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

DEC - 3 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione (Dazomet): Review of Toxicology Data .

Tox. Chem. No: 840
P.C. Code: 035602

Submission: S478165
DP Barcode: D210051

FROM: Timothy F. McMahon, Ph.D., Pharmacologist 11/24/95
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Virginia Dietrich/ Ron Kendall - PM 51
Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y. M. Ioannou 11/28/
Review Section I, Toxicology Branch II
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and

Stephanie Irene, Ph.D., Acting Branch Chief
Toxicology Branch II
Health Effects Division (7509C) Stephanie R. Irene 12-1-95

Registrant: BASF Corporation

Action Requested: Review of acute and subchronic neurotoxicity studies; review of dermal sensitization and 21-day dermal toxicity studies.

I. General

The registrant (BASF Corporation) has submitted acute and subchronic neurotoxicity studies to the Environmental Protection Agency, Office of Pesticide Programs, for review as part of the re-registration of dazomet. In addition, a dermal sensitization study and a 21-day dermal toxicity study were retrieved and reviewed, as these data had not been previously sent to Toxicology Branch II for review. The results of these reviews are presented below in executive summary format.

II. Review of Data

A) MRID # 434653-02: Dazomet - Acute Oral Neurotoxicity Study in Wistar Rats

In an acute neurotoxicity study (MRID # 434653-02), Wistar Chbb: THOM (SPF) rats (10/sex/group) were orally gavaged once with Dazomet in 0.5% aqueous carboxymethylcellulose at doses of 0 (vehicle only), 50, 150 and 450 mg/kg body weight (a.i. equivalents: 50, 130, and 450 mg/kg) for males and 0, 15, 50, and 150 mg/kg body weight (a.i. equivalents: 13, 50, and 130 mg/kg) for females. The animals were observed for mortality and clinical signs of toxicity for 14 days post-dosing. Compound-related decreases in body weight were noted in mid-(7.0%) and high-(12.7%) dose males at day 7; the body weight gains for the same dose groups were 34.2% and 58.6%, respectively. A dose-dependent increase in clinical signs (half closure of eyelids, salivation, lacrimation, impaired activity in open field, changes in fur, reduced number of rearings) and impairment of motor activity was seen in males and/or females at all dose levels. These effects were reversible by observation Day 7. **No treatment-related gross or neuropathological findings were present. The NOEL for systemic toxicity = 50 mg/kg in males; not established (>HDT) in females. The LOEL for systemic toxicity = 150 mg/kg in males based on decreased body weight and body weight gain.**

Based on the findings of this study (screening battery), the LOELs for neurobehavioral effects were established at 50 mg/kg in males (FOB findings and reduced number of rearings) and 15 mg/kg in females (reduced number of rearings and decreased motor activity).

The study is classified as **Acceptable** and **satisfies** the requirements (81-8) for an acute neurotoxicity in rats.

B) **MRID 434653-01: Dazomet - Subchronic Oral Neurotoxicity Study in Wistar Rats.**

In a subchronic neurotoxicity study, Dazomet (>96.3%) was administered to Wistar rats (10/sex/group) at dietary levels of 0, 50, 200, and 400 (females) or 450 ppm (males) (0, 4, 15/16 and 34/36 mg/kg/day, respectively). The rats were observed for 91 days; clinical observation, body weight, functional observation battery, and motor activity data were collected. Neuropathological evaluations were performed on 5 rat/sex/group. The remaining animals were subjected to gross- and histopathological examinations of the liver.
Treatment-related effects included fatty degeneration of the liver in 3/10 low-dose males. In addition, increased liver weights were noted in mid-dose males and high-dose males and females. A significant decrease in mean body weight and body weight gain was noted for high-dose males and females. **No treatment-related neurobehavioral or neuropathological effects were observed.**
Based on the findings of this study (fatty degeneration of the liver) the LOELS were established at 50 ppm (4 mg/kg/day) in male and 200 ppm (16 mg/kg/day) in females. The NOEL was not established in males and established at 50 ppm (4 mg/kg/day) in females.

This study is classified as **Acceptable** and **satisfies** the §82-7 guideline requirement for subchronic neurotoxicity study in rats.

C) **MRID 402991-01: 21-Day Dermal Toxicity Study in Rabbits**

In a 21-day dermal toxicity study (MRID # 402991-01), Hra:(NZW)SPF rabbits received dermal applications of either 0, 10, 100 or 1000 mg/kg of Dazomet, six hours per day, five days per week for 21 days. Ten animals/sex/group (including 5/sex/group satellite animals) were assigned to 0-, and 10-mg/kg/day group; and 5 animals/sex/group to the 100- and 1000-mg/kg/day groups. All animals were sacrificed after 3 weeks except the satellite animals which were sacrificed after 6 weeks. The test material was applied on 0.4% w/v on carboxymethylcellulose in purified water daily at a volume-dosage of 1 to 3 ml/kg body weight.

There were no differences between the control and treated groups in any of the parameters measured.

The systemic toxicity LOEL is >1000 mg/kg/day males and females; the systemic toxicity NOEL is ≥ 1000 mg/kg/day males and females.

The dermal toxicity LOEL is >1000 mg/kg/day for males and females; the dermal toxicity NOEL is ≥ 1000 mg/kg/day for males and females.

The study is **Acceptable** and **satisfies** the guideline requirements for a 21-day dermal toxicity study (82-2) in the rabbit.

D) **MRID 470145-05: Report of the Maximization Test for the Sensitizing Potential of Dazomet in Guinea Pig.**

In a dermal sensitization study (MRID # 470145-05) using the Magnusson and Kligman test, twenty female Dunkin-Hartley albino guinea pigs received six intradermal injections (in groups of two per animal) in the clipped flank area, at induction doses of 0.1 ml of Freund's adjuvant/water (1:1) with or without test substance (5% formulation), and 0.1 ml of 5% test formulation in olive oil. Controls (10/group) received the same injections but without test substance. Skin reactions were recorded after 24 hours. One week after intradermal injections (during percutaneous induction phase), animals were exposed to 5% test formulation in olive oil under occulded dressing for 48 hours and skin reactions were recorded after 24 hours. During two topical challenge doses (one after 19 days and another after 26 days following intradermal induction), 60% test formulation in olive oil or olive oil alone, was applied to each animal under occluded dressing for 24 hours. Skin reactions were recorded at 24, 48, and 72 hours post-dosing. One of the two control groups received olive oil alone during the first challenge applications.

After intradermal induction, erythema and edema were observed in control and test animals. After percutaneous induction with the test substance in olive oil, in addition to erythema and edema, incrustation resulting from the intradermal induction was observed. The treatment of the control group with olive oil caused the same symptoms. The first and second challenge with a 60% test suspension in olive oil did not cause any skin reactions in control and surviving test animals (6 of them died of pneumonia during the study).

The chemical's ability to produce dermal sensitization could not be determined with assurance due to the lack of positive control data.

The study is classified as **Supplementary Data** and **does not satisfy** the requirements (81-6) for a dermal sensitization study in guinea pigs. The study may be upgraded if accceptable positive control data are provided.

DAZOMET Dermal Sensitization (81-6)

Reviewed by: Sanjivani B. Diwan, Ph.D. Sanjivani Diwan, Date: 10/15/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou, Date: 10/18/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization/Guinea Pigs (81-6)

EPA ID NUMBERS:
DP BARCODE: D210051
P. C. CODE: 035602
MRID NUMBER: 470145-05
SUBMISSION No.: S478165

TEST MATERIAL: Dazomet
Synonym: Tetrahydro-3.5-dimethyl-2H-1.3.5-thiadiazine-2-thione

STUDY NUMBER: Project No. 30H318/85

TESTING FACILITY: BASF Aktiengesellschaft, Ludwigshafen/Rhein, FRG

SPONSOR: The Dazomet Task Force consisted of the following:

BASF Wyandotte Chemical Corp., Parsippany, NJ
Buckman Laboratories, Inc., Memphis, TN
Calgon Corporation, Pittsburgh, PA
Stauffer Chemical Company., Richmond, CA
Vinings Chemical Company, Atlanta, GA

TITLE OF REPORT: Report of the Maximization Test for the Sensitizing Potential of Dazomet in Guinea pig

AUTHOR: H.P. Gelbke?

REPORT ISSUED: December 20, 1985

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID # 470145-05) using the Magnusson and Kligman test, twenty female Dunkin-Hartley albino guinea pigs received six intradermal injections (in groups of two per animal) in the clipped flank area, at induction doses of 0.1 ml of Freund's adjuvant/water (1:1) with or without test substance (5% formulation), and 0.1 ml of 5% test formulation in olive oil. Controls (10/group) received the same injections but without test substance. Skin reactions were recorded after 24 hours. One week after intradermal injections (during percutaneous induction phase), animals were exposed to 5% test formulation in olive oil under occulded dressing for 48 hours and skin reactions were recorded after 24 hours. During two topical challenge doses (one after 19 days and another after 26 days following intradermal induction), 60% test formulation in olive oil or olive oil alone, was applied to each animal under occluded dressing for 24 hours. Skin reactions were recorded at 24, 48, and 72 hours post-dosing.

One of the two control groups received olive oil alone during the first challenge applications.

After intradermal induction, erythema and edema were observed in control and test animals. After percutaneous induction with the test substance in olive oil, in addition to erythema and edema, incrustation resulting from the intradermal induction was observed. The treatment of the control group with olive oil caused the same symptoms. The first and second challenge with a 60% test suspension in olive oil did not cause any skin reactions in control and surviving test animals (6 of them died of pneumonia during the study).

The chemical's ability to produce dermal sensitization could not be determined with assurance due to the lack of positive control data.

The study is classified as **Supplementary Data** and **does not satisfy** the requirements (81-6) for a dermal sensitization study in guinea pigs. The study may be upgraded if accceptable positive control data are provided.

I. MATERIALS

A. Test Material

Name: Dazomet
Synonym: Tetrahydro-3.5-dimethyl-2H-1.3.5-thiadiazine-2-thione
Purity: 98.2%
Substance Number: 85/318
Batch Number: 26-5297
Description: White powder
Storage Conditions: at room temperature

Structure:

Vehicle(s): Freund's Ajuvant/water (1:1)
Olive oil DAB 8 (hereafter referred to as olive oil)

A fresh formulation of the test material was prepared either as 0.1 ml Freund's adjuvant with test substance in emulsified water in a ratio of (1:1) or as 5% or 60% test suspension in olive oil.

B. Test Animals

Species: Pirbright White guinea pigs, Dunkin-Hartley HOE DHPK [SPF-LAC] BO
Source: Lippische Versuchstiersucht, Hagemann GmbH & Co. KG, Extertal 1, FRG
Age: 6-8 weeks at dosing
Weight: Females - 253 to 300 g at dosing
Housing: Five per cage
Food and water: Diet (Kliba 341, 4mm) and tap water *ad libitum*
Environmental Conditions: Temperature: 20-40^{0}C
Relative Humidity: 30-70%
Photoperiod: 12 hours light/dark
Acclimation Period: at least 7 days

II. METHODS

The study was conducted by maximization test using modified Magnusson and Klingman method.

Preliminary Test

In a preliminary test, the sensitization potential of Dazomet was investigated in guinea pigs (4/group; sex unspecified). Test formulations were prepared fresh and concentrations were determined in weight/weight. During intradermal induction phase, guinea pigs received 5% test suspension in olive oil or formulation in Freund's adjuvant/water (1:1) or olive oil alone. During percutaneous

induction as well as two challenge phases, the animals received 60% test formulation in olive oil or olive oil alone. During challenge 6phases, test substance was applied 2 times for 24 hours within a period of 96 hours. The treated areas were evaluated and scored for signs of erythema and edema at 24 and 48 hours using the following scale.

Grade	Reaction to Treatment
0	No erythema or edema
1	Very slight (barely perceptible) erythema or edema
2	Well-defined erythema or slight edema (with edges of area well defined by definite raising)
3	Moderate to severe erythema or moderate edema (raised approx. 1 mm)
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth) or severe edema (raised more than 1 mm and extending beyond the area of exposure)

In the preliminary trial, there was no evidence of dermal irritation with the 60% test formulations at 24 or 48 hours, therefore, the 60% formulation was selected for both the induction and challenge doses in the main study.

Main study

The animals were assigned to four groups as summarized below.

Groups (No. of animals)	Intradermal induction	Percutaneous induction	First Challenge	Second Challenge
Control 1 (10)	Freund's adjuvant/ water or olive oil only	60% in olive oil or olive oil only	60% in olive oil or olive oil	60% in olive oil or olive oil
Control (10)	Freund's adjuvant/ water or olive oil only	60% in olive oil or olive oil only	Olive oil only	60% in olive oil or olive oil
Test Group (20)	Freund's adjuvant/ water (1:1), 5% test formu- lation emulsified in water or 5% in olive oil	60% in olive oil or olive oil only	60% in olive oil or olive oil only	60% in olive oil or olive oil

Induction Phases

During intradermal induction, six injections in groups of two per animal were administered in the clipped flank area as follows: Test groups received two injections each of 0.1 ml Freund's adjuvant/water (1:1) in the front row; 2 injections each of 0.1 ml of test substance suspension in olive oil in the middle row; and 2 injections each of 0.1 ml Freund's adjuvant with test substanace emulsified with water (1:1). Control animals received similar injections with formulating agent but without the test substance. Skin reactions were recorded 24 hours after beginning of intradermal phase. Due to slight skin reactions at injection sites, they were treated with 10% formulation of sodium dodecyl sulfate in vaseline 24 hours before percutaneous induction.

During percutaneous induction phase (one week after intradermal induction), 0.5 mm thick layer (containing 0.3 g) of the test substance formulation was applied to the skin under occlusive dressing for 48 hours. Evaluations for signs of dermal irritation were made at 48 hours post application.

Challenge Phase

Fourteen days following the last induction dose, the first challenge doses of the test and control chemicals were applied to test sites and the second challenge dose was applied one week later. The following procedure was used:

For the first challenge phase, an occluded topical application of 0.15 g of test substance formulation was applied as first challenge dose to both the test and control group 1. In addition olive oil was applied as a vehicle. Control group 2 received olive oil only.

For the second challenge phase, both the test and control groups 1 and 2 received applications with 0.15 g of the test substnace formulation which were followed by application of olive oil as a vehicle.

After twenty-four hour exposure period, the application sites were examined at 24, 48, and 72 hours post-dosing using the scale stated above. Significant erythematous reaction (Grade 1 or above) in at least 15% of the animals was considered to be positive.

Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality.

III. RESULTS

After intradermal induction erythema and edema were observed in control and test animals that received Freund's adjuvant. Injection of test formulation in Freund's adjuvant also caused distinct erythema and edema in test animals, whereas test animals injected with test formulation in olive oil only showed distinct erythema. Percutaneous induction with test formulation in olive oil caused incrustation in addition to erythema and edema. The control animals receiving olive oil also showed same reactions. After first and second challenge applications, no skin reactions were noted in the test and control animals.

IV. STUDY DEFICIENCIES

The major deficiency in this study is the lack of data on positive controls.

In addition, the following minor deficiencies were noted:

- Six test animals died of pneumonia during the course of the study.
- The stability of the test substance in olive oil was not tested and concentration analyses were not provided.

However, these minor deficiencies do not negatively impact upon the outcome of the study.

IV. CONCLUSIONS

The chemical's ability to produce dermal sensitization could not be determined with aaaurance due to the lack of positive control data.

The study is classified as **Supplementary Data** and **does not satisfy** the requirements (81-6) for a dermal sensitization study in guinea pigs. The study may be upgraded if the acceptable positive control data are provided.

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DAZOMET Acute Neurotoxicity (81-8)

Reviewed by: Sanjivani B. Diwan, Ph.D. Sanjivani Diwan Date: 11/2/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Robert F. Fricke, Ph.D. Robert Fricke Date: 2 NOV 95
Section II, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Neurotoxicity/Rats (§81-8)
EPA ID NUMBERS:	DP BARCODE: D210051 P. C. CODE: 035602 MRID NUMBER: 434653-02 SUBMISSION No.: S478165 CASWELL No.: 840
TEST MATERIAL:	Dazomet Synonym:Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
STUDY NUMBER:	94/10800
TESTING FACILITY:	BASF Aktiengesellschaft Ludwigshafen, Germany
SPONSOR:	BASF Corporation, Research Triangle Park, NC
TITLE OF REPORT:	Dazomet - Acute Oral Neurotoxicity Study in Wistar Rats
AUTHOR:	W. Mellert
REPORT ISSUED:	September 16, 1994

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID # 434653-02), Wistar Chbb: THOM (SPF) rats (10/sex/group) were orally gavaged once with Dazomet in 0.5% aqueous carboxymethylcellulose at doses of 0 (vehicle only), 50, 150 and 450 mg/kg body weight (a.i. equivalents: 50, 130, and 450 mg/kg) for males and 0, 15, 50, and 150 mg/kg body weight (a.i. equivalents: 13, 50, and 130 mg/kg) for females. The animals were observed for mortality and clinical signs of toxicity for 14 days post-dosing. Compound-related decreases in body weight were noted in mid-(7.0%) and high-(12.7%) dose males at day 7; the body weight gains for the same dose groups were 34.2% and 58.6%, respectively. A dose-dependent increase in clinical signs (half closure of eyelids, salivation, lacrimation, impaired activity in open field, changes in fur, reduced number of rearings) and impairment of motor activity was seen in males and/or females at all dose levels. These effects were reversible by observation Day 7. **No treatment-related gross or neuropathological findings were present.**

NOEL for systemic toxicity = 50 mg/kg in males; not established (>HDT) in females.
LOEL for systemic toxicity = 150 mg/kg in males based on decreased body weight and body weight gain

Based on the findings of this study (screening battery), the LOELs for neurobehavioral effects were established at 50 mg/kg in males (FOB findings and reduced number of rearings) and 15 mg/kg in females (reduced number of rearings and decreased motor activity).

The study is classified as **Acceptable** and **satisfies** the requirements (81-8) for an acute neurotoxicity in rats.

A. MATERIALS

1. Test Material: Dazomet
Chemical Name: Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
Purity: ≥96.3%
Batch Number: 92-1
Description: Light-yellow granular powder
Storage Conditions: At room temperature

2. Vehicle: 5% Aqueous carboxymethyl cellulose

3. Test Animals: Species: Rat
Strain: Wistar (Chbb: THOM (SPF))
Source: Karl Thomae GmbH, Biberach/Riss, FRG.
Age at Dosing: Males and females - 42 days
Weight at Dosing: Males - 184 to 215 g; Females -140 to 183 g
Housing: One rat per stainless steel cage
Environmental Conditions: Temperature: 20-24°C
Relative Humidity: 30-70%
Photoperiod: 12 hours light/dark
Air Changes: Not reported
Food and Water: Pelleted standard Kliba 343 (from Kliba, Klingentalmuehle AG, Kaiseraugst, Switzerland) Rat/Mouse/Hamster Diet and water *ad libitum* except during motor activity measurements
Acclimation Period: Up to fifteen days

B. STUDY DESIGN and METHODS:

1. Dose-Range Finding Studies: Prior investigations included two acute oral studies and a kinetic and metabolism study. In addition, the peak time of effect was also determined. During the first acute study, Sprague-Dawley rats (5/sex/group) were once gavaged with 320, 400, 500, 640, 800, and 1000 mg/kg in 4% carboxymethylcellulose (CMC) and observed for mortality and clinical signs for 7 days. For the second study, Sprague-Dawley rats (10/sec/group) were gavaged once at dose levels of 147, 215, 316, 464, 562, and 681 mg/kg in 5% CMC and observed for mortality and clinical signs for 14 days. For the metabolism study, rats received ^{14}C-labeled Dazomet in aqueous CMC at 10 and 100 mg/kg body weight. In a "peak finding study", male and female Wistar rats (5/sex/dose) were gavaged once with Dazomet at 450 mg/kg and 150 mg/kg, respectively.

2. Main Study:

a. Animal assignment and treatment: Animals were assigned to the test groups in Table 1 using computer generated randomization procedure. The doses for the main study were selected based on the results of range-finding studies. The test animals received corresponding doses (10 ml/kg) once by gavage while the control animals received vehicle only. Each animal received a single dose of the test solution on a mg/kg body weight basis.

Table 1. Animal Assignment to Study Groups.

Dose Group	Dose Level in mg/kg Male	Female	Number Assigned Male	Female
Control	0	0	10	10
Low	50	15 (13)	10	10
Mid	150 (130)	50	10	10
High	450	150 (130)	10	10

*Values in parentheses represent the doses corrected for percent purity of active ingredient

b. Dosing Preparations: The dosing solutions were prepared fresh prior to administration. The weighed amount of the test substance was placed in a beaker and an appropriate volume of 5% aqueous carboxymethylcellulose solution was added and mixed using a high speed sonicator for approx. 1 minute. The dosing solution was mixed on a magnetic stirrer to achieve homogeneity. The concentration analyses were conducted on samples from the three dosing solutions.

3. Analytical Chemistry: The purity of the test substance and homogeneity of the dosing solutions were confirmed prior to dosing. Three samples, each from the top, middle and bottom portions, of the high and low male dosing solutions were analyzed for homogeneity. Concentration analyses were performed on all concentrations administered to female rats. Stability of the dosing solution over 4 hours (before and at the end of the study) was confirmed. All samples were frozen until analyzed.

4. Observations

a. Clinical signs: Animals were observed at least once daily and once during weekends for signs of toxicity and

mortality. A detail examination was conducted once weekly.

b. Body weights/Food Consumption: Body weight and food consumption of animals were recorded prior to group assignment and at Day 0 (day of dosing) and at weekly intervals, thereafter.

5. Neurobehavioral Evaluations: Neubehavioral tests consisted of the Functional Observational Battery (FOB) and evaluation of motor activity. Tests were performed 7 days prior to dosing, on Day 0 (1 hr after dosing) and on Days 7 and 14 post-dosing.

a. Motor activity: Motor activity was measured using the Multi-Varimex-System with 4 infrared beams per cage. Animals were monitored individually over a 60 minute session, consisting of 12, 5-minute intervals.

b. Functional Observational Battery: The following parameters were evaluated for the presence or absence of finding, ranked based on severity and degree of effect:

Home Cage Observations:
- Posture/behavior
- Urination/defecation
- Convulsions/tremors
- General observations

Sensorimotor/Reflex Response Observations:
- Hyperesthesia
- Abdominal tension
- Palpebral closure
- Winking reflex
- Pupil size
- Pupillary reflex
- Pinna reflex
- Startle response (Audition)
- Olfaction
- Tail pinch response (pain perception)
- Righting response (coordination of movements
- Vision (visual placing response)

Open field Observations:
- Appearance of fur
- Skin color
- Posture
- Salivation
- Respiration
- Arousal/Activity
- Vocalization
- Lacrimation
- Convulsion/tremors
- Bizarre behavior
- Gait impairment
- Exophthalmus
- Number of rearings within 2 minutes

Measured Responses:
- Landing foot spread
- Fore/hindlimb grip strength

6. Historical Data: Data from validation studies for a neurotoxicity screen in rats using positive and negative control materials were provided (study No. 20C0062/92044, Vol.IV, included as pages C025-C62. The positive control substances tested consisted of acrylamide, trimethyltin chloride, 3,3"-iminodiproprionitrile, nomifensin and diazepam. These studies demonstrated the ability of the performing

laboratory to evaluate neurological effects.

7. Sacrifice and Pathology: At the end of the study, 5 animals/sex/group showing the most distinct neurological signs, were selected for neuropathological evaluations. In the absence of any symptoms the first five animals of the test groups were selected and sacrificed by perfusion fixation and subjected to neuropathological examinations of the central and peripheral nervous system. The remaining animals were anesthetized with CO_2 and sacrificed with no further examinations.

8. Statistical Evaluations: Homogeneous (parametric) data were evaluated using ANOVA with significance assessed using the F-distribution. Significance of ANOVA findings were followed by pair-wise comparisons between the control and treatment groups using Dunnett's test. Nonparametric data were evaluated using Kruskal-Wallis-h-test; the significant findings were followed by pairwise comparison between the control and treatment groups using Mann-Whitney-U-test.

III. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

IV. RESULTS

A. Dose-Range Finding Studies: During the first acute oral study, 0, 20, 30, 50, 70 and 100% mortality was noted in rats at dose levels of 320, 400, 500, 640, 800, and 1000 mg/kg, respectively. Clinical signs observed over 7-day observation period at 800 and 1000 mg/kg included dyspnea, abdominal position, tremors and red colored urine; similar but less severe signs were noted at 500 and 640 mg/kg while the two lower doses produced nonspecific symptoms in few animals. In the second acute study, the incidence of mortality was 0, 10, 10, 20, 60 and 90% in rats dosed at 147, 215, 316, 464, 562, and 681 mg/kg, respectively. The clinical signs observed over 14-day observation period including dyspnea, apathy, staggering, piloerection and poor general health were noted at all dose levels. These studies established the LD_0 in male and female rats at 320 mg/kg and 147 mg/kg, respectively.

In the metabolism study, 15-49% radioactivity was excreted via the urine within 24 hours and 52-78% by 120-168 hours; 21-33% radioactivity was excreted in the expired air within 24 hours. The peak plasma concentration was achieved within 1 hr. The plasma concentration in females (9-16.7 μg equivalent Dazomet/ml) was greater than in males (1.6-2.1 μg equivalent Dazomet/ml) examined up to 168 hrs post-dosing; plasma half-life was 61 and 67-71 hrs in males and females, respectively. The peak effect as indicated by clinical signs including lethality, ataxia, salivation, eyelid discharge and poor general state was noted within 1 to 5 hrs post-dosing.

Thus, based on the lower LD_0 value and higher plasma peak levels the female rats appear to be more sensitive to Dazomet than males.

B. Main Study:

1. Analytical Chemistry: The purity of the test material analyzed prior to initiation and at termination of study was 98% and 96.3%, respectively. The concentration analyses revealed that the achieved concentrations for the 15, 50, 150 and 450 mg/kg dose groups were 87.1,96.6, 85.5 and 101.0% of target, respectively. The homogeneity was confirmed at 50 and 450 mg/kg/day. The dose levels of 15 (for females) and 150 mg/kg (for males and females) were corrected for percent active ingredient; thus the actual doses administered were 13 and 130 mg/kg, respectively. The stability analyses indicated that the compound was stable in vehicle over 4 hour period (101.9-101.4% of nominal).

2. Clinical Signs and Mortality: During the general clinical observations no abnormal signs or mortality were detected.

3. Body Weight and Body Weight Gain: The mean body weight for high-pose males was significantly below control (13% on day 7 and 8% on day 14). The body weight for mid-dose males was 7% below control on day 7 (Table 2A). The mean body weight gain of high-dose males was also below control (59% on day 7 and 24% on day 14); the body weight gain for mid-dose males was about 34% below control on day 7 (Table 2B). No changes were observed in females at any dose level.

Table 2 A: Mean Body Weight Data for Male Rats[a]

Day	Dose Level (mg/kg)			
	0	50	150	450
0	197.0	196.3	196.8	195.0
% Change	--	-0.4	-0.11	-1.0
7	247.3	244.7	229.9*	215.8**
% Change	--	-1.1	-7.0	-12.7
14	289.8	292.0	279.7	265.6**
% Change	--	+0.7	-3.5	-8.3

[a]Data extracted from Study No.94/10800, Table 3, p. 56
*p ≤0.05, **p ≤0.01

Table 2 B: Mean Body Weight Gain Data for Male Rats[a]

Day	Dose Level (mg/kg)			
	0	50	150	450
7	50.3	48.4	33.1**	20.8**
% Change	--	-3.7	-34.2	-58.6
14	92.8	95.7	82.9	70.6**
% Change	--	+4.3	-10.6	-23.9

[a]Data extracted from Study No.94/10800, Table 5, p. 58
**p ≤0.01

4. Functional Observation Battery (FOB) and Motor Activity: Compound and dose-related increased incidence of clinical signs and impairment of motor activity were seen within several hours of dosing at all dose levels (Table 3).

a. Functional Observation Battery: A dose-related increase in the incidences of FOB findings were observed in male and/or female of rats at low-, mid- and high-dose levels. The findings were evaluated for the presence or absence of observation, and degree of effect and were ranked based on severity. The clinical signs consisted of half closure of eyelids, salivation , impaired (decreased) activity in the open field , lacrimation and changes in fur (i.e. urine stain on anogenital fur (Table 3A). Impaired gait (rank 1) noted in one mid-dose male was considered incidental. The number of rearings were significantly reduced in all treated males and/or females

at low- (26% in males), mid- (31-33%) and high- (11.8-14.5) dose levels (Table 3B). No abnormal signs were seen during examinations on day 7 and 14. No compound-related adverse changes in sensorimotor and reflex tests were observed.

Table 3A. Functional Observational Batterya. Results at 1-5 hrs post-dosing.

Observation	Dose Levels, mg/kg (Males/Females)			
	0/0	50/15	150/50	450/150
Males (N = 10)				
Half closed eyelids	0	1	7	8
Salivation, moderate	0	2	9	10
Decreased activity	0	3	6	10
Lacrimation, slight	0	2	8	8
Females (N = 10)				
Half closed eyelids	0	0	3	10
Anogenital stains	0	0	1	5
Salivation, moderate	0	0	3	8
Decreased activity	0	0	4	6
Lacrimation, slight	0	0	6	9

[a]Data were extracted from Study No.94/10800, Tables 10-34; p. 68-87.

Table 3 B: Mean Number of Rearings in Rats on Day 0[a]

Sex	Dose Level, mg/kg (Males/Females)			
	0/0	50/15	150/50	450/150
Males	4.2	1.1**(26.2)[b]	1.3**(31.0)	1.4* (33.3)
Females	7.6	4.5	0.9***(11.8)	1.1***(14.5)

[a]Data summarized from Study No. 94/10800, Table 5, pp. 88-89; Number of rearings measured within 1-5 hrs post-dosing.
[b]The numbers in parentheses represent the % of control
*p≤0.5; **p≤0.01; ***p≤0.001

b. Motor activity: The overall motor activity on day 0 for all intervals combined was significantly decreased compared to controls in mid- and high-dose males (>20%) and in all treated females (13.9-77.4%) (Table 3C). Although significant decreases in motor activity occurred

in mid- and high-dose males on day 7 because of lack of dose-response they were considered incidental.

Table 3C. Effect of Dazomet on Total Motor Activity in Rats on Day 0[a]

Dose Groups, mg/kg (Males/Females;10/sex)	No. Beam Interruptions(%)	
	Males	Females
Control	83 (100)	205 (100)
50/15	51 (61.5)	159*(77.4)
150/50	17**(20.6)	69**(33.4)
450/150	17**(20.5)	29**(13.9)

[a]Total beam interruptions over 12 5-min. intervals; the numbers in parentheses represent % of control; the data are extracted from Study No.94/10800, Tables 43 and 44,pp. 96-97.
*p≤0.05;**p≤0.02;***p≤0.002

5. Sacrifice and Pathology: There were no compound-related pathological findings in the central and peripheral nervous system. The incidental findings included internal hydrocephalus in one male at 450 mg/kg and a single axonal degeneration in the proximal sciatic nerve was noted in two control males and one male from the 450 mg/kg dose group.

V. DISCUSSIONS/CONCLUSIONS: In this neurotoxicity study, Wistar rats (10/sex/group) were orally gavaged once with Dazomet at doses of 0 (vehicle only), 50, 150, or 450 mg/kg for males and 0, 15, 50, or 150 mg/kg for females. Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted 7 days prior to dosing, Day 0 (within 1-5 hrs post-dosing), and Days 7 and 14. On Day 15, animals were euthanized and neuropathological examinations were performed on control and treated animals (5/sex/group).

All animals survived until terminal sacrifice without any abnormal signs during general clinical observations. In mid- and high-dose males, biologically and statistically significant decreases in body weight (7.0% and 12.7%, respectively) and body weight gain (34.2% and 59%, respectively) were noted on day 7 post-dosing.

Neurobehavioral evaluation revealed increased incidence of clinical signs such as half closure of eyelids, salivation, lacrimation, impaired activity in open field, anogenital staining, reduced number of rearings and decreased motor

activity in a dose-dependent manner. These symptoms were reversible by day 7. Comprehensive neuropathological examinations of the central and peripheral nervous system revealed no compound-related effects.

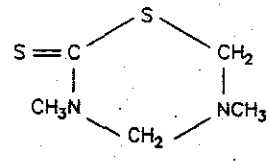
NOEL for systemic toxicity = 50 mg/kg in males; not established (>HDT) in females.
LOEL for systemic toxicity = 150 mg/kg in males based on decreased body weight and body weight gain

Based on the findings of this study (screening battery), the LOELs for neurobehavioral effects were established at 50 mg/kg in males (FOB findings and reduced number of rearings) and 15 mg/kg in females (reduced number of rearings and decreased motor activity). The NOEL was not established (<50 mg/kg for males and <15 mg/kg in females)

The study is classified as **Acceptable** and **satisfies** the requirements (81-8) for an acute neurotoxicity study in rats.

Reviewed by: Sanjivani B.Diwan, Ph.D. Sanjivani B. Diwan, Date: 11/9/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Robert F. Fricke, Ph.D. Robert F. Fricke, Date: 11/9/95
Section II, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Subchronic Neurotoxicity Study in Rats (§82-7)
EPA ID NUMBERS:	DP BARCODE: 210051 P. C. CODE: 035602 MRID NUMBER: 434653-01 SUBMISSION No.: S478165 CASWELL No.: 840
TEST MATERIAL:	Dazomet Synonym:Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
STRUCTURE:	
STUDY NUMBER:	94/10799
TESTING FACILITY:	BASF Aktiengesellschaft Ludwigshafen, Germany
SPONSOR:	BASF Corporation, Research Triangle Park, NC
TITLE OF REPORT:	Dazomet - Subchronic Oral Neurotoxicity Study in Wistar Rats
AUTHOR:	W. Mellert
REPORT ISSUED:	September 23, 1994

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study, Dazomet (>96.3%) was administered to Wistar rats (10/sex/group) at dietary levels of 0, 50, 200, and 400 (females) or 450 ppm (males) (0, 4, 15/16 and 34/36 mg/kg/day, respectively). The rats were observed for 91 days; clinical observation, body weight, functional observation battery, and motor activity data were collected. Neuropathological evaluations were performed on 5 rat/sex/group. The remaining animals were subjected to gross- and histopathological examinations of the liver.

Treatment-related effects included fatty degeneration of the liver in 3/10 low-dose males. In addition, increased liver weights were noted in mid-dose males and high-dose males and females. A significant decrease in mean body weight and body weight gain was noted for high-dose males and females. **No treatment-related neurobehavioral or neuropathological effects were observed.**

Based on the findings of this study (fatty degeneration of the liver) the LOELS were established at 50 ppm (4 mg/kg/day) in male and 200 ppm (16 mg/kg/day) in females. The NOEL was not established in males and established at 50 ppm (4 mg/kg/day) in females.

This study is classified as **Acceptable** and **satisfies** the §82-7 guideline requirement for subchronic neurotoxicity study in rats.

I. MATERIALS:

1. Test Material: Dazomet
Chemical Name: Tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
Purity: ≥96.3%
Batch Number: 92-1
Description: Light-yellow granular powder
Storage Conditions: At room temperature

2. Vehicle: 5% Aqueous carboxymethyl cellulose

3. Test Animals: Species: Rat
Strain: Wistar Chbb: THOM (SPF)
Source: Karl Thomae GmbH, Biberach/Riss, FRG.
Age at Dosing: Males and females - 42 days
Weight at Dosing: Males - 151 to 199 g; Females -138 to 166 g
Housing: One per stainless steel cage
Environmental Conditions: Temperature: 20-24°C
Relative Humidity: 30-70%
Photoperiod: 12 hours light/dark
Air Changes: Not reported
Food and Water: Pelleted standard Kliba 343 (Kliba, Klingentalmuehle AG, Kaiseraugst, Switzerland) rat/mice/hamster maintenance Diet and water *ad libitum* except during motor activity measurements
Acclimation Period: Fifteen days

B. STUDY DESIGN:

1. Animal Assignment:

Following a period of acclimation, the rats were assigned to study groups via a body weight-dependant randomization scheme (Table 1).

Table. Animal Assignment

Test Group [a]	Dietary Level (ppm)	Number Assigned	
		Males	Females
1 (Control)	0	10	10
2 (Low)	50	10	10
3 (Mid)	200	10	10
4 (High)	450/400[a]	10	10

a Male and female rats received 450 and 400 ppm, respectively

2. Justification of Dose Level Selection

The dose levels were selected on the basis of the results of three earlier feedings studies in rats as follows:

In a test study, Dazomet was administered orally to rats (3/sex/dose) at 0, 200, 800 and 3,200 ppm for 24 days. The clinical signs observed at the two highest dose level included reduced general condition associated with severely impaired food consumption and body weights ($\geq$29%) and ataxia. Death of all females occurred at 3,200 ppm. The NOEL was 200 ppm.

In a 4-week study, Wistar rats (5/sex/dose) were fed Dazomet in diet at nominal concentrations of 0, 20, 60, 180 and 540 ppm (actual conc.: 0, 17, 51, 153 or 459 ppm, respectively). Dose-related signs of toxicity observed at the two highest concentrations included decrease in body weight ($\geq$11%) and food consumption in both sexes, and neurological symptoms in females (hind limb paresis, strutting and waddling gait, impaired posture of the forelimb, less coordinated swimming behavior; no morphological changes in the gross- and histopathology were observed. Increase in liver weights and fatty degeneration of the liver and decrease in creatinine in both sexes and decrease in glutamate oxalacetate transaminase, glutamate pyruvate transaminase (GPT), and plasma cholinesterase in females. The overall effects were less pronounced at 153 ppm. No mortalities were observed. At 51 ppm, reduced GPT activity was noted in females only; the NOEL was 17 ppm.

In another study, Dazomet was fed to Wistar rats (10/sex/dose) for 3 months at dietary concentrations of 0, 20, 60, 180 and 360 ppm. Changes in clinical chemistry parameters noted at the 180 ppm and greater were as follows: decrease in total protein (in both sexes at 360 ppm; in males at 180 ppm), and creatine, potassium and albumin in females and triglycerides in males (at 360 ppm). In addition, an increase in liver weight and fatty degeneration in both sexes at 180, and 360 ppm and in males at 60 ppm were observed. The NOEL for males and females was 20 and 60 ppm, respectively.

Based upon these findings, the dosages for the subchronic neurotoxicity study were set at 50, 200, and 450 (males) and 400 (females) ppm.

3. Test Diet Formulation, Administration, and Analysis

A measured amount of test substance was thoroughly mixed with a small amount of basal diet in a beaker. This mixture was blended with basal diet in a BOSCH mixer for 3 min. Basal diet was then added to this

premix to yield desired dietary concentrations. Control diet consisted of unmixed basal diet. Test diets were prepared at least twice weekly and stored at 4°C. The homogeneity and stability were proven in previous studies. Animals received the appropriate diets *ad libitum* throughout the study except during motor activity measurements; fresh diet was provided at intervals of two days.

The purity of the test substance and the homogeneity of test diets were confirmed before the start of the study. The concentration analyses and stability of the test substance in the diet at room temperature and over 4 days at 4°C were confirmed prior to study initiation.

C. Observations

1. Clinical Signs: Animals were observed twice daily and once during weekend for signs of toxicity and mortality. A detail examination was conducted once weekly.

2. Body Weights/Food Consumption: Body weights of animals were recorded prior to exposure (Day -7), at Day 0 (day of dosing) and at weekly intervals, thereafter. In addition, body weights were also recorded on the days when functional observational batteries were conducted. Food consumption was recorded weekly during the study period.

3. Neurobehavioral Tests/Motor Activity: Neurobehavioral tests consisted of the Functional Observational Battery (FOB) and evaluation of motor activity. These tests were performed 7 days prior to dosing (Day -7), on Day 22, Days 50 and 85.

   a. Functional Observational Battery: The following parameters were evaluated:

Home Cage Observations:
- Posture/behavior
- Urination/defecation
- Convulsions/tremors
- General observations

Sensorimotor/Reflex Response Observations:
- Hyperesthesia
- Abdominal tension
- Palpebral closure
- Winking reflex
- Pupil size
- Pupillary reflex
- Pinna reflex
- Startle response (Audition)
- Olfaction
- Tail pinch response (pain perception)
- Righting response (coordination of movements
- Vision (visual placing response)

Open field Observations:
- Fur
- Skin color
- Posture
- Salivation
- Respiration
- Arousal/Activity
- Vocalisation
- Lacrimation
- Convulsion/tremors
- Bizarre behavior
- Gait impairment
- Exophthalmus
- Number of rearings within 2 minutes

Measured Responses:
- Landing foot spread
- Fore/hind limb grip strength

b. Motor activity: Motor activity evaluation began immediately following FOB testing by placing animals in polycarbonate cages with bedding. No food or water was supplied during the measurements. Motor activity was measured using the Multi-Varimex-System with 4 infrared beams per cage. Animals were monitored individually over a 90 minute session, consisting of 18, 5-minute intervals.

4. Postmortem Observations:

a. Sacrifice and Pathology: All animals not scheduled for neuropathology were sacrificed by decapitation under CO_2-anesthesia and were necropsied and subjected to gross pathology. All gross lesions and liver were preserved in neutral buffered formaldehyde, processed through paraffin, sectioned and then stained with hematoxylin and eosin for microscopic examination.

b. Neuropathology: Five rats/sex/group scheduled for neuropathological examination were anesthetized with Nembutal and sacrificed by perfusion fixation. Tissues were processed in the following manner:

The following portions of the central and peripheral nervous systems were processed through paraffin, sectioned and then stained with hematoxylin and eosin:

Brain* (frontal lobe, parietal lobe with diencephalon, midbrain, Pons, cerebellum with pons, medulla oblongata)
Spinal cord (cervical [C3-C6] and lumbar [L1-L4] swellings)
Gasserian ganglia (s. trigeminale)
One hind limb
All gross lesions*

The following portions of the peripheral nervous systems were embedded in epoxyresin, sectioned, and then stained with toluidine blue:

Dorsal root ganglion* (C3-C6; L1-L4)
Dorsal root fibers* (C3-C6; L1-L4)
Ventral root fibers* (C3-C6; L1-L4)
Both cross- and longitudinal sections of the following:
Proximal sciatic nerve*
Tibial nerve*
Sural nerve*
Gastrocnemius muscle

*These tissues for both low- and mid-dose rats were preserved in neutral buffered formaldehyde; brain weights were determined.

D. Data Analysis

1. Statistical Analyses: Body weight data (parametric) were analyzed using ANOVA with significance assessed using the F-distribution. Significance of ANOVA findings were followed by pair-wise comparisons between the control and treatment groups using Dunnett's test. FOB and motor activity data (nonparametric) were evaluated using Kruskal-Wallis-H-test; the significant findings were followed for pairwise comparison between the control and treatment groups using Mann-Whitney-U-test or Wilcoxon-test. P values $\leq$ 0.05 were considered significant.

2. Historical Data: Data from validation studies for a neurotoxicity screen in rats using positive and negative control materials were provided (study No. 94/10799, vol. IV; pages 464-511). The positive control substance tested consisted of acrylamide, trimethyltin chloride, 3,3"-iminodiproprionitrile, nomifensin and diazepam. These studies demonstrated the ability of the performing laboratory to evaluate neurological effects.

II. COMPLIANCE: The following signed and dated statements were included in the report:
- Data Confidentiality Claims Statement (none claimed)
- GLP Compliance Certification (FR Germany and OECD GLPs)
- Quality Assurance Statement

The Flagging Statement was not required.

III. RESULTS

A. Analytical Chemistry: Purity of the test substance, determined before the study and at study termination, was 98% and 96.3%, respectively; the test substance was stable at room temperature for at least 6 months. Stability analyses of diet preparations demonstrated stability for 64 hours when stored at ambient temperatures and for period of 4 days at 4°C. Analyses of top, middle, and bottom samples of these same low-and high-dose formulations demonstrated that Dazomet was homogeneously distributed in the diet when prepared in the manner described; the mean values of each of the three levels were within an acceptable range (93.5-111.4%) of the target concentrations.

B. Mortality and Clinical Signs: No treatment-related deaths occurred. The incidental clinical signs were observed in low-dose group and consisted of alopecia in one male and microphthalmia in one female. No clinical signs were noted in other test groups.

C. Body Weight: Body weight and body weight gain data are summarized in Tables 2A and 2B. The mean body weights for high-dose males and females were consistently lower compared to controls throughout the study period; for high-dose males it was lower on days 49, 63-77 and 91(≥6%) resulting in overall decrease in body weight gain of 12% over 91 days. For high-dose females, the mean body weight was below control on day 21 (9%) with overall decrease in body weight gain of 24% over 91 days.

Table 2A. Mean (±S.D.) Body Weight and Body Weight Change Data (g)

Interval	0 ppm	50 ppm	200 ppm	450/400 ppm
Males				
Body Weight (g)				
Day 0	187±6	184±13	190±4	188±7
Day 7	238±7	237±8	240±9	232±7
Day 21	318±12	316±11	324±16	305±14
Day 35	369±20	366±12	377±21	353±21
Day 49	418±21	405±14	417±25	392±23*
Day 63	445±24	433±17	446±29	418±25*
Day 77	471±25	461±19	474±31	441±28*
Day 91	494±31	482±21	491±33	457±31*
Days 0 to 91	306±31	298±26	301±30	269±27*
Females				
Body Weight (g)				
Day 0	151±6	151±8	153±5	153±8
Day 7	176±9	172±12	175±5	169±10
Day 21	208±14	203±13	202±8	189±18**
Day 35	232±22	225±20	222±8	209±21
Day 49	244±24	240±22	237±11	223±26
Day 63	256±29	250±25	248±11	235±24
Day 77	269±26	260±23	256±13	244±26
Day 91	278±28	268±29	265±14	250±28
Days 0 to 91	127±24	117±22	112±12	97±24 **

*Statistically significantly different from control value, $p \leq 0.05$.
**Statistically significantly different from control value, $p \leq 0.01$.
Note: Data were extracted from Study No. 94/10799, Tables 5-8 and 11-14; pages 60-63 and 66-69.

The body weight determinations conducted during the FOB tests also confirmed the above findings; the decrease in body weight gain from Day -7 to 85 for both sexes was over 9% (Table 2B).

Table 2B. Mean (±S.D.) Body Weight and Body Weight Change Data (g)

Interval	0 ppm	50 ppm	200 ppm	450/400 ppm
Males				
Body Weight (g)				
Day - 7	136± 4	136± 4	137± 4	135± 4
Day 22	322±13	319±10	328±16	307±16*
Day 50	416±22	407±13	420±27	393±22
Day 85	482±34	472±20	482±34	449±28
Days -7 to 85	346± 2	336± 3	345± 1	313±8[a]
Females				
Body Weight (g)				
Day - 7	126± 3	125± 4	125± 3	126± 5
Day 22	209±14	205±16	202± 8	188±19*
Day 50	244±24	240±22	237±12	223±25
Day 85	273±28	264±26	260±13	245±26
Day - 7 to 85	147± 6	139± 2	134± 9	118± 7[b]

aDecrease in body weight gain = 9%; calculated by the reviewer
bDecrease in body weight gain = 20%; calculated by the reviewer
*Significantly different from control value, $p \leq 0.05$
Note: Data were extracted from Study No. 94/10799, Tables 9 and 10; pages 64-65.

C. Food Consumption and Test Substance Intake: No compound-related effects on food consumption were noted in either sexes at any dose level. Mean actual weekly test substance consumption data are summarized in Table 3.

Table 3. Mean Weekly Test Substance Intake (mg/kg/day)

Sex	50 ppm	200 ppm	450/400 ppm
Males	3.8	14.9	34.31
Females	4.3	16.5	36.21

Note: Data were extracted from Study No. 94/10799, Tables 15 -18, pp. 70-73.

D. Functional Observation Battery (FOB): During the FOB, no compound-related findings were observed in Home Cage and Open field observations as well as Sensorimotor and Reflex tests. The only significant effect noted in high-dose

females was significantly reduced landing foot-spread on Day 22; no change was noted from day -7 test. Because of lack of dose-response this finding was not considered to be treatment-related. Although a significant increase was noted in males at 50 ppm on Day 85, it was not considered an adverse effect. No other treatment-related findings were noted in FOB testing.

E. Motor Activity Data: A significant reduction in motor activity was observed in low-dose females as revealed by decrease in the total number of counts on day 22 testing interval. No significant differences in motor activity levels were noted for treated rats at any other dose group, as compared to control. Therefore, the above finding was not considered to be compound-related. Interval data were not provided.

F. Postmortem Data

1. Organ Weights/Macroscopic Pathology: The mean absolute and relative liver weights of rats at necropsy are summarized in Table 4. Among mid- and high-dose males and females, the absolute liver weights were slightly higher compared to controls (3–9%). There were significant increases in relative liver weights in mid- and high-dose males (≥9%) and females (≥12%). Histopathology of the liver revealed fatty degeneration of the liver in 3 males at low-dose, 5/5 males and 3/5 females at mid-dose, and 5/5 males and 4/5 females at high-dose. There were no other treatment-related findings noted at necropsy.

Table 4. Mean (± S.D.) Absolute (g) and Relative (g/kg b.w.) Liver Weights and histopathological changes*

Observations	0 ppm	50 ppm	200 ppm	450/400 ppm
Males				
Absolute wt	16.00±2.08	14.52±1.51	17.13±2.86	17.42±2.44
Relative wt.	3.39±0.28	3.18±0.25	3.70±0.48	4.00±0.46
Fatty changes	0/5	3/5	5/5	5/5
Females				
Absolute wt	7.56±1.08	6.94±0.87	7.77±0.44	8.03 ±0.52
Relative wt.	2.87±0.17	2.97±0.16	3.20±0.11*	3.61±0.34*
Fatty changes	0/5	0/5	3/5	4/5

*Data were extracted from Study No. 94/10799, pages 336-339 and 346–347.
*Significantly different from control value, $p \leq 0.05$.

2. Microscopic pathology: No treatment-related lesions of the nervous system were noted in high-dose animals. For that reason, tissues from the low- and mid-dose groups were not examined. A small number of

2. Microscopic pathology: No treatment-related lesions of the nervous system were noted in high-dose animals. For that reason, tissues from the low- and mid-dose groups were not examined. A small number of incidental lesions were identified in the peripheral nerves from the high-dose and control animals, but were not attributed to treatment. Axonal degeneration is considered to be a common spontaneous lesion in subchronic neurotoxicity studies in rats.

III. DISCUSSION

A. Investigator's/Reviewer's Conclusions

Dietary administration of Dazomet to male and female Wistar rats at levels of 50, 200, or 450/400 ppm resulted in significantly decrease in mean body weight and mean body weight gain (males and females at 450/400 ppm), increased liver weights and fatty changes in the liver (3/10 males at 50 ppm; 5/5 males and 3/5 females at 200 ppm, and 5/5 males and 4/5 females at 450/400 ppm).

No neurobehavioral or neuropathological effects were noted at the highest dose tested.

Based on the findings of this study (fatty degeneration of the liver) the LOELS were established at 50 ppm (4 mg/kg/day) in male and 200 ppm (16 mg/kg/day) in females. The NOEL was not established in males and established at 50 ppm (4 mg/kg/day) in females.

This study is classified as **Acceptable** and **satisfies** the §82-7 guideline requirement for subchronic neurotoxicity study in rats.

B. Study Deficiencies: None noted.

ATTACHMENT

Results of Landing Foot-Spread Test (cm)- Mean Data (±S.D.)

Sex	Interval (Day)	0 ppm	50 ppm	200 ppm	450/400 ppm
Males:	-7	10.0±1.0	10.3±0.8	9.6±1.0	9.6±0.7
	22	13.2±0.8	13.2±1.0	12.7±1.2	12.8±0.8
	50	13.0±1.3	13.3±1.1	12.5±1.3	12.6±1.2
	85	12.2±1.0	12.9±0.5*	11.4±1.3	12.2±1.3
Females:	-7	9.2±0.9	9.6±1.3	9.4±0.7	9.2±0.9
	22	11.1±1.1	10.8±0.8	10.4±0.9	9.2±1.2**
	50	10.3±1.2	10.0±1.0	9.6±1.0	9.7±1.1
	85	10.2±1.0	10.2±1.0	9.9±1.0	9.3±0.9

*Significantly different from control value, $p \leq 0.05$.
**Significantly different from control value, $p \leq 0.02$.
Note: Data were extracted from Study No. 94/10799, Tables 53 and 54, pages 108 and 109.

Neuropathology Data - Incidence of axonal degeneration

Tissue	Males (dose in ppm)		Females (dose in ppm)	
	0	450	0	400
No. examined	5	5	5	5
Sciatic nerve	2	1	1	0
(proximal)	1	0	0	1
Sural nerve (notch)	2	0	0	0
Tibial nerve				

Note: Data were extracted from Study No. 94/10799, pages 344 and 345.

DAZOMET 21-Day Dermal Study (82-2)

Reviewed by: Sanjivani Diwan, Ph.D. Sanjivani Diwan, Date: 10/18/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou, Date: 10/18/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity/Rabbits (82-2)

EPA ID NUMBERS: DP BARCODE: D210051
P. C. CODE: 035602
MRID NUMBER: 402991-01
SUBMISSION No.: S478165

TEST MATERIAL: Dazomet
Synonym: Tetrahydro-3.5-dimethyl-2H-1.3.5-thiadiazine-2-thione

STUDY NUMBER: Project ID No. HLA 6220-100

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI

SPONSOR: Dazomet Task Force

TITLE OF REPORT: 21-Day Dermal Toxicity Study in Rabbits

AUTHOR: K.M. MacKenzie

REPORT ISSUED: June 17, 1987

EXECUTIVE SUMMARY: In a 21-day dermal toxicity study (MRID # 402991-01), Hra:(NZW)SPF rabbits received dermal applications of either 0, 10, 100 or 1000 mg/kg of Dazomet, six hours per day, five days per week for 21 days. Ten animals/sex/group (including 5/sex/group satellite animals) were assigned to 0-, and 10-mg/kg/day group; and 5 animals/sex/group to the 100- and 1000-mg/kg/day groups. All animals were sacrificed after 3 weeks except the satellite animals which were sacrificed after 6 weeks. The test material was applied on 0.4% w/v on carboxymethylcellulose in purified water daily at a volume-dosage of 1 to 3 ml/kg body weight.

There were no differences between the control and treated groups in any of the parameters measured.

The systemic toxicity LOEL is >1000 mg/kg/day males and females; the systemic toxicity NOEL is ≥1000 mg/kg/day males and females.

The dermal toxicity LOEL is >1000 mg/kg/day for males and females; the dermal toxicity NOEL is ≥1000 mg/kg/day for males and females.

The study is **Acceptable** and **satisfies** the guideline requirements for a 21-day dermal toxicity study (82-2) in the rabbit.

I. MATERIALS

A. Test Material

Name: Dazomet
Synonym:Tetrahydro-3.5-dimethyl-2H-1.3.5-thiadiazine-2-thione
Purity: 99%
Batch Number: 87/001
Sample Number: 70105068
Description: white powder, as cited in MRID# 470145-05
Storage Conditions: At room temperature

B. Vehicle: 0.4% Aqueous carboxymethylcellulose

C. Administration: Dermal

D. Test Animals:

Species: Rabbit
Species: Hra:(NZW)SPF
Source: Hazleton Research Products, Inc., Denver, PA
Age: Young adult males and females
Weight: Males - Approximately 2,293-2,642 g;
Females - approximately 2,001-2,486 g at initiation of dosing
Housing: Individually in stainless steel cages
Environmental Conditions:
Temperature: target of 70±3°F
Relative humidity: target of 50±20%
Photoperiod: 12 hours light/dark
Air changes: Not reported
Food and Water: Diet (Rabbit Chow #5325) and tap water *ad libitum*
Acclimation Period: 13 days

II. METHODS

A. Preparation of Dosing Substance

The daily dose for each animal was calculated based upon its most recent body weight. Chemical analyses were performed by the Sponsor.

B. Dosage and Administration

Dosage Groups

The animals were assigned by computer randomization to the following treatment groups:

Test Group	Dose Applied (mg/kg/day)	# Males	# Females
1 (Control)	0	10[b]	10
2	10	10[b]	10[b]
3	100	5	5
4	1000	5	5

[a]Dosages were selected based on the results of a pilot range-finding study.
[b]Five animals/sex were designated satellite animals and were observed for 24 days post-treatment for reversibility, persistence, or delayed occurrence of toxic effects.

Administration

Twenty-four hours prior to the initiation of dosing, the fur from the dorsal area of the trunk of each rabbit was clipped. This procedure was repeated as needed thereafter. Twenty-one repeated doses of test material or vehicle were applied to each animal once daily, five days per week, for three weeks at dose volume ranging from 1 to 3 mL. The test material was spread evenly over approximately 10% of the total body surface area after the vehicle was applied to the back of each animal. Control animals received the same amount of vehicle. A gauze dressing was secured in place with nonirritating tape, covered with Saran Wrap and Elastoplast tape. The animals were fitted with a collar to prevent ingestion of the test substance and disruption of the wrappings. After each exposure period, the dressings were removed and the application site was washed with warm water and dried with paper towels. Formulations were prepared fresh before each administration. All animals were sacrificed after 21 days of treatment except for the satellite animals which were observed for 24 days post-treatment before being sacrificed.

C. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies:

Signs of systemic toxicity, mortality and morbidity - At least twice daily for moribundity and mortality, and at least once daily for clinical signs.
Dermal irritation - once before each application, on the day of terminal sacrifice, and three times/week during post-treatment period (satellite group)
Body weights - at study initiation, weekly thereafter, and at

study termination
Food consumption - weekly intervals
Hematology and clinical chemistry - just prior to euthanasia after 3 weeks on test and on the satellite animals after post-treatment period of 6 weeks
Gross necropsy - all animals
Organ weights - brain, kidneys, liver, testes and epididymides from all animals
Histopathology - skin, kidneys, liver, and lesions from all animals (except satellite animals)

D. Pathological Parameters

Hematology and clinical chemistry evaluations were performed on all animals including satellite animals. Blood was drawn from the retro-orbital sinus. The CHECKED (X) hematology parameters were examined.

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Total plasma protein (TP)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc. (MCHC)
X Mean corpuscular volume (MCV)

* EPA guideline requirement

The CHECKED (X) clinical chemistry evaluations were done.

Electrolytes:
X Calcium*
X Chloride*
__Magnesium
X Phosphorus*
X Potassium*
X Sodium*

Enzymes:
__Alkaline phosphatase
__Cholinesterase
__Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)*
X Serum aspartate aminotransferase (also SGOT)*

Other:
X Albumin*
X Blood creatinine*
X Blood urea nitrogen*
__Cholesterol
X Globulins
X Glucose*
X Total Bilirubin*
X Total Protein*
__Triglycerides
X Creatine phosphokinase*

* EPA guideline requirement

Approximately 24 hours after the last treatment, the animals were sacrificed by carbon dioxide inhalation. The following CHECKED (X) tissues were examined histologically. The addition, the organs CHECKED(XX) from treated and control animals (including satellite animals) were weighed.

Digestive System	Cardiovasc./Hemat.	Neurologic
__Tongue	__Aorta	XXBrain
__Salivary glands	__Heart	__Periph. nerve
__Esophagus	__Bone marrow	__Spinal cord
__Stomach	__Lymph nodes	__Pituitary
__Duodenum	__Spleen	__Eyes (Optic nerve)
__Jejunum	__Thymus	Glandular
__Ileum	Urogenital	__Adrenals
__Cecum	XXKidneys*	__Lacrimal gland
__Colon	__Urinary bladder	__Mammary gland
__Rectum	XXTestes	__Parathyroids
XXLiver*	XXEpididymides	__Thyroids
__Gall bladder	__Prostate/urethra	Other
__Pancreas	__Seminal vesicle	__Bone
Respiratory	__Ovaries	__Skeletal muscle
__Trachea	__Uterus	X Skin+
__Lung	__Vagina	XXAll gross lesions and masses

* EPA guideline requirement
\+ Treated and untreated skin were examined

Histological examinations were done on the above tissues from the control and high dosage groups. No histological examinations were performed on the satellite animals.

E. Statistical Analyses

The following procedures were utilized in analyzing the numerical data:

- initial body weight, food consumption, clinical chemistry, absolute and relative organ weight, and hematology (except erythrocyte morphology) -- ANOVA, Dunnet's t-test, Kruskal-Wallis H-test ANOVA, Nemenyi-Kruskal-Wallis, Wilcoxon-Mann-Whitney two-sample rank test or ANOVA

F. Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality. Any deviations from the protocol were

appropriately reported.

III. RESULTS

A. Administered Dosage

The concentration analyses were not provided.

B. Mortality/Clinical Observations and Systemic Toxicity

There were no treatment-related mortality, clinical signs of dermal irritation or systemic toxicity seen in treated animals. One male in the 1000 mg/kg dose group was found moribund on Day 6. No other deaths occurred during the study. In addition to this male, one male each at 10 and 100 mg/kg and one female at 1000 mg/kg exhibited one or more clinical signs of toxicity. These included anorexia, bloated abdomen, hypoactivity, mucoid diarrhea, cyanosis and few feces (Refer to the attachment for p. 27 from the study). The incidence of these signs was higher at 1000 mg/kg/day (20%) compared to control (0%) and other dose groups (5% at 10 mg/kg/and 10% at 100 mg/kg), and appeared to be dose-related (Table 2). However, there were no treatment-related changes in the body weight gain, food consumption, and clinical chemistry or histopathological examinations. Therefore, the clinical signs observed were not considered to be toxicologically significant.

C. Body Weight and Body Weight Gain

Mean body weights and body weight gains of the treated animals did not significantly differ from those of the controls.

D. Food Consumption

There were no significant differences between the treated and control groups in mean daily food consumption.

E. Clinical Pathology

There were no treatment-related changes in the hematology or clinical chemistry parameters examined. For 21-day study group, males from the 100 mg/kg dose group had significantly higher globulin; males from the 1000 mg/kg dose group had significantly lower aspartate aminotransferase. The creatinine values were lower in males from 10 and 1000 mg/kg dose groups. In the satellite group, males from 10 mg/kg group had higher absolute eosinophil count and lower glucose and albumin while females had lower total protein values. The above changes were not dose-related or associated with pathological effects and, therefore, were not toxicologically important. These findings were considered as normal biological variations.

TABLE 1. Incidence of Clinical signs[a]

Test Group (mg/kg/day)	#Males with clinical signs/# of males treated	# Females with clinical signs/# of females treated	Incidence (%) of clinical signs
1 (Control)	0/10	0/10	0
10	1/10	0/10	5
100	1/5	0/5	10
1000	1/5	1/5	20

[a]The table includes the data for satellite animals
[b]Data extracted from study No. 6220-100, table 1 and appendix B

F. Necropsy Findings

Gross Necropsy

There were no treatment-related changes on gross necropsy examination of the animals.

Organ Weights

There were no treatment-related changes on gross necropsy examination of the animals.

Histopathology

There were no treatment-related histopathological findings involving skin observed.

G. Conclusion from Study Report

The study report concludes that the No-Observed-Effect Level (NOEL) is greater than 1000 mg/kg.

IV. DISCUSSION/CONCLUSIONS

In this 21-day dermal toxicity study (MRID # 402991-01), 5-10 Hra: (NZW)SPF rabbits/sex/group were treated topically with dosages of either 10, 100 or 1000 mg/kg of Dazomet in 0.4% aqueous carboxymethylcellulose (vehicle) for 6-hours per day, five days/week for 21 days. The control group of 5 rabbits/sex received vehicle only. There were no differences between the control and treated groups in any of the parameters measured.

The systemic toxicity LOEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg/day for males and females.

The dermal toxicity LOEL is >1000 mg/kg/day for males and females; the dermal toxicity NOEL is ≥1000 mg/kg/day for males and females.

Table 1

Summary of Antemortem Observations[a]

Observation	Dazomet (mg/kg) 0	10	100	1,000	Post-Treatment 0	10
Males						
Few feces	0	1	1	0	0	1
Anorexic	0	1	1	1	0	1
Bloated abdomen	0	1	0	1	0	1
Hypoactive	0	1	1	1	0	1
Diarrhea	0	0	1	0	0	1
Mucoid diarrhea	0	0	0	1	0	1
Cyanotic	0	0	0	1	0	1
Moribund sacrifice	0	0	0	1	0	1
Females						
Few feces	0	0	0	1	0	1
Anorexic	0	0	0	1	0	1

a This table indicates the number of animals in which a condition was observed without regard to the specific nature, severity, reversibility, number of incidences per animal, or length of time the condition persisted.

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PC Code:	**035602**
HED File Code	**13000 Tox Reviews**
Memo Date:	**12/03/95**
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