(8/26/02)

DATA EVALUATION RECORD

METHYLENE BIS(THIOCYANATE)

STUDY TYPE: 90-DAY INHALATION - RAT [870.3465 (82-4)]
MRID 45366301

Prepared for

Antimicrobials Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: 90-day inhalation – Rat; OPPTS 870.3465 (§82-4)

DP BARCODE: D275408
P.C. CODE: none

SUBMISSION CODE: S598303
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TEST MATERIAL (PURITY): Methylene bis(thiocyanate) (99.7%, a.i)

SYNONYMS: none

CITATION: Jones, L.J., Blagden, S.M., Blackwell, M.P., and Brooks, P.N. (1999) Methylene bisthiocyanate (MBT): Ninety day repeated exposure inhalation toxicity study in the rat. Safepharm Laboratories Limited, P.O. Box No. 45, Derby DE1 2BT UK. Project No. 071/621. December 9, 1999. MRID 45366301. Unpublished.

SPONSOR: Rodia Inc., 4851 Lake Brook Drive, Glen Allen, VA 23060

Rodia Consumer Specialties Limited, 210-222 Hadley Road West, Oldbury, West Midlands B86 0NN U.K.

EXECUTIVE SUMMARY: In a subchronic inhalation study (MRID 45366301), methylene (bis)thiocyanate (MBT; 99.7%, a.i, Batch No. X1125) was administered by nose-only exposure to groups of 10 male and 10 female Sprague-Dawley Crl:CD®BR rats at concentrations of 0, 0.04, 0.20, or 0.99 mg/m^3 for 6 hours per day, 7 days per week for 13 weeks. For exposure, the test material was formulated as a 2% w/w solution in anhydrous polyethylene glycol (PEG) 400. As part of a functional observational battery (FOB), rats were subjected to a detailed clinical observation before initiation of treatment and weekly thereafter; motor activity, fore- and hind-limb grip strength, and sensory reactivity were assessed during week 12. All observations were done following completion of the 6-hour exposure period. The animals were monitored continuously during exposure. Body weight was recorded on day 0, weekly, and prior to sacrifice. Blood was collected for hematology and clinical chemistry measurements on day 91 (the day after the final exposure).

All animals survived to scheduled termination. Noisy respiration was heard in 10/10 males and 10/10 females administered the high concentration. The first incidence of noisy respiration occurred on day 4 for males and day 6 for females and persisted throughout the study. On several days, decreased respiratory rates, frequent sneezing, labored respirations, hunched posture, or piloerection were observed from 1-3 high-concentration males and females. Blisters on the soles of the feet were observed on 4-7 males beginning on day 76 and on 1-3 females beginning on day 75. These clinical signs were not observed in animals in the control or other

treated groups. No treatment-related clinical signs of toxicity were observed in the low- or mid-concentration groups.

No statistically significant differences on absolute body weights were noted between the treated and control groups of either sex. Body weight gains by the high-concentration males were significantly ($p \leq 0.05$ or 0.01) less than those of the vehicle controls for weeks 1-3, 6, 9, and 11. Overall weight gain by the high-concentration males was 68% of the control level resulting in a mean final absolute body weight for this treated group 82% of the control value. Weekly body weight gains by the treated female groups were not affected by treatment.

Weekly mean food consumption by the high-concentration males was 77-84% of the control amounts throughout the study with the exception of week 9 (99% of controls). Weekly mean food consumption by the high-concentration females was 81-90% of the control amounts for weeks 2, 3, 7, and 9-13. Food consumption by the low- and mid-concentration groups was similar to the controls throughout the study. Weekly food efficiency values were similar between the treated and control groups throughout the study.

Abnormal findings during open field observations were similar to those described under clinical signs. No other treatment-related behavioral changes were observed during the FOB. No treatment-related differences were found between the treated and control groups of either sex for any sensory reactivity or startle response assessments, fore- and hind-limb grip strengths, or motor activity.

No ophthalmoscopic lesions were found on any animal. Hematology, clinical chemistry, and urinalysis parameters were not affected by treatment. Differences in absolute and relative organ weights of the high-concentration males corresponded with reduced final body weights of these animals.

Gross necropsy was unremarkable with the exception of blistered feet observed on 7/10 males and 2/10 females administered the highest concentration. Microscopically, subepithelial inflammatory cell infiltrates were observed in the feet of 4/10 and 2/10 males and females, respectively, in the high concentration groups compared with 1/10 and 0/10 control males and females, respectively. In addition at the highest concentration, epithelial ulceration was seen in the foot of one male and subepithelial fibrosis was found on the feet of one male and one female. All treated male groups had a decreased incidence of globular accumulations of eosinophilic material in the renal tubules compared with the controls. The incidence (severity) of globular eosinophilic accumulations in the control, low-, mid-, and high-concentration groups was 8/10 (1.875), 3/10 (1.333), 3/10 (1.667), and 1/10 (1.00), respectively. The relationship of the foot and kidney lesions to treatment is uncertain but can not be discounted.

The systemic toxicity LOAEL is 0.99 mg/m^3 based on noisy respiration, other clinical signs of respiratory abnormalities, and blisters on the feet from males and females and decreased body weight gains of males. The systemic toxicity NOAEL is 0.20 mg/m^3.

This study is considered **Acceptable/Guideline** and does satisfy the requirements for a subchronic inhalation toxicity study in rats (OPPTS 870.3465 [§82-4]).

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COMPLIANCE: Signed and dated GLP, Data Confidentiality, and Quality Assurance statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test substance: MBT

Description: yellow granular solid
Batch #: X1125
Purity: 99.7%
Stability of compound: responsibility of the sponsor; expiry date, June 1999
CAS #: not given
Structure: not given

2. Vehicle control

The test material was formulated as a 2% w/w solution in anhydrous polyethylene glycol (PEG) 400.

3. Test animals

Species: rat
Strain: Sprague-Dawley Crl:CD®BR
Age and weight at study initiation: approximately 6-8 weeks; males: 148-201 g, females: 142-189 g
Source: Charles River (UK) Limited, Margate, Kent
Housing: Except during exposure, animals were housed in groups of up to four by sex in polypropylene grid-floor cages suspended over trays lined with absorbent paper.
Diet: Rat and Mouse SQC Expanded Diet No. 1 (Special Diets Services Limited, Witham, Essex, UK) was available *ad libitum*, except during exposure.
Water: Mains water was available *ad libitum*, except during exposure.
Environmental conditions:
Temperature: 21±2°C
Humidity: 55±15%
Air changes: at least 15/hour
Photoperiod: 12 hour light/dark cycle
Acclimation period: 15 days

B. STUDY DESIGN

1. In life dates

Start: September 16, 1998; end: December 15, 1998

2. Animal assignment

Animals were randomly assigned to the exposure groups listed in Table 1. Exposures were nose-only for 6 hours/day for 90 days. Two control groups were used in this study. An air control group was exposed to air only at the same chamber flow rates used for the treatment groups. A vehicle control group was exposed to the vehicle only (corresponding to the concentration of vehicle at the highest concentration level).

TABLE 1: Study design

Test Group	Target conc. (mg/m³)	Anal. conc. (mg/m³)	Males	Females
Control-1 (air)	0	0	10	10
Control-2 (vehicle)	0.00	0	10	10
Low	0.04	0.04	10	10
Mid	0.20	0.20	10	10
High	1.00	0.99	10	10

Data taken from text table p.21, MRID 45366301.

3. Exposure concentrations selection rationale

The exposure concentrations were selected based on the results of a range-finding study. Groups of 3 male and 3 female rats were exposed by nose-only to analytical concentrations of 0, 0.2, 1.1, or 4.6 mg MBT/m³ for 6 hours/day for 14 days. The control group was exposed to vehicle (5% PEG 400) and no air control group was used. All high-concentration animals were killed *in extremis* following exposure on day 5. Clinical signs in the high-concentration animals included gasping, labored and noisy respirations, decreased respiratory rates, and marked weight loss. Noisy respirations were occasionally noted in mid-concentration animals beginning on day 6. At necropsy, enlargement and/or pallor of the lungs was seen in high-concentration females. No treatment-related microscopic lesions were found in any animal. Therefore nominal concentrations of 0.04, 0.20, and 1.0 mg/m³ were chosen for the current study.

4. Exposure chamber/aerosol generation

Exposures were nose-only with each rat individually held in a tapered polycarbonate restraining tube fitted onto a single tier of the exposure chamber.

The test material was administered as a 2% w/w formulation in anhydrous PEG 400. Atmospheres were generated in each exposure chamber prior to insertion of the animals on each day. The cylindrical exposure chamber had a volume of approximately 30L. Chamber atmospheres were generated by passing compressed air through a concentric jet nebulizer. Chamber air flow was maintained at 20 L/min.

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5. Analysis of test atmosphere

Temperature and relative humidity inside the exposure chambers were measured by an electronic thermometer/humidity meter and recorded every 30 minutes during the exposure period. Results were presented in graphical form only. Generally temperatures ranged from 19-23°C and relative humidity ranged from 30-55% during the first half of the study and 20-45% during the last half of the study. Oxygen content was 19.8-20.6%.

The analytical concentrations of the test article in the exposure chambers were determined by high-pressure liquid chromatography (HPLC). The samples were taken through a glass liquid impinger containing acetonitrile. Sampling of the test material level in each group was performed once during each daily exposure. Results are in Table 1 above.

In addition, the generated aerosols were continuously monitored using a laser photometer in order to determine stability of the chamber concentrations. Generally, good temporal stability was demonstrated over the 6-hour exposure period. On some occasions the atmospheres in the high-concentration chambers showed a tendency to increase over the exposure period, but the variation was not sufficient to affect the study. Concentrations of the vehicle in the vehicle control and high-concentration chambers were similar as measured by a gravimetric method.

The particle size of the generated aerosols was measured weekly using a cascade impactor. The overall mean mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) for the formulation (determined gravimetrically) for the vehicle control, low-, mid-, and high-concentration atmospheres was 3.687±1.060, 2.469±0.714, 3.091±0.461, and 3.398±0.637 µm, respectively. The MMAD for the test article (determined by chemical analysis) for the low-, mid-, and high-concentration atmospheres was 4.974±2.317, 3.290±0.869, and 3.311±0.659 µm, respectively.

6. Statistics

Data from the treated groups was compared to the vehicle control group; data from the air control group was also compared to the vehicle control group. Hematology, clinical chemistry, organ weight, body weight gain, quantitative functional performance, and sensory reactivity data were assessed for dose-response relationships by linear regression analysis followed by one-way ANOVA incorporating Levene's test for homogeneity of variance. When variances were homogeneous, pairwise comparisons were done using Dunnett's test; for unequal variances, the data were analyzed with Kruskal-Wallis and Mann-Whitney U tests. Histopathology data were analyzed using Chi-squared for the incidence of lesions and Kruskal-Wallis for comparison of severity grades.

C. METHODS

1. Observations

All animals were continuously monitored during exposure. Clinical observations were recorded prior to the start of exposure, three hours after the start of exposure, and on removal from the chamber at six hours.

2. Body weight

Body weight was recorded on day 0, weekly, and at sacrifice.

3. Food consumption

Food consumption was determined for each cage group weekly during the study.

4. Functional observational battery (FOB)

Rats were subjected to a detailed clinical observation before initiation of treatment and weekly thereafter. Motor activity, fore- and hind-limb grip strength, and sensory reactivity were assessed during week 12. All observations were done following completion of the 6 hour exposure period.

a. Home Cage and Handling Observations

Observations in the home cage or while handling were not specified beyond clinical observations described above.

b. Open Field Observations

Once a week, animals were observed in an open-arena with the following parameters evaluated: tremors, twitches, convulsions, gait, transfer arousal, stereotypical and bizarre behavior, piloerection, exophthalmia, lachrymation, hyper/hypothermia, skin color, respiration, palpebral closure, defecation, urination, salivation, and tail elevation.

c. Sensorimotor Tests/Reflexes

During week 12, the rats were subjected to the following sensorimotor or reflex tests: grasp response, vocalization, finger approach, touch escape, startle reflex, blink reflex, toe and tail pinch, pupil reflex, and grip strength (forelimb and hindlimb).

5. Motor activity

Motor activity measurements were assessed for each animal during week 12 using automated activity monitors. The evaluation period was thirty minutes for each animal. The percentage of time each animal was active and mobile was recorded for the overall thirty minute period and also during the final six minute period (considered to be the asymptotic period).

6. Ophthalmoscopic examinations

The eyes of all control and high-concentration animals were examined before treatment and before termination using an indirect ophthalmoscope with 0.5% Tropicamide.

7. Clinical Chemistry

Blood was collected for hematology and clinical chemistry measurements from the lateral tail vein of all rats on day 91 (the day after the final exposure). Rats were not fasted overnight prior to collection. The CHECKED (X) parameters were evaluated:

a. 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*		Blood cell morphology
X	(Activated thromboplastin time)		Red cell distribution width
	(Clotting time)		
X	(Prothrombin time)		

*Required for subchronic studies based on OPPTS 870.3465 Guidelines.

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b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Total Cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)	X	Total serum protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Sorbitol dehydrogenase		
X	Alanine aminotransferase (also SGPT)		
X	Aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic toxicity studies based on OPPTS 870.3465 Guidelines.

8. Urinalysis

A limited urinalysis was performed on all vehicle control and high-concentration animals. Urine was collected on day 86 for males and day 84 for females by placing the animals in metabolism cages following the exposure period until a sample was collected. Water was available during collection. The CHECKED (X) parameters were measured:

X		X	
	Appearance	X	Glucose
	Volume	X	Ketones
	Specific gravity	X	Bilirubin
X	pH	X	Blood
	Sediment (microscopic)		Urobilinogen
X	Protein		Reducing substances

Urinalysis is not required for subchronic studies.

9. Sacrifice and pathology

All surviving animals were killed by exsanguination following an intravenous overdose of sodium pentobarbitone and subjected to gross necropsy. The CHECKED (X) tissues were collected from all animals and preserved in 10% buffered formalin. All tissues from the control and high-concentration animals were examined macroscopically. In addition, tissues from the foot were examined from all animals in all groups. The (XX) organs were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Oral tissues	X	Aorta*	XX	Brain*
X	Tongue	XX	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)
X	Esophagus*	X	Lymph nodes*	X	Pituitary*
X	Stomach*	XX	Spleen*	X	Eyes* (optic n.)
X	Duodenum*	XX	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	Liver*+	XX	Epididymides	X	Thyroids*
X	Pancreas*	X	Prostate		Coagulation glands
		X	Seminal vesicle		
	RESPIRATORY	XX	Ovaries		OTHER
X	Trachea*		Oviducts	X	Bone*
X	Lung*+	X	Uterus*	X	Skeletal muscle*
X	Nasal cavity*		Cervix	X	Skin*
X	Pharynx		Vagina	X	Feet
X	Larynx			X	All gross lesions and masses*

* Required for subchronic toxicity studies based on OPPTS 870.3465 Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

All animals survived to scheduled termination. Noisy respirations were heard from 10/10 males and 10/10 females administered the high concentration. The first incidence of noisy respirations occurred on day 4 for males and day 6 for females and persisted throughout the study. On several days, decreased respiratory rates, frequent sneezing, labored respirations, hunched posture, or piloerection were observed from 1-3 high-concentration males and females. Blisters on the soles of the feet were observed on 4-7 males beginning on day 76 and on 1-3 females beginning on day 75. These clinical signs were not observed in animals in the control or other treated groups. No treatment-related clinical signs of toxicity were observed in the low- or mid-concentration groups. Wet fur and red or brown staining around the eyes and nose were common findings in animals of all treated and control groups.

B. BODY WEIGHT AND WEIGHT GAIN

Selected body weight and body weight gain data are given in Table 2. No statistically significant differences in absolute body weights were noted between the treated and control groups of either sex. However, body weight gains by the high-concentration males were significantly ($p \leq 0.05$ or 0.01) less that those of the vehicle controls for weeks 1-3, 6, 9, and 11. Overall weight gain by the high-concentration males was 68% of the control level resulting in a mean final body weight for this treated group 82% of the

control value. Weekly body weight gains by the treated female groups were occasionally less than or greater than those of the controls but no dose- or time-related trends were apparent.

TABLE 2. Mean body weights and body weight gains (g) (± SD) for male and female rats exposed to MBT for 13 weeks

Study day	0 mg/m³ (vehicle)	0.04 mg/m³	0.20 mg/m³	0.99 mg/m³
Males				
0	179 ± 10	180 ± 6	178 ± 13	182 ± 12
7	217 ± 10	211 ± 6	211 ± 18	208 ± 16
14	255 ± 13	247 ± 10	245 ± 22	231 ± 18
28	321 ± 24	316 ± 15	304 ± 30	281 ± 25
42	371 ± 32	359 ± 17	346 ± 39	318 ± 27
70	425 ± 37	419 ± 30	404 ± 49	357 ± 34
90	455 ±38	452 ± 36	429 ± 50	371 ± 36 (82)[a]
Weight gain week 1	38 ± 6	31* ± 5	33 ± 6	26** ± 6 (68)
Weight gain week 2	38 ± 5	36 ± 6	34 ± 8	23** ± 3 (61)
Weight gain week 1-6[b]	192	179	168	136 (71)
Weight gain week 1-13[b]	276	272	251	189 (68)
Females				
0	156 ± 9	163 ± 7	167 ± 13	163 ± 9
7	174 ± 9	181 ± 7	187 ± 12	179 ± 11
14	190 ± 9	195 ± 7	205 ± 15	192 ± 13
28	216 ± 9	224 ± 13	232 ± 15	218 ± 12
42	232 ± 13	242 ± 13	253 ± 20	239 ± 17
70	254 ± 16	264 ±14	278 ± 22	248 ± 15
90	264 ± 17	275 ± 16	288 ± 23	257 ± 14
Weight gain week 1	18 ± 4	19 ± 6	20 ± 5	15 ± 5
Weight gain week 2	16 ± 4	14 ± 6	18 ± 4	13 ± 3
Weight gain week 1-6[b]	76	79	86	76
Weight gain week 1-13[b]	108	112	121	94

Data taken from Tables 35-38, pp. 124-127, MRID 45366301.
[a]Number in parentheses is percent of control; calculated by the reviewer.
[b]Calculated by reviewer from group means.
Significantly different from vehicle control: *p≤0.05; **p≤0.01.

C. FOOD CONSUMPTION

Weekly mean food consumption by the high-concentration males was 77-84% of the control amounts throughout the study with the exception of week 9 (99% of controls). Weekly mean food consumption by the high-concentration females was 81-90% of the

control amounts for weeks 2, 3, 7, and 9-13 and 92-98% of the control amounts for the remaining weeks. Food consumption by the low- and mid-concentration groups was similar to the controls throughout the study. Weekly food efficiency values were similar between the treated and control groups throughout the study. The study author noted that visual inspection of the water bottles did not reveal any treatment-related effects on water consumption.

D. FUNCTIONAL OBSERVATION BATTERY (FOB)

1. Open field

Abnormal findings during open field observations were similar to those described above under clinical signs. High-concentration males and females had increased incidences of respiratory pattern changes and hunched posture. No other treatment-related behavioral changes were observed.

2. Grip strength

Fore- and hind-limb grip strengths were similar between the treated and control groups for both males and females.

3. Other FOB endpoints

No treatment-related differences were found between the treated and control groups of either sex for any sensory reactivity or startle response assessments.

E. MOTOR ACTIVITY

Motor activity data are given in Table 3. No differences were noted between the treated and control groups in overall activity or in activity during the final 20% of the trial.

TABLE 3. Motor activity for male and female rats exposed to MBT for 13 weeks				
Endpoint	**0 mg/m³ (vehicle)**	**0.04 mg/m³**	**0.20 mg/m³**	**0.99 mg/m³**
Males				
Overall % Activity % Mobile Activity	63.1 ± 10.3 16.9 ± 5.6	58.2 ± 14.9 16.4 ± 5.7	47.9 ± 25.9 12.8 ± 10.4	48.2 ± 14.5 9.8 ± 4.7
Final 20% of trial % Activity % Mobile Activity	56.3 ± 19.1 13.3 ± 5.1	44.4 ± 28.7 10.3 ± 9.1	41.5 ± 24.8 9.6 ± 9.4	47.7 ± 21.8 10.3 ± 8.7
Females				
Overall % Activity % Mobile Activity	59.9 ± 19.2 18.8 ± 8.5	55.3 ± 15.8 15.6 ± 7.6	67.2 ± 10.7 18.7 ± 4.9	49.7 ± 18.6 11.1 ± 6.7
Final 20% of trial % Activity % Mobile Activity	45.7 ± 27.8 12.8 ± 9.0	57.4 ± 13.4 15.1 ± 8.3	55.4 ± 21.4 12.9 ± 7.2	42.3 ± 28.6 8.8 ± 8.2

Data taken from Tables 31 and 32, pp. 118 and 119, respectively, MRID 45366301.

F. OPHTHALMOSCOPIC EXAMINATION

No ophthalmoscopic lesions were found in any animal.

G. CLINICAL PATHOLOGY

1. Hematology

High-concentration males had a statistical increase in prothrombin time and high-concentration females had a statistically greater RBC count as compared with the vehicle controls. These differences were not biologically significant (<10%) and are considered incidental to treatment.

2. Clinical chemistry

No treatment-related changes in any clinical chemistry parameter were noted for either sex. Statistically significant differences between the treated and control groups were sporadic, not concentration-related, not consistent between sexes, and not biologically significant.

H. URINALYSIS

Urinalysis endpoints were similar between the high concentration and vehicle control groups of both sexes.

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I. SACRIFICE AND PATHOLOGY

1. Organ weight

Selected organ weight data are given in Table 4. Terminal body weights of the high-concentration males were 81% of the vehicle control level. In the high-concentration males, significant ($p \leq 0.05$ or 0.01) differences in organ weights from the control group included decreased absolute kidney, liver (relative wt. also), spleen, thymus, and epididymides weights and increased relative (to body weight) adrenal, brain, heart, and testes weights. For females administered the highest concentration, absolute spleen weight and absolute and relative thymus weights were significantly ($p \leq 0.05$ or 0.01) decreased compared with those of the controls. The only statistically significant difference noted in the low- and mid-concentration groups was a decrease in relative liver weight of the mid-concentration males which was not considered to be biologically significant.

TABLE 4: Terminal body weight and organ weight data of male and female rats exposed to MBT for 13 weeks

Organ	0 mg/m³	0.04 mg/m³	0.20 mg/m³	0.99 mg/m³
Males				
Terminal body wt. (g)	457 ± 39	456 ± 36	428 ± 52	372 ± 36
Brain				
absolute (g)	1.979 ± 0.093	2.024 ± 0.068	1.991 ± 0.132	2.006 ± 0.252
relative (% b.wt.)	0.435 ± 0.036	0.446 ± 0.034	0.471 ± 0.062	0.544** ± 0.080
Kidneys				
absolute (g)	2.543 ± 0.366	2.611 ± 0.237	2.532 ± 0.277	2.214* ± 0.217
relative (% b.wt.)	0.555 ± 0.046	0.573 ± 0.040	0.593 ± 0.041	0.597 ± 0.049
Liver				
absolute (g)	14.618 ± 2.182	13.889 ± 1.843	12.541 ± 2.231	10.798** ± 1.457
relative (% b.wt.)	3.190 ± 0.302	3.033 ± 0.185	2.915* ± 0.238	2.899* ± 0.218
Spleen				
absolute (g)	0.712 ± 0.137	0.657 ± 0.126	0.685 ± 0.143	0.540* ± 0.087
relative (% b.wt.)	0.156 ± 0.025	0.144 ± 0.024	0.160 ± 0.023	0.145 ± 0.013
Thymus				
absolute (g)	0.459 ± 0.079	0.430 ± 0.061	0.407 ± 0.071	0.323** ± 0.087
relative (% b.wt.)	0.101 ± 0.019	0.094 ± 0.011	0.095 ± 0.014	0.086 ± 0.019
Testes				
absolute (g)	3.679 ± 0.311	3.716 ± 0.263	3.556 ± 0.565	3.626 ± 0.270
relative (% b.wt.)	0.808 ± 0.068	0.817 ± 0.057	0.835 ± 0.129	0.981** ± 0.101
Epididymides				
absolute (g)	1.582 ± 0.112	1.623 ± 0.158	1.497 ± 0.182	1.419* ± 0.113
relative (% b.wt.)	0.348 ± 0.039	0.357 ± 0.034	0.351 ± 0.035	0.384 ± 0.043
Females				
Terminal body wt. (g)	264 ± 17	275 ± 17	286 ± 22	257 ± 13
Brain				
absolute (g)	1.913 ± 0.065	1.887 ± 0.115	1.873 ± 0.070	1.877 ± 0.078
relative (% b.wt.)	0.729 ± 0.061	0.688 ± 0.064	0.657 ± 0.051	0.732 ± 0.063
Liver				
absolute (g)	8.801 ± 0.715	8.455 ± 0.577	8.682 ± 0.647	8.090 ± 1.141
relative (% b.wt.)	3.344 ± 0.195	3.072 ± 0.150	3.041 ± 0.235	3.134 ± 0.295
Spleen				
absolute (g)	0.556 ± 0.167	0.486 ± 0.058	0.521 ± 0.066	0.424* ± 0.049
relative (% b.wt.)	0.210 ± 0.058	0.177 ± 0.021	0.183 ± 0.025	0.165 ± 0.015
Thymus				
absolute (g)	0.382 ± 0.038	0.398 ± 0.053	0.396 ± 0.050	0.300** ± 0.027
relative (% b.wt.)	0.145 ± 0.012	0.145 ± 0.019	0.139 ± 0.026	0.117** ± 0.008

Data taken from Tables 49-52, pp. 138-141, MRID 45366301.
Significantly different from vehicle control: *p<0.05; **p<0.01.

2. Gross pathology

At necropsy, blistered feet were observed on 7/10 males and 2/10 females administered the highest concentration. No other treatment-related gross lesions were noted in any animal. Incidental findings included a yellow epididymal mass in one control male, small testis in one mid-concentration male, and dark areas on the lungs in one control female.

3. Microscopic pathology

a) Non-neoplastic - Treatment-related microscopic lesions were found in the feet of high-concentration males and females and in the kidneys of the high-concentration males. Subepithelial inflammatory cell infiltrates were observed in the feet of 4/10 and 2/10 males and females, respectively, in the high concentration groups compared with 1/10 and 0/10 control males and females, respectively. In addition at the highest concentration, epithelial ulceration was seen in the foot of one male and subepithelial fibrosis was found in the feet of one male and one female. All treated male groups had a decreased incidence of globular accumulations of eosinophilic material in the renal tubules compared with the controls. The incidence (severity) of globular eosinophilic accumulations in the control, low-, mid-, and high-concentration groups was 8/10 (1.875), 3/10 (1.333), 3/10 (1.667), and 1/10 (1.00), respectively.

No definitive treatment-related lesions were observed in the respiratory tracts of males or females exposed to the test article. In the high-concentration groups, minimal or slight goblet cell hypertrophy was seen in the lungs of 3/10 males and in the nasal turbinates of 4/10 females, and food particles were found in the ventral pouch of the larynx of 4/10 females. These findings were not observed in any vehicle control animals, however, goblet cell hypertrophy in the nasal cavity was seen in 4/10 air control females.

b) Neoplastic - No neoplastic lesions were found in any animal.

III. DISCUSSION

A. REVIEWER'S DISCUSSION

No deaths occurred during the study. For the low- and mid-concentration males and females, no treatment-related clinical signs of toxicity were observed and body weight, body weight gain, and food consumption were similar to their respective control levels throughout the study. Abnormal respirations were observed from the high-concentration animals during exposure and during the FOB. Although the first incidences of noisy respirations were noted during the first week of exposure, the severity did not appear to worsen throughout the study. No histopathological lesions were found in the respiratory tracts of these animals that corresponded with the clinical observations.

High-concentration males had significantly reduced body weight gains as a result of reduced food consumption throughout the study. The lower body weight gain resulted in

lower absolute body weights although statistical significance was not attained. For the high-concentration females, reductions in food consumption during some weeks did not affect body weight gain. Food efficiency values for the treated males and females were similar to the controls.

Evidence for neurotoxicity was not found in any treated group as assessed by the FOB and motor activity. Treatment with the test article did not cause any alterations in hematology and clinical chemistry parameters or ophthalmoscopic lesions. At necropsy, decreases in absolute organ weights and increases in relative organ weights of the high-concentration males were considered due to lower final body weights for these animals. Reduced organ weights for the high-concentration females were not considered biologically significant.

Blisters on the soles of the feet of several high-concentration males and females were observed during clinical examinations and at necropsy. Inflammation was confirmed by microscopic examination for most of these animals. The blisters may have been caused by contact with urine while the animals were in the restraining tubes, however, no changes in urinalysis parameters were found. Because urinalyses were conducted on freshly collected urine samples, the results are considered accurate. Although the mechanism of this lesion is not known, it is considered to be treatment-related.

All treated male groups had decreased incidence and severity of globular accumulations of eosinophilic material in the renal tubules compared with the controls. A reduction in this lesion may represent an alteration in metabolism, but the mechanism is unknown. However, the relevance of this finding is limited since eosinophilic accumulations in the kidney are specific to the male rat.

Therefore, the systemic toxicity LOAEL is 0.99 mg/m^3 based on noisy respiration, other clinical signs of respiratory abnormalities, and blisters on the feet from males and females and decreased body weight gains of males. The systemic toxicity NOAEL is 0.20 mg/m^3.

B. STUDY DEFICIENCIES

No major deficiencies were identified in the conduct of the study. Positive control data from the testing facility for neurotoxicity testing was not included and the animals were not tested prior to initiation of exposure to establish a baseline. However, this was not intended to be a full neurotoxicity study and these data are useful in the overall assessment of the toxicity of the test article.