	0.25	100	+
	48	0.0625	10	+
	48	0.125	26	+
	48	0.25	Toxic	Toxic
KBr	24	0	4	
	24	2.0	4	−
	24	4.0	10	+
	24	6.0	39	+
	48	2.0	3	−
	48	4.0	12	+
	48	6.0	76	+

KBr was negative after up to five IP injections at doses in the range of 62.5 to 500 mg/kg body weight. Induction of micronuclei by KBrO₃ was compared in male ddY mice and a mutagen-sensitive mouse strain (designated as MS) after a single IP administration (70), and positive results were obtained in both; a higher susceptibility of the sensitive strain to KBrO₃ was also confirmed. No sex differences were evident in ddY mice for the induction of micronuclei by KBrO₃ after a single IP administration (71).

Silk Worm Test

Mutagenicity testing using silk worms was reported to be negative (2).

Absorption, Distribution, Excretion and Metabolism

In Vivo Studies

Male Wistar rats were given KBrO₃ IG at a dose of 50 mg/kg body weight as BrO_3^-, and the levels of BrO_3^- and Br^- in various organs were examined (72). Each organ was homogenized in water and freeze-dried and then assayed using ion chromatography method (28). As shown in Table 13, approximately 30% of BrO_3^- was detected in the urine 24 hr after the treatment. The levels of Br^- were increased significantly in the plasma, RBC, kidney, pancreas, stomach, small intestine, and urine. Thus it is evident that the BrO_3^- given orally was absorbed and partly excreted, unchanged in the urine; the remainder was at least partly reduced to Br^-. However, it was not clear whether the increased Br^- all originated via degradation of BrO_3^- or if redistribution in the body might play a role. Also it is unknown whether BrO_3^- or Br^- was not totally extracted because of binding with insoluble fractions, proteins, etc., during sample preparation or for other reasons. Clarification of these points will require further study including the development of a microassay for biological specimens.

Time-related changes in the levels of BrO_3^- after the IG administration of KBrO₃ (100 mg/kg body weight) are illustrated in Figures 15 and 16. It is evident that

Table 13. BrO₃ and Br⁻ levels in tissues and feces 24 hr after IG administration of KBrO₃ to male Wistar rats.

Samples[a]	BrO₃		Br⁻	
	Control	KBrO₃-treated	Control	KBrO₃-treated
Plasma	ND[b]	ND	51.4 ± 0.9	187.1 ± 0.3*
RBC	ND	ND	47.2 ± 2.0	289.0 ± 1.0*
Spleen	ND	ND	51.3 ± 22.1	102.1 ± 13.9
Kidney	ND	ND	12.7 ± 1.7	87.4 ± 4.6*
Liver	ND	ND	30.6 ± 1.2	42.6 ± 7.1
Pancreas	ND	ND	13.6 ± 1.8	32.1 ± 3.3*
Stomach	ND	ND	36.1 ± 4.6	113.5 ± 14.1*
Small intestine	ND	ND	34.6 ± 0.1	62.5 ± 3.5*
Urine	ND	1729.9 ± 11.4*	212.6 ± 6.5	1314.1 ± 4.1*
Feces	ND	14.1 ± 0.3*	13.0 ± 0.3	12.3 ± 0.3

[a] Units in plasma, RBC, tissues = micrograms per gram; in urine, feces = micrograms per 24 hr.
[b] ND = < 2.5 μg/mL (urine, plasma), < 5 μg/g (tissues).
*$p < 0.01$ as compared to control (mean ± SD).
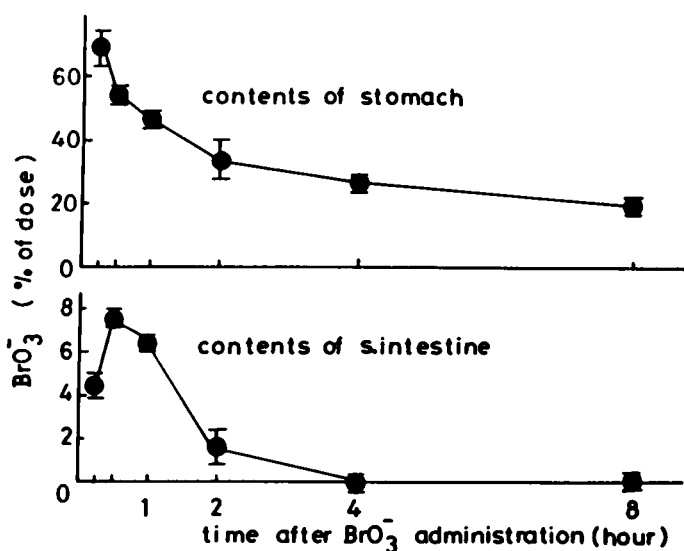
FIGURE 15. Levels of BrO₃⁻ in stomach and small intestine contents after IG administration of KBrO₃.

FIGURE 16. Levels of BrO₃⁻ in plasma and urine after IG administration of KBrO₃.
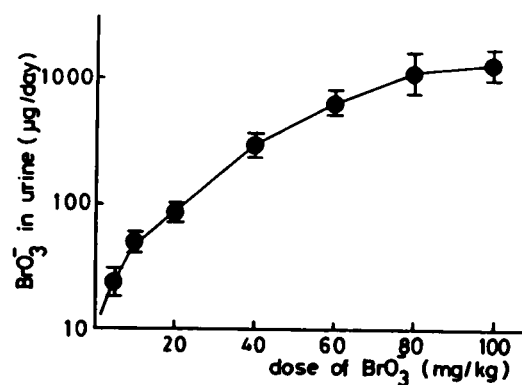
FIGURE 17. Changes in the levels of urinary excretion of BrO₃⁻ after IG administration of KBrO₃.

BrO₃ was rapidly absorbed and degraded in the stomach, small intestine, plasma, and urine within 2 to 4 hr. Dose-response studies further revealed that no BrO₃ was detectable in the urine of rats given KBrO₃ at doses lower than 2.5 mg/kg body weight. However, at doses higher than 5 mg/kg body weight, a dose-related increase in the levels of BrO₃ excreted into the urine was apparent (Fig. 17). Therefore, it is probable that BrO₃ could have exerted direct effects on the renal tubular epithelial cells at doses higher than 5 mg/kg; this finding could help to explain the mechanism of carcinogenicity of this compound in the kidney.

In Vitro Studies

The decomposition of BrO₃ in various rat tissues was examined in vitro to cast light on the mechanism of in vivo biodegradation (73).
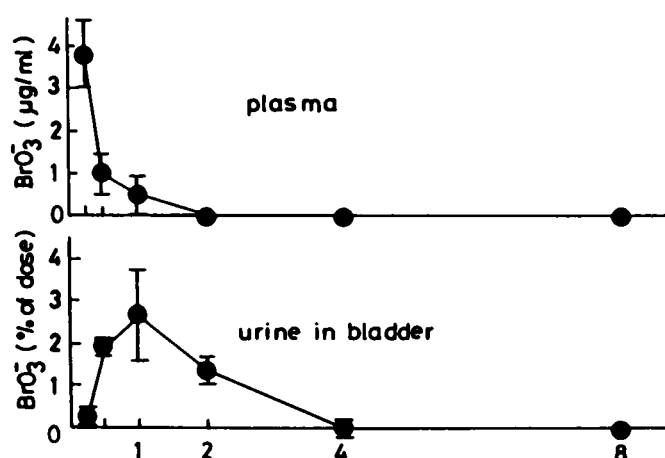
As shown in Table 14, no degradative activity was

observed with human saliva. On the other hand, rat liver and kidney homogenates and RBCs showed strong activity for biodegradation of BrO₃ when incubated at 37°C for 3 min. The activities still remained at the same

Table 14. Degradation of BrO₃⁻ in vitro by human saliva and rat tissue homogenates.

	BrO_3, ppm, before incubation	BrO_3, ppm, after incubation[a]	Recovery, %
Saline	10	6.96 ± 0.40	100.0
Saliva	10	6.25 ± 0.40	89.9
Saline	5	4.30 ± 0.60	100.0
Liver	5	Not discernable	0
		(0.03 ± 0.07)	(Trace)
Kidney	5	0.03 ± 0.04	Trace
		(0.07 ± 0.03)	(Trace)
Spleen	5	0.46 ± 0.22	10.7
Stomach	5	1.30 ± 0.17	30.2
Small intestine	5	0.42 ± 0.05	9.8
Plasma	5	3.68 ± 0.18	85.6
		(4.20 ± 0.52)	(97.7)
RBC	5	0.02 ± 0.02	Trace
		(0.94 ± 0.08)	(21.9)
Gastric juice	5	0.79 ± 0.12	18.4

[a] Concentration of BrO_3 after incumbation with heat treated homogenate, plasma or hemolysate (100°C, 5 min); mean ± SD of three experiments. Incubation at 37°C, 3 min.

levels after heat treatment at 100°C for 5 min. Subsequently, supernatants from tissues showing degradative activity were fractionated by gel filtration. High and low molecular weight liver fractions—the latter containing GSH and other -SH compounds—were examined. The activity of the former disappeared after heating, whereas that of the latter was retained. Hemolyzed RBCs, kidney, small intestinal mucosa, and stomach tissues could be also fractionated to give two similar components.

SH compounds such as cysteine, glutathione (GSH), and ergothioneine were found to have BrO₃⁻ degradative activity (Table 15) and simultaneous analysis of residual BrO₃⁻ and yielded Br⁻ in the presence of GSH revealed a near stoichiometric response, as shown in Figure 18. From these data it is highly probable that GSH is intimately involved in the degradation of BrO₃⁻. Moreover, Br⁻ is yielded in the GSH-mediated reaction that corresponds well to the fact that Br⁻ concentration increased in organs and urine of rats after an oral administration of BrO₃⁻.

# Studies on the Mechanism of KBrO₃ Carcinogenesis

Toxicological Studies

Lipid peroxide (LPO) in tissues is mainly generated by the oxidative deterioration of cell membrane polyunsaturated fatty acids by active oxygen species (74).

Table 15. Degradation of BrO₃⁻ by SH compounds in vitro.[a]

	Concentration of BrO_3^-, ppm		
	5	10	20
Cysteine	0	13.0	47.0
Glutathione	0	0.9	47.5
Ergothioneine	20.9	35.2	59.5

[a] Numbers denote percent of initial concentration after incubation at 37°C for 30 min with 0.1 mM of each SH compound.
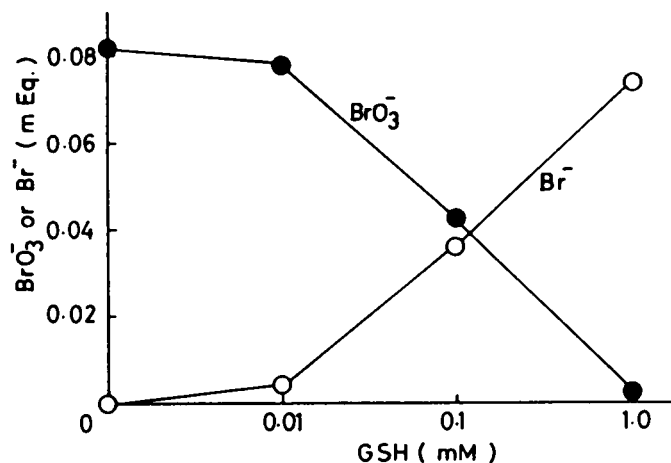
FIGURE 18. Stoichiometric degradation of BrO_3^- to Br^- by glutathione.

The protective role of cysteine and GSH and the deleterious influence of diethylmaleate (DEM), a GSH-depleter, on cellular oxidative damage and LPO formation are well known (75).

Lipid Peroxidation in the Kidney. Changes in the levels of LPO in the kidney of male F344 rats, CDF_1, BDF_1, and $B6C3F_1$ mice, and Syrian golden hamsters were studied after IV administration of $KBrO_3$. Alteration in levels of malondialdehyde (MDA, nmole/g wet tissue) was used as an index of LPO according to the procedure using thiobarbituric acid (TBA) (76). The TBA method has been used extensively to measure LPO levels in the liver, and its recent application to the kidney allowed dose-dependent and time-dependent changes to be shown in response to exogenous chemicals (77–79).

As illustrated in Figure 19, LPO levels were significantly increased in rats given $KBrO_3$ without them receiving accompanying cysteine treatment. However, when the rats were also given IP injections of cysteine
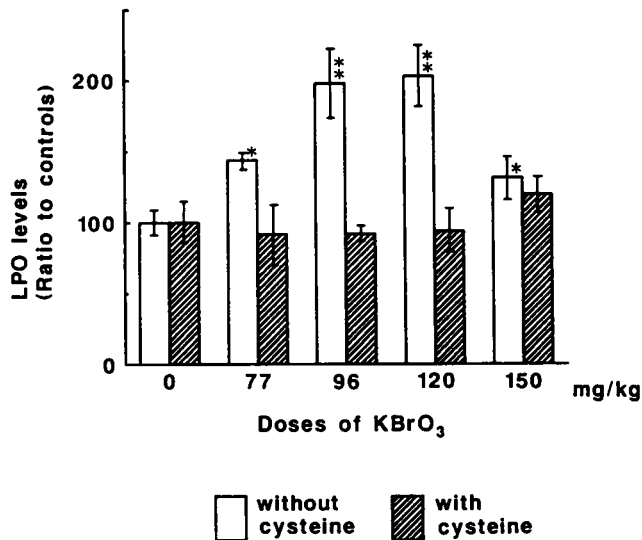
FIGURE 19. Levels of LPO in the kidneys of male F344 rats 24 hr after a single IV administration of $KBrO_3$ at doses of 77, 96, 120, or 150 mg/kg with or without cysteine treatment. Each bar represents the mean ± SD of four rats. **p < 0.01 and *p < 0.05 as compared to controls.
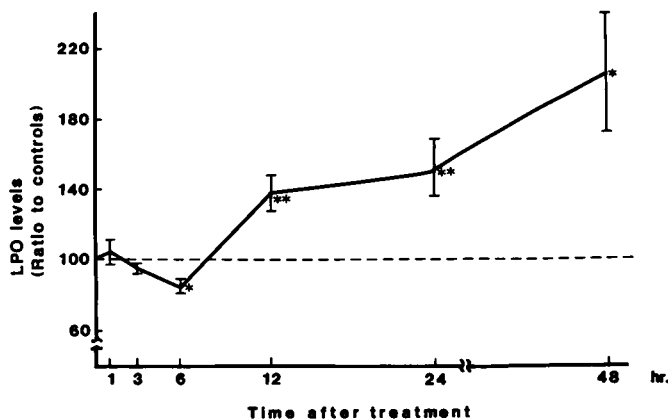

FIGURE 20. Changes in the levels of LPO in the kidneys of male F344 rats at 2, 4, 6, 12, 24, and 48 hr after a single IV administration of 120 mg/kg of $KBrO_3$. The levels of LPO are expressed as ratios to concurrent control values sacrificed at the same experimental time points. Each point represents the mean ± SD of five rats. **p < 0.01 and *p < 0.05 as compared to controls.

(400 mg/kg) 30 min before and after IV treatment with $KBrO_3$, this change was inhibited. In contrast, when DEM (0.7 mL/kg) was administered IP 1 hr before IV treatment by $KBrO_3$ (20 mg/kg), LPO levels were significantly elevated ($KBrO_3$ alone; 214.6 ± 15.6 versus DEM + $KBrO_3$; 347.7 ± 64.9, $p < 0.05$). Figure 20 shows the time-dependence of LPO elevation in rats receiving a single $KBrO_3$ treatment of 120 mg/kg body weight. After an initial significant decrease at 6 hr, the levels of LPO were found to be significantly increased at 12, 24, and 48 hr. However, LPO levels in the kidneys of mice and hamsters did not demonstrate any equivalent increase at this dose level of $KBrO_3$.

Therefore, dose- and time-related significant in-

creases in the levels of LPO were apparent in the kidneys of male rats given $KBrO_3$ at a dose of 77 mg/kg body weight or above. These overall data strongly show a particularly strong potential for oxidative damage in the kidney of rats; this finding might explain the observed species differences in susceptibility to $KBrO_3$ carcinogenicity to the kidney.

Effects of GSH, Cysteine, and DEM Treatment on Mortality. In rats given $KBrO_3$ IV without GSH treatment, all animals died within 3 days at a dose as low as 108 mg/kg (18). On the other hand, when animals were also treated with GSH, mortality was greatly reduced and no animals died at doses lower than 214 mg/kg $KBrO_3$ (Table 16). Similar results were observed with cysteine treatment (Table 17), and—again in contrast—when rats were pretreated with DEM (Table 18), mortality was significantly increased.

Changes in Serum Biochemistry. As shown in Table 19, the levels of NPN, BUN, and creatinine were significantly increased in a dose-related manner in male F344 rats after a single IV administration of $KBrO_3$ (18). Elevation was first observed between 3 and 6 hr, peaking at 24 hr before returning to normal at 48 hr (Table 20). However, the increase in values for these parameters was significantly inhibited in rats treated with GSH or cysteine (Table 21). DEM treatment had the opposite effect, bringing about a significant further elevation over $KBrO_3$-alone levels (Table 22).

Table 16. Effect of glutathione (GSH) treatment on mortality of male F344 rats given IV injection of $KBrO_3$.

Doses of $KBrO_3$, mg/kg	Number of animals dead (days)[a]	
	GSH-treated[b]	Saline-treated[b]
300	8 (2)	8 (1)
268	6 (3)	8 (1)
239	2 (2)*	8 (1)
214	0[†]	8 (1)
170	0[†]	8 (1)
136	0[†]	8 (2)
108	0[†]	8 (3)
86	0	0

[a] Eight animals were used for each group and observed for 14 days.
[b] GSH (800 mg/kg) or saline was injected IP 30 min before and after $KBrO_3$ administration.
*$p < 0.01$.
[†]$p < 0.001$ compared to saline-treated controls.

Table 17. Effect of cysteine treatment on mortality of male F344 rats given IV injection of $KBrO_3$.

Doses of $KBrO_3$, mg/kg	Number of animals dead (days)[a]	
	Cysteine-treated[b]	Saline-treated[b]
169	0*	5 (1–2)
130	0*	5 (2)
100	0	0
77	0	0

[a] Five animals were used for each group and observed for 7 days.
[b] Cysteine (400 mg/kg) or saline was injected IP 30 min before and after $KBrO_3$ administration.
*$p < 0.01$ compared to saline-treated controls.

Table 18. Effect of diethyl maleate (DEM) treatment on mortality in male F344 rats given IV injection of KBrO₃.

Doses of KBrO₃, mg/kg	Number of animals dead (days)[a]	
	DEM-treated[b]	Saline-treated[b]
49	4 (1–2)*	0
29	4 (1–2)*	0
17	0	0
10	0	0

[a] Five animals were used for each group and observed for 7 days.
[b] DEM (0.7 mL/kg) or saline was injected IP 60 min before KBrO₃ administration.
*$p < 0.05$ compared to saline-treated controls.

Table 19. Dose-response studies of serum biochemistry in male F344 rats given a single IV injection of KBrO₃.

Doses of KBrO₃, mg/kg	NPN, mg/dL ± SD	BUN, mg/dL ± SD	Creatinine, mg/dL ± SD
0	30.8 ± 2.2	14.6 ± 1.3	0.3 ± 0.1
77	79.1 ± 11.4*	52.9 ± 8.4‡	0.5 ± 0.1‡
96	182.0 ± 45.7‡	154.6 ± 32.2§	2.0 ± 0.5‡
120	231.5 ± 11.1*	194.7 ± 7.2*	2.8 ± 0.3*
150	264.0 ± 17.6†	221.3 ± 20.2†	4.0 ± 0.6†

[a] Three rats per dose were sacrificed 24 hr after IV injection of KBrO₃.
†$p < 0.01$.
*$p < 0.001$.
‡$p < 0.05$.
§$p < 0.02$ compared to saline-treated controls.

Table 20. Time course studies of serum biochemistry in male F344 rats given a single IV injection of KBrO₃[a]

Time after KBrO₃, injection, hr[a]	NPN, mg/dL ± SD	BUN, mg/dL ± SD	Creatinine, mg/dL ± SD
0	33.7 ± 5.1	15.6 ± 1.9	0.3 ± 0.0
1	30.9 ± 1.6	15.0 ± 1.0	0.4 ± 0.1
3	35.7 ± 1.2	19.7 ± 1.3‡	0.5 ± 0.1‡
6	58.4 ± 5.8*	35.9 ± 6.9*	0.5 ± 0.1‡
12	79.5 ± 4.3†	51.1 ± 3.0†	0.5 ± 0.1‡
24	210.7 ± 12.0†	169.6 ± 6.4†	2.3 ± 0.2*
48	37.5 ± 0.5	17.2 ± 1.4	0.4 ± 0.1

[a] Three rats were sacrificed sequentially after IV injection of KBrO₃ (120 mg/kg).
*$p < 0.01$.
†$p < 0.001$.
‡$p < 0.05$ compared to saline-trated controls.

Formation of 8-Hydroxydeoxyguanosine (8-OH-dG) in Rat Kidney DNA

8-OH-dG is one of the DNA-damaged products formed *in vitro* and *in vivo* by oxygen radical-forming agents, such as reducing agents, asbestos-H_2O_2, polyphenol-Fe^{3+} -H_2O_2 and radiation (*80–82*). It was therefore considered of interest to determine the relationship between 8-OH-dG formation in tissue DNA and the carcinogenic potential of an oxidizing agent like KBrO₃ (*19*).

As shown in Figure 21, the 8-OH-dG in kidney DNA of male F344 rats increased 4-fold up to 6 residues/10⁵ deoxyguanosine (dG), 24 hr after a single IG KBrO₃ administration. After 48 hr, a slight reduction towards

Table 21. Effect of GSH or cysteine treatment on serum biochemistry in male F344 rats given a single IV injection of KBrO₃.[a]

Treatment	NPN, mg/dL ± SD	BUN, mg/dL ± SD	Creatinine, mg/dL ± SD
Saline	28.1 ± 0.8	16.0 ± 1.5	0.3 ± 0.1
KBrO₃	193.8 ± 18.6*	177.5 ± 9.2†	2.5 ± 0.1†
KBrO₃ + GSH	81.8 ± 38.3#	67.9 ± 40.5‡	0.9 ± 0.4‖
KBrO₃ + cysteine	33.4 ± 11.9§	18.3 ± 6.2§	0.4 ± 0.0§

[a] Three rats per dose were sacrificed 24 hr after IV injection of KBrO₃ (120 mg/kg). GSH (800 mg/kg) or cysteine (400 mg/kg) were injected IP 30 min before and after KBrO₃ administration.
*$p < 0.01$.
†$p < 0.001$.
‡$p < 0.05$ compared to saline-treated controls.
§$p < 0.001$.
‖$p < 0.01$.
#$p < 0.05$ compared to KBrO₃-treated controls.

Table 22. Effect of DEM treatment on serum biochemistry in male F344 rats given a single IV injection of KBrO₃.[a]

Treatment	NPN, mg/dL ± SD	BUN, mg/dL ± SD	Creatinine, mg/dL ± SD
Saline	28.1 ± 0.8	16.0 ± 1.5	0.3 ± 0.1
KBrO₃	28.6 ± 2.3	15.9 ± 1.6	0.4 ± 0.1
KBrO₃ + DEM	284.0 ± 14.1*,†	246.8 ± 24.0*,‡	2.6 ± 1.0

[a] Three rats per dose were sacrificed 24 hr after IV injection of KBrO₃ (15 mg/kg). DEM (0.7 mL/kg) was injected IP 60 min before KBrO₃ administration.
*$p < 0.001$.
†$p < 0.001$.
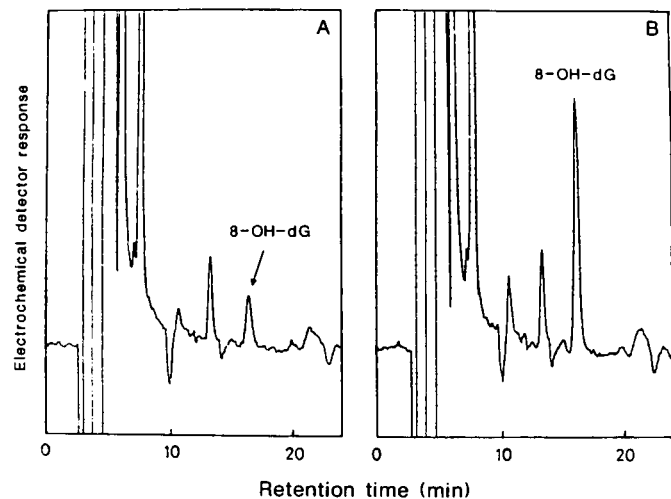‡$p < 0.05$ compared to KBrO₃-treated controls.

FIGURE 21. Analysis of 8-OH-dG using an HPLC-ECD system. *A)* untreated rat kidney DNA, *B)* rat kidney DNA, 24 hr after IG KBrO₃ treatment.

normal levels was observed (Fig. 22), suggesting the presence of repair enzymes for 8-OH-dG in the rat kidney. A slight increase (~ 50%) was observed in the liver, but not significant.

Analysis of 8-OH-dG levels in rats after continuous oral administration of 500 ppm KBrO₃ revealed a significant increase in the kidney after 12 weeks of treat-
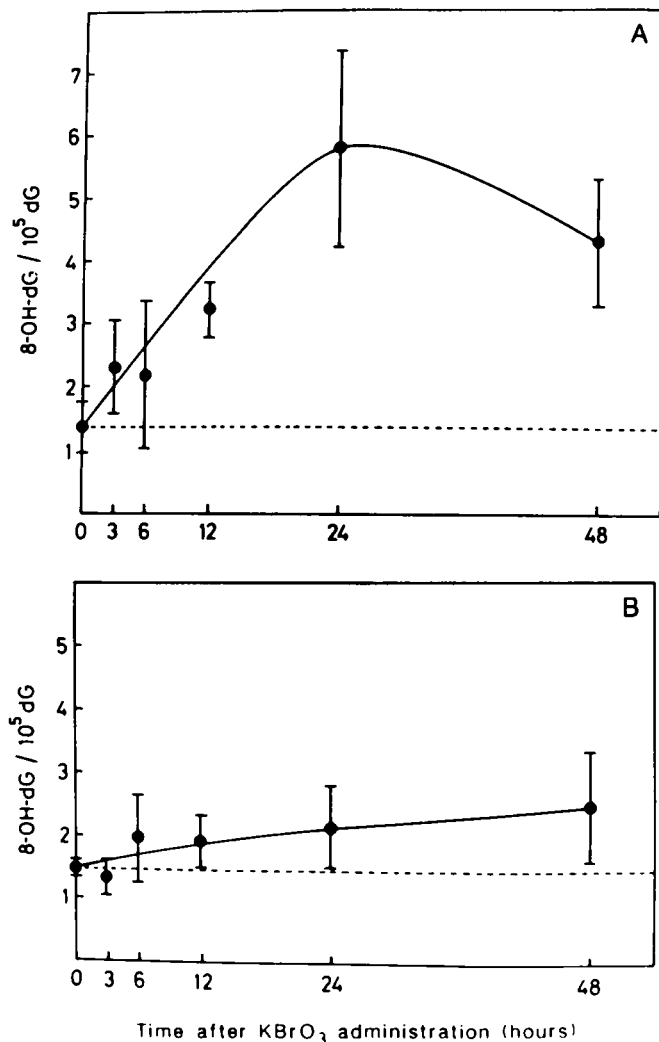
FIGURE 23. Quantity of 8-OH-dG in tissue DNA following oral administration of $KBrO_3$. A) Kidney, B) liver. Points and bars represent mean values and SD for three independent analyses from three separate rats.

FIGURE 22. Quantity of 8-OH-dG in tissue DNA following a single IG $KBrO_3$ treatment. A) Kidney, B) liver. Points and bars represent mean values and SD for three independent analyses from three separate rats.

FIGURE 24. Levels of 8-OH-dG formation in DNA by $KBrO_3$ and/ or methyl linolenate in vitro.

ment, as shown in Figure 23. However, the levels (2-3 residues/10^5 dG) were much lower than in the single application experiment and no elevation was apparent in the liver. These results are compatible with the fact that the latter organ was not found to be a target organ in long-term carcinogenicity studies of $KBrO_3$. The above data clearly showed a positive correlation between the formation of 8-OH-dG in DNA and $KBrO_3$ carcinogenesis and also strongly implicated an involvement of oxygen radicals in the underlying processes. $KBrO_3$ was incubated with DNA in vitro at 37°C for 20 hr, and the formation of 8-OH-dG was not observed (Kasai, personal communication), although it was associated with a markedly enhanced induction of 8-OH-dG by methyl linolenate (Fig. 24). Therefore it is conceivable that in the latter case, $KBrO_3$ produced LPO from methyl linolenate, which resulted in the increased formation of 8-OH-dG from DNA in vitro. The possibility of artificial nonspecific formation of 8-OH-dG by
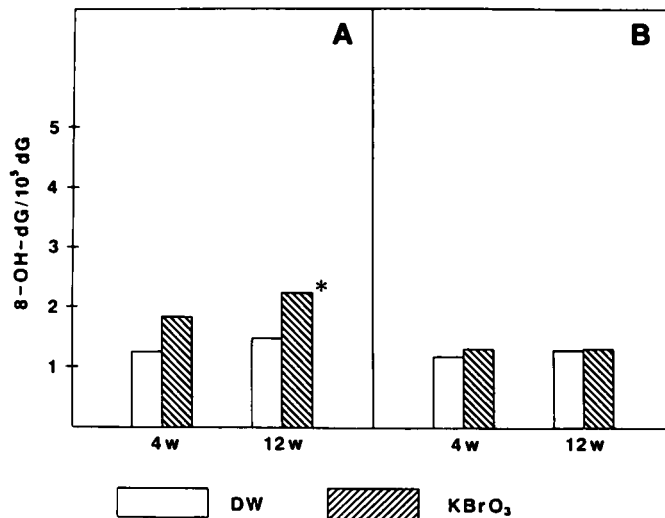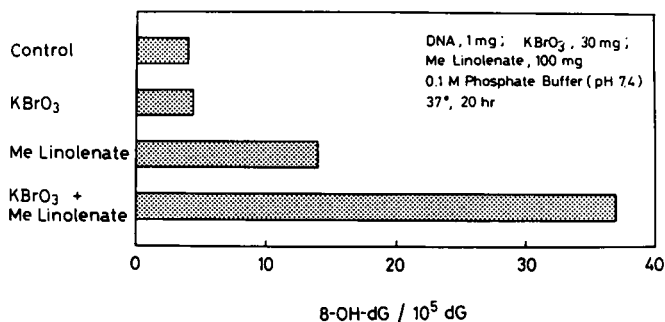
$KBrO_3$ during DNA isolation can be ruled out by the in vitro findings and also as shown previously, by the fact that BrO_3^- was not detected in the kidney 24 hr after IG administration of $KBrO_3$ (72). The 8-OH-dG produced in DNA can thus be considered as one lesion with possible direct involvement in carcinogenesis together with other DNA alterations such as strand scission or thymine glycol formation. At present, however, it is not clear which of these DNA lesions is the most important with regard to $KBrO_3$ carcinogenesis.

General Discussion and Summary

Effects

Although negative results were reported in Great Britain after administration of bread basal diets made from flour treated with $KBrO_3$ at levels of 75 or 50 ppm (49,50), chemical analysis revealed that almost all the

additive is converted to KBr during the normal British baking process (33,34); the actual exposure was therefore negligible. In contrast, $KBrO_3$ is fairly stable when dissolved in water (6), and the concentrations administered were considered as the actual dose levels ingested by the animals.

The carcinogenicity of $KBrO_3$ was clearly established in F344 rats after long-term oral administration in the drinking water at doses of 500 and 250 ppm (6), with significantly higher incidences of RCTs in both sexes and mesotheliomas in males being induced. Subsequent dose-response studies (14) confirmed the generation of RCTs even at the 125 ppm level, and these further demonstrated induction of thyroid follicular adenomas and adenocarcinomas in males given 500 ppm. It has therefore been concluded that tubular epithelial cells of the kidney, mesothelial cells of the peritoneum, and follicular epithelial cells of the thyroid are target cells for $KBrO_3$-carcinogenesis.

While the incidences of RCTs in mice (3 strains) and hamsters were relatively low after long-term oral treatment (11,5), the fact that the RCTs spontaneous develop in these species is very rare suggests that $KBrO_3$ might possess cross-species kidney carcinogenic potential. The weak response might be related to the finding that resistant species are less susceptible than rats to the toxicity of $KBrO_3$.

In most cases current chronic bioassays are incapable of distinguishing between complete carcinogens, incomplete carcinogens (pure initiators), and promoters (83). There is no doubt that $KBrO_3$ can act as a mutagen from the results of both chromosome aberration and micronucleus tests, although the activity is only very weak in some microbial assays (61). After a single IG (600 mg/kg body weight) administration of $KBrO_3$ in vivo, RCTs were observed in 4 of 41 rats after 87 weeks (Kurokawa, unpublished data). This fact, taken together with the significant eventual rate of RCT induction (50%) when the compound was administered in drinking water at 500 ppm for only 13 weeks (17), strongly suggests a positive initiating action of $KBrO_3$.

Furthermore, a promoting action of $KBrO_3$ in renal tumorigenesis was clearly evident in the two-stage carcinogenesis model investigated (5). The threshold level of $KBrO_3$ for promotion of RCTs appeared to be 15 to 30 ppm in the drinking water (8), although no effects on tumors of the liver (5,59), skin (7), or GI tract (Kurokawa, unpublished data) were observed. The available data suggest that $KBrO_3$ should be classified as a complete carcinogen, possessing both initiating and promoting activities for the rat kidney.

A close similarity between $KBrO_3$-related toxicological findings in experimental animals and man has been noted. Disturbance of functions accompanied with the histopathological changes in the kidney and the inner ear have been observed in common in acute $KBrO_3$ intoxication (39,41). It should further be borne in mind that the morphological features of RCTs induced by $KBrO_3$ in rats, mice, and hamsters are very similar to those observed in humans, being essentially the same as those caused by application of other renal carcinogens (6,14). The nephrotoxic action of $KBrO_3$ in animals was found to be reversible in the subacute toxicity (10) and the limited duration protocol experiments (17). In addition, RCTs and DF were induced by $KBrO_3$ in rats at doses < 125 ppm, in which chronic nephropathic changes were only very slight. Therefore, the carcinogenic action of $KBrO_3$ is not dependent on its nephrotoxicity.

Although the occurrence of periarteritis in the pancreas and accelerated aging pathology of the adrenals were observed in rats fed on bread made from flour treated with $KBrO_3$ alone or with $KBrO_3$ and other oxidizing chemicals (49,50), these findings seem to be the effects of the bread diet, since they were not observed after oral administration of $KBrO_3$ even at high dose levels.

Mechanisms

The oxidizing properties of $KBrO_3$ are the reasons for its use as a food additive and industrial chemical. Recently, carcinogenic and promoting potentials of several oxidizing chemicals have been revealed by various in vivo and in vitro studies. In mouse skin carcinogenesis, for example, benzoyl peroxide was found to be a potent promoter (84–86) and weak complete carcinogen (7). The same compound is also suspected as a causative agent for skin cancer in man (87,88). Hydrogen peroxide, lauroyl peroxide, decanoyl peroxide, cumene peroxide, and sodium chlorite were all demonstrated to be promoters in the skin system, albeit relatively weak (7,84,89). Hydrogen peroxide given orally proved to be a carcinogen inducing duodenal tumors in mice (90) and a promoter for the development of intestinal tumors (91) and forestomach papillomas in rats (92). Disturbance of cellular communication, activation of protein kinase C and H-ras oncogene, and induction of DNA strand breaks by oxidizing chemicals have also been recently reported (93–97).

It is generally accepted that the carcinogenic and promoting action of these compounds is caused by generated active oxygen species (98–101). Furthermore, studies on oxidant chemicals such as paraquat (102), ozone (103), and NOx (104), have clearly demonstrated that they all induce LPO in their target organs, and the induction of LPO and clastogenic activity are now considered to be the main factors underlying the carcinogenic and/or promoting effects shown by these agents.

Based on the oxidizing property of $KBrO_3$, the levels of kidney LPO were examined in animals administered this compound (18). The findings of significant increases in kidney LPO levels in both a dose-dependent and time-dependent manner in rats, but not mice or hamsters, seem to imply a possible relationship between LPO formation in the kidney and the species differences in the renal toxicity and carcinogenicity of $KBrO_3$.

In addition, a protective role of cysteine and GSH

against cellular oxidative damage and LPO formation is well documented. In $KBrO_3$-treated rats, treatment with cysteine or GSH was similarly associated with a protective effect in terms of mortality and various biochemical parameters indicative of nephrotoxicity and the appearance of lipofuscin pigments, which are considered to be induced by active oxygen radicals (18). The fact that GHS and cysteine decreased the numbers of eosinophilic bodies in the renal tubular cells also implies that they are the result of cellular oxidative damage by active oxygen radicals (48). On the other hand, treatment with DEM, a GSH depleter, resulted in an exacerbation of these lesions. From in vitro studies it was found that homogenates of kidney, liver, and RBC possess degradative activities for BrO_3^-. In studies of homogenate supernatant by fractionation, GSH was identified as an involved factor and SH-group compounds showed direct degradative activity when incubated in vitro with BrO_3^- (73). Thus, it is possible that protective agents could be depleted by $KBrO_3$ administration leading to overload and toxicity with high doses.

The fact that 8-OH-dG, a DNA-damaged product formed by oxygen-radical generating agents, was detected in the kidney of rats treated with $KBrO_3$ is noteworthy in this respect (19). Significantly increased levels of 8-OH-dG were observed after either a single IG dose or continuous oral administration of $KBrO_3$ in the kidney, but not in the liver. In contrast, the noncarcinogenic oxidizing agents sodium chlorite and sodium hypochlorite had no effect on 8-OH-dG formation (19). These results are therefore in agreement with the hypothesis that formation of 8-OH-dG in tissue DNA is closely related to organ specificity in carcinogenesis. On the other hand, incubation of $KBrO_3$ directly with DNA in vitro did not result in 8-OH-dG generation, although it did increase the level of 8-OH-dG produced by methyl linolenate.

In summary, we suggest that $KBrO_3$ produces LPO from unsaturated fatty acids in vivo through its oxidizing actions, and the genotoxic activity of $KBrO_3$ may be the result of DNA damage by LPO and/or active oxygen radicals generated in the process of LPO formation. Recently we found that hydroxy radicals were generated in vitro by $KBrO_3$ using electron spin resonance (Kurokawa, unpublished data).

Meanwhile, further research will be needed to clarify the mechanism of action of $KBrO_3$ for induction of peritoneal mesotheliomas and thyroid follicular cell tumors.

Risk Assessment, Regulatory Status, and Future Prospects

Toxicologic studies of $KBrO_3$ were reviewed by the JECFA in 1964 (22) and 1979 (24). As a result, it was evaluated as one of the safe-to-use food additives and listed within Class A (1). However, since more recent studies provided strong evidence of its carcinogenicity, it was decided at a 1983 meeting that the previous acceptance for the treatment of flour used for baking products should be changed to a temporary acceptance with a maximum treatment level of 75 mg $KBrO_3$ per kg of flour, provided that bakery products prepared from such treated flour could be shown to contain only negligible residues of $KBrO_3$ (25). No acceptable level was allocated for use in other foods. In 1982, the Ministry of Health and Welfare of Japan had already decided to lower the maximum treatment level of $KBrO_3$ for flour from 50 mg/kg to 30 mg/kg. At the same time, the use of $KBrO_3$ for the improvement of fish paste products was banned (26).

The residual levels of $KBrO_3$ at currently acceptable flour treatment doses have been reported to be negligible in bread (32,34). In fact, no carcinogenic action was detectable after feeding bread-based diets in long-term bioassays (49,50). Therefore, in consideration of the fact that almost all $KBrO_3$ added to the flour is converted to KBr during the bread-baking process (34), future concern should be directed toward the toxicological effects of KBr in humans (105); so far, no promoting and only weak mutagenic activities have been demonstrated for this compound (8,62).

Recently the concept of a virtually safe dose (VSD) has been proposed as a useful parameter for risk assessment, especially for genotoxic carcinogens (106, 107). The VSD values, based on data for RCTs from the dose-response studies (Table 6) estimated by different models at a risk level of 10^{-6}, are listed in Table 23 (12–14). The VSD value of 0.950 ppm $KBrO_3$ was obtained for RCTs by the Probit model with an independent background, with the largest p-value (0.898), which indicates a good fit.

In the similar evaluation process to IARC, which has been adopted by the U.S. Environmental Protection Agency (EPA) (108), this compound will probably be included in Group B2, because of sufficient evidence from animal studies and no data from epidemiologic studies. Alternatively, $KBrO_3$ can be classified as a compound showing "clear evidence of carcinogenicity," ac-

Table 23. VSD calculated for RCTs at a risk level of 10^{-6}.

Lesion	Type of count	VSD values by models[a]			
		Probit	Logit	Weibul	Gamma multihit
Renal cell tumor	Chi-square value	1.627	2.155	2.472	2.693
	p-Value	0.898	0.827	0.781	0.747
	VSD[b]	0.950	0.160×10^{-1}	0.481×10^{-2}	0.182×10^{-2}

[a] All models are with independent background.
[b] $KBrO_3$ dose in ppm, in the drinking water.

cording to the categorization used by the National Toxicology Program (109).

Although active oxygen radicals have been anticipated to play an important role in the carcinogenic process on the basis of various in vitro studies (110), the numbers of in vivo models in which this hypothesis could be confirmed are limited. In the case of KBrO₃, there is increasing evidence to suggest that active oxygen species are actually involved in its carcinogenic and toxic effects. Therefore we believe that KBrO₃ could provide a key for future investigation of this intriguing area of carcinogenesis research (111).

Conclusion

KBrO₃ exerts nephrotoxic and ototoxic effects in experimental animals as well as in man. KBrO₃ is a genotoxic carcinogen inducing renal cell tumors, mesotheliomas, and thyroid follicular cell tumors in rats. KBrO₃ is a complete carcinogen having both initiating and promoting activities for the development of renal cell tumors. It is highly probable that active oxygen radicals are involved in the demonstrated carcinogenic and toxic effects. Commercial bread made from flour treated with KBrO₃ is not carcinogenic in experimental animals, probably because almost all of the KBrO₃ is converted to KBr during the bread-baking process. KBrO₃ is a useful new compound for analyzing the roles played by active oxygen radicals in carcinogenesis, both in vivo and in vitro.

REFERENCES

1. Odashima, S. Cooperative programme on long-term assays for carcinogenicity in Japan. In: Molecular and Cellular Aspects of Carcinogen Screening Tests, IARC Publication No. 27 (R. Montesano, H. Bartsch, and L. Tomatis, Eds.), International Agency for Research on Cancer, Lyon, France, 1980, pp. 315–322.
2. Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M. Jr., Sasaki, M., and Sugiyama, T. Cooperative programme on short-term assays for carcinogenicity in Japan. In: Molecular and Cellular Aspects of Carcinogen Screening Tests, IARC Scientific Publications No. 27 (R. Montesano, H. Bartsch, and L. Tomatis, Eds.), International Agency for Research on Cancer, Lyon, France, 1980, pp. 330–333.
3. Kurokawa, Y., Hayashi, Y., Maekawa, A., Takahashi, M., and Kokubo, T. Induction of renal cell tumors in F-344 rats by oral administration of potassium bromate, a food additive. Gann 73: 335–338 (1982).
4. Ohno, Y., Onodera, H., Takamura, N., Imazawa, T., Maekawa,

A., and Kurokawa, Y. Carcinogenicity testing of potassium bromate in rats [in Japanese]. Bull. Natl. Inst. Hyg. Sci. 100: 93–100 (1982).
5. Kurokawa, Y., Takahashi, M., Kokubo, T., Ohno, Y., and Hayashi, Y. Enhancement by potassium bromate of renal tumorigenesis initiated by N-ethyl-N-hydroxyethylnitrosamine in F-344 rats. Gann 74: 607–610 (1983).
6. Kurokawa, Y., Hayashi, Y., Maekawa, A., Takahashi, M., Kokubo, T., and Odashima, S. Carcinogenicity of potassium bromate administrated orally to F344 rats. J. Natl. Cancer Inst. 71: 965–972 (1983).
7. Kurokawa, Y., Takamura, N., Matsushima, Y., Imazawa, T., and Hayashi, Y. Studies on the promoting and complete carcinogenic activities of some oxidizing chemicals in skin carcinogenesis. Cancer Lett. 24: 299–304 (1984).
8. Kurokawa, Y., Aoki, S., Imazawa, T., Hayashi, Y., Matsushima, Y., and Takamura, N. Dose-related enhancing effect of potassium bromate on renal tumorigenesis in rats initiated with N-ethyl-N-hydroxyethylnitrosamine. Jpn. J. Cancer Res. (Gann) 76: 583–589 (1985).
9. Kurokawa, Y. Overview on the toxicity and carcinogenicity of potassium bromate [in Japanese]. Kosankinbyo Kenkyuzasshi 37: 139–149 (1985).
10. Onodera, H., Tanigawa, H., Matsushima, Y., Maekawa, A., Kurokawa, Y., and Hayashi, Y. Eosinophilic bodies in the proximal renal tubules of rats given potassium bromate [in Japanese]. Bull. Natl. Inst. Hyg. Sci. 103: 15–20 (1985).
11. Takamura, N., Kurokawa, Y., Matsushima, Y., Imazawa, T., Onodera, H., and Hayashi, Y. Long-term oral administration of potassium bromate in male Syrian golden hamsters. Sci. Rep. Res. Inst. Tohoku Univ., Ser. C. 32: 43–46 (1985).
12. Hayashi, Y., Kurokawa, Y., and Maekawa, A. Risk evaluation of tumor-inducing substances in foods. In: Diet, Nutrition and Cancer (Y. Hayashi, M. Nagao, T. Sugimura, S. Takayama, L. Tomatis, L. W. Wattenberg, and G. N. Wogan, Eds.), Japan Scientific Societies Press, Tokyo, 1986, pp. 295–303.
13. Hayashi, Y., Kurokawa, Y., Maekawa, A., and Takahashi, M. Strategy of long-term animal testing for quantitative evaluation of chemical carcinogenicity. In: New Concepts and Developments in Toxicology (P. L. Chambers, P. Gehring, and F. Sakai, Eds.), Elsevier Science Publishers, New York, pp. 383–391 (1986).
14. Kurokawa, Y., Aoki, S., Matsushima, Y., Takamura, N., Imazawa, T., and Hayashi, Y. Dose-response studies on the carcinogenicity of potassium bromate in F344 rats after long-term oral administration. J. Natl. Cancer Inst. 77: 977–982 (1986).
15. Kurokawa, Y., Takayama, S., Konishi, Y., Hiasa, Y., Asahina, S., Takahashi, M., Maekawa, A., and Hayashi, Y. Long-term in vivo carcinogenicity test of potassium bromate, sodium hypochlorite and sodium chlorite conducted in Japan. Environ. Health Perspect. 69: 221–235 (1986).
16. Matsushima, Y., Takamura, N., Imazawa, T., Kurokawa, Y., and Hayashi, Y. Lack of carcinogenicity of potassium bromate after subcutaneous injection to newborn mice and newborn rats. Sci. Rep., Tohoku Univ., Ser.-C. 33: 22–26 (1986).
17. Kurokawa, Y., Matsushima, Y., Takamura, M., Imazawa, T., and Hayashi, Y. Relationship between the duration of treatment and the incidence of renal cell tumors in male F344 rats administered potassium bromate. Jpn. J. Cancer Res. (Gann) 78: 358–364 (1987).
18. Kurokawa, Y., Takamura, N., Matsuoka, C., Imazawa, T., Matsushima, Y., Onodera, H., and Hayashi, Y. Comparative studies on lipid peroxidation in the kidney of rats, mice and hamsters and on the effect of cysteine, glutathione and diethyl maleate treatment on mortality and nephrotoxicity after administration of potassium bromate. J. Am. Coll. Toxicol. 6: 489–501 (1987).
19. Kasai, H., Nishimura, S., Kurokawa, Y., and Hayashi, Y. Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ. Carcinogenesis 12: 1959–1961 (1987).
20. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Ra-

diation IARC Publication No. 40, World Health Organization/ IARC, Lyon, France, 1986, pp. 207–220.

21. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. IARC, Lyon, France, 1987, Suppl. 7., p. 70.

22. Expert Committee on Food Additives. Seventh Report on the Specifications for the Identity and Purity of Food Additives and Their Toxicological Evaluation: Emulsifiers, Stabilizers, Bleaching and Maturing Agents. World Health Organization Technical Report, Series 281, Geneva, 1964, p. 164.

23. Food and Agriculture Organization-World Health Organization. List of Additives Evaluated for the Safety-in-Use in Food, First Series. World Health Organization, Geneva, 1973, p. 26.

24. Food and Agriculture Organization—World Health Organization. Guide to the Safe Use of Food Additives, Second Series. World Health Organization, Geneva, 1979, p. 60.

25. Expert Committee on Food Additives. Twenty-seventh Report. Evaluation of Certain Food Additives and Contaminants. World Health Organization, Geneva, 1983, p. 27.

26. The Ministry of Health and Welfare Japan. The Japanese Standards of Food Additives, 5th edition. The Ministry of Health and Welfare, Tokyo, 1986, p. 433.

27. Norris, J. A. Toxicity of home permanent waving and neutralizer solutions. Food Cosmet. Toxicol. 3: 93–97 (1965).

28. Oikawa, K., Saito, H., Sakazume, S., and Fujii, M. Behavior of bromate in bread by ion chromatography. Chemosphere 11: 953–961 (1982).

29. Watanabe, I., Tanaka, R., and Kashimoto, T. Determination of potassium bromate by ion chromatography [in Japanese]. J. Food Hyg. Soc. Japan. 23: 135–141 (1982).

30. Hidaka, T., Kirigaya, T., Kamijo, M., Suzuki, Y., and Kawamura, T. Behavior of potassium bromate added to bread and fish paste products during preparation [in Japanese]. J. Food Hyg. Soc. Jpn. 24: 383–389 (1983).

31. Yamamoto, A., Matsunaga, A., Sekiguchi, H., Hayakawa, K., and Miyazaki, M. Determination of potassium bromate in bread and Kamaboko by photometric ion chromatography [in Japanese]. Eisei Kagaku. 31: 47–50 (1985).

32. Mohri, T., Nishioka, C., Ishikawa, H., and Kuroda, H. Determination of potassium bromate in food by high performance liquid chromatography [in Japanese]. J. Food Hyg. Soc. Jpn. 26: 260–265 (1985).

33. Bushuk, W., and Hlynka, I. Disappearance of bromate during baking of bread. Cereal Chem. 37: 573–576 (1960).

34. Thewlis, B. H. The fate of potassium bromate when used as a breadmaking improver. J. Sci. Food. Agric. 25: 1471–1475 (1974).

35. Oinuma, T. 8 cases of death by intoxication of potassium bromate [in Japanese]. Nichidaiishi 33: 759–766 (1974).

36. Gradus (Ben-Ezer), D., Rhoads, M., Bergstrom, L. B., and Jordan, S. C. Acute bromate poisoning associated with renal failure and deafness presenting as hemolytic uremic syndrome. Am. J. Nephrol. 4: 188–191 (1984).

37. Kuwahara, T., Ikehara, Y., Kanatsu, K., Doi, T., Nagai, H., Nakayashiki, H., Tamura, T., and Kawai, C. Two cases of potassium bromate poisoning requiring long-term hemodialysis therapy for irreversible tubular damage. Nephron 37: 278–280 (1984).

38. Dunsky, I. Potassium bromate poisoning. Am. J. Dis. Child. 74: 734–743 (1947).

39. Matsumoto, I. Clinical and experimental studies on ototoxicity of bromate [in Japanese]. Otol. Fukuoka 19: 220–236 (1973).

40. Quick, C. A., Chole, R. A., and Mauer, S. M. Deafness and renal failure due to potassium bromate poisoning. Arch. Otolaryngol. 101: 494–495 (1975).

41. Mizushima, N. Experimental study on the ototoxicity of the bromate [in Japanese]. Nichidaiishi 37: 1057–1082 (1978).

42. Onoue, M., Uchida, K., Takahashi, T., Kusano, N., and Mutai, M. Relationship between some biochemical measurements and histopathological changes in age-related kidney lesions of rats [in Japanese]. Exp. Anim. 27: 405–412 (1978).

43. Uchida, K., Onoue, M., Takahashi, T., Kusano, N., and Mutai,

M. Histopathological findings of age-related kidney lesions in inbred strain Fischer-344/Yit rats [in Japanese]. Exp. Anim. 29: 45–54 (1980).

44. Kanerva, R. L., Ridder, G. M., Stone, L. C., and Alden, C. L. Characterization of spontaneous and decalin-induced hyaline droplets in kidneys of adult male rats. Fed. Chem. Toxic. 25: 63–82 (1987).

45. Loury, D. J., Smith-Oliver, T., and Butterworth, B. E. Assessment of the covalent binding potential of 2,2,4-trimethylpentane to rat α_{2u}-globulin. Toxicol. Appl. Pharmacol. 88: 44–56 (1987).

46. Olson, M. J., Garg, B. D., Murty, C. V. R., and Roy, A. K. Accumulation of α_{2u}-globulin in the renal proximal tubules of male rats exposed to unleaded gasoline. Toxicol. Appl. Pharmacol. 90: 43–51 (1987).

47. Tanigawa, H., Mitui, M., Shimazaki, I., Maekawa, A., Onodera, H., and Kurokawa, Y. Sex difference in the appearance of eosinophilic bodies in rats given potassium bromate. In: Proceedings of the 15th Annual Japanese Toxicologic Society, Sendai, 1988.

48. Umemura, T., Takada, K., Sai, K., Kaneko, T., and Kurokawa, Y. Sex difference in the rat renal toxicity after p-dichlorobenze treatment. In: Proceedings of the 15th Annual Japanese Toxicologic Society, Sendai, 1988.

49. Fisher, N., Hutchinson, J. B., Hardy, J., Ginocchio, A. V., and Waite, V. Long-term toxicity and carcinogenicity studies of the bread improver potassium bromate. 1. Studies in rats. Food Cosmet. Toxicol. 17: 33–39 (1979).

50. Ginocchio, A. V., Waite, V., Hardy, J., Fisher, N., Hutchinson, J. B., and Berry, R. Long-term toxicity and carcinogenicity studies of the bread improver potassium bromate. 2. Studies in mice. Food Cosmet. Toxicol. 17: 41–47 (1979).

51. Littlefield, N. A., Farmer, J. H., and Gaylor, D. W. Effects of dose and time in a long-term low-dose carcinogenic study. J. Environ. Pathol. Toxicol. 3: 17–34 (1979).

52. Littlefield, N. A., and Gaylor, D. W. Influence of total dose and dose rate in carcinogenicity studies. J. Toxicol. Environ. Health 15: 545–550 (1985).

53. Kurokawa, Y., Matsushima, Y., and Hayashi, Y. Long-term oral administration of potassium bromate to mice. In: Proceedings of the 46th Annual Meeting of the Japanese Cancer Association, Tokyo, 1987.

54. Ward, J. M., Goodman, D. G., Squire, R. A., Chu, K. C., and Linhart, M. S. Neoplastic and non-neoplastic lesions in aging B6C3F1 mice. J. Natl. Cancer Inst. 63: 849–854 (1979).

55. Schmidt, R. E., Eason, R. L., Hubbard, G. B., Young, J. T., and Eisenbrandt, D. L. Urinary System in Pathology of Aging Syrian Hamsters. CRC Press, Inc., Boca Raton, FL, 1983, pp. 89–106.

56. Fukushima, A., Hirose, M., Hagiwara, A., Hasegawa, R., and Ito, N. Inhibitory effect of 4,4'-diaminodiphenylmethane on liver, kidney and bladder carcinogenesis in rats ingesting N-ethyl-N-hydroxyethylnitrosamine or N-butyl-N-(4-hydroxybutyl)nitrosamine. Carcinogenesis 2: 1033–1037 (1981).

57. Hirose, M., Shirai, T., Tsuda, H., Fukushima, S., Ogiso, T., and Ito, N. Effect of phenobarbital, polychlorinated biphenyl and sodium saccharin on hepatic and renal carcinogenesis in unilaterally nephrectomized rats given N-ethyl-N-hydroxyethylnitrosamine orally. Carcinogenesis 2: 1299–1302 (1981).

58. Hiasa, Y., Ohshima, M., Kitahori, Y., Konishi, N., Fujita, T., and Yuasa, T. β-Cyclodextrin: promoting effect on the development of renal tubular cell tumors in rats treated with N-ethyl-N-hydroxyethylnitrosamine. J. Natl. Cancer Inst. 69: 963–967 (1982).

59. Ito, N., Tsuda, H., Tatematsu, M., Inoue, T., Tagawa, Y., Aoki, T., Uwagawa, S., Ogiso, T., Masui, T., Imaida, K., Fukushima, S., and Asamoto, M. Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats—an approach for a new medium-term bioassay system. Carcinogenesis 9: 387–394 (1988).

60. Tsuda, H., Fukushima, S., Imaida, K., Kurata, Y., and Ito, N. Organ-specific promoting effect of phenobarbital and saccharin in induction of thyroid, liver and urinary bladder tumors in rats

after initiation with N-nitrosomethylurea. Cancer Res. 43: 3292–3296 (1983).

61. Ishidate, M. Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., and Matsuoka, A. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22: 623–636 (1984).

62. Ishidate, M. Jr., Yoshikawa, K., and Sofuni, T. Studies on the mutagenicity of potassium bromate and other oxidizing chemicals. In: Proceedings of the 41st Annual Meeting of the Japanese Cancer Association, 1982.

63. Levin, D. E., Hollstein, M. C., Christman, M. F., Schwiers, E. A., and Ames, B. N. A new Salmonella tester strain (TA102) with A:T base pairs at the site of mutation detects oxidative mutagens. Proc. Natl. Acad. Sci. USA 79: 7445–7449 (1982).

64. Ishidate, M. Jr., and Yoshikawa, K. Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation—a comparative study on mutagens and carcinogens, Arch. Toxicol. Suppl. 4: 41–44 (1980).

65. Ishidate, M. Jr., Sofuni, T., and Yoshikawa, K. Chromosomal aberration tests in vitro as a primary screening tool for environmental mutagens and/or carcinogens. Gann Monogr. Cancer Res. 27: 95–108 (1981).

66. Ishidate, M. Jr. Data Book of Chromosomal Aberration Tests In Vitro, revised edition. L. I. C. Inc., Tokyo, 1987, p. 334.

67. Sasaki, M., Sugimura, K., Yoshida, M. A., and Abe, S. Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. Kromosomo II: 20: 574–584 (1980).

68. Fujie, K., Shimazu, H., Matsuda, M., and Sugiyama, T. Acute cytogenetic effects of potassium bromate on rat bone marrow cells in vivo. Mutat. Res. 206: 455–458 (1988).

69. Hayashi, M., Kishi, M., Sofuni, T., and Ishidate, M. Jr. Micronucleus tests with mice on 39 food additives and 8 miscellaneous chemical substances. Food. Chem. Toxicol. 26: 487–500 (1988).

70. Hayashi, M., Sofuni, T., and Ishidate, M. Jr. High-sensitivity in micronucleus induction of a mouse strain (MS). Mutat. Res. 105: 253–256 (1982).

71. The collaborative study group for the micronucleus test. Sex difference in the micronucleus test. Mutat. Res. 172: 151–163 (1986).

72. Fujii, M., Oikawa, K., Saito, H., Fukuhara, C., Onosaka, S., and Tanaka, T. Metabolism of potassium bromate in rats. I. In vivo studies. Chemosphere 13: 1207–1212 (1984).

73. Tanaka, K., Oikawa, K., Fukuhara, C., Saito, H., Onosaka, S., Min, K. S., and Fujii, M. Metabolism of potassium bromate in rats. II. In vitro studies. Chemosphere 13: 1213–1219 (1984).

74. Bus, J. S., and Bibson, J. E. Lipid peroxidation and its role in toxicology. Rev. Biochem. Toxicol. 1: 125–149 (1979).

75. Casini, A. F., Pompelia, A., and Comporti, M. Glutathione depletion, Lipid peroxidation, and liver necrosis following bromobenzene and iodobenzene intoxication. Toxicol. Pathol. 12: 295–299 (1984).

76. Ohkawa, H., Ohishi, N., and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351–358 (1979).

77. Yonaha, M., Saito, M., and Sagai, M. Stimulation of lipid peroxidation by methyl mercury in rats. Life Sci. 32: 1507–1514 (1983).

78. Fukino, H., Hirai, M., Hsueh, Y. M., and Yamane, Y. Effect of zinc pretreatment on mercuric chloride-induced lipid peroxidation in the rat kidney. Toxicol. Appl. Pharmacol. 73: 395–401 (1984).

79. Sunderman, F. W., Jr., Marzouk, A., Hopfer, S. M., Zaharia, O., and Reid, M. C. Increased lipid peroxidation in tissues of nickel chloride-treated rats. Ann. Clin. Lab. Sci. 15: 229–236 (1985).

80. Kasai, H., and Nishimura, S. Hydroxylation of guanine in nucleosides and DNA at the C-8 position by heated glucose and oxygen radical-forming agents. Environ. Health Perspect. 67: 111–116 (1986).

81. Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A., and Tanooka, H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 7: 1849–1851 (1986).

82. Floyd, R. A., Watson, J. J., Harris, J., West, M., and Wong, P. K. Formation of 8-hydroxydeoxyguanosine, hydroxy free radical adduct of DNA in granulocytes exposed to the tumor promoter, tetradecanoylphorbolacetate. Biochem. Biophys. Res. Commun. 137: 841–846 (1986).

83. Goldsworthy, T. L., and Pitot, H. C. An approach to the development of a short-term whole-animal bioassay to distinguish initiating agents (incomplete carcinogens), promoting agents, complete carcinogens, and noncarcinogens in rat liver. J. Toxicol. Environ. Health 16: 389–402 (1985).

84. Slaga, T. J., Klein-Szanto, A. J. P., Triplett, L. L., and Yotti, L. P. Skin tumor-promoting activity of benzoyl peroxide, a widely used free radical-generating compound. Science 213: 1023–1025 (1981).

85. O'Connell, J. F., Klein-Szanto, A. J. P., DiGiovanni, D. M., Fries, J. A. W., and Slaga, T. J. Enhanced malignant progression of mouse skin tumors by the free-radical generator benzoyl peroxide. Cancer Res. 46: 2863–2865 (1986).

86. Schweizer, J., Loehrke, H., Edler, L., and Goerttler, K. Benzoyl peroxide promotes the formation of melanotic tumors in the skin of 7,12-demethylbenz(a)anthracene-initiated Syrian golden hamsters. Carcinogenesis 8: 479–482 (1987).

87. Jones, G. R. N. Skin cancer: risk to individuals using the tumour promoter benzoyl peroxide for acne treatment. Human Toxicol. 4: 75–78 (1985).

88. Jackson, E. M. Benzoyl peroxide: an old drug with new problems. J. Toxicol. Cutaneous Ocul. Toxicol. 5: 163–165 (1986).

89. Klein-Szanto, A. J. P., and Slaga, T. J. Effects of peroxides on rodent skin: epidermal hyperplasia and tumor promotion. J. Invest. Dermatol. 79: 30–34 (1982).

90. Ito, A., Watanabe, H., Naito, M., and Naito, Y. Induction of duodenal tumors in mice by oral administration of hydrogen peroxide. Gann 72: 174–175 (1981).

91. Hirota, N., and Yokayama, T. Enhancing effect of hydrogen peroxide upon duodenal and upper jejunal carcinogenesis in rat. Gann 72: 811–812 (1981).

92. Takahashi, M., Hasegawa, R., Furukawa, F., Toyoda, K., Sato, H., and Hayashi, Y. Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with N-methyl-N'-nitro-N-nitrosoguanidine. Jpn. J. Cancer Res. (Gann) 77: 118–124 (1986).

93. Lawrence, N. J., Parkinson, E. K., and Emmerson, A. Benzoyl peroxide interferes with metabolic co-operation between cultured human epidermal keratinocytes. Carcinogenesis 5: 419–421 (1984).

94. Gindhart, T. D., Srinivas, L., and Colburn, N. H. Benzoyl peroxide promotion of transformation of JB6 mouse epidermal cells: inhibition by ganglioside G_t but not retinoic acid. Carcinogenesis 6: 309–311 (1985).

95. Donnelly, T. E., Jr., Pelling, J. C., Anderson, C. L., and Dalbey, D. Benzoyl peroxide activation of protein kinase C activity in epidermal cell membranes. Carcinogenesis 8: 1871–1874 (1987).

96. Pelling, J. C., Fischer, S. M., Neades, R., Strawhecker, J., and Schweickert, L. Elevated expression and point mutation of the Ha-ras protooncogene in mouse skin tumors promoted by benzoyl peroxide and other promoting agents. Carcinogenesis 8: 1481–1484 (1987).

97. Hartley, J. A., Gibson, N. W., Kilkenny, A., and Yuspa, H. Mouse keratinocytes derived from initiated skin or papillomas are resistant to DNA strand breakage by benzoyl peroxide: a possible mechanism for tumor promotion mediated by benzoyl peroxide. Carcinogenesis 8: 1827–1830 (1987).

98. Fisher, S. M., Floyd, R. A., and Copeland, E. S. Meeting Report. Workshop report from the division of research grants. National Institute of Health. Oxy radicals in carcinogenesis-A chemical pathology study section workshop. Cancer Res. 48: 3882–3887 (1988).

99. Ames, B. N. Dietary carcinogens and anticarcinogens. Science 221: 1256–1264 (1983).

100. Nishimura, S., and Ames, B. N. U.S.-Japan conference on Ox-

ygen radicals in cancer. Jpn. J. Cancer Res. (Gann) 77: 843–848 (1986).

101. Halliwell, B. Oxidants and humans disease: some new concepts. Fed. Am. Soc. Exp. Biol. J. 1: 358–364 (1987).

102. Bus, J. S., and Gibson, J. E. Paraquat: model for oxidant-initiated toxicity. Environ. Health Perspect. 55: 37–46 (1984).

103. Menzel, D. B. Ozone: an overview of its toxicity in man and animals. J. Toxicol. Environ. Health 13: 183–204 (1984).

104. Morrow, P. E. Toxicological data on NO$_x$: an overview. J. Toxicol. Environ. Health 13: 205–227 (1984).

105. van Leeuwen, F. X. R., and Sangster, B. The toxicology of bromide ion. CRC Crit. Rev. Toxicol. 18: 189–213 (1987).

106. Clayson, D. B. Dose relationships in experimental carcinogenesis: dependence on multiple factors including biotransformation. Toxicol. Pathol. 13: 119–127 (1985).

107. Sielken, R. L. Jr. Some issues in the quantitative modeling portion of cancer risk assessment. Regul. Toxicol. Pharmacol. 5: 175–181 (1985).

108. Federal Register. 185: 51 (1986).

109. Haseman, J. K., Tharrington, E. C., Huff, J. E., and McConnell, E. E. Comparison of site-specific and overall tumor incidence analyses for 81 recent National Toxicology Program carcinogenicity studies. Regul. Toxicol. Pharmacol. 6: 155–170 (1986).

110. Marnett, L. J. Peroxyl free radicals: potential mediators of tumor initiation and promotion. Carcinogenesis 8: 1365–1373 (1987).

111. Chemical Induction of Cancer. Structural Bases and Biological Mechanisms, Vol. IIIC. Natural, Metal, Fiber, and Macromolecular Carcinogens (Y. Woo, D. Y. Lai, and M. F. Argus, Eds.), Academic Press, Inc., New York, 1988, p. 505.