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DATA EVALUATION REPORT

STUDY TYPE: 28-day dietary range-finding study- rats

Guideline: 82-7/82-1 TOX. CHEM. NO.: 194

Shaughnessy No.: 011101

MRID NO.: 427242-01

TEST MATERIAL: Busan 11-M1

SYNONYMS: barium metaborate monohydrate

STUDY NUMBER: WIL-94043

SPONSOR: Buckman Laboratories International, Inc.

TESTING FACILITY: WIL Research Laboratories, Inc., Ashland, OH

TITLE OF REPORT: A 28-Day Dietary Range-Finding Study of Busan 11-M1 in Rats

AUTHOR: IC Lamb

REPORT ISSUED: March 10, 1993

QUALITY ASSURANCE: Both a quality assurance statement and a GLP compliance statement were provided.

CONCLUSIONS: Under the conditions of the study, administration of Busan 11-M1 to rats at dose levels of 0, 1000, 5000, 10000, and 15000 ppm for 28 days resulted in reduced body weight/gains in rats of both sexes at the 15000 ppm dose level throughout the study and to some extent in males at the 10000 ppm dose level, with concomitant decreases in food consumption. Other findings include decreases in several hematology and clinical chemistry parameters and dose-related decreases in liver and kidney weights, which were statistically significant only in males. There were apparent dose-related decreases in testes and ovarian weight, and relative brain weights were increased in both sexes at the highest dose tested. A no-effect dose (NOEL) can be set at 5000 ppm (459 mg/kg) for males/10000 ppm (984 mg/kg) for females, and the LEL at 10000 ppm (881 mg/kg) for males/15000 ppm (1285 mg/kg) for females, based on reduced body-weight/gain/food consumption, gait abnormalities, changes in hematology and clinical chemistry parameters, decreased testes, liver, and kidney weights in males, decreased ovarian

weight, and increased relative brain weight in both sexes. The dose levels chosen for the definitive subchronic/neurotoxicity study, based on the current study, were 1000, 5000, and 10000 ppm.

Classification: Acceptable. This is a range-finding study, and it does not satisfy the guideline requirements (82-1/82-7) for a subchronic toxicity/neurotoxicity study nor was it intended to.

MATERIALS:

1. Test Compound: Busan 11-M1; Description: white powder; Batch #: Lot # 1-9769; Purity: 94.3%, assumed 100% pure for dose calculation purposes; Source: Buckman Laboratories, Memphis, TN; CAS # 13701-59-2.

2. Test Animals: Species: rat; Strain: Sprague-Dawley Crl:CD®BR; Age: 31 days old on receipt; 12-day acclimation period; ≈ 6 weeks old at study start; Weight: males 173-206 g, females 127-161 g; Source: The Charles River Breeding Laboratories, Inc., Portage, Michigan.

3. Statistics: Body weights/gains, food consumption, clinical pathology values, absolute/relative organ weights: one-way analysis of variance (ANOVA); if significant, Dunnett's test was used to compare the control and treated groups. All statistical tests were performed using a Digital MicroVAX with appropriate programming.

B. STUDY DESIGN

1. Methodology: Thirty-two males and 32 females were allocated to the various groups using a computer randomization procedure (based on body weight stratification in a block design). Five rats/sex/group were allocated to control and four dose groups. The control groups received the basal laboratory diet (see below). Busan 11-M1 was administered via the diet at dose levels of 0, 1000, 5000, 10000, and 15000 ppm (control groups received basal diet only). The rats were housed individually during the study and were fed Purina® Certified Rodent Chow® #5002 in meal form ad libitum, except during the period of fasting prior to blood collection. Water was available ad libitum.

Dose preparation: Test diets were prepared weekly and stored at room temperature. The appropriate amount of Busan 11-M1 (assumed 100%) was mixed with the feed. The diet preparations were analyzed for homogeneity (prior to study), 14-day stability (prior to study), and test material concentration (samples collected on day of preparation).

RESULTS

The test material diets were found to be homogeneously mixed and stable, based on target recoveries and the day 49 analyses. No information was found in the report regarding the concentrations of Busan 11-M1 attained.

2. Clinical Observations: The rats were observed twice daily for mortality and/or moribundity, and detailed clinical observations were recorded on a daily basis. Weekly, all rats

were placed in a Plexiglas® open-field arena, and any changes in arousal level and gait were recorded. Observations included changes in skin, fur, eyes, and mucous membranes, respiratory, circulatory, autonomic, and central nervous system function, somatomotor activity, and behavior patterns, among others. Individual body weights and food consumption (g/rat/day and g/kg/day) were recorded weekly (from one week prior to study initiation). The mean amounts of Busan 11-M1 consumed (mg/kg/day) by each group and sex were calculated from the mean food consumption (g/kg/day) and the appropriate concentration of Busan 11-M1 in the food (ppm).

RESULTS

Survival and Clinical Observations: All rats survived to study termination. At the highest dose level (15000 ppm), both sexes displayed gait abnormalities (rocking, lurching, or swaying) at each of the weekly evaluations. Soft feces were noted for both sexes at the highest dose level, mainly during the first week of dosing. No other clinical signs appeared to be related to treatment.

Body Weight and Food Consumption: Males at 15000 ppm displayed significantly reduced body weights compared to the controls from week 1 on, and males at the 10000 ppm dose level displayed a smaller reduction in body weight compared to the controls, which attained statistical significance at weeks 2 and 4. Females at the highest dose level also displayed reduced body weight compared to the control females, but statistical significance was not attained (see Table 1, below). Body-weight gains were reduced similarly, and when compared to the initial body weight, males at the 10000 and males and females at the 15000 ppm dose levels displayed statistically significant decreases in body-weight gain for each week (see Table 2, below).

Table 1. Body Weight (% of control)

Week/Dose (ppm)	1000	5000	10000	15000
MALES				
-1	99	100	99	101
0	99	100	99	99
1	100	99	92	80**
2	99	97	91*	73**
3	99	98	91	71**
4	99	97	90*	68**
FEMALES				
-1	100	100	102	99
0	100	101	101	101
1	101	101	100	90
2	101	104	103	91
3	103	105	103	89
4	103	106	101	88

* $p < 0.05$; ** $p < 0.01$

Table 2. Body-Weight Gains [grams (% of control)]

Interval/Dose (ppm)	0	1000	5000	10000	15000
MALES					
-1-0	62	62	62	61	59 (95)
0-1	55	55	53	38** (64)	7** (13)
1-2	53	50	45 (85)	44 (83)	23** (43)
2-3	30	31	34	28 (93)	13** (43)
3-4	33	31	28 (85)	27 (82)	12** (36)
0-2	108	105	98	81** (75)	30** (28)
0-3	138	136	132	109** (79)	43** (31)
0-4	171	167	160	136** (80)	55** (32)
FEMALES					
-1-0	33	33	35	32	35
0-1	20	22	21	20	3** (15)
1-2	20	20	23	24	19 (95)
2-3	10	14	14	11	5 (50)
3-4	10	11	13	9	8 (80)
0-2	40	42	45	44	23* (58)
0-3	50	56	59	55	28** (56)
0-4	61	67	72	63	36** (59)

* $p<0.05$; ** $p<0.01$

There was a dose-related decrease in food consumption (g/rat/day and/or g/kg/day) in males at the 10000 and 15000 ppm dose levels throughout the study, and females at the 15000 ppm dose level also displayed a decrease during most of the study. Food consumption was comparable among the 1000 [♂&♀], 5000 [♂&♀], and 10000 [♀] ppm dose groups compared to their respective controls.

Table 3. Food Consumption

Interval/Group/Dose	0 ppm	1000 ppm	5000 ppm	10000 ppm	15000 ppm
Grams/rat/day					
MALES					
-1-0	23	22	22	22	23
0-1	26	25	25	21**	16**
1-2	27	25	25	22**	17**
2-3	28	26	26	24**	17**
3-4	28	26	27	24**	17**
FEMALES					
-1-0	17	18	18	18	17
0-1	17	18	17	17	12**
1-2	17	18	18	19	15
2-3	18	19	19	18	15*
3-4	19	19	20	19	14**
Grams/kg/day					
MALES					
-1-0	144	140	139	139	140
0-1	118	115	112	101**	80**
1-2	97	94	92	89*	81**
2-3	89	84	84	84	76**
3-4	81	77	79	78	69**
FEMALES					
-1-0	135	138	141	141	135
0-1	112	115	109	106	83**
1-2	98	100	100	104	94
2-3	96	96	96	94	87
3-4	92	91	93	90	79**

The average amount of Busan 11-M1 consumed by each group is

listed below.

Table 4. Busan Intake

Dietary Level (ppm)	Average Calculated Busan 11-M1 Consumed (mg/kg/day)	
	MALES	FEMALES
1000	93	101
5000	459	498
10000	881	984
15000	1149	1285

3. Clinical Pathology

Clinical pathologic parameters were evaluated for all rats in the study following 28 days of test material administration. Blood was collected (following an overnight fast; via the inferior vena cava) at necropsy. The CHECKED (X) parameters were evaluated.

Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
X	Blood clotting measurements	X	Red cell morphology
	(Thromboplastin time)		
X	(Activated partial thromboplastin time)		
X	(Prothrombin time)		

Serum Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous	X	Cholesterol
X	Potassium	X	Globulin
X	Sodium	X	Glucose
	Iron		Phospholipids
	Enzymes	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total Protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
	Creatine kinase (CK)	X	A/G ratio
	Lactate dehydrogenase (LAD)		Triiodothyronine, total T3
X	Serum alanine aminotransferase		Thyroxine, total T4
X	Serum aspartate aminotransferase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GLDH)		
	Ornithine carbamyltransferase (OCT)		

RESULTS

Hematology: Both sexes of the high-dose group displayed decreased mean red blood cell count (93% of control but not statistically significant), hemoglobin, and hematocrit values. Mid- and high-dose males also displayed concomitant decreases in MCV and MCH and a dose-related decrease in white cell counts. There was a dose-related increase in segmented neutrophils (%) and a dose-related decrease in lymphocytes (% and absolute numbers) in males at the 10000 and 15000 ppm dose levels (see Table 5, below).

Table 5. Hematology Results

Parameter Group Dose (ppm)	Hematology Values 0	1000	5000	10000	15000
MALES					
RBC [mil/µL]	8.1	7.7	8.1	8.2	7.6
HGB [g/dL]	16.5	15.8	15.9	15.5	14.4**
HCT [%]	49.8	48.4	48.3	46.9	42.5**
MCV [μ^3]	61.7	63.0	59.5	57.1**	56.4**1
MCH [µµg]	20.4	20.6	19.6	18.9*	9.1*
WBC [thous/µL]	15.1	14.7	14.2	7.1**	5.6**
Segmented					
[%]	8	10	8	17**	21**
[thous/µL]	1.3	1.4	1.1	1.2	1.2
Lymphocyte					
[%]	86	85	89	78*	76*
[thous/µL]	13.1	12.5	12.7	5.6**	4.2**
FEMALES					
RBC [mil/µL]	8.13	8.1	8.0	8.1	7.6
HGB [g/dL]	16.0	16.4	15.6	15.4	14.9*
HCT [%]	47.5	48.1	46.0	45.9	43.0**
WBC [thous/µL]	9.3	9.5	9.6	11.6	8.4
Segmented					
[%]	14	7	9	11	18
[thous/µL]	1.3	0.6	0.8	1.2	1.4
Lymphocyte					
[%]	83	87	87	83	78
[thous/µL]	7.7	8.3	8.4	9.6	6.7

* $p<0.05$; ** $p<0.01$

Serum Chemistry: Blood urea nitrogen values were increased in males at the 10000 and 15000 ppm dose levels and in females at the 15000 ppm dose level. Decreased total protein and globulins values were observed in males at the 15000 ppm dose level compared to the control values, and phosphorus values were increased in these males. Additionally, cholesterol and triglyceride levels were decreased in males at the mid- and high-dose levels, although the decrease was not dose-related in either case (see Table 6, below).

Table 6. Serum Chemistry Data

Parameter Group Dose (ppm)	Serum Chemistry Values				
	0	1000	5000	10000	15000
MALES					
BUN [mg/dL]	10.8	11.7	11.7	16.4**	18.4**
Total protein [g/dL]	6.0	5.7	5.8	5.3**	5.3**
Globulin [g/dL]	2.4	2.2	2.3	2.0**	1.9**
Cholesterol [mg/dL]	40	38	36	20**	27*
Triglycerides [mg/dL]	53	37	39	22**	26**
Phosphorus [mg/dL]	9.5	9.4	9.4	10.4	11.5**
FEMALES					
BUN [mg/dL]	14.0	14.7	12.6	13.8	19.0**

* $p<0.05$; ** $p<0.01$;

4. Ophthalmological Examinations

No parameters were monitored.

5. Gross Necropsy

All rats were subjected to a complete necropsy examination, which consisted of an examination of the external surface, all orifices, and cranial, thoracic, abdominal, and pelvic cavities including the viscera. The following organs were weighed: adrenals, brain, kidneys, liver, and ovaries/testes.

RESULTS

There were no apparent treatment-related lesions observed in either sex.

Organ Weights: There was a dose-related decrease in absolute and relative-to-brain liver and testes weights in males at the 10000 and 15000 ppm dose levels, and absolute (15000 ppm) and relative-to-brain kidney weight (10000 and 15000 ppm) compared to male control values. Relative-to-body testes weight was decreased at the high-dose level also. Females at the 15000 ppm dose level displayed decreased absolute and relative-to-brain ovarian weights, compared to the female control values. Liver and kidney (absolute and relative-to-brain) weights were decreased in females at the 15000 ppm dose levels, although these decreases did not attain statistical significance. Relative brain weights were increased in males at the 10000 ppm dose level and in both sexes at the 15000 ppm dose level. Other differences in organ weights are shown in the table below.

Organ-Weight Data

Organ/Group/Dose	0 ppm	1000 ppm	5000 ppm	10000 ppm	15000 ppm
MALES - FBW♦	334	334	325	302* (90)	224** (67)
Liver					
absolute♦	12.42	11.26	11.84	10.01** (81)	7.30** (59)
relative-body♥	3.72	3.38	3.65	3.31	3.25
relative-brain♥	643.5	594.2	587.9	497.0** (77)	386.2** (60)
Testes					
absolute	3.40	3.15	3.11	2.84* (84)	1.82** (54)
relative-body	1.024	0.947	0.955	0.941 (92)	0.813* (79)
relative-brain	176.0	166.4	154.8	140.8** (80)	96.3** (55
Kidneys					
absolute	3.11	3.08	3.13	2.79 (90)	2.24** (72)
relative-body	0.933	0.924	0.964	0.925	1.002
relative-brain	160.9	162.6	155.6	138.2** (86)	118.9** (74)
Adrenals					
absolute	0.061	0.065	0.069	0.061	0.051(85)
relative-body	0.018	0.019	0.021	0.020	0.023 (123)
relative-brain	3.107	3.406	3.421	3.028	2.720 (88)
Brain					
absolute	1.93	1.90	2.01	2.02	1.89
relative-body	0.580	0.569	0.620	0.670** (116)	0.844** (146)
FEMALES - FBW♦	190	194	199	191	164 (86)
Liver					
absolute	6.97	7.21	7.29	6.86	5.82 (84)
relative-body	3.66	3.72	3.66	3.59	3.56
relative-brain	386.8	394.5	405.5	376.7	330.1 (85)
Kidneys					
absolute	1.85	1.92	1.98	1.93	1.68 (91)
relative-body	0.975	0.988	0.999	1.015	1.026
relative-brain	102.9	105.0	110.5	106.0	95.2 (93)
Adrenals					
absolute	0.077	0.074	0.073	0.072	0.063 (81)
relative-body	0.041	0.038	0.037	0.037	0.038 (93)
relative-brain	4.282	4.024	4.064	3.948	3.547 (83)
Brain					
absolute	1.80	1.83	1.79	1.82	1.76
relative-body	0.951	0.946	0.907	0.959	1.080* (114)
Ovaries					
absolute	0.131	0.138	0.129	0.121 (93)	0.095* (72)
relative-body	0.069	0.071	0.065	0.063 (91)	0.058 (84)
relative-brain	7.295	7.516	7.195	6.655 (91)	5.379* (74)

♦ grams; ♥ grams/100 grams; * $p<0.05$; ** $p<0.01$; ♣ FBW=final body weight

8. Histopathology

The following organs/tissues (CHECKED (X)) were preserved from all animals at terminal sacrifice. Histological examination of the liver, kidneys, testes, ovaries, thyroid, parathyroid, brain, lungs, and gross lesions was performed on all control and high-dose (15000 ppm) rats. The testes were examined from all male rats in the 1000, 5000, and 10000 ppm dose groups.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue		Aorta	X	Brain (fore-, mid-, & hind)
	Salivary glands	X	Heart		Periph. nerve (sciatic)
X	Esophagus		Bone marrow		Spinal cord
X	Stomach	X	Lymph nodes♥	X	Pituitary
X	Duodenum	X	Spleen		Eyes (optic nerve)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenal gland
X	Cecum	X	Kidneys		Lacrimal gland
X	Colon		Urinary bladder		Mammary gland ♀♀
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate		Other
	Pancreas	X	Seminal vesicle		Bone (sternebrae)
	Respiratory	X	Ovaries		Skeletal muscle
	Trachea	X	Uterus		Skin
X	Lung (w/ bronchi)	X	Vagina	X	All gross lesions
	Nose	X	Oviducts		
	Pharynx		Bone marrow smears		
	Larynx				

♥ mesenteric

RESULTS

The only treatment-related change observed occurred in the testes at the high-dose level. All males in this group displayed degeneration of the germinal epithelium of the testes, and the presence of giant (multinucleated) cells was observed in one of these males.

DISCUSSION

The objective of the study was to evaluate the possible toxic effects of Busan 11-M1 administered to rats in the diet for a 28-day period. No adverse effects were observed on survival among the groups of either sex. Gait abnormalities (rocking, lurching, or swaying) were displayed by all rats at the 15000 ppm dose level (both sexes) at each of the weekly evaluations. Additionally, soft feces were observed at this dose level (both sexes) mainly during the first week of dosing. Body weight and body-weight gains were decreased for both sexes at the 15000 ppm dose level and in males at 10000 ppm. At termination, a significant decrease in body weight was observed in males at the 10000 (90% of control value) and 15000 ppm (67% of control value) dose levels. Females at 15000 ppm displayed a decrease that was 86% of the control value, but statistical significance was not attained (n=5). Food consumption was decreased at the 10000 ppm (♂♂) and 15000 ppm (♂♂ & ♀♀) dose levels. Several hematology (↓ RBC, HGB, HCT, MCV, MCH, WBC, lymphocytes, ↑ segmented neutrophils) and clinical chemistry (↑ BUN, ↓ total protein, cholesterol, globulin, triglycerides, phosphorus) parameters were affected

by treatment at the 10000 (♂♂) and 15000 ppm (♂♂ & ♀♀) dose levels. At the 15000 ppm dose level, significant decreases in hemoglobin and hematocrit were displayed by both sexes, and there was a dose-related ↓ in MCV, MCH, WBC, segmented neutrophils, and lymphocytes in the 10000 and 15000 ppm males. With the exception of the ↑ BUN values observed at 15000 ppm in both sexes, the effects on clinical chemistry parameters occurred only in males at the 10000 and 15000 ppm dose levels (dose-related). Males at the 10000 and 15000 ppm dose levels displayed decreased liver (absolute and relative to brain), testes [absolute and relative to brain/body (15000 ppm dose only)]; kidney (absolute and relative to brain) weights and increased relative brain weight. Females displayed decreased absolute and relative-to-brain ovarian weight and increased relative brain weight at the 15000 ppm dose level. No compound-related gross lesions were observed. Microscopically, all males at the 15000 ppm dose level displayed degeneration of the germinal epithelium of the testes, and one also displayed giant (multinucleated) cells in the testes.

CONCLUSION

Under the conditions of the study, administration of Busan 11-M1 to rats at dose levels of 0, 1000, 5000, 10000, and 15000 ppm for 28 days resulted in reduced body weight/gains in rats of both sexes at the 15000 ppm dose level throughout the study and to some extent in males at the 10000 ppm dose level, with concomitant decreases in food consumption. Other findings include decreases in several hematology and clinical chemistry parameters and dose-related decreases in liver and kidney weights, which were statistically significant only in males. There were apparent dose-related decreases in testes and ovarian weight, and relative brain weights were increased in both sexes at the highest dose tested. A no-effect dose (NOEL) can be set at 5000 ppm (459 mg/kg) for males/10000 ppm (984 mg/kg) for females, and the LEL at 10000 ppm (881 mg/kg) for males/15000 ppm (1285 mg/kg) for females, based on reduced body-weight/gain/food consumption, gait abnormalities, changes in hematology and clinical chemistry parameters, decreased testes, liver, and kidney weights in males, decreased ovarian weight, and increased relative brain weight in both sexes. This is a range-finding study, and it does not satisfy the guideline requirements (82-1/82-7) for a subchronic toxicity/ neurotoxicity study nor was it intended to. The dose levels chosen for the definitive subchronic/neurotoxicity study, based on the current study, were 1000, 5000, and 10000 ppm.