**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
WASHINGTON, D.C. 20460

JAN 29 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Methylene (Bis) Thiocyanate: Review of Toxicology Data.

**EPA Identification Numbers:**

P.C. Code: 068102
DP Barcode: D239689
Submissions: S531280
MRID: 44367101.
ID# 068102

**TO:** Marshall Swindell / Martha Terry
PM Team # 33
Regulatory Management Branch I
Antimicrobials Division (7510W)

**FROM:** Timothy F. McMahon, Ph.D. 1/24/98
Senior Toxicologist, RASSB
Antimicrobials Division (7510W)

**THRU:** Winston Dang, Ph.D. 1/27/98
Team Leader, Team One
RASSB/AD (7510W)

and

Norm Cook, Chief
RASSB/AD
01.29.98

**Registrant:** Rohm and Haas Company

**Action Requested:** Review of a subchronic inhalation toxicity study conducted with the technical grade of methylene (bis) thiocyanate.

**Background**

The registrant (Rohm and Haas Company) has submitted a subchronic inhalation toxicity study on methylene (bis) thiocyanate for review. A summary of the findings of the review are shown below.

CITATION: Bernacki, H.J., Jr., J.S. Ferguson and L.G. Lomax. (1995) Methylene (bis)thiocyanate. Thirteen-week inhalation toxicity study in rats. Rohm and Haas Company, Spring House, PA 19477-0904. Laboratory Report No. 94R-087. May 30, 1995. MRID 44367101. Unpublished.

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 44367101), ten young adult Crl:CD BR rats/sex/dose were exposed via nose-only inhalation to an aerosolized solution of 0.5% (w/w) methylene (bis)thiocyanate (98.1% a.i.) in propylene glycol. The rats were exposed to 0 (air control group and propylene glycol control group), 0.015, 0.23 or 2.1 mg/$m^3$ methylene (bis)thiocyanate (equivalent to 0, 0.000015, 0.00023 or 0.0021 mg/L) for 6 hours/day, 5 days/week for 13 weeks, for a total of 65 exposure days.

One male rat at the 2.1 mg/l dose died during exposure at wek 5 and no explanation for the death was ascertained. Exposure-related effects were limited to the 2.1 mg/$m^3$ exposure groups, and included irritation to the respiratory tract. Respiratory noise was observed in 1/10 males and 6/10 females during several initial study weeks. The most anterior section of the nasal cavity in 10/10 males and 9/10 females was chronically inflamed (slight); damaged epithelium was replaced by stratified squamous epithelium along the ventral meatus. The nasal cavities of 3/10 males exhibited hyperplasia of the respiratory/transitional epithelium along the lateral wall and nasoturbinate and maxilloturbinate tips. Body weights in males were reduced ($p<0.05$) during all study weeks compared to the air controls; final body weight gains were reduced 18% compared to the air controls. Body weights in females were reduced ($p<0.05$) by 7-9% during weeks 6-12 compared to the air controls. Food consumption was reduced ($p<0.05$) in males during all study weeks (9-22%), and in females mainly during the latter weeks (8-14%) compared to the air control groups. No treatment-related effects were observed in the 0.23 or 0.015 mg/$m^3$ exposure groups. No treatment-related differences in ophthalmology, hematology, clinical chemistry, organ weights or gross histopathology were observed. Urine was not collected. No neoplastic tissue was observed. **The LOEL for**

**this study is 2.1 mg/m$^3$ (0.0021 mg/L), based on decreased body weights and food consumption, and histopathological changes in the nasal cavity in both sexes. The NOEL is 0.23 mg/m$^3$ (0.00023 mg/L).**

This subchronic inhalation toxicity study in rats is classified **acceptable (§82-4)** and satisfies the Subdivision F guideline requirement for a subchronic inhalation toxicity study in rodents.

# DATA EVALUATION RECORD

METHYL (BIS)THIOCYANATE

Study Type: 82-4; Subchronic Inhalation Toxicity Study in Rats

Work Assignment No. 3-27 (MRID 44367101)

Prepared for
Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Joan L. Harlin, M.S.

Signature: Joan L. Harlin
Date: 12/4/97

Secondary Reviewer
Kathleen P. Ferguson, Ph.D.

Signature: Kathleen P. Ferguson
Date: 12/4/97

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date:

Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date:

Disclaimer

This Data Evaluation Report may have been altered by the Antimicrobial Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Tim McMahon, Ph.D. _____
RASSB/AD

Work Assignment Manager: Tim McMahon, Ph.D. _____
RASSB/AD

DATA EVALUATION RECORD

STUDY TYPE: 13-Week subchronic toxicity [inhalation] - rat
OPPTS Number: 870.3465 OPP Guideline Number: §82-4

DP BARCODE: D239689 SUBMISSION CODE: None
P.C. CODE: 68102 TOX. CHEM. NO.: 565

TEST MATERIAL (PURITY): Methylene (bis)thiocyanate (98.1% a.i.)

SYNONYMS: MBT

CITATION: Bernacki, H.J., Jr., J.S. Ferguson and L.G. Lomax. (1995) Methylene (bis)thiocyanate. Thirteen-week inhalation toxicity study in rats. Rohm and Haas Company, Spring House, PA 19477-0904. Laboratory Report No. 94R-087. May 30, 1995. MRID 44367101. Unpublished.

SPONSOR: Rohm and Haas Company, 727 Norristown Road, Box 904, Spring House, PA 19477-0904.

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 44367101), ten young adult Crl:CD BR rats/sex/dose were exposed via nose-only inhalation to an aerosolized solution of 0.5% (w/w) methylene (bis)thiocyanate (98.1% a.i.) in propylene glycol. The rats were exposed to 0 (air control group and propylene glycol control group), 0.015, 0.23 or 2.1 mg/$m^3$ methylene (bis)thiocyanate (equivalent to 0, 0.000015, 0.00023 or 0.0021 mg/L) for 6 hours/day, 5 days/week for 13 weeks, for a total of 65 exposure days.

There were no exposure-related deaths. Exposure-related effects were limited to the 2.1 mg/$m^3$ exposure groups, and included irritation to the respiratory tract. Respiratory noise was observed in 1/10 males and 6/10 females during several initial study weeks. The most anterior section of the nasal cavity in 10/10 males and 9/10 females was chronically inflamed (slight); damaged epithelium was replaced by stratified squamous epithelium along the ventral meatus. The nasal cavities of 3/10 males exhibited hyperplasia of the respiratory/transitional epithelium along the lateral wall and nasoturbinate and maxilloturbinate tips. Body weights in males were reduced ($p<0.05$) during all study weeks compared to the air controls; final body weight gains were reduced 18% compared to the air controls. Body weights in females were reduced ($p<0.05$) by 7-9% during weeks 6-12 compared

to the air controls. Food consumption was reduced ($p<0.05$) in males during all study weeks (9-22%), and in females mainly during the latter weeks (8-14%) compared to the air control groups. No treatment-related effects were observed in the 0.23 or 0.015 $mg/m^3$ exposure groups. No treatment-related differences in ophthalmology, hematology, clinical chemistry, organ weights or gross histopathology were observed. Urine was not collected. No neoplastic tissue was observed. **The LOEL for this study is 2.1 $mg/m^3$ (0.0021 mg/L), based on decreased body weights and food consumption, and histopathological changes in the nasal cavity in both sexes. The NOEL is 0.23 $mg/m^3$ (0.00023 mg/L).**

This subchronic inhalation toxicity study in rats is classified **acceptable (§82-4)** and satisfies the Subdivision F guideline requirement for a subchronic inhalation toxicity study in rodents.

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



6

# I. MATERIALS AND METHODS

## A. MATERIALS:

1. Test Material: Methylene (bis)thiocyanate
   Description: Yellow crystalline solid
   Lot #: 949097
   Purity: 98.1% a.i.
   Stability of compound: Reported to be stable over the duration of the study when stored at room temperature.
   CAS #: 6317-18-6
   Structure: Not provided

2. Vehicle and/or positive control: Propylene glycol

3. Test animals: Species: Rat
   Strain: Crl:CD BR
   Age and weight at study initiation: Approximately 6 weeks of age; males, 158.7-205.3 g; females, 138.5-178.2 g
   Source: Charles River-Kingston, Stone Ridge, NY
   Housing: Individually housed in stainless steel wire-mesh cages suspended above absorbent paper liners
   Diet: PMI Certified Rodent Chow 5002 (Ralston Purina Co., St. Louis, MO), ad libitum, except during the inhalation exposures and pre-necropsy fasting periods.
   Water: Filtered tap water, ad libitum
   Environmental conditions during non-exposure periods:
   Temperature: Approximately 23 C
   Humidity: 40-70%
   Air changes: Not reported
   Photoperiod: 12-Hour light/dark cycle
   Acclimation period: Approximately 2 weeks

## B. STUDY DESIGN:

1. In life dates: September 6-December 9, 1994

2. Animal assignment

Animals that were considered healthy were assigned to the test groups in Table 1 using a computer-based randomization procedure based on body weight.

Table 1: Study design.[a,b]

| Test group | Chamber Airflow (L/min) | Nominal Conc. (mg/L)[c] | Analytical Conc. (mg/L)[c] | MMAD[d] ($\mu$m) | GSD[e] ($\mu$m) | Rats/ sex |
|---|---|---|---|---|---|---|
| 1 Air Control | 400 | 0 | 0 | --- | --- | 10 |
| 2 Vehicle Control | 250-400 | 0 | 0 | 4.2 | 4.8 | 10 |
| 3 Low | 350-425 | 0.00002 | 0.000015 | 3.0 | 4.3 | 10 |
| 4 Mid | 410 | 0.00020 | 0.00023 | 3.6 | 5.7 | 10 |
| 5 High | 400 | 0.0020 | 0.0021 | 3.4 | 3.2 | 10 |

[a] Data were obtained from page 21 and Tables 1 and 3, pages 30, 31, and 34-37 of the study report.

[b] Dose levels were selected based on the results of a 2-week range-finding inhalation toxicity study (Rohm and Haas Report No. 94R-086) in which male and female rats were exposed to filtered air, propylene glycol aerosol or to methylene (bis)thiocyanate at 0.02, 0.37 or 2.1 mg/m$^3$ air for 6 hours/day for a total of 10 exposures. In the 0.37 mg/m$^3$ exposure group, food consumption was decreased. In the 2.1 mg/m$^3$ exposure group, body weight and food consumption were decreased, and nasal cavities were mildly inflamed in the most anterior section. Based on these results, nominal concentrations of 0.02, 0.2 and 2.0 mg/m$^3$ were selected for this 13-week inhalation study.

[c] The reviewer converted the aerosol concentrations from mg/m$^3$, as reported in the study report, to mg/L using inhalation conversions.

[d] MMAD = Mass median aerodynamic diameter.

[e] GSD = Geometric standard deviation.

## 3. Exposure conditions

A 0.5% (w/w) solution of methylene (bis)thiocyanate was prepared by dissolving 20 g of the test substance in 4000 g of propylene glycol. The solution was heated to approximately 60 C and stirred for 90 minutes. Test solution (approximately 4 L) was prepared one day prior to each exposure, except the solution used on Monday was prepared on the previous Friday. It was reported that the test solution was stable for up to 7 days (Biocides Research Analytical Group Report No. B-94-170).

The test atmosphere (aerosol) was generated via a metering pump into the fluid port of an all-glass nebulizer. Compressed air (20-30 psi) was directed through a jet situated in close proximity at a 90-degree angle to the fluid port to disperse the fluid stream into a fine aerosol. Aerosol that impacted on the inside of the nebulizer drained into a waste container. Suspended

aerosol from the nebulizer was directed through a glass manifold to the inlet of the exposure chamber. Chamber aerosol concentration was achieved by varying both the rate at which the solution was pumped to the nebulizer and the chamber airflow rate. Total airflow through the exposure chambers was maintained at 350-425 L/min for the treatment groups (equivalent to 16.8-20.4 turnovers/hour), and at 250-400 L/min (equivalent to 12-19.2 turnovers/hour) for the air and vehicle control groups (Table 1). The exposure chamber airflow rate was adjusted to achieve 99% of the maximum aerosol concentration ($t_{99}$) within 30 minutes. Exposure time was defined as the time that the test substance was first introduced into the chamber atmosphere until generation of the test material was terminated.

The animals received a 6-hour/day nose-only inhalation exposure under dynamic conditions in 1250-L stainless steel, glass, and plexiglas chambers. Exposures were conducted for 5 days/week for 13 weeks, for a total of 65 exposures. During exposure, the animals were individually housed in PVC nose-only restraining tubes (5.1 cm O.D. x 20 cm length) attached to the front of the exposure chamber. As the males grew larger, they were individually housed in larger PCV tubes (6.4 cm O.D. x 20 cm length). The animal positions were rotated daily throughout the study. Following exposure, the animals were kept in the chambers for a period of time equal to or greater than the calculated $t_{99}$.

Aerosol generator and airflow parameters in the vehicle control and high methylene (bis)thiocyanate chambers were set to yield approximately equivalent total aerosol concentrations. Gravimetric samples were collected approximately three times during each exposure to monitor the total aerosol concentration in these chambers. A glass fiber filter (25 mm) held in a plastic filter holder was connected in series to a calibrated flowmeter and vacuum source controlled by a solenoid. For each sample, chamber atmosphere was drawn through a preweighed filter for 10 minutes at a flow rate of 6 L/minute. Each filter was reweighed after sampling, and the total aerosol concentration was calculated by dividing the weight change of the filter by the total volume of air sampled (60 L). The nominal test concentrations are reported in Table 1.

The test atmosphere concentration was determined analytically by collecting one impinger sample from the vehicle control and methylene (bis)thiocyanate exposure chambers on each day of exposure. Each impinger solution



9

contained 12 mL buffered water. The air control chamber was sampled weekly to confirm the lack of test material in the control atmosphere. Airflow rates and durations for impinger sample collection are reported in Table 2. The impinger solutions were analyzed using HPLC. The measured test concentrations are reported in Table 1.

Table 2. Airflow rates and durations for impinger sample collection.

| Test Group | Flow rates (L/min)[b] | Sampling Time | |
|---|---|---|---|
| | | Min/Hr | Total Duration (min) |
| 1 Air Control | 0.50 or 0.40 | 60[c] | 330 |
| 2 Vehicle Control | 0.50 | 60[c] | 330 |
| 3 Low | 0.50 or 0.40 | 60[c] | 330 |
| 4 Mid | 0.49 or 0.40 | 29 | 174 |
| 5 High | 0.48 | 5 | 30 |

[a] Information obtained from page 16 of the study report.
[b] Sampling flow rate was decreased during the study for groups 1, 3, and 4 to eliminate excessive bubbling in the impingers.
[c] Sampled continuously for 5.5 hours (330 minutes).

Particle size distribution of aerosols from the test material and vehicle control chambers was measured weekly using a PC-2 QCM Cascade Impactor. The data were analyzed using a log-probit regression analysis to obtain mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). For the vehicle control and treatment exposure chambers, MMADs were 3.0-4.2 $\mu$m and mean GSDs were 3.2-5.7 $\mu$m (Table 1). Particle size samples were not collected from the air control chamber.

During exposure, the temperature of the test chamber was 20.8-23.9 C and the humidity was 60.7-90.5%. Exposure chamber airflow rates were set to provide at least 12 air changes per hour. The oxygen content of the chamber airflow was not reported.

4. Statistics

Body weight, cumulative body weight gain, food consumption, hematology and clinical chemistry parameters, and organ weights were analyzed for normality

and homogeneity of group variances using one-way analysis of variance (ANOVA). Pairwise comparisons between group means were made using Duncan's multiple range procedure conducted at the 95% confidence level.

C. METHODS

1. Observations

All animals were examined twice daily, before and after each exposure, for signs of ill health or reaction to treatment. On weekends, when no exposures occurred, the animals were examined once daily for moribundity and mortality. Thorough physical examinations were performed weekly beginning one week prior to exposure initiation, and included evaluation of external structures, posture, gait, and behavior. Gross abnormalities in respiration and body temperature were noted.

2. Body weight

All animals were weighed weekly beginning one week prior to study initiation.

3. Food and compound intake

Food consumption for each animal was determined weekly beginning one week prior to study initiation, and was reported as g/animal/day.

4. Blood

Blood samples were collected from the abdominal aorta of each animal just prior to exsanguination. The animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined in all samples analyzed.

a. 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* | | |
| | Blood clotting measurements* | | |
| | (Thromboplastin time) | | |
| | (Clotting time) | | |
| | (Prothrombin time) | | |

* Required for subchronic toxicity studies.

b. Clinical Chemistry

| | ELECTROLYTES | | OTHER |
|---|---|---|---|
| X | Calcium* | X | Albumin* |
| X | Chloride | X | Blood creatinine |
| | Iron | X | Blood urea nitrogen* |
| | Magnesium | X | Total Cholesterol |
| X | Phosphorus* | X | Globulins |
| X | Potassium | X | Glucose* |
| X | Sodium* | X | Bilirubin |
| | | X | Total protein (TP)* |
| | | X | Triglycerides |
| | ENZYMES | X | A/G Ratio |
| X | Alkaline phosphatase* | | |
| | Cholinesterase | | |
| | Creatine phosphokinase | | |
| | Lactic acid dehydrogenase* | | |
| X | Serum alanine aminotransferase* | | |
| X | Serum aspartate aminotransferase* | | |
| X | Gamma glutamyl transferase | | |

* Required for subchronic toxicity studies.

5. Urinalysis*

Urine was not collected during the study.

6. Sacrifice and Pathology

Following blood collection, all animals were anesthetized with an intraperitoneal injection of sodium pentobarbital and sacrificed by exsanguination. The bodies of all animals were subject to gross pathological examination. The following CHECKED (X) tissues were collected from all animals, including those that died prior to scheduled sacrifice. The (XX) organs were weighed from all animals rats during the scheduled necropsy. Blood and organ weights were not obtained from the two rats that died prematurely.

| | DIGESTIVE SYSTEM | | CARDIOVASC./HEMAT. | | NEUROLOGIC |
|---|---|---|---|---|---|
| | Tongue | X | Aorta* | X | Brain* |
| X | Salivary glands* | X | Heart* | X | Sciatic nerve* |
| X | Esophagus* | X | Bone marrow* (sternum) | X | Spinal cord (3 levels) |
| X | Stomach* | | | | |
| X | Duodenum* | X | Lymph nodes* | X | Pituitary* |
| X | Jejunum* | X | Spleen* | X | Eyes (optic n.)* |
| X | Ileum* | X | Thymus* | | |
| X | Cecum* | | | | |
| X | Colon* | | | | GLANDULAR |
| X | Rectum* | | UROGENITAL | | |
| XX | Liver*+ | | | XX | Adrenal gland* |
| X | Pancreas* | XX | Kidneys*+ | X | Coagulating gland |
| | | X | Urinary bladder* | X | Lacrimal gland |
| | | XX | Testes*+ | X | Harderian gland |
| | RESPIRATORY | X | Epididymides | X | Mammary gland |
| | | X | Prostate gland | X | Thyroids/parathyroids* |
| X | Trachea* | X | Seminal vesicle | | |
| XX | Lung* | X | Ovaries*+ | | OTHER |
| | Pharynx | X | Uterus* and cervix | | |
| X | Larynx | X | Vagina | X | Bone* (femur, sternum) |
| X | Nasal cavity | | | X | Muscle* (skeletal) |
| X | Bronchi | | | X | Skin* |
| | | | | X | All gross lesions and masses* |

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic studies.

Microscopic examinations were conducted on all CHECKED (X) tissues from the air control, vehicle control, and 2.1 mg/m$^3$ exposure groups, and from both animals that died prematurely. Respiratory tracts (nasal cavity, trachea and lungs with mainstem bronchi) and gross lesions (except forelimb alopecia) were examined in the 0.015 and 0.23 mg/m$^3$ exposure groups only.

## II. RESULTS

### A. Observations

1. Mortality - The report stated that no treatment-related deaths occurred during the study. However, two males (one each in the control and 2.1 mg/m$^3$ exposure groups) died during exposure at weeks 2 and 5, respectively. The control male was found to have a fractured femur that may have contributed to its death, while the cause of death in the high dose male could not be determined.

2. Clinical Signs - In the 2.1 mg/m$^3$ exposure groups, respiratory noise was noted in 1/10 males and 6/10 females during weeks 2 and 3 only. After wek 3, these signs were not present. All dose groups including controls demonstrated red-stained eyes and muzzle immediately after exposure, but these were attributes to restraint in the nose-only tubes. There were no data provided in the report in summary or individual format listing the clinical observations in treated and control animals in this study. This is considered a deficiency and should not be repeated in future submissions.

### B. Body weight and body weight gain

In the 2.1 mg/m$^3$ group males, body weights were significantly ($p<0.05$) reduced during all study weeks compared to the air controls; by study termination, mean body weights were 64.2 g lower and mean body weight gains were 18% lower than the air control values (Table 3). In the 2.1 mg/m$^3$ group females, body weights were 7-9% lower ($p<0.05$) during weeks 6-12 compared to the air controls. No other differences in body weights or body weight gains were considered to be treatment-related in any treatment group. Decreased body weight changes ($p<0.05$) in the 2.1 and 0.23 mg/m$^3$ and vehicle exposure group females compared to the air control females during the final study week were exaggerated because 6/10 air control females lost weight during that week.

Table 3. Body weights (g) of rats following 4 and 13 weeks of inhalation exposure to methylene (bis)thiocyanate.[a]

| Treatment rate ($mg/m^3$) | Body Weight (g) | | | Body Weight Gain (g) | |
|---|---|---|---|---|---|
| | 0 weeks | 4 weeks | 13 weeks | Total (g) | % of air control gain |
| **Males** | | | | | |
| 0[b] | 181.2 | 344.4 | 547.3 | 366.1 | --- |
| 0[c] | 181.9 | 348.2 | 529.2 | 347.3 | -5 |
| 0.015 | 180.5 | 345.3 | 524.3 | 343.8 | -6 |
| 0.23 | 176.9 | 341.1 | 530.4 | 353.5 | -3 |
| 2.1 | 187.5 | 320.6*@ | 489.4*@ | 301.9 | -18 |
| **Females** | | | | | |
| 0[b] | 158.1 | 218.1 | 276.7 | 118.6 | --- |
| 0[c] | 155.5 | 214.0 | 281.2 | 125.7 | +6 |
| 0.015 | 159.8 | 223.8 | 291.2 | 131.4 | +11 |
| 0.23 | 160.6 | 209.6 | 270.8 | 110.2 | -7 |
| 2.1 | 157.9 | 207.6 | 265.5@ | 107.6 | -9 |

[a] Data obtained from Table 6, pages 42-43 of the study report.
[b] Air control group.
[c] Vehicle control group.
* Significantly different from air controls, $p<0.05$.
@ Significantly different from vehicle controls, $p<0.05$.

C. Food consumption and compound intake

Males in the 2.1 $mg/m^3$ exposure group consumed 9-22% less food ($p<0.05$) than the air control males throughout the study, and 9-17% less food ($p<0.05$) than the vehicle control males during several study weeks. Females in the 2.1 $mg/m^3$ exposure groups consumed 8-14% less food ($p<0.05$) than the air control females during weeks 1 and 7-11. Food consumption by the 2.1 $mg/m^3$ exposure and vehicle control group females did not differ significantly except during the first week when the exposed females consumed 6% less food ($p<0.05$). No differences in food consumption by the 0.23 or 0.015 $mg/m^3$ exposure groups were considered to be treatment-related.



15

D. Ophthalmoscopic examination

No treatment-related ophthalmoscopic abnormalities were observed.

E. Blood work

1. Hematology - No treatment-related differences in hematology parameters were observed in any treatment group.

2. Clinical Chemistry - There were no significant treatment-related effects on clinical chemistry parameters except in high dose males, where an increase in activity of alkaline phosphatase was observed in relation to the naive and vehicle control (29 and 43%, respectively). There were no associated microscopic findings in the liver of male rats at this dose or increased liver enzyme activities in the corresponding exposed females. Increased inorganic phosphorus ($p<0.05$) in all male exposure groups were not dose-related and lacked associated histopathological alterations. All other statistically significant differences were considered incidental or toxicologically insignificant.

F. Sacrifice and Pathology

1. Organ weight - No treatment-related differences in organ weights were observed in any treatment group. The 2.1 $mg/m^3$ group males had decreased absolute liver, lung, and kidney weights that were considered to be a result of depressed terminal body weights. Relative weights for these organs were similar to the control weights and no associated microscopic changes were observed.

2. Gross pathology - No treatment-related gross postmortem differences were observed in any treatment group. All abnormalities appeared to occur randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Treatment-related microscopic changes were limited to the nasal cavity of rats in the 2.1 $mg/m^3$ exposure groups. In the most anterior portion of the nasal cavity in 10/10 males and 9/10 females, the stratified squamous epithelium was extended along the ventral meatus to replace damaged respiratory epithelium; slight chronic inflammation was also observed. In addition, 3/10 males had hyperplasia of the respiratory/transitional epithelium along

the lateral wall and on the tips of the nasoturbinates and maxilloturbinates. No other treatment-related histopathological changes were observed in any other exposure group.

b) Neoplastic - No neoplastic tissue was observed.

## III. DISCUSSION

### A. Investigator's Conclusions

The study authors concluded that the NOEL for this study is 0.23 mg/m$^3$. Treatment-related effects observed in the 2.1 mg/m$^3$ exposure groups were decreased body weights in males and decreased food consumption and histopathological evidence of irritation in the nasal cavities in both sexes.

### B. Reviewer's Discussion

Male and female rats exposed to methylene (bis)thiocyanate at 2.1 mg/m$^3$ via nose-only inhalation exposure were adversely affected by treatment. Respiratory noise was present in 1/10 males and 6/10 females during several initial study weeks, indicating that the test substance irritated the airway passages of the animals. In the nasal cavities of all rats exposed to this aerosol concentration, except for one female, respiratory epithelium in the most anterior section was damaged; stratified squamous epithelium that extended along the ventral meatus replaced the damaged epithelium. Slight chronic inflammation was also observed in these nasal cavities. Additional evidence of respiratory insult was hyperplasia of the respiratory/transitional epithelium along the lateral wall and tips of the nasoturbinates and maxilloturbinates. Significant decreases in body weights and food consumption in both sexes are also considered to be treatment-related. The fact that the vehicle control rats also consumed less food than the air control rats suggests that propylene glycol contributed to reduced food consumption in the 2.1 mg/m$^3$ exposure groups.

None of the effects observed in rats in the 0.23 or 0.015 mg/m$^3$ exposure groups appeared to be related to treatment. Differences that were statistically significant were incidental, not dose-related, lacked histopathological changes and/or were considered toxicologically insignificant.

Based on these observations, we agree with the study authors' conclusions that the LOEL for this study is 2.1 mg/m$^3$ (0.0021 mg/L), and that the NOEL is 0.23 mg/m$^3$ (0.00023 mg/L).

## IV. STUDY DEFICIENCIES

The oxygen content of the chamber airflow was not reported. However, this minor deficiency does not affect interpretation of the study results.