THIRTEEN-WEEK SUBACUTE TOXICITY STUDY OF 4-BROMOPHENYL CHLOROMETHYL SULFONE (BCS) IN RATS

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Accepted July 18, 1985

Abstract.....Four groups of 12 Wistar SPF rats of both sexes were fed diets containing 4-bromophenyl chloromethyl sulfone (BCS) at the levels of 0, 20, 100 or 500 ppm for 13 weeks to evaluate the subacute toxicity. Males and females in the 500 ppm group showed increases in plasma protein and calcium and a decreased A/G ratio. The values of plasma total cholesterol and uric acid increased in this female group. In addition, significant increases in both absolute and relative weights were seen in the liver, thyroid and adrenal of this group of both sexes, together with the increased kidney weight in males. All males and females in the 500 ppm group had centrilobular hepatocellular swelling of the liver consisting of proliferation of the smooth endoplasmic reticulum. Males of this group also showed increased incidences of hyalin droplet degeneration of the proximal tubular epithelium and focal tubular atrophy of the kidney. The maximum no-effect level of BCS was determined to be 100 ppm (males : 6.25 mg/kg/day, females : 6.80 mg/kg/day).

Key words : 4-bromophenyl chloromethyl sulfone , Subacute study , rat , hepatocellular swelling.

INTRODUCTION

The herbicide benthicarb (thiobencarb, S-4-chlorobenzyl N, N-diethylthiocarbamate) is widely used in rice paddy fields in Japan to control various weeds (Kumiai Chemical Industries Company, 1977). In 1976, a phytotoxicity of rice plants was found in some paddy fields in Toyama Prefecture where benthicarb was applied. This phytotoxicity was reported to be attributable to the dechlorinated product of benthicarb in soil (Ishikawa et al., 1980). As a safener against the phytotoxicity caused by benthicarb, 4-bromophenyl chloromethyl sulfone (BCS) was developed by Kumiai Chemical Industries Co., Ltd, in 1982. Small amount of this chemical incorporated into the formulated product of benthicarb is effective for the control of the phytotoxicity

of rice plants and this chemical has no biological activities to any kinds of the crops and weeds.

As for the safety evaluation of BCS, although no toxicological data have been published, following information obtained by Kumiai Chemical Industries is available : oral LD₅₀ values in rats and mice, more than 5,000 mg/kg body weight ; no irritating responses to the eye and skin in rabbits ; no mutagenic activities on bacteria (*S. typhimurium* and *E. Coli*) ; LC₅₀ values in carp and daphnia, more than 30 ppm. The subacute toxicity of this chemical, however, has not been evaluated. The present study was carried out in rats to provide subacute data appropriate to the safety evaluation of BCS.

MATERIALS AND METHODS

Test materials. 4-bromophenyl chloromethyl sulfone (BCS) used in this study was supplied by Kumiai Chemical Industries, Co., Ltd., Tokyo, with the following information : purity, 99.5% (Lot No. RDT-204) ; appearance, colorless acicular crystal ; melting point, 149.5°C ; solubility, miscible in N, N-dimethylformamide (43 g/100 g) and dimethyl sulfoxide (29 g/100 g), but immiscible in water (30 ppm). The structural formula is illustrated in Fig. 1. BCS was incorporated into the pulverized diet M (Oriental Yeast Co., Ltd., Tokyo), at concentrations of 0, 20, 100 and 500 ppm. The stability of this chemical in test diets was determined on the samples of 2 different test diets (30 and 1,000 ppm) formulated in a range finding study of BCS. The results of this analysis indicated that BCS in the test diets was stable when stored at a room temperature for 14 days after preparation. Therefore, preparation of test diets was performed once a week. Determination of homogeneity of BCS in the test diet performed prior to initiation of the present study on the samples of each level taken from the top, middle and bottom of the mixer revealed that mixture of the test diets was sufficient (Table 1).

Fig. 1 Structural formula of BCS

Samples of the test diets of each dose level were also analyzed chemically to ascertain the concentration of BCS in the diet prior to initiation of the study and at week 7. As shown in Table 1, the analyzed results showed that actual level of BCS in each test diet was approximately equal to the nominal concentration.

Animals and handling. Specific-pathogen-free (SPF) Wistar rats of both sexes were obtained at 4 weeks of age from Shizuoka Agriculture Cooperative Association for

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Table 1. Homogeneity and concentration of BCS in diets.

Week of formulation	Nominal Concent. (ppm)	Sample	Actual Concent. (ppm)	C. V. (%)
Homogeneity				
Pretest	20	Top	21.7	1.8
		Middle	22.3	5.4
		Bottom	17.9	3.9
	100	Top	91.2	4.2
		Middle	82.8	2.2
		Bottom	85.9	7.7
	500	Top	480	4.1
		Middle	470	2.5
		Bottom	476	1.3
Concentration				
Pretest	0		1.25	—
	20		20.6	3.7
	100		86.6	4.7
	500		475	2.6
At week 7	0		1.25	—
	20		19.3	4.6
	100		101	6.9
	500		501	2.1

Laboratory Animals, Shizuoka. They were kept in a barrier-sustained animal room maintained at $24\pm1^{\circ}\text{C}$ and with a relative humidity of $55\pm5\%$ and an illumination cycle of 14 hours light and 10 hours dark. A positive air pressure was maintained with 12 air changes per hour. The rats were acclimatized to the environmental conditions for a week before treatment. They were housed in groups of four of the same sex of the control and treatment groups, in wire-mesh stainless steel cages (width 310 mm $\times$ depth 440 mm $\times$ height 230 mm).

Experimental procedure. Groups of 12 male and 12 female rats were given a diet containing 0, 20, 100 or 500 ppm BCS for 13 weeks. The dose levels were determined based on the following results of a preliminary 3-week range finding study which was conducted at dietary levels of 0, 30, 100, 300 and 1,000 ppm of this chemical : Significant increases in the liver weight were seen in the males treated with 100 ppm or more and in the females with 300 ppm or more. Diets and tap-water (Kodaira-Shi) were available to the rats *ad libitum*. Analyses of the basal diet and drinking water for contaminants were performed once during the treatment by Oriental Yeast Co., Ltd. and Japan Medical Foods Association, Tokyo, respectively and showed that concentrations of contaminants likely to affect the results of the present study were not observed.

The rats were observed daily for abnormalities of condition. Individual body weights were recorded weekly during the treatment. Food and water consumption of each cage was measured twice a week. Food efficiency of each group was calculated from the ratio of mean body weight gain to mean food consumption. Chemical intake was calculated from the food consumption, body weight and dose level.

At week 13, urinalysis was carried out on urine samples of all the animals which were collected by pressing the lumbosacral region. Specific gravity of the urine was determined with a Handy Refractometer (Atago Optical Instrument Inc., Tokyo), and pH, protein, glucose, ketones, and occult blood were examined by Labstix® (Miles-Sankyo Co., Ltd., Tokyo).

All surviving animals at termination were lightly anesthetized with ether and laparotomized, and blood samples were withdrawn from the posterior vena cava using syringes. A part of the samples were poured into cups with EDTA and subjected to the hematological examination. Following parameters were examined with Coulter Counter® Model SP (Japan Scientific Instrument Co., Ltd., Tokyo); hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Platelet) and leukocyte count (WBC). Differential leukocyte count was calculated from leukocyte count and the percentage found on blood smears stained with May-Grünwalds and Giemsa. Plasma samples obtained from the terminal heparinized blood were analyzed for total protein (TP), albumin (Alb), globulin (Glob), albumin/globulin ratio (A/G), alkaline phosphatase (AIP), blood urea nitrogen (BUN), glucose, total cholesterol (T. Chol), lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ -glutamyl transpeptidase (GG-TP), total bilirubin (T. Bil), direct bilirubin (D. Bil), calcium (Ca) and uric acid (UA) with a blood biochemical autoanalyzer Hitachi 726 (Nissei Sangyo Co., Ltd., Tokyo). Sodium (Na) and potassium (K) were determined on serum separated from the terminal blood samples with Hitachi Flame Photometer 205D (Nissei Sangyo Co., Ltd.).

All surviving animals were killed by cutting the posterior vena cava and removing the diaphragm after blood sampling, and autopsied. Any abnormalities were recorded and the brain, pituitary, thyroids with parathyroids, heart, thymus, liver, spleen, kidneys, adrenals and gonads (testes/ovaries) were weighed. Relative organ weights were calculated from the ratios of organ weight to body weight. Specimens of the following organs and tissues in addition to those weighed were preserved in neutral buffered 10% formalin; spinal cord (cervical, thoracic and lumbar), sciatic nerve, bone including marrow (from the sternum, femur and tibio-femoral joint, and vertebra), lymph nodes (cervic, mesenteric), aorta, tongue, salivary glands, esophagus, stomach, pancreas, small and large intestines, head (including nasal cavity, paranasal sinuses, oral cavity, nasopharynx and middle ears), larynx, trachea, lung, urinary bladder, accessory genital organs, eyes and Harderian glands, skeletal muscle (M. triceps surae), skin (lumbodorsal), mammary gland (abdominal), and all gross lesions. Wax-embedded

5- μ m sections of these tissues from all the animals were stained with hematoxylin and eosin, and examined microscopically. Electron microscopic observation was performed on the liver from several animals. The sampled livers were fixed with 3% glutaraldehyde in phosphate buffer, post-fixed with 2% osmium tetroxide, dehydrated in alcohol, and embedded in Epon 812. Ultrathin sections were made using an ultramicrotome LKB-8800 (Marubun Co., Ltd., Tokyo), stained with uranyl acetate and lead citrate, and examined with an electron microscope Hitachi H-600 (Nissei Sangyo, Co., Ltd.).

Statistical analysis. Data on the incidences of pathological lesions were analyzed by Fisher's exact test. Other data were analyzed using Student's *t* test.

RESULTS

There were no abnormalities of condition attributable to the feeding and no deaths. No statistically significant differences in body weight were seen between the treated groups and control group (Fig. 2). Males in the 500 ppm group showed an increasing tendency of food consumption and the overall mean was 8% higher than that in the control (Table 2). There were no consistent trends in food efficiency in the treated groups of both sexes during the study and the overall mean values were comparable to the controls (Table 2). Males in the 500 ppm group revealed a tendency of increased water consumption during the study and the overall mean was approximately 11% higher than that in the control. On the contrary, decreasing trend of water consumption was observed in females of the 500 and 100 ppm groups, and the overall mean was 9% and 7% less than that in the control, respectively (Table 2).

The average daily intake of BCS in the treated groups was higher in the first 4 weeks than during the remainder of the study. The overall mean daily intake of this chemical in each treated group was shown in Table 2.

Urinalysis showed no noteworthy changes related to the treatment.

Hematological examination revealed a slight but significant increase in platelet count in the 500 ppm group of both sexes (Table 3). Males of this group showed decreased values of MCV and MCH which might be associated with a significant increase in erythrocyte count. These values, however, were within normal ranges in our laboratory.

In blood biochemistry (Table 4), males and females in the 500 ppm group disclosed significant increases in total protein, albumin, globulin and calcium and decreases in A/G ratio, total and direct bilirubin and plasma enzymes (LDH, AIP, GOT, GPT and GGPT). In females of this group, uric acid and total cholesterol increased significantly, and blood urea nitrogen decreased. A significant decrease in A/G ratio was also found in both sexes in the 100 ppm group. Females in this group also revealed a mild but significant decrease in direct bilirubin. In addition, significant fluctuations in several parameters, unrelated to the dose level, were sporadically noted in the groups treated with 100 ppm or less.

At autopsy, enlargement of the liver was observed in 10 of 12 males and 4 of 12

females in the 500 ppm group, and the incidences were statistically significant as compared to the controls. Among these animals, 6 males and 3 females had dark color of the liver. This change was also seen in 2 of 12 females of the 100 ppm group, but this incidence was not statistically significant.

Fig. 2 Group mean body weights in rats fed diets containing BCS for 13 weeks.

Table 2. Food and water consumption, chemical intake and food efficiency in rats fed diets containing BCS for 13 weeks.

Sex	Dose group (ppm)	Food consumption average (g/day/rat)	Food efficiency average	Chemical intake		Water consumption average (ml/day/rat)
				average (mg/kg/day)	total (g/rat)	
Males	0	15.3	16.3	—	—	24.9
	20	15.3	16.1	1.24	0.0279	24.2
	100	15.8	16.5	6.25	0.144	25.5
	500	16.6	15.9	32.5	0.755	27.6
Females	0	10.6	9.9	—	—	21.1
	20	10.4	9.7	1.35	0.0190	20.6
	100	10.7	9.9	6.80	0.0973	19.6
	500	10.8	9.4	34.9	0.492	19.3

Organ weight analysis showed significant increases in absolute and relative weights of the liver, thyroid and adrenal in both sexes of the 500 ppm group (Table 5). In this group, absolute and relative weights of the kidney increased in males and the relative

kidney weight also increased in females. In addition, there were significant increases in relative weights of the brain, thymus and heart in females of the 500 ppm group. However, these changes were not accompanied by the increased absolute weights. In other treated groups, no changes related to the dose level were seen.

On histopathological examination, centrilobular hepatocellular swelling of the liver was observed in all the animals in the 500 ppm group. This change was characterized by slight to moderate swelling of the hepatocellular cytoplasm and no abnormalities were detected in the nucleus. Electron microscopic studies of the liver from several animals of the 500 ppm group showed proliferation of the smooth endoplasmic reticulum and marked decreases of glycogen granules in the periportal hepatocyte (Photos. 1-6).

Table 3 Hematology in rats fed diets containing BCS for 13 weeks.

Sex	Dose (ppm)	No. of animals examined	Ht (%)	Hb (g/dl)	RBC (mill./cmm)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet (1000/cmm)	WBC (1000/cmm)
Males	0	12	39.3±1.3	15.0±0.4	8.27±0.29	47.3±0.3	18.2±0.5	38.4±1.1	911±132	6.6±0.7
	20	12	40.4±1.6	15.3±0.5	8.50±0.32	47.4±0.3	18.0±0.4	37.9±0.8	978±115	6.9±1.1
	100	11	39.8±1.7	15.0±0.5	8.43±0.35	47.2±0.2	17.9±0.4	37.8±1.0	957± 91	6.9±0.8
	500	12	39.6±0.8	14.9±0.3	8.48±0.18*	46.6±0.3***	17.6±0.3**	37.6±0.4	1017± 38*	6.8±0.7
Females	0	12	37.4±1.2	14.5±0.4	7.39±0.23	50.4±0.4	19.6±0.4	38.9±0.9	886± 69	4.8±0.5
	20	12	38.0±1.4	14.7±0.4	7.52±0.28	50.5±0.6	19.6±0.4	38.8±0.7	869± 80	4.8±0.6
	100	12	37.9±1.5	14.7±0.6	7.40±0.45	51.2±1.4	19.9±0.6	38.8±0.5	855± 98	5.2±0.7
	500	12	37.8±1.2	14.5±0.4	7.50±0.20	50.3±0.5	19.4±0.3	38.5±0.6	1003±102**	5.2±0.5

Each value represents Mean±S. D.

*, **, ***: significantly different from the control at 5, 1 and 0.1% level of probability, respectively

Table 4-1 Blood biochemistry in rats fed diets containing BCS for 13 weeks.

Sex	Dose (ppm)	No. of animals examined	LDH (U/l)	AlP (U/l)	GOT (U/l)	GPT (U/l)	GGTP (U/l)	T. Bil (mg/dl)	D. Bil (mg/dl)
Males	0	12	80±18	539±32	81±17	39±13	2.3±0.3	0.17±0.03	0.12±0.02
	20	12	95±30	536±58	95±37	50±23	2.0±0.4*	0.17±0.05	0.12±0.03
	100	12	114±86	536±55	87±18	42± 8	2.8±1.0	0.19±0.10	0.13±0.06
	500	12	61± 7**	482±39***	56± 5***	25± 2**	1.9±0.2***	0.13±0.03*	0.09±0.02**
Females	0	12	59±18	424±53	74± 9	35± 7	2.6±0.2	0.13±0.01	0.08±0.01
	20	12	61±24	446±42	84±19	41± 9	2.3±0.2**	0.13±0.03	0.08±0.02
	100	12	56±14	468±39*	76±13	36± 9	2.5±0.4	0.12±0.01	0.07±0.01*
	500	12	35± 7***	361±38**	51± 6***	24± 4***	2.1±0.3***	0.11±0.02*	0.06±0.01***

Each value represents Mean±S. D.

*, **, ***: significantly different from the control at 5, 1 and 0.1% level of probability, respectively

Table 4-2 Blood biochemistry in rats fed diets containing BCS for 13 weeks.

Sex	Dose (ppm)	No. of animals examined	TP (g/dl)	Alb (g/dl)	Glob (g/dl)	A/G ratio	BUN (mg/dl)	UA (mg/dl)	T. Chol (mg/dl)	Ca (mg/dl)
Males	0	12	6.14±0.08	3.14±0.06	3.00±0.06	1.05±0.03	22.0±1.0	1.0±0.2	55±3	10.6±0.1
	20	12	6.24±0.12*	3.17±0.10	3.07±0.08*	1.03±0.04	22.1±1.6	1.2±0.3	58±6	10.6±0.3
	100	12	6.19±0.18	3.13±0.10	3.06±0.10	1.02±0.03*	22.8±2.1	1.3±0.5	56±3	10.5±0.2
	500	12	6.57±0.12***	3.30±0.07***	3.28±0.09***	1.01±0.03**	22.5±1.2	1.1±0.2	52±4	10.8±0.1***
Females	0	12	6.18±0.26	3.30±0.15	2.88±0.13	1.15±0.04	22.4±1.6	0.8±0.2	79±6	10.2±0.2
	20	12	6.23±0.27	3.32±0.15	2.91±0.13	1.14±0.04	21.5±1.7	0.8±0.1	79±10	10.2±0.2
	100	12	6.13±0.17	3.23±0.12	2.91±0.08	1.11±0.04*	21.8±1.2	0.9±0.2	76±7	10.1±0.2
	500	12	6.64±0.23***	3.43±0.11*	3.21±0.12***	1.07±0.02***	20.6±1.6*	1.0±0.1**	98±10***	10.5±0.3*

Each value represents Mean±S.D.
*, **, *** : significantly different from the control at 5, 1 and 0.1% level of probability, respectively

Photo. 1 Liver from a male rat in the 0 ppm group. Showing normal structure of the hepatocytes. $\times 350$.

Photo. 2 Liver from a male rat in the 500 ppm group. Showing marked swelling of the hepatocytes in the centrilobular region. $\times 350$

Photo. 3 Liver from a male rat in the 0 ppm group. The ultrastructure of hepatocytes showing normal configuration of organelles. $\times 5,600$.

Photo. 4 Liver from a male rat in the 500 ppm group. Marked decreases in glycogen granules and proliferation of the endoplasmic reticulum in the centrilobular hepatocytes. $\times 5,600$.

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Photo. 5 Liver from a male rat in the 0 ppm group. Detail of the normal hepatocyte. $\times 21,000$.

Photo. 6 Liver from a male rat in the 500 ppm group. Detail of the centrilobular hepatocyte showing proliferation of the endoplasmic reticulum. $\times 21,000$.

Table 5 Absolute and relative organ weights in rats fed diets containing BCS for 13 weeks.

	Dose group (ppm)	Body weight (g)	Thyroids (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)
Males	0	335 $\pm$ 16	19.5 $\pm$ 3.0 (0.0058 $\pm$ 0.0009)	11.24 $\pm$ 0.66 (3.36 $\pm$ 0.15)	2,096 $\pm$ 118 (0.63 $\pm$ 0.04)	42.7 $\pm$ 3.3 (0.013 $\pm$ 0.001)
	20	334 $\pm$ 17	18.1 $\pm$ 4.6 (0.0054 $\pm$ 0.0013)	11.46 $\pm$ 0.74 (3.43 $\pm$ 0.18)	2,098 $\pm$ 90 (0.63 $\pm$ 0.02)	46.6 $\pm$ 4.0* (0.014 $\pm$ 0.002)*
	100	344 $\pm$ 21	20.3 $\pm$ 3.6 (0.0059 $\pm$ 0.0010)	11.82 $\pm$ 1.08 (3.43 $\pm$ 0.23)	2,149 $\pm$ 122 (0.62 $\pm$ 0.03)	43.7 $\pm$ 3.5 (0.013 $\pm$ 0.001)
	500	346 $\pm$ 23	23.5 $\pm$ 3.1** (0.0068 $\pm$ 0.0007)**	15.33 $\pm$ 1.31*** (4.43 $\pm$ 0.20)***	2,346 $\pm$ 170*** (0.68 $\pm$ 0.04)**	50.1 $\pm$ 6.3** (0.015 $\pm$ 0.002)**
Females	0	192 $\pm$ 8	12.5 $\pm$ 2.5 (0.0065 $\pm$ 0.0013)	6.50 $\pm$ 0.44 (3.38 $\pm$ 0.18)	1,318 $\pm$ 74 (0.69 $\pm$ 0.03)	49.3 $\pm$ 3.6 (0.026 $\pm$ 0.001)
	20	190 $\pm$ 12	14.5 $\pm$ 3.8 (0.0077 $\pm$ 0.0020)	6.51 $\pm$ 0.53 (3.43 $\pm$ 0.22)	1,294 $\pm$ 112 (0.68 $\pm$ 0.07)	51.5 $\pm$ 5.6 (0.027 $\pm$ 0.003)
	100	193 $\pm$ 6	11.4 $\pm$ 2.4 (0.0059 $\pm$ 0.0013)	6.52 $\pm$ 0.48 (3.38 $\pm$ 0.23)	1,285 $\pm$ 72 (0.67 $\pm$ 0.04)	49.8 $\pm$ 3.6 (0.026 $\pm$ 0.002)
	500	188 $\pm$ 7	15.5 $\pm$ 2.3** (0.0082 $\pm$ 0.0012)**	8.07 $\pm$ 0.55*** (4.29 $\pm$ 0.31)***	1,344 $\pm$ 86 (0.72 $\pm$ 0.04)*	53.4 $\pm$ 4.6* (0.028 $\pm$ 0.003)**

Each value represents mean $\pm$ S. D. for 12 animals.

Values in parenthesis represent relative organ weight; (organ weight/body weight) $\times$ 100

*, **, ***: significantly different from the control at 5, 1 and 0.1% level of probability, respectively

Hyaline droplet degeneration of the renal proximal tubular epithelium was seen in 11 of 12 males in the 500 ppm group. The incidence was statistically significant as compared to that in the control (3 of 12 males), and the severity of this degeneration was more remarkable than the control. All males in the 500 ppm group had focal tubular atrophy in the kidney which is identical to the early lesion of the chronic progressive nephrosis (Gray, 1977) found frequently in untreated aging rats. No statistically significant increases in the incidence of this lesion were found in males of the groups treated with 100 ppm or less as compared to the control. In addition, although several spontaneous lesions were sporadically noted in treated group, the incidences of these lesions did not exceed those in the control.

DISCUSSION

In the present study, distinct toxicological effects were observed in the liver from both sexes in the 500 ppm group. These effects were characterized by the morphological evidence of centrilobular hepatocellular swelling accompanied by the autopsy findings of enlargement and/or dark color of the liver and increases in the liver absolute and relative weights. This hepatocellular swelling was confirmed ultrastructurally to be caused by proliferation of the smooth endoplasmic reticulum which might be related to the synthesis of drug metabolizing enzymes. These morphological evidences may suggest that this hepatic change is not a severe destructive change inducing hepatocellular necrosis.

In the kidney, hyaline droplet degeneration of the proximal tubular epithelium and focal tubular atrophy showing increased incidences in males of the 500 ppm group are spontaneous lesions found in untreated rats (Gray, 1977, Greaves and Faccini, 1984). The severity of the hyaline droplet degeneration in this group, however, was more remarkable than the control and the kidney absolute and relative weights increased significantly in males of the 500 ppm group. These findings might indicate that this chemical exerts some effects to the kidney of this male group. Increased water consumption noted in males of the high dose group might be also associated with the renal effect of this chemical, since animals with renal injuries generally exhibit increased water consumption. On the contrary, decreasing trends of water consumption observed in females treated with 100 ppm or more could not be regarded as a treatment-related change, since the rates of these decreases were minimal and no marked morphological changes were detected in the kidney of these female groups.

A significant increase in platelet count was noted in both sexes in the 500 ppm group. However, no morphological abnormalities indicating toxic injuries were detected microscopically in the blood smear preparation and hematopoietic system. The cause of the increased platelet count is unknown.

In blood biochemistry, increases in total protein, albumin and globulin and a decrease in A/G ratio observed in both sexes in the 500 ppm group were considered to be attributable to the feeding of this chemical, since similar changes were noted in animals

of the 1,000 and 300 ppm groups in the 3-week range finding study performed previously in our institute. Males and females of the 100 ppm group in the present study also revealed a decrease in A/G ratio. Decreased A/G ratio in the 500 ppm group was ascribed to the results in which the increasing rate of globulin was higher than that of albumin. However, in the 100 ppm group, no statistically significant differences were noted in the determined values of total protein and albumin, and the fluctuations of albumin and globulin were not consistent with those in the 500 ppm group. Therefore, decreased A/G ratio in the 100 ppm group could not be regarded as a treatment-related change. The value of calcium also increased in both sexes in the 500 ppm group. In general, higher values of plasma total protein are known to cause simultaneously higher calcium values, since calcium in plasma not only exists as an ion but also is chelated with plasma protein (Fujita, 1980). The higher calcium value in the 500 ppm group is assumed to be a secondary effect following the increased plasma protein observed.

Increased values of plasma total cholesterol and uric acid noted in females of the 500 ppm group might indicate the hepatotoxicity (Kaneko and Cornelius, 1971) and nephrotoxicity (Benjamin and McKelvie, 1978), respectively. However, significant increases in total cholesterol could not be found in males of this group in spite of the fact that hepatocellular swelling was seen morphologically in both sexes of the high dose group, and no histological changes suggestive of nephrotoxicity were seen in the female treated groups. The evaluation as to whether the increased total cholesterol and uric acid are related to the treatment of this chemical could not be determined.

Significantly increased organ weights of the thyroid and adrenal observed in both sexes in the 500 ppm group were assumed to be related to the treatment, since both absolute and relative weights of these organs increased coincidentally and these fluctuations were the same fashion as those in the liver and kidney. No changes suggestive of toxic changes, however, could be seen histologically in the thyroid and adrenal.

In conclusion, the feeding of diets containing 500 ppm BCS induced several toxicological effects in the liver and kidney of rats. The maximum no-effect level was determined to be 100 ppm (males : 6.25 mg/kg/day, females : 6.80 mg/kg/day).

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