Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 6/10/93
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 6/21/93
Tox. Branch II, Head Section II (H7509C)
Tertiary Reviewer: William F. Sette, Ph.D. William Sette 7/7/93
Science Support and Special Review Section (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: combined oral 13-week toxicity/neurotoxicity - rats

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

Shaughnessy No.: 011101

MRID NO.: 427478-01

TEST MATERIAL: Busan 11-M1

SYNONYMS: barium metaborate monohydrate

STUDY NUMBER: WIL-94044

SPONSOR: Buckman Laboratories International, Inc.

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

TITLE OF REPORT: A Combined Oral Subchronic (13 Week) Toxicity and Neurotoxicity Study of Busan 11-M1 in Rats

AUTHOR: IC Lamb

REPORT ISSUED: April 14, 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 via the diet to rats for 90 days at dose levels of 0, 1000, 5000, and 10000 ppm resulted in reduced body weight/gains in rats of both sexes at the high-dose level throughout the study and to some extent in females at the mid-dose level, with concomitant decreases in food consumption. Other findings include decreases in several hematology [RBC, HGB, HCT; high-dose level (both sexes)] and clinical chemistry [total protein, cholesterol, globulin; mid- and high-dose males] parameters, decreased liver and testes weights (absolute and relative) and decreased relative (to brain) kidney weight in the high-dose males, increased relative brain weight in females at the mid-dose level and in both sexes at the high-dose level, increased relative (to body) kidney weight in the high-dose females, and small and/or soft testes with aspermatogenesis in males at the high-dose level. Additionally, there was an absence of spermatocytes in the epididymal tubules at the high-dose. With regard to the neurotoxicity phase of the study, there were no

differences in brain weight or brain dimensions in the rats perfused at necropsy, and no treatment-related neuropathological lesions were observed between the high-dose and control rats at the microscopic examination of perfused tissues (forelimbs were not examined). An apparent treatment-related decrease in one of the parameters of the functional observational battery (forelimb grip strength) was observed in the high-dose males at week 3, and a small stimulatory effect (increased ambulatory activity) was noted at week 3 in mid-dose females and in both sexes at the high dose. A no-effect dose (NOEL) can be set at 1000 ppm (70 mg/kg ♂♂/80 mg/kg ♀♀), and the LEL can be set at 5000 ppm (349 mg/kg ♂♂/406 mg/kg ♀♀), based on reduced body-weight/gain in females; clinical chemistry parameters in males; increased ambulatory activity in females, and decreased relative liver weights in males. A decrease in forelimb grip strength was observed in the high-dose males at week 3. The dose levels tested are adequate.

Classification: Core Minimum. This study satisfies the guideline requirements (82-1) for a subchronic toxicity study in rodents and (82-7) for a neurotoxicity study.

A. 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 International, Inc.; CAS # 13701-59-2.

2. Test Animals: Species: rat; Strain: Sprague-Dawley Crl:CD®BR; Age: 43 days; Weight: males 155-227 g, females 123-175 g; Source: The Charles River Breeding Laboratories, Inc., Portage, Michigan.

3. Statistics: Body weights/gains, food consumption, clinical pathology values, absolute/relative organ weights, brain dimensions: one-way analysis of variance (ANOVA); if significant, Dunnett's test was used to compare the control and treated groups. Histopathological findings: one-tailed Kolmogorov-Smirnov test. The above tests were performed by a Digital® MicroVAX 3400 computer with appropriate programming. Continuous FOB and locomotor activity data: two-way repeated measures ANOVA; if significant treatment or treatment-time interactions occurred, a one-way ANOVA was conducted at each time point and when significant at a time point, Dunnett's multiple T-test was conducted. FOB parameters yielding scalar (ordinal) or descriptive data: repeated measures SAS CATMOD procedure; if significant, Fisher's Exact test or Dunnett's test was performed [performed by a Digital ® Micro VAX 3400 computer with appropriate programming]. All statistical tests for FOB and Locomotor Activity data were performed using a personal computer installed with SAS/STAT statistical software.

B. STUDY DESIGN

1. Methodology: Sixty males and 60 females were allocated to the various groups using a computer randomization procedure (based on body weight; variation did not exceed ± 20% of group mean for each sex). At randomization, 15 males and 15 females were placed into each group. Ten animals/sex/group were allocated to the subchronic toxicity evaluation phase of the study, and the remaining 5/sex/group were allocated to the subchronic neurotoxicity evaluation phase of the study. Additionally, 5 rats/sex/group from the subchronic toxicity portion of the study were combined with the rats in the neurotoxicity portion of the study for the purposes of conducting the Functional Observational Battery (FOB) and Locomotor Activity (LA) assessments. After randomization into study groups, these 10 rats/sex/group were then randomized into four study replicates to allow sufficient time for the reasonable conduct of the FOB and LA assessments. Each dose group and sex were equally [≈] represented within a study replicate. Busan 11-M1 was administered via the diet at dose levels of 0, 1000, 5000, and

10000 ppm (control groups received basal diet only). The rats were housed individually during the study and were fed Purina® Certified Rodent Chow® #5002 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 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 for weeks 0, 1, 2, 3, 7, and 11).

RESULTS

The test material diets were found to be homogeneously mixed, stable over a 14-day interval, based on target recoveries, and to contain the appropriate concentration of Busan 11-M1. The mean target recoveries are shown below.

Table 1. Mean Target Recoveries (%)

| Week/Dose (ppm) | 1000 | 5000 | 10000 |
|---|---|---|---|
| 1 | 103.3 | 102.4 | 103.8 |
| 2 | 104.4 | 101.2 | 104.1 |
| 3 | 99.8 | 102.9 | 102.6 |
| 4 | 111.1 | 105.1 | 104.1 |
| 8 | 93.6 | 99.4 | 106.7 |
| 12 | 109.3 | 97.4 | 89.9 |

2. Clinical Observations: The rats were observed twice daily for mortality and/or moribundity, and detailed clinical observations were recorded on a daily basis. Individual body weights were recorded weekly (from one week prior to study initiation), on treatment days when FOB and LA evaluations were performed, and prior to the scheduled sacrifice. Food consumption (individual) was 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: Two low-dose males were euthanized in extremis; one was hypoactive and unkempt, displaying whole body tremors, lacrimation, constricted/dilated pupils, soft stool, decreased urination and clear material around mouth and neck on day of sacrifice (during week 7). The other sacrifice was due to an apparent mechanical trauma. Neither death was attributed to treatment since no deaths occurred at either the 5000 or 10000 ppm dose level. All other rats survived to study termination. There were no

clinical signs observed that could be attributed to treatment.

Body Weight and Food Consumption: The high-dose rats of both sexes displayed significant reductions in body weight compared to their respective controls throughout most of the study (♀♀ from week 5 on and ♂♂ from week 2 on). Mid-dose females displayed a significant decrease in body weight compared to the control during weeks 8 and 12 (see Table 2, below). Body-weight gains were significantly reduced throughout the study at the high-dose level for both sexes compared to their respective control values. Mid-dose females also displayed decreased body-weight gains, although statistical significance was reported only for the week 0-8 interval (see Table 3, below).

Food consumption on a g/rat/day basis was decreased (1-4 grams) at the high-dose level for both sexes throughout the study, although statistical significance was not always attained. Mid-dose females also displayed a slight decrease. On a g/kg/day basis, consumption was comparable among the male groups, but the high-dose females displayed reduced consumption during the first weeks of the study.

Table 2. Body Weight (% of control)

| Week/Dose (ppm) | 1000 | 5000 | 10000 |
|---|---|---|---|
| **FEMALES** | | | |
| -1 | 99 | 99 | 100 |
| 0 | 102 | 97 | 101 |
| 1 | 101 | 95 | 97 |
| 2 | 102 | 96 | 96 |
| 3 | 101 | 95 | 95 |
| 5 | 100 | 92 | 92* |
| 6 | 98 | 93 | 91* |
| 8 | 98 | 92* | 91* |
| 11 | 99 | 92 | 90** |
| 12 | 99 | 92* | 90* |
| 13 | 100 | 92 | 90* |
| **MALES** | | | |
| -1 | 101 | 101 | 101 |
| 0 | 99 | 101 | 101 |
| 1 | 101 | 101 | 93 |
| 2 | 100 | 100 | 90* |
| 3 | 100 | 100 | 91* |
| 4 | 99 | 99 | 89** |
| 6 | 99 | 100 | 89** |
| 9 | 100 | 100 | 89* |
| 10 | 102 | 100 | 90* |
| 13 | 101 | 98 | 88** |

* p <0.05; ** p <0.01

Table 3. Body-Weight Gains [grams (% of control)]

| Interval/Dose (ppm) | 0 | 1000 | 5000 | 10000 |
|---|---|---|---|---|
| **FEMALES** | | | | |
| 0-1 | 23 | 21 | 19 (83%) | 15** (65%) |
| 0-2 | 44 | 44 | 40 (91%) | 35* (80%) |
| 0-3 | 62 | 60 | 56 (90%) | 49** (79%) |
| 0-5 | 91 | 88 | 77 (85%) | 70** (77%) |
| 0-6 | 101 | 93 | 87 (86%) | 76** (75%) |
| 0-8 | 114 | 105 | 98* (86%) | 88** (77%) |
| 0-12 | 140 | 134 | 121* (86%) | 109** (78%) |
| 0-13 | 141 | 137 | 122 (87%) | 110** (78%) |
| **MALES** | | | | |
| 0-1 | 49 | 52 | 49 | 30** (61%) |
| 0-2 | 104 | 105 | 103 | 74** (71%) |
| 0-3 | 140 | 141 | 139 | 108** (77%) |
| 0-5 | 198 | 200 | 198 | 160** (81%) |
| 0-6 | 225 | 223 | 225 | 176** (78%) |
| 0-13 | 323 | 325 | 312 | 238** (83%) |

* p<0.05; ** p<0.01

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 | 70 | 80 |
| 5000 | 349 | 406 |
| 10000 | 707 | 794 |

3. Clinical Pathology - **Subchronic Toxicity Evaluation Only**

Clinical pathologic parameters (listed below) were evaluated for all surviving rats of this phase of the study at study termination. Blood was collected (following an overnight fast) from the inferior vena cava at necropsy. The CHECKED (X) parameters were evaluated.

Hematology

| | | | |
|---|---|---|---|
| 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/estimate | | Reticulocyte count |
| X | Blood clotting measurements | X | Red cell morphology |
| | (Thromboplastin time) | | |
| X | (Activated partial thromboplastin time) | | |
| X | (Prothrombin time) | | |
| | Nucleated erythrocytes normoblasts | | |

Serum Chemistry

| X | Electrolytes: |
|---|---|
| X | Calcium |
| X | Chloride |
| | Magnesium |
| X | Phosphorous |
| X | Potassium* |
| X | Sodium |
| | Iron |

| X | Other: |
|---|---|
| X | Albumin |
| X | Blood creatinine |
| X | Blood urea nitrogen |
| X | Cholesterol |
| X | Globulins |
| X | Glucose |
| | Phospholipids |
| X | Total bilirubin |
| X | Total serum Protein (TP) |
| | Triglycerides |
| X | A/G ratio |
| | Triiodothyronine, total T3 |

Enzymes

| | |
|---|---|
| X | Alkaline phosphatase (ALK) |
| | Cholinesterase (ChE) |
| | Creatine kinase (CK) |
| X | Lactate dehydrogenase (LAD) |
| X | Serum alanine aminotransferase |
| X | Serum aspartate aminotransferase |
| X | Gamma glutamyl transferase (GGT) |
| | Glutamate dehydrogenase (GLDH) |
| | Ornithine carbamyltransferase (OCT) |
| | Serum protein electrophoresis |
| | Thyroxine, total T4 |

RESULTS

Hematology: Both sexes of the high-dose group displayed decreased mean red blood cell count, hemoglobin, and hematocrit values, which may be treatment-related since similar decreases were reported at 15000 ppm in the range-finding study. High-dose females also displayed concomitant decreases in MCV and MCH (see Table 5, below).

Table 5. Hematology Results

| Parameter<br>Group<br>Dose (ppm) | Hematology Values | | | |
|---|---|---|---|---|
| | 0 | 1000 | 5000 | 10000 |
| **MALES** | | | | |
| RBC [mil/µL] | 9.01 | 8.77 | 8.66 | 8.20** (91)♦ |
| HGB [g/dL] | 15.9 | 15.4 | 15.3 | 14.8** (93) |
| HCT [%] | 48.5 | 47.1 | 47.0 | 44.9** (93) |
| **FEMALES** | | | | |
| RBC [mil/µL] | 8.87 | 8.75 | 8.57 | 8.37 |
| HGB [g/dL] | 16.5 | 16.1 | 15.5 | 15.0** (91) |
| HCT [%] | 50.2 | 48.8 | 47.3 | 45.3** (90) |
| MCV [$\mu^3$] | 56.7 | 55.7 | 55.2 | 54.2** (96) |
| MCH [µµg[ | 18.6 | 18.4 | 18.1 | 17.9** (96) |

** $p<0.01$; ♦ (% of control value)

Serum Chemistry: Males at the mid- and high-dose levels displayed decreased total protein and globulins values compared to the control values, and the A/G ratio at the high-

dose was significantly increased compared to the control value. Additionally, cholesterol values were decreased in males at the mid- and high dose levels. The total protein and cholesterol values were said to be within the historical control data ranges at the testing facility, but since a similar decrease in these parameters was observed in the range-finding study, these differences were considered to be potential effects of treatment (see Table 6, below).

Table 6. Serum Chemistry Data

| Parameter<br>Group<br>Dose (ppm) | Serum Chemistry Values | | | |
|---|---|---|---|---|
| | 0 | 1000 | 5000 | 10000 |
| **MALES** | | | | |
| A/G ratio | 1.10 | 1.17 | 1.17 | 1.27** (115) |
| Total protein [g/dL] | 6.6 | 6.5 | 6.1* (92)♦ | 6.0** (91) |
| Cholesterol [mg/dL] | 51 | 48 | 33** (65) | 31** (61) |

* p<0.05; ** p<0.01; ♦ (% of control value)

4. Ophthalmological Examinations - **All animals**

Ocular examinations were performed on all animals prior to study initiation (week -2) and close to study termination (week 12) using a hand-held slit lamp and an indirect ophthalmoscope after induction of mydriasis with 1.0% topical tropicamide hydrochloride.

RESULTS

There were no treatment-related findings.

5. Behavioral Investigations - **Subchronic Neurotoxicity Evaluation Only**

The behavioral investigations incorporated a functional observational battery (FOB) and a test for locomotor activity (LA). Both tests were conducted prior to study initiation and during the 4th, 8th, and 13th weeks of the test material exposure period (study weeks 3, 7, and 12, respectively). Testing was performed "blind" by the same technicians. All animals (10/group/sex) were observed for the following parameters (see below).

**Functional Observational Battery (FOB)**

1. Home cage observations: (a) posture; (b) convulsions/tremors; (c) feces consistency; (d) biting; and (e) palpebral (eyelid) closure.
2. Observations during handling: (a) cage removal ease; (b) piloerection; (c) lacrimation/chromodacryorrhea; (d) salivation; (e) red/crusty deposits; (f) palpebral closure; (g) eye prominence; (h) fur appearance; (i) ease of handling

rat in hand; (j) respiratory rate/character; (k) mucous membranes/eye/skin color; and (l) muscle tone.
3. Observations in the open field: evaluated over a 2-minute observation period. (a) mobility; (b) rearing; (c) convulsions/tremors; (d) bizarre/stereotypic behavior; (e) time to first step; (f) gait; (g) arousal; (h) urination/ defecation;(i) gait score; and (j) backing.
4. Sensory observations: (a) approach response; (b) startle response; (c) pupil response; (d) forelimb extension; (e) air righting reflex; (f) touch response; (g) tail pinch response; (h) eyeblink response; (i) hindlimb extension; and (j) olfactory orientation.
5. Neuromuscular observations: (a) hindlimb extensor strength; (b) hindlimb foot splay; (c) grip strength-hind and forelimb; and (d) rotarod performance.
6. Physiological observations: (a) catalepsy; (b) body temperature; and (c) body weight.

**Locomotor Activity (LA)**

Locomotor activity, recorded after the completion of the FOB, was measured automatically using the Digiscan 'Micro" Animal Activity System (Omnitech Electronics, Inc.). This personal computer-controlled system utilizes a series of infrared photobeams surrounding a clear plastic, rectangular cage to quantify an individual animal's motor activity. The testing of treatment groups was done according to replicate sequence. Each rat was tested separately. The activity system was operated in the Stagger Start mode of operation, and data were collected in one-minute epochs (print intervals); test session duration was 41 minutes. Animal placement into cage initiated the data collection process, but since the first epoch was often incomplete due to animal placement, the first minute of data was deleted for each rat. The remaining 40 minutes of data collection (divided into 10-minute subsessions) were complied for data presentation. Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of one or two adjacent photobeams) and ambulatory motor activity (interruption of three or more consecutive photobeams).

RESULTS

**Functional Observational Battery (FOB):** Home cage activity: No differences were reported among the groups for either sex during the pre-test period and weeks 3, 7, and 12. Handling observations: No differences were reported among the groups of either sex at any time point. Open field observations: During week 12, one high-dose female was observed walking on tiptoes, which was described as a slight, but definite, alteration in gait. Since this was an isolated occurrence and the rat had

shown no apparent alterations in neuromuscular or sensory observations throughout the study, the author did not consider this to indicate a neurotoxic effect. No other remarkable differences were reported. Sensory and Neuromuscular observations: No remarkable differences were reported among the groups in both sexes during the pre-test period and during weeks 3,7, and 12; however, TB II notes that high-dose males displayed a significant decrease in forelimb grip strength compared to the control group at week 3 (see Table 7, below). Supporting evidence that this decrease may be related to treatment is the 11% decrease noted at the mid-dose level in males at the same time point. Physiological observations: No effects were observed on catalepsy or body temperature at any dose level for either sex. Mean body weights of mid-dose females and high-dose males and females were decreased relative to their respective control values during the 4th (9-10%), 8th (11-13%, and 13th (9-14%) week.

Table 7. Neuromuscular Observations

| Parameter/Sex/Dose | 0 ppm | 1000 ppm | 5000 ppm | 10000 ppm |
|---|---|---|---|---|
| **MALES** | | | | |
| forelimb grip strength (g) | | | | |
| pretest | 480 | 508 (106)♦ | 498 (104) | 506 (105) |
| 3 weeks | 1053 | 1053 (100) | 936 (89) | 883* (84) |
| 7 weeks | 1097 | 1134 (103) | 1101 (100) | 1140 (104) |
| 12 weeks | 1224 | 1188 (97) | 1190 (97) | 1176 (96) |
| hindlimb grip strength (g) | | | | |
| pretest | 363 | 350 (96) | 384 (106) | 383 (106) |
| 3 weeks | 879 | 880 (100) | 828 (94) | 845 (96) |
| 7 weeks | 973 | 1028 (106) | 992 (102) | 973 (100) |
| 12 weeks | 949 | 1037 (109) | 940 (99) | 958 (101) |
| rotarod performance (sec) | | | | |
| pretest | 79 | 53 (67) | 72 (91) | 44 (55) |
| 3 weeks | 92 | 79 (85) | 64 (69) | 40 (43) |
| 7 weeks | 63 | 85 (135) | 69 (109) | 22 (35) |
| 12 weeks | 60 | 54 (91) | 50 (84) | 18 (30) |
| hindlimb footsplay (mm) | | | | |
| pretest | 58 | 56 (97) | 47 (81) | 56 (97) |
| 3 weeks | 67 | 66 (98) | 54 (81) | 55 (82) |
| 7 weeks | 69 | 62 (89) | 62 (90) | 62 (89) |
| 12 weeks | 75 | 62 (83) | 70 (94) | 57 (76) |
| **FEMALES** | | | | |
| forelimb grip strength (g) | | | | |
| pretest | 460 | 446 (97) | 486 (106) | 481 (105) |
| 3 weeks | 993 | 939 (95) | 897 (90) | 928 (93) |
| 7 weeks | 1083 | 1113 (103) | 993 (92) | 1050 (97) |
| 12 weeks | 1114 | 1131 (102) | 1163 (104) | 1098 (99) |
| hindlimb grip strength (g) | | | | |
| pretest | 329 | 325 (99) | 324 (98) | 317 (96) |
| 3 weeks | 784 | 789 (101) | 748 (95) | 743 (95) |
| 7 weeks | 885 | 912 (103) | 869 (98) | 872 (99) |
| 12 weeks | 845 | 840 (99) | 832 (98) | 814 (96) |
| rotarod performance (sec) | | | | |
| pretest | 42 | 52 (124) | 62 (148) | 45 (107) |
| 3 weeks | 86 | 67 (78) | 71 (82) | 62 (72) |
| 7 weeks | 102 | 79 (78) | 74 (73) | 75 (74) |
| 12 weeks | 89 | 95 (107) | 108 (121) | 82 (92) |
| hindlimb footsplay (mm) | | | | |
| pretest | 54 | 51 (94) | 54 (100) | 55 (102) |
| 3 weeks | 62 | 57 (92) | 50 (81) | 56 (90) |
| 7 weeks | 55 | 59 (107) | 55 (100) | 64 (116) |
| 12 weeks | 59 | 64 (108) | 50 (85) | 65 (110) |

* $p<0.05$; ♦ (% of control value)

**Locomotor Activity (LA):** Mean ambulatory and total motor activity values were similar among the groups for both sexes during the pre-test period and during weeks 3, 7, and 12, with two exceptions. During the week 3 evaluation, a significant increase in mean ambulatory activity was displayed by the high-dose males (subsession 2) and the mid- and high-dose females (subsession 1) compared to their respective control values. The mean <u>total</u> (4 subsessions combined) ambulatory activity values for these groups during the week 3 evaluation were not statistically significant, and the increases were not observed in more than one subsession (see Table 7, below).

Table 7. Motor Activity Counts

| Parameter Group/Week Dose | Mean Motor Activity Counts (total counts-4 subsessions) | | | |
|---|---|---|---|---|
| | 0 | 1000 | 5000 | 10000 |
| **MALES** | | | | |
| Total | | | | |
| pretest | 1407 | 1296 (92)♦ | 1348 (96) | 1442 (102) |
| 3 | 1556 | 1527 (98) | 1830 (118) | 1939 (125) |
| 7 | 1501 | 1567 (104) | 1659 (111) | 1615 (108) |
| 12 | 1141 | 1739 (152) | 1273 (112) | 1510 (132) |
| Ambulatory | | | | |
| pretest | 807 | 706 (86) | 794 (98) | 842 (104) |
| 3 | 1011 | 957 (95) | 1208 (119) | 1291 (128) |
| 7 | 998 | 1009 (101) | 1087 (109) | 1056 (106) |
| 12 | 773 | 1092 (141) | 863 (112) | 1018 (132) |
| **FEMALES** | | | | |
| Total | | | | |
| pretest | 1661 | 1503 (90) | 1704 (103) | 1696 (102) |
| 3 | 1785 | 1676 (94) | 1945 (109) | 1906 (107) |
| 7 | 1509 | 1498 (99) | 2033 (135) | 1784 (118) |
| 12 | 1537 | 1608 (105) | 1707 (111) | 1529 (99) |
| Ambulatory | | | | |
| pretest | 975 | 912 (94) | 1018 (104) | 954 (98) |
| 3 | 1196 | 1111 (93) | 1271 (106) | 1270 (106) |
| 7 | 1004 | 940 (94) | 1332 (133) | 1200 (120) |
| 12 | 1000 | 1066 (107) | 1084 (108) | 1006 (101) |

♦ (% of control value)

6. <u>Gross Necropsy of Rats Selected for Neuropathologic Evaluation</u>

At scheduled sacrifice, five rats/sex/group were euthanized and then perfused <u>in situ</u> for neuropathological evaluation. The central and peripheral tissues were dissected and preserved. Brain weight (excluding olfactory bulbs) and brain dimensions (length and width) were recorded. Any observable gross changes, abnormal coloration, or lesions of the brain and spinal cord were recorded.

<u>RESULTS</u>

There were no treatment-related changes reported in brain weight, length, or width, and no gross lesions were observed.

7. Gross Necropsy-Rats Selected for Subchronic Toxicity Evaluation

All rats killed in extremis and all survivors were subjected to a complete necropsy examination, which consisted of an examination of the external surface, all orifices, and $T_3$cranial, thoracic, abdominal, and pelvic cavities including the viscera. The following organs were weighed: adrenals, brain, kidneys, liver, lungs, and ovaries/testes.

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

♥ mesenteric; ♦ submaxillary; ♣ vastus medialis; ♠ cervical, mid-thoracic, lumbar;

RESULTS

The low-dose male that displayed hypoactivity and was euthanized had a reddened cervical lymph node and reddened lacrimal glands. The other low-dose male euthanized showed a compound fracture of the maxilla, an intramuscular hemorrhage associated with the fracture, red foamy contents in the trachea, dark red contents in the stomach and duodenum, and a white area on the liver. At terminal sacrifice, 9 out of ten high-dose males displayed small testes and 7 displayed soft testes. Enlarged lymph nodes were observed in 1 low- and mid- and 3 high-dose males and in 2 low- and high-dose females. These latter findings and others are not considered to be treatment-related.

Organ Weights: High-dose males displayed significant decreases in absolute and relative liver and testes weights, compared to the control values. Mid-dose females and high-dose rats of both sexes displayed increased relative brain weights compared to their respective controls. High-dose males displayed a

significant decrease in relative (to brain weight) kidney weights compared to the control value, and high-dose females displayed increased relative (to body weight) kidney weights. Other differences in organ weights are shown in Table 8 below.

Table 8. Organ-Weight Data

| Organ/Group/Dose | 0 ppm | 1000 ppm | 5000 ppm | 10000 ppm |
|---|---|---|---|---|
| **MALES - FBW♦** | 480 | 491 | 477 | 433* |
| Liver | | | | |
| absolute♦ | 15.54 | 16.00 | 14.19 | 12.14** |
| relative-body♥ | 3.232 | 3.277 | 2.981 | 2.805** |
| relative-brain♥ | 767.8 | 772.7 | 656.7** | 581.2** |
| Testes | | | | |
| absolute | 3.54 | 3.48 | 3.58 | 1.39** |
| relative-body | 0.743 | 0.712 | 0.754 | 0.317** |
| relative-brain | 174.4 | 167.6 | 165.6 | 66.4** |
| Kidneys | | | | |
| absolute | 3.67 | 3.66 | 3.73 | 3.29 |
| relative-body | 0.765 | 0.750 | 0.782 | 0.760 |
| relative-brain | 180.6 | 176.4 | 172.6 | 157.5* |
| Adrenals | | | | |
| absolute | 0.0661 | 0.0701 | 0.0747 | 0.0742 |
| relative-body | 0.014 | 0.014 | 0.016 | 0.017* |
| relative-brain | 3.252 | 3.373 | 3.453 | 3.562 |
| Brain | | | | |
| absolute | 2.03 | 2.08 | 2.16** | 2.09 |
| relative-body | 0.427 | 0.426 | 0.455 | 0.485** |
| **FEMALES - FBW♦** | 271 | 262 | 243* | 243* |
| Liver | | | | |
| absolute | 8.94 | 7.93 | 7.54* | 8.02 |
| relative-body | 3.303 | 3.031 | 3.111 | 3.293 |
| relative-brain | 470.6 | 406.7* | 393.4* | 411.9 |
| Kidneys | | | | |
| absolute | 2.19 | 2.12 | 2.05 | 2.15 |
| relative-body | 0.810 | 0.812 | 0.847 | 0.886* |
| relative-brain | 115.4 | 108.9 | 106.9 | 110.4 |
| Brain | | | | |
| absolute | 1.91 | 1.95 | 1.92 | 1.95 |
| relative-body | 0.710 | 0.749 | 0.794* | 0.805** |

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

8. Histopathology-Rats Selected for Subchronic Toxicity Evaluation

The following organs/tissues (CHECKED (X)) were preserved from all animals at terminal sacrifice. Histological examinations of all tissues/organs collected were performed for all rats dying on test and all control and high-dose rats. The lungs, liver, kidneys, testes, epididymides, and gross lesions were examined from all rats in the low- and mid-dose groups.

RESULTS

Treatment-related changes were observed in the testes and epididymides of the high-dose males, which consisted of aspermatogenesis in the testes of 10/10 males (one mild/9 severe), and no spermatocytes were present in the tubules of the epididymides in 9 of the 10 males examined. There were no other lesions observed that could be attributed to treatment.

9. Histopathology of Rats Selected for Neuropathological Evaluation

The nerve tissues were embedded in plastic or paraffin, as appropriate, sectioned, then stained with hematoxylin and eosin. The following nerve tissues were sampled for a qualitative histopathological examination from rats in the control and high-dose groups.

**Central Nervous System Tissues** [embedded in paraffin]

Brain - forebrain, center of cerebrum, midbrain, cerebellum and pons, and the medulla oblongata
Spinal cord - at cervical swellings $C_3$ - $C_8$ and at lumbar swellings $T_{13}$ - $L_4$
Gasserian ganglion/Trigeminal nerves
Lumbar dorsal root ganglion at $T_{13}$ - $L_4$
Lumbar dorsal root fibers at $T_{13}$ - $L_4$
Lumbar ventral root fibers at $T_{13}$ - $L_4$
Cervical dorsal root ganglion at $C_2$ - $C_8$
Cervical dorsal root fibers at $C_2$ - $C_8$
Cervical ventral root fibers at $C_2$ - $C_8$
Optic nerves
Eyes

**Peripheral Nervous System Tissues** [embedded in plastic]

Sciatic nerve - mid-thigh region and at sciatic notch
Sural nerve
Tibial nerve
Peroneal nerve
Forelimbs [preserved for potential future examinations]
Tail [preserved for potential future examinations]

RESULTS

There were no microscopic lesions observed in the central or peripheral nervous system tissues examined that could be attributed to treatment. NOTE: The forelimbs were not examined.

Positive Control Data

Validation studies performed to provide evidence that (1) the motor activity system employed in the conduct of the Motor Activity Test meets EPA's criteria regarding the ability to detect both increases and decreases in activity; reliability across devices and across days per device; (2) procedures employed in the FOB and LA tests detect endpoints indicative of neurotoxicity; (3) personnel performing FOB observations demonstrate similar observations and detect the major behavioral signs associated with the positive control test material toxicity. Additionally, historical control data from several studies performed at the testing facility were

provided.

DISCUSSION

No adverse effects were observed following the administration of Busan 11-M1 to rats for ≥ 91 days at dose levels of 0, 1000, 5000, and 10000 ppm with respect to survival, clinical signs, and ocular lesions, and no apparent differences were noted between treated and control rats of either sex with respect to the locomotor activity evaluations (except as noted below). Additionally, no differences were displayed in brain weight or brain dimensions in the rats perfused at necropsy, and there were no treatment-related neuropathological lesions observed between the high-dose and control rats at the microscopic examination of perfused tissues. One parameter (forelimb grip strength) in the functional observational battery appeared to be affected by treatment; males at the high-dose level displayed a significant decrease (84% of the control value) in forelimb grip strength at the 3-week interval, and the mid-dose males also displayed a decrease (11% of control value), although statistical significance was not attained. An examination of the individual data suggests that the effect is real, based on the fact that the decrease was displayed by most of the rats in the two groups and is not due to outliers. The significance of this effect is lessened in light of the fact that it occurred in only one sex at one time point and was not accompanied by any other changes in the FOB or any microscopic lesions, but forelimbs were <u>not</u> examined. Additionally, at week 3, a small but genuine stimulatory effect was observed in the mid-dose females and in both sexes at the high-dose level. With regard to the subchronic phase of the study, there was a treatment-related decrease in body weight and body-weight gain at the mid- (females) and high-dose (both sexes) levels throughout the study, and food consumption (on a g/animal/day basis) was decreased in these same groups. At the high-dose level, decreases in RBC, hemoglobin, and hematocrit were displayed by both sexes, and mid- and high-dose males displayed decreased total protein, globulin, and cholesterol values. Males at the high-dose level displayed decreased liver (absolute and relative to brain/ body), testes (absolute and relative to brain/body), and kidney (relative to brain) weights. Relative, but not absolute, brain weight was increased in both sexes at the high-dose level and in mid-dose females (dose-related), and relative (to body) kidney weight was increased in the high-dose females. Macroscopically, small and/or soft testes were observed in 9 out of 10 high-dose males. Aspermatogenesis was displayed in the testes of all 10 high-dose males, and there was an absence of spermatocytes in the epididymal tubules of 9 out of 10 high-dose males. The author stated that similar microscopic changes in the testes and epididymides have been observed in studies with boron, a component of Busan 11-M1. No microscopic changes were observed in the liver, kidney, or brain to correlate with the organ weight findings.

CONCLUSION

Under the conditions of the study, administration of Busan 11-M1 to rats at dose levels of 0, 1000, 5000, and 10000 ppm for at least 91 days resulted in reduced body weight/gains in rats of both sexes at the high-dose level throughout the study and to some extent in females at the mid-dose level, with concomitant decreases in food consumption. Other findings include decreases in several hematology [RBC, HGB, HCT; high-dose level (both sexes)] and clinical chemistry [total protein, cholesterol, globulin; mid- and high-dose males] parameters, decreased liver and testes (absolute and relative) weights and relative (to brain) kidney weight in the high-dose males, increased relative brain weight in females at the mid-dose level and in rats of both sexes at the high-dose level, increased relative (to body) kidney weight in the high-dose females, and small and/or soft testes with aspermatogenesis in males at the high dose. Additionally, there was an absence of spermatocytes in the epididymal tubules at this dose level.

With regard to the neurotoxicity phase of the study, no differences were displayed in brain weight or brain dimensions in the rats perfused at necropsy, and there were no treatment-related neuropathological lesions observed between the high-dose and control rats at the microscopic examination of the perfused tissues that were examined. No microscopic examination of the forelimbs was performed. An apparent treatment-related decrease in one of the parameters in the functional observational battery (forelimb grip strength) was observed in males at the high-dose level at week 3, and a small stimulatory effect was noted (increased ambulatory activity counts) at week 3 in mid-dose females and in both sexes at the high-dose level. A no-effect dose (NOEL) can be set at 1000 ppm (70 mg/kg ♂♂/80 mg/kg ♀♀), and the LEL can be set at 5000 ppm (349 mg/kg ♂♂/406 mg/kg ♀♀), based on reduced body-weight/gain in females; clinical chemistry parameters in males; increased ambulatory activity in females, and decreased relative liver weights in males. Forelimb grip strength was decreased in the high-dose males at week 3. The dose levels tested are adequate. This study is classified Core Minimum, and it satisfies the guideline requirements (82-1) for a subchronic toxicity study in rodents and (82-7) for a neurotoxicity study.