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Subacute Toxicity Study of 40 kGy Irradiated Ready-to-Eat *Bulgogi*

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Abstract

The wholesomeness of 40 kGy irradiated ready-to-eat (RTE) *bulgogi* was evaluated by subacute toxicity studies (body weight, food consumption, organ weight, hematology, serum biochemistry, and histopathological examination) with groups of 40 male and female ICR mice fed the agent at dietary levels of 5% for 90 days. There were no treatment-related adverse effects with regard to body weight, food consumption, organ weight, hematology, serum biochemistry, and histopathology. The no-observed-adverse-effect-level (NOAEL) was also determined to be greater than dietary level of at least 5% (3900 mg/kg body weight/day for males, 3500 mg/kg body weight/day for females) for samples under the present experimental conditions. These results suggest that, under these experimental conditions, RTE *bulgogi* irradiated at 40 kGy did not show any toxic effects.

Key words: *bulgogi*, high-dose irradiation, subacute toxicity, ICR mice

INTRODUCTION

Gamma irradiation has become an effective sterilization method that is widely used for food, agricultural and hygienic products (1,2). It has been confirmed that irradiation inactivates pathogenic or saprogenic microorganisms selectively and that the original qualities of the irradiated food are quite stable (3,4). Furthermore, gamma irradiation is convenient for industrial application (5).

It has been reported that when the gamma irradiation below 10 kGy was applied to ready-to-cook *bulgogi* and *bulgogi* sauce, there were significant positive effects on the improvement of hygienic quality and elongation of shelf-life (6-8). However, studies on the wholesomeness of high-dose irradiated *bulgogi*, which was the most important consideration for the consumer acceptance and industrial application, have not been conducted.

The wholesomeness of irradiated foods has been evaluated in many *in vitro* and *in vivo* systems, and the results showed that food irradiation was the cleanest and safest method for food preservation (5). At the Joint Expert Committee on the wholesomeness of irradiated food in 1980, it was concluded that no toxicological hazards or specific nutritional or microbiological problems had been found in any food commodity irradiated up to an overall average dose of 10 kGy (9). In spite of these conclusions, many consumers and managers working in food industries require a safety insurance against each irradiated item (10).

Even though the 10 kGy dose has been used to make *bulgogi* and *bulgogi* sauce free from microbes (6-8), evaluating the wholesomeness of high-dose irradiated samples at more than 10 kGy is also necessary to address concerns regarding human consumption. Thus, the purposes of this study were to evaluate the wholesomeness of 40 kGy irradiated ready-to-eat (RTE) *bulgogi* using subacute toxicity studies, such as body weight, food consumption, organ weight, hematology, serum biochemistry, and histopathology, in ICR mice fed the agent at dietary levels of 5% for 90 days.

MATERIALS AND METHODS

Materials

Korean beef (tenderloin), soy sauce, sugar, salt, onion, green onion, garlic, sesame, red wine, and black pepper powder were purchased from local stores. The tenderloin from three Korean beefs was obtained after 25 hr of slaughter, and cut into slices ($2 \times 2 \times 0.8$ cm) with a knife. Xanthan gum powder, vitamin-C and α-tocopherol were obtained from a company that manufactures food additives (MSC Co., Gyeongnam, Korea).

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Table 1. Ingredients in *bulgogi* sauce and *bulgogi*

Sample	Raw materials	Ratio (%)
Sauce	Soy sauce	8.00
	Onion	4.00
	Green onion	1.60
	Garlic	1.40
	Sesame	0.50
	Sugar	4.00
	Xanthan gum	0.50
	Water	30.00
Marinated beef	Beef (tenderloin)	40.00
	Red wine	9.40
	Salt	0.25
	Black pepper powder	0.35
Sum		100.00

Table 2. Ingredients in feeds containing 0 kGy and 40 kGy irradiated *bulgogi*

Ingredient (%)	AIN93G	0 kGy	40 kGy
Casein	20	15	15
Bulgogi powder	0	5	5
Corn starch	39.7	38.62	38.62
Dyetrose	13.2	13.2	13.2
Sucrose	10	0	0
Cellulose	5	6.2	6.2
Soybean oil	7	6.88	6.88
Mineral mix	3.5	3.5	3.5
Vitamin mix	1	1	1
L-cystine	0.3	0.3	0.3
Choline bitartrate	0.3	0.3	0.3
Total	100	100	100

Preparation of *bulgogi*

Bulgogi sauces were prepared with ingredients listed in Table 1. The sauce ingredients were put into a pot and boiled at 100°C for 20 min. At this time, a pre-made xanthan gum solution (mix of sugar, xanthan gum and water) was added to the boiling sauce, and then the mixture was continuously boiled at 100°C for 10 min with gentle agitation. The prepared sauce was cooled to room temperature. The recipe for marinated beef was prepared according to Table 1. Sliced beef was marinated with wine, NaCl, and black pepper powder at 4°C for 1 hr. The marinated beef was placed on a preheated pan at about 170°C and cooked for about 9 min until the meat reached a temperature of approximately 78°C. After cooling at room temperature for 20 min, the cooked beef was mixed with prepared *bulgogi* sauce at a 1:1 ratio. The 100 g of *bulgogi* were individually placed into an aluminium-laminated low-density polyethylene bag (Al-LDPE, Sunkyung Co., Ltd., Seoul, Korea). Al-LDPE has the physical properties that water and gas are not transmittable, with a melting point and density of 120°C and 0.92 g/cm^3, respectively. Each sample was packaged under vacuum condition (300 mmHg).

Irradiation of *bulgogi*

Gamma irradiation at 0 and 40 kGy was conducted using a cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, Ontario, Canada) at the Korea Atomic Energy Research Institute (Jeounbuk, Korea). The source strength was approximately 300 kCi with a dose rate of 10 kGy/hr. Dosimetry was conducted using 5-mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany).

Diet preparation

The *bulgogi* samples irradiated with 0 and 40 kGy were lyophilized and pulverized. The feeding of laboratory animals was carried out using a standard feed AIN93G, and the ingredients of feeds showed in Table 2. The subacute toxicity study of 40 kGy irradiated *bulgogi* was conducted based on the methods described by Kang et al. (11).

Animals and dietary administration

Male and female Crj:CD-1 (ICR) mice, 5 week old, were used after a 1 week acclimatization period. On the day before study initiation, mice were allocated to four groups of each sex, for a total of eight groups (10 mice/group each sex). During this period, and throughout the experiment, the mice were housed individually in plastic caes with cedar chip bedding, and were kept under controlled temperature conditions (25±1°C) and relative humidity (55±5%), with a 12-hr light/dark cycle. Male or female mice were given diets containing *bulgogi* powder (5%) for 12 weeks.

Clinical observation, body weight, and food consumption

During the experimental period, the mice were observed daily and clinical signs and mortality were recorded. Body weight and food intakes were measured weekly throughout the study. At the end of administration, the mice were killed by decapitation. Blood was collected for hematology and biochemical analysis.

Tissue preparation and organ weight

Gross observations were made at autopsy, and recorded. At terminal sacrifice, the following organs (liver, spleen, kidney (left and right), testis/ovary, lung, and heart) from each mouse were weighed.

Hematology analysis

At the end of week 12, the mice were killed by decapitation. Blood was collected for hematology and biochemical analysis. Blood for hematology was collected into tubes treated with EDTA dipotassium. Hematological estimations were carried out using an automatic analyzer (Sysmex F820, Symex Co., Ltd., Hyogo,

Japan) for white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), and platelet (PLT). Blood cell morphology, including differential white blood cell percentages (stab form neutrophils (ST), segmented neutrophils (SEG), lymphocytes (LY), monocytes (MO), eosinophils (EOS), basophils (BA)), was assessed for all animals using staining and microscopy techniques.

Serum biochemistry analysis

The following clinical chemistry measurements were made on sera obtained by centrifugation of the aforementioned blood samples: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CREA), direct bilirubin (D-BIL), glucose (GLUC), total bilirubin (T-BIL), triglyceride (TG), and total protein (TP). All parameters were determined using an automatic analyzer, model 7070 (Hitachi Ltd., Tokyo, Japan).

Histopathologic examination

Gross observations were made at autopsy, and recorded. At terminal sacrifice, the fresh organs were fixed in 10% phosphate-buffered formalin, embedded in paraffin wax. The paraffin wax were cut into 7 μm sections, stained with hematoxylin and eosin, and then examined under the light microscopy (×250) (Opticphot-2, Nikon, Tokyo, Japan) for histological examination.

Statistical analysis

Data were examined for equal variance and normal distribution prior to statistical analysis. Mean values were compared by the general linear model procedures of the SAS® 9.2 (SAS Institute, Cary, NC, USA). Tukey's multiple range tests were used to compare least squared means among treatments at α=0.05.

RESULTS

Body weight and food consumption

No deaths were observed in all laboratory animals of control, 0 kGy and 40 kGy groups during the course of the experiment of 12 weeks. The initial body weight (male, 32～33; female 26), final body weight (male, 43～44; female, 40), body weight gain (male, 10～11; female, 13～14) and food consumption (male, 3.8～4.0; female, 3.3～3.7) had no significant difference in groups of control, 0 kGy and 40 kGy (Table 3).

Organ weight

Relative organ weights in groups of control, 0 kGy and 40 kGy showed no significant difference in liver (male, 4.0～4.1; female, 3.7～3.8), spleen (male, 0.2; female, 0.2), kidney (male, 0.6; female, 0.4), testis (0.5), ovary (0.3～0.4), lung (male, 0.4～0.5; female, 0.4～0.5) and heart (male, 0.4～0.5; female, 0.3) at week 12 (Table 4).

Leukocyte values

The leukocyte values of the ICR mice administered with feed contained 40 kGy irradiated *bulgogi* were evaluated after week 12. The significant differences of 0 kGy

Table 3. Effects of 40 kGy irradiated *bulgogi* on body weight and food consumption (mean±standard deviation) of ICR mouse

Sex	Group	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Food intake (g/day)
Male	Control	32.64±0.91^{NS}	43.52±4.67^{NS}	10.97±1.61^{NS}	3.84±0.37^{NS}
	0 kGy	33.29±1.25	43.86±3.81	10.68±2.07	3.89±0.45
	40 kGy	32.81±1.24	44.13±2.96	11.34±1.13	4.01±0.13
Female	Control	26.45±2.11^{NS}	40.28±5.32^{NS}	13.86±3.62^{NS}	3.35±0.48^{NS}
	0 kGy	26.28±1.47	40.92±6.21	14.75±1.98	3.32±0.31
	40 kGy	26.19±1.56	40.75±4.93	14.69±3.25	3.76±0.49

^{NS}Not significant.

Table 4. Effects of 40 kGy irradiated *bulgogi* on the organ weight (mean±standard deviation) of ICR mouse

(unit: g/100 g body weight)

Sex	Group	Liver	Spleen	Kidney (left)	Kidney (right)	Testis/ovary	Lung	Heart
Male	Control	4.07±0.36^{NS}	0.25±0.04^{NS}	0.63±0.07^{NS}	0.62±0.08^{NS}	0.58±0.10^{NS}	0.49±0.03^{NS}	0.51±0.13^{NS}
	0 kGy	4.29±0.35	0.25±0.06	0.59±0.07	0.60±0.06	0.56±0.13	0.48±0.08	0.48±0.08
	40 kGy	4.14±0.40	0.22±0.04	0.61±0.10	0.59±0.10	0.57±0.13	0.50±0.09	0.49±0.08
Female	Control	3.78±0.32^{NS}	0.26±0.07^{NS}	0.43±0.10^{NS}	0.45±0.09^{NS}	0.38±0.12^{NS}	0.45±0.09^{NS}	0.37±0.08^{NS}
	0 kGy	3.83±0.58	0.28±0.06	0.40±0.04	0.42±0.08	0.39±0.11	0.45±0.07	0.37±0.03
	40 kGy	3.86±0.46	0.30±0.08	0.45±0.12	0.46±0.11	0.41±0.14	0.51±0.09	0.38±0.08

^{NS}Not significant.

Table 5. Analysis of leukocytes (mean±standard deviation) in ICR mouse treated orally with 40 kGy irradiated *bulgogi* (unit: K/mL)

Sex	Group	WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Male	Control	5.21±1.51^{NS}	3.30±0.46^{a}	3.04±0.60^{a}	0.16±0.07^{NS}	0.04±0.02^{NS}	0.02±0.01^{NS}
	0 kGy	3.46±1.13	2.37±0.73^{ab}	2.28±1.07^{ab}	0.15±0.04	0.03±0.01	0.01±0.01
	40 kGy	3.75±1.47	2.01±0.52^{b}	1.94±0.38^{b}	0.14±0.06	0.03±0.02	0.02±0.01
Female	Control	5.49±1.36^{NS}	2.15±0.58^{a}	4.91±1.34^{a}	0.28±0.06^{NS}	0.07±0.04^{NS}	0.01±0.01^{NS}
	0 kGy	3.50±0.72	1.02±0.41^{b}	2.32±0.79^{b}	0.19±0.04	0.03±0.01	0.01±0.00
	40 kGy	3.57±1.28	1.09±0.48^{ab}	2.84±1.16^{ab}	0.17±0.08	0.03±0.02	0.01±0.00

^{a,b}Means within the same column with different letters were significantly different ($p<0.05$). ^{NS}Not significant.

and 40 kGy feeding groups were not observed in WBC (male, 3.4～3.7; female, 3.5), NE (male, 2.0～2.3; female, 1.0), LY (male, 1.9～2.2; female, 2.3～2.8), MO (male, 0.1; female, 0.1), EO (male, 0.03; female, 0.03) and BA (male, 0.01～0.02; female, 0.01) (Table 5).

Erythrocyte and thrombocyte values

The erythrocyte and thrombocyte values in ICR mice treated orally with 0 and 40 kGy irradiated *bulgogi* had no significant differences in RBC (male, 9.0～9.1; female, 9.4～9.6), Hb (male, 11.1; female, 11.5～12.1), HCT (male, 49.6～50.0; female, 50～56), MCV (male, 54.2～55.6; female, 54.1～58.2), MCH (male, 12.1～12.3; female, 12.2～12.5), MCHC (male, 22.4～22.6; female, 21.6～22.8), RDW (male, 18.8～19.5; female, 18.3～18.4) and PLT (male 911～1170; female, 1132～1226) (Table 6).

Serum biochemical values

The serum biochemical values in ICR mice treated orally with 0 and 40 kGy irradiated *bulgogi* were not considered to be biologically significant, since other related parameters were normal in serum biochemistry (Table 7). These parameters, including ALB (male, 3.7～3.8; female, 3.8～4.0), ALP (male, 40.6～52.6; female, 77.6～79.4), ALT (male, 45.4～50.2; female, 31.5～35.5), AST (male, 101～133; female, 131～136), BUN (male, 34.1～35.9; female, 25.1～27.1), CHOL (male, 190～204;

Table 6. Analysis of erythrocytes and thrombocytes (mean±standard deviation) in ICR mouse treated orally with 40 kGy irradiated *bulgogi* (unit: g/100 g body weight)

Sex	Group	RBC (M/mL)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/mL)
Male	Control	8.70±0.51^{NS}	11.03±0.73^{NS}	51.26±5.95^{NS}	58.99±6.19^{NS}	12.63±0.30^{NS}	21.86±2.03^{NS}	19.13±0.67^{NS}	1302±223^{NS}
	0 kGy	9.04±0.83	11.19±1.17	50.08±5.66	55.66±6.23	12.38±0.70	22.41±1.75	18.89±1.14	1170±214
	40 kGy	9.12±0.47	11.10±0.75	49.60±7.03	54.28±5.62	12.18±0.47	22.65±2.11	19.54±2.13	911±168
Female	Control	9.40±0.47^{NS}	11.81±0.65^{NS}	54.80±4.94^{NS}	58.35±5.08^{NS}	12.58±0.46^{NS}	21.69±1.76^{NS}	18.36±0.35^{NS}	1263±381^{NS}
	0 kGy	9.66±0.36	12.11±0.69	56.30±6.37	58.21±5.34	12.55±0.46	21.68±1.66	18.48±0.70	1226±184
	40 kGy	9.40±0.41	11.54±0.48	50.80±3.71	54.16±4.71	12.29±0.50	22.83±1.60	18.31±0.68	1132±94

^{NS}Not significant.

Table 7. Analysis of serum biochemical (mean±standard deviation) in ICR mouse treated orally with 40 kGy irradiated *bulgogi*

Sex	Group	ALB (g/dL)	ALP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	CHOL (mg/dL)
Male	Control	3.67±0.14^{NS}	46.53±16.49^{NS}	59.78±16.58^{NS}	149.80±48.26^{NS}	25.14±3.12^{b}	197.02±43.16^{NS}
	0 kGy	3.74±0.37	44.63±12.86	45.49±19.18	101.10±23.69	35.96±6.38^{a}	204.75±51.78
	40 kGy	3.80±0.29	52.62±19.26	50.52±7.05	133.11±20.69	34.12±2.02^{ab}	190.26±15.54
Female	Control	3.97±0.33^{NS}	91.85±37.14^{NS}	38.98±10.53^{NS}	181.50±45.72^{NS}	23.08±2.01^{NS}	133.65±40.56^{NS}
	0 kGy	4.08±0.22	79.41±20.25	31.57±6.03	131.34±24.31	25.10±3.76	121.46±34.16
	40 kGy	3.83±0.18	77.60±28.91	35.55±14.57	136.06±22.99	27.11±5.04	105.45±22.29
Sex	Group	CREA (mg/dL)	D-BIL (mg/dL)	GLUC (mg/dL)	T-BIL (mg/dL)	TG (mg/dL)	TP (g/dL)
Male	Control	0.45±0.11^{NS}	0.72±0.13^{NS}	46.93±10.54^{NS}	0.74±0.13^{a}	134.93±38.19^{NS}	5.12±0.13^{NS}
	0 kGy	0.54±0.19	0.75±0.29	54.42±19.20	0.69±0.22^{ab}	114.63±30.58	5.38±0.60
	40 kGy	0.62±0.06	0.57±0.06	70.13±16.61	0.53±0.04^{b}	134.98±23.78	5.36±0.49
Female	Control	0.64±0.07^{NS}	0.75±0.27^{NS}	44.03±25.98^{NS}	0.63±0.20^{NS}	149.72±52.14^{NS}	5.61±0.32^{NS}
	0 kGy	0.72±0.06	0.71±0.13	65.43±13.81	0.62±0.13	133.63±35.39	5.86±0.36
	40 kGy	0.63±0.03	0.50±0.08	57.16±17.39	0.46±0.07	118.25±30.92	5.45±0.14

^{a,b}Means within the same column with different letters were significantly different ($p<0.05$). ^{NS}Not significant.

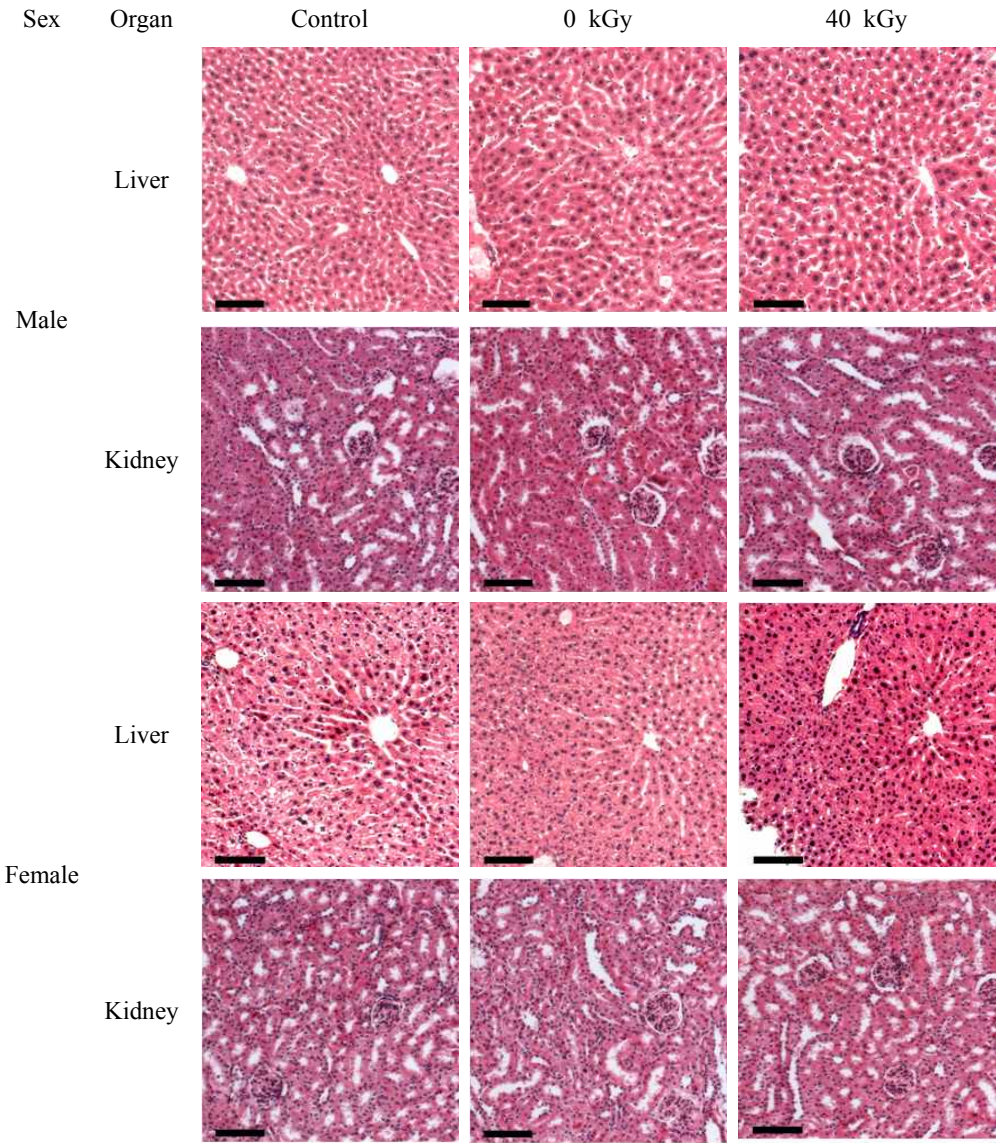

Fig. 1. Histopathological examination of the ICR mouse administered with 40 kGy-irradiated *bulgogi* for 3 months (×250). Scale bars=100 μm.

female, 105～121), CREA (male, 0.5～0.6; female, 0.6～0.7), D-BIL (male, 0.5～0.7; female, 0.5～0.7), GLUC (male, 54～70; female, 57～65), T-BIL (male, 0.5～0.6, female, 0.4～0.6), TG (male, 114～134; female, 118～133) and TP (male, 5.3; female, 5.4～5.8), showed no significant difference between 0 kGy and 40 kGy feeding groups.

Histopathological examination

Pathological examinations of the organs such as liver and kidney on a gross basis indicated that there were no detectable abnormalities (Fig. 1). No alterations were seen in the microscopic examination of the internal organs; the degeneration (tissue atrophy, necrosis, exfoliation and inflammation) of cellular appearances were unremarkable in both groups and sexes.

DISCUSSION

Many subchronic studies on safety have been conducted in rat, mice, dogs, pigs and chickens (5). These studies examined the safety and nutritional adequacy of a variety of dietary items and complete laboratory diets treated with high-dose irradiation. The vast majority of these studies reported no toxic effects in laboratory animals after consumption of high-dose irradiated foods (2,12). Additional carcinogenicity bioassays without reproductive components have been reported for rats and mice (13,14). This large collection of carcinogenicity data is unique in the assessment of all food-related treatments and processes. No irradiation-related increases in tumours occurred in any of the studies that involved administering high-dose irradiated foods or diets to rats

or mice (9). Chronic studies in dogs, conducted for durations of 2～4 years, reported no adverse findings attributable to high-dose irradiated food such as fruit, vegetable, tuna, meat and meat products (15). In a non-human primate study in which high-dose irradiated peaches (27.9 and 55.8 kGy) were fed to rhesus monkeys for a duration of two years, there were no adverse findings in monkeys (5).

The appearance, behavior, mortality, and food consumption of ICR mice administered to feeds containing 40 kGy-irradiated *bulgogi* (5%) were not affected compared to the non-irradiated control during the experimental periods. Although minor changes in biochemical parameters were observed, they were not dose-dependent and were not affected by gamma irradiation. These results indicate that 40 kGy irradiated *bulgogi* did not show any subacute toxic effects under these experimental conditions.

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