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

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **CYROMAZINE:** Review of two acute inhalation toxicity studies and a data waiver request for the 21 day dermal study requirement.

EPA DP Barcode D220405, D220406, D220409; EPA Submission Barcode S495936, S495938, S495941; EPA Pesticide Chemical Code 121301; Toxicology Chemical Number 167B.

TO: George LaRocca/Linda Deluise, PM 13
Insecticide-Rodenticide Branch
Registration Division (7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/30/96
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (7509C)

THRU: Jess Rowland, M.S. Jess Rowland 9/3/96
Acting Section Head, Review Section I
and
Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y M Ioannou 9/10/96
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Ciba Crop Protection, Ciba-Geigy Corporation
P.O. Box 18300, Greensboro, NC 27419-8300

Action Requested: Review of two acute inhalation toxicity studies and a data waiver request for the 21 day dermal requirement. [Cyromazine, TRIGARD 75W]

Recommendation: TB II reviewed the following acute inhalation toxicity studies:

I. *ACUTE INHALATION TOXICITY STUDY IN RATS* (STILLMEADOW INCORPORATED for Ciba Plant Protection, Ciba-Geigy Corporation, Study No. 0971-94, August 4, 1994, EPA MRID# 43799901). The following is the executive summary from the review:

In an acute inhalation study (MRID# 43799901), young adult HSD:SD Rats from Harlan Sprague-Dawley, Inc. (Houston TX) were exposed to either 0, 0.744 or 3.60 mg/L Cyromazine Technical (96.5% a.i.; Batch GP-930910) administered as a generated aerosol using a GEM T Trost Air Mill exposing rats in a nose only dynamic flow

inhalation chamber for 4 hours.

The LC_{50} for Cyromazine Technical is greater than 0.744 mg/L for 4 hours (the particle size for the 3.60 mg/L dose was unacceptable). This is Toxicity Category III.

The study is classified as Acceptable and satisfies the guideline requirement (§81-3) for an acute inhalation toxicity study in rats.

II. *ACUTE INHALATION TOXICITY STUDY IN RATS* (STILLMEADOW INCORPORATED for Ciba Plant Protection, Ciba-Geigy Corporation, Study No. 2068-95, September 7, 1995, EPA MRID# 43799902). The following is the executive summary from the review:

In an acute inhalation study (MRID# 43799902), young adult HSD:SD Rats from Harlan Sprague-Dawley, Inc. (Houston TX) were exposed to either 2.39 or 2.83 mg/L Trigard 75W (75.8% a.i.; Batch/lot not provided) administered as a generated aerosol using a GEM T Trost Air Mill exposing rats in a nose only dynamic flow inhalation chamber for 4 hours.

The LC_{50} for Trigard 75W is greater than 2.39 mg/L for 4 hours (the particle size for the 2.83 mg/L dose was unacceptable). This is Toxicity Category IV.

The study is classified as Acceptable and satisfies the guideline requirement (§81-3) for an acute inhalation toxicity study in rats.

TBII also reviewed the request for a waiver of the 21-day dermal toxicity study requirement (§82-2) for the registration of Cyromazine. The registrant submitted 2 studies to support the data waiver request. They claimed that the first study had not established a NOEL; however, that was for dermal irritation and the Agency looks for systemic toxicity in this study, a NOEL for dermal irritation is not required. For the second study the registrant claimed that the presence of infection in the animals used in the repeat study may be a problem. Although the investigators showed evidence of a low grade infection or parasite migration in the liver, kidneys, and lungs in animals of all groups, there was a lack of toxicity noted. This observation did not affect the outcome of the study. TBII determined that these two studies are acceptable and fulfill the data requirement (§82-2) for a 21-day dermal toxicity study in rabbits.

The following are the executive summaries from the reviews:

I. *CYROMAZINE TECHNICAL 21-DAY DERMAL TOXICITY STUDY IN RABBITS* (Ciba-Geigy Pharmaceuticals Research, Toxicology/Pathology Division, Chemical Evaluation Subdivision, Safety Evaluation Facility for Agricultural Division, CIBA-GEIGY Corporation, Toxicology/Pathology Report No. 152-84; MIN 842009, August 16, 1984, EPA MRID# 44060902)

In a 21-day dermal toxicity study (MRID# 44060902), HAR:PF/CF (NZW) BR Albino Rabbits (5/sex/dose) from H.A.R.E. Rabbits for Research (531 Burnt Meadow Road, Hewitt, N.J. 07421) were exposed to either 0, or 2010 mg/kg/day Cyromazine Technical (96% a.i.).

No treatment related systemic toxicity was noted. **The Systemic Toxicity NOEL is equal to or greater than 2010 mg/kg/day and the Systemic Toxicity LOEL is greater than 2010 mg/kg/day.**

Dermal irritation was slightly increased over control. **The Dermal Toxicity NOEL is less than 2010 mg/kg/day and the Dermal Toxicity LOEL is equal to or less than 2010 mg/kg/day.**

The study is classified as Acceptable and satisfies the guideline requirement (§82-2) for a 21-day dermal toxicity study in rabbits.

II. *CYROMAZINE TECH (CGA 72662) 21-DAY DERMAL TOXICITY STUDY IN RABBITS* (STILLMEADOW, Inc. for CIBA-GEIGY Corporation, Agricultural Division, Laboratory Study Number 3805-85, May 18, 1992, EPA MRID# 44060901).

In a 21-day dermal toxicity study (MRID# 44060901), New Zealand White Rabbits (5-7 males/dose; 5 females/dose) from Ray Nichols Rabbitry (Lumberton, Texas) were exposed to either 0, 50, 200, or 2000 mg/kg/day Cyromazine Technical (94.6% a.i.; Batch FL 850478).

No treatment related systemic toxicity was noted. **The Systemic Toxicity NOEL is equal to or greater than 2000 mg/kg/day and the Systemic Toxicity LOEL is greater than 2000 mg/kg/day.**

No dermal irritation was noted. **The Dermal Toxicity NOEL is equal to greater than 2000 mg/kg/day and the Dermal Toxicity LOEL is greater than 2000 mg/kg/day.**

The study is classified as Acceptable and satisfies the guideline requirement (§82-2) for a 21-day dermal toxicity study in rabbits.

3.1

I. Toxicology Profile for Cyromazine Technical and Formulations (CFR §158.340)

Technical: Cyromazine
Use Pattern: food
Action Type: data waiver request

This compound is a registered active ingredient. The following data were submitted prior to this application.

	Required	Satisfied
§81-1 Acute Oral Toxicity	Yes	Yes
§81-2 Acute Dermal Toxicity	Yes	Yes
§81-3 Acute Inhalation Toxicity	Yes	Yes[1]
§81-4 Primary Eye Irritation	Yes	Yes
§81-5 Primary Dermal Irritation	Yes	Yes
§81-6 Dermal Sensitization	Yes	Yes
§82-1(a) Subchronic Oral (rodent)	Yes	Yes[2]
§82-1(b) Subchronic Oral (non-rodent)	Yes	Yes
§82-2 21 day dermal - rat	Yes	Yes[1]
§83-1(a) Chronic Toxicity (rodent)	Yes	Yes
§83-2(b) Chronic Toxicity (non-rodent)	Yes	Yes[3]
§82-2(a) Oncogenicity - rat	Yes	Yes
§82-2(b) Oncogenicity - mouse	Yes	Yes
§83-3(a) Teratology - rat	Yes	Yes
§83-3(b) Teratology - rabbit	Yes	Yes
§83-4 Multigeneration Reproduction	Yes	Yes
§84-2(a) Mutagenicity - Gene Mutation	Yes	Yes
§84-2(b) Mutagenicity - Structural Chromosomal Aberration	Yes	Yes
§84-4 Mutagenicity - Other Genotoxic Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes
§85-2 Dermal Penetration	Yes	Yes

[1] = Reviewed in this memorandum
[2] = Satisfied by an acceptable chronic/oncogenicity study
[3] = Satisfied by an acceptable 6-month dog study

Formulation: Trigard 75W

	Required	Satisfied
§81-1 Acute Oral Toxicity	Yes	Yes
§81-2 Acute Dermal Toxicity	Yes	Yes
§81-3 Acute Inhalation Toxicity	Yes	Yes[1]
§81-4 Primary Eye Irritation	Yes	Yes
§81-5 Primary Dermal Irritation	Yes	Yes
§81-6 Dermal Sensitization	Yes	Yes

[1] = Reviewed in this memorandum

Formulation: Cyromazine Formulation (Larvadex) 0.3 % premix

	Required	Satisfied
§81-1 Acute Oral Toxicity	Yes	Yes
§81-2 Acute Dermal Toxicity	Yes	Yes
§81-3 Acute Inhalation Toxicity	Yes	**NO**
§81-4 Primary Eye Irritation	Yes	Yes
§81-5 Primary Dermal Irritation	Yes	Yes
§81-6 Dermal Sensitization	Yes	**NO**

Formulation: Cyromazine Formulation (Larvadex) 5.0 %

	Required	Satisfied
§81-1 Acute Oral Toxicity	Yes	Yes
§81-2 Acute Dermal Toxicity	Yes	Yes
§81-3 Acute Inhalation Toxicity	Yes	Yes
§81-4 Primary Eye Irritation	Yes	Yes
§81-5 Primary Dermal Irritation	Yes	Yes
§81-6 Dermal Sensitization	Yes	Yes

II. Data Gaps:

The are no data gaps for the technical cyromazine at this time.

The following studies with the end use products (not for cyromazine as Armour) are data gaps:

§81-3 Acute inhalation toxicity in rats (0.3%)
§81-6 Dermal sensitization - guinea pig (0.3%)

III. Actions Being Taken to Obtain Additional Information or Clarification

HED defers to the Registration Division for further action to obtain these missing data. Cyromazine is not on List A, B, C, or D for reregistration under FIFRA 88.

IV. Reference Dose (RfD):

The reference dose is 0.0075 mg/kg/day based on a 6 month feeding study in the dog with a NOEL of 0.75 mg/kg/day and a LOEL of 7.5 mg/kg/day (based on hematological changes) with an uncertainty factor of 100.

V. Pending Regulatory Actions:

None at this time.

VI. Toxicologic Issues Pertinent to This Request:

A. New toxicology Data on Cyromazine

New data discussed in this memo.

B. Carcinogenicity and Mutagenicity

The HED Carcinogenicity Peer Review Committee classified Cyromazine as a Group E, no evidence of carcinogenicity. Available mutagenicity data was negative.

CYROMAZINE

ACUTE INHALATION TOXICITY-RAT

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/6/96
Senior Pharmacologist, Review Section I, TBII (7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YMI 8/7/96
Section Head, Review Section I, TBII (7509C)

DATA EVALUATION RECORD

Study Type: Acute Inhalation Toxicity
Species: Rat Guideline: §81-3

EPA Numbers: EPA MRID# 43799901
EPA Pesticide Chemical Code 121301
Toxicology Chemical No. 167B
EPA DP Barcode D220405
EPA Submission Barcode S495936

Test Material: Cyromazine Technical (Batch GP-930910)
Synonyms: FL-931759

Title of Report: ACUTE INHALATION TOXICITY STUDY IN RATS

Sponsor: Ciba Plant Protection, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419-8300

Testing Facility: STILLMEADOW INCORPORATED
12852 Park One Drive, Sugar Land TX 77478

Study Number: 0971-94

Author(s): Mark S. Holbert, B.S.

Report Issued: August 4, 1994

Executive Summary: In an acute inhalation study (MRID# 43799901), young adult HSD:SD Rats from Harlan Sprague-Dawley, Inc. (Houston TX) were exposed to either 0, 0.744 or 3.60 mg/L Cyromazine Technical (96.5% a.i.; Batch GP-930910) administered as a generated aerosol using a GEM T Trost Air Mill exposing rats in a nose only dynamic flow inhalation chamber for 4 hours.

The LC_{50} for Cyromazine Technical is greater than 0.744 mg/L for 4 hours (the particle size for the 3.60 mg/L dose was unacceptable). This is Toxicity Category III.

The study is classified as Acceptable and satisfies the guideline requirement (§81-3) for an acute inhalation toxicity study in rats.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, GLP COMPLIANCE STATEMENT, and an AFFIRMATION OF QUALITY ASSURANCE statement was provided.

A. Materials and Methods:

1. **Test compound:** Cyromazine Technical FL-931759
   Description - fine white powder
   Batch GP-930910
   Purity - 96.5% a.i.
   Contaminants - none reported

2. **Vehicle(s):** none used

3. **Test animals:** Species: Rat
   Strain: HSD:SD
   Age: young adult
   Weight: males: 179-225 g; females: 195-242 g
   Source: Harlan Sprague-Dawley, Inc.,Houston TX
   Acclimation Period: at least 4 days

Animal Husbandry (scanned from the investigators report, page 7)

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	One per cage
Animal Room Conditions:	Temperature Range: 67-77°F Humidity Range: 30-80% 12-hour light/dark cycle 10-12 air changes/hour
Transfer to Clean Cages:	Weekly
Litter Pan Lining:	Paper and aspen bedding
Litter Pan Lining Change:	Three times weekly
Food:	Purina Formulab Chow #5008, available *ad libitum;* except during the exposure period
Water Type:	Municipal water supply from automatic water system, available *ad libitum* except during the exposure period

Animal husbandry and housing at STILLMEADOW, Inc. comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No 86-23, revised 1985). No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

B. Study Design

This study was designed to assess the acute inhalation toxicity potential of Cyromazine Technical when administered as a generated aerosol using a GEM T Trost Air Mill exposing rats in a nose only dynamic flow inhalation chamber.

1. Procedures:

The following procedures were utilized in the conduct of this study (scanned from pages 7-9 of the investigators report); they met the guideline requirements:

Prestudy Testing

Trial assays were conducted using different methods of aerosolizing the test material into the exposure chamber in attempts to determine which method(s) would produce the highest concentration and a mass median aerodynamic diameter (MMAD) between 1 and 4 µm.

Exposure Chamber

A 500 L nose-only stainless steel, dynamic flow inhalation chamber was utilized in this experiment (Diagram 1). The body of the chamber has 25 ports in 5 rows. Polycarbonate cones are inserted into 10 designated individual ports. The test material is introduced through the opening in the top of the chamber. The bottom section has a corresponding air outlet and a drain valve for cleaning the chamber. The individual polycarbonate cones (tubes) are tapered at one end to fit the shape of the animal's head and the back portion is sealed with a polycarbonate cap. The cones containing the animals fit tightly into the ports, and are sealed with "O" rings.

Generation of Test Atmosphere

The aerosol was generated during the 0.744 mg/L level by a Gem T Trost Air Mill which aspirated the test material from a motorized revolving disc delivery system coupled to the mill, and elutriated the resulting aerosol through a baffling chamber. The aerosol was generated during the 3.60 mg/L level in the same manner, but without the baffling chamber. These methods were selected based on results of the trial assays. For both levels, the concentrated aerosol was then diluted with filtered air and drawn into the exposure chamber. Air flow into the chamber was maintained through the use of a calibrated critical orifice at a rate of 11.7 or 13.8 air changes per hour. Air flow was recorded at 30 minute intervals during the exposure period, and was sufficient to ensure an oxygen content of at least 19% of the exposure atmosphere. Temperature and humidity were recorded at 30 minute intervals during the exposure period from a Taylor wet bulb/dry bulb hygrometer located in the exposure chamber. The control group was exposed to room air under similar environmental and experimental conditions as the test groups.

Test Material Administration

The test animals were exposed to an aerosol generated from the undiluted test material (fine powder) for a period of four hours. During the exposure period, the animals were individually housed in polycarbonate containers inserted into a 500 L stainless steel nose-only chamber when T-99 was achieved. A maximum of

10 animals were exposed during any given exposure period. The animals were returned to their stock laboratory cages at the termination of the exposure period.

Determination of Concentration

The concentration of test material in the exposure atmosphere was determined analytically once per hour (taken from the breathing zone of the animals), and nominally at the end of each exposure. The analytical determination was made using a Tracor Model 560 gas chromatograph (Appendix A) according to methods provided by the Sponsor. A standard curve is prepared by weighing 2 known samples of the total formulation product and bringing the samples to a known volume With solvent.

Dilutions of the samples are prepared using solvent. After the dilutions are prepared, an Internal Standard (IS) is added to each standard, correcting for any variation in injection volume into the gas chromatograph (GC). Each standard is injected into the GC at a known volume and the response (area) for the IS peak and test material (TM) peak recorded. An adjusted response is calculated by dividing the TM response by the IS response, then multiplying by the IS concentration. A standard curve (linear regression) is calculated from the TM standard concentrations and adjusted response. Unknown samples taken are treated identical to standards, the adjusted response added to the standard curve, and the unknown sample concentration calculated. The nominal concentration was determined by dividing the loss in weight of the test material after each exposure by the total volume of air which passed through the chamber.

Particle Size Distribution

Particle size, taken from the breathing zone of the animals, was determined at least twice during each test exposure, using an Andersen cascade impactor, at a rate of 28.3 L/minute for a duration of 1-2 minutes. The MMAD and percentage of particles collected were calculated from these data.

In-life Observations

Observations for mortality and signs of pharmacologic and/or toxicologic effects were made twice on the day of exposure and at least once daily thereafter for 15 to 17 days (day of exposure considered Day 0). Due to chamber design, the animals could be observed only for mortality during the exposure period. Individual body weights were recorded just prior to the inhalation exposure, on Day 7 and on the day of termination.

Postmortem Observations

At study termination, each surviving animal was euthanized by an overexposure to CO2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/6/96
Senior Pharmacologist, Review Section I, TBII (7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YMI 8/7/96
Section Head, Review Section I, TBII (7509C)

DATA EVALUATION RECORD

Study Type: Acute Inhalation Toxicity
Species: Rat Guideline: §81-3

EPA Numbers: EPA MRID# 43799902
EPA Pesticide Chemical Code 121301
Toxicology Chemical No. 167B
EPA DP Barcode D220406
EPA Submission Barcode S495938

Test Material: Trigard 75W (Batch/lot not provided)
Synonyms: FL-950053

Title of Report: ACUTE INHALATION TOXICITY STUDY IN RATS

Sponsor: Ciba Plant Protection, Ciba-Geigy Corporation, P.O. Box 18300, Greensboro, NC 27419-8300

Testing Facility: STILLMEADOW INCORPORATED
12852 Park One Drive, Sugar Land TX 77478

Study Number: 2068-95

Author(s): Mark S. Holbert, B.S.

Report Issued: September 7, 1995

Executive Summary: In an acute inhalation study (MRID# 43799902), young adult HSD:SD Rats from Harlan Sprague-Dawley, Inc. (Houston TX) were exposed to either 2.39 or 2.83 mg/L Trigard 75W (75.8% a.i.; Batch/lot not provided) administered as a generated aerosol using a GEM T Trost Air Mill exposing rats in a nose only dynamic flow inhalation chamber for 4 hours.

The LC_{50} for Trigard 75W is greater than 2.39 mg/L for 4 hours (the particle size for the 2.83 mg/L dose was unacceptable). This is Toxicity Category IV.

The study is classified as Acceptable and satisfies the guideline requirement (§81-3) for an acute inhalation toxicity study in rats.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, GLP COMPLIANCE STATEMENT, and an AFFIRMATION OF QUALITY ASSURANCE statement was provided.

C. Results:

1. Exposure Conditions:

For the 0.744 mg/L dose group, over the 4 hour exposure period the Mean Temperature was 68°F (20°C), the Relative Humidity was 88% and the Air Flow was 115 ppm. For the 3.60 mg/L dose group, the Mean Temperature was 72°F (22°C), the Relative Humidity was 70% and the Air Flow was 115 ppm.

For the 0.744 mg/L dose group, over the 4 hour exposure period the Mean Exposure Concentration was 0.7443 mg/L and the Nominal Concentration was 4.51 mg/L. For the 3.60 mg/L dose group, the Mean Exposure Concentration was 3.597 mg/L and the Nominal Concentration was 45.4 mg/L.

The Mass Median Aerodynamic Diameter (MMAD) for the 0.744 mg/L (744 mg/m^3) dose group was 3.92 µm and for the 3.60 mg/L (3600 mg/m^3) dose group was 6.68 µm. **For the 3.60 mg/L dose, the MMAD was rather high (>5.0 µm); however, the lower dose of 0.744 mg/L has an acceptable MMAD.**

2. Mortality/Estimated LC_{50}

No mortality was noted in the study; therefore the LC_{50} is greater than 0.744 mg/L.

3. Body Weights

No treatment related effects were noted in the supplied data (group mean and individual animal data).

4. Clinical Signs

The 0.744 and 3.60 mg/L dose groups exhibited a decrease in activity and piloerection in all animals at the 4.5 hour, 6.0 hour and 2 day observations, additionally the 3.60 mg/L dose group exhibited nasal discharge at the 4.5 hour time point. These observations were most likely due to the direct exposure to the compound rather than a toxic effect of the compound. No effects were noted in the control group. The investigators provided group summary and individual animal data.

5. Necropsy Findings

Pathological examination revealed mottled red lungs in control and test animals and multiple red foci on the lungs of the 0.744 mg/L dose group males. The pathologist concluded that the red foci may be treatment related.

A. Materials and Methods:

1. **Test compound:** Trigard 75W FL-950053
   Description - gray-tan powder
   Batch/Lot not provided
   Purity - 75.8% a.i.
   Contaminants - none reported

2. **Vehicle(s):** none used

3. **Test animals:** Species: Rat
   Strain: HSD:Sprague Dawley
   Age: young adult
   Weight: males: 267-284 g; females: 204-228 g
   Source: Harlan Sprague-Dawley, Inc.,Houston TX
   Acclimation Period: At least five days

Animal Husbandry (scanned from the investigators report, page 7)

Cage Type:	Suspended, wire bottom, stainless steel
Housing:	One per cage
Environmental Controls Set to	Maintain:
	•Temperature Range: 72° ± 5°F
	•Humidity Range: 30-80%
	•12-hour light/dark cycle
	•10-12 air changes/hour
Transfer to Clean Cages:	Weekly
Litter Pan Lining:	Paper and aspen bedding
Litter Pan Lining Change:	Three times weekly
Food:	Purina Formulab Chow #5008, available *ad libitum* except during the exposure period
Water Type:	Municipal water supply from automatic water system, available *ad libitum* except during the exposure period

Animal husbandry and housing at STILLMEADOW, Inc. comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No 86-23, revised 1985). No contaminants were expected to have been present in the feed or water which would have interfered with or affected the results of the study.

B. Study Design

This study was designed to assess the acute inhalation toxicity potential of Trigard 75W when administered as a generated aerosol using a GEM T Trost Air Mill exposing rats in a nose only dynamic flow inhalation chamber.

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1. Procedures:

The following procedures were utilized in the conduct of this study (scanned from pages 8-9 of the investigators report); they met the guideline requirements:

Prestudy Testing

Trial assays were conducted to determine which method(s) of aerosolizing the test material into the exposure chamber would produce an acceptable concentration and mass median aerodynamic diameter (MMAD).

Exposure Chamber

A 500 L nose-only stainless steel, dynamic flow inhalation chamber was utilized in this experiment (Diagram 1). The body of the chamber has 25 ports in 5 rows. Polycarbonate cones are inserted into 10 designated individual ports. The test material is introduced through the opening in the top of the chamber. The bottom section has a corresponding air outlet and a drain valve for cleaning the chamber. The individual polycarbonate cones (tubes) are tapered at one end to fit the shape of the animal's head and the back portion is sealed with a polycarbonate cap. The cones containing the animals fit tightly into the ports, and are sealed with "O" rings.

Generation of Test Atmosphere

The aerosol was generated by a Gem T Trost Air Mill which aspirated the test material from a motorized revolving disc delivery system coupled to the mill, then elutriated the resulting aerosol through a baffling chamber. The concentrated aerosol was then diluted with filtered air and drawn into the exposure chamber. Air flow into the chamber was maintained through the use of a calibrated critical orifice at a rate of 15.5 air changes per hour. Air flow was recorded at 30 minute intervals during the exposure period, and was sufficient to ensure an oxygen content of at least 19% of the exposure atmosphere. Temperature and humidity were recorded at 30 minute intervals during the exposure period from a Taylor wet bulb/dry bulb hygrometer located in the exposure chamber.

Test Material Administration

Healthy albino rats were released from quarantine. Five males and five females per each of two exposure levels were selected for testing. The test material was sifted prior to exposure. The animals were exposed to an aerosol generated from the undiluted test material (fine powder) for a period of four hours. During the exposure period, the animals were individually housed in polycarbonate containers inserted into a 500 L stainless steel nose-only chamber when 99% concentration (T-99) was attained. A maximum of 10 animals were exposed daring any given exposure period. At the termination of the exposure period, the animals were w ash-d and returned to their stock laboratory cages.

Determination of Concentration

The concentration of test material in the exposure atmosphere (taken from the breathing zone of the animals) was determined gravimetrically at least once per hour, and nominally at the end of each exposure. The gravimetric concentration was determined by passing a known volume of exposure air through a pre-weighed filter and dividing the amount of test material deposited on the filter by the volume of air which passed through the filter. The nominal concentration was determined by dividing the loss in weight of the test material after each exposure by the total volume of air which passed through the chamber.

Particle Size Distribution

Particle size, taken from the breathing zone of the animals, was determined twice during each exposure, using an Andersen cascade impactor, at a rate of 28.3 L/minute for a duration of 0.5 minute. The MMAD and particle size distributions are calculated from these data.

In-life Observations

Observations for mortality and signs of pharmacologic and/or toxicologic effects were made frequently on the day of exposure and at least once daily thereafter for 14 days (day of exposure considered Day 0). Individual body weights were recorded just prior to the inhalation exposure, on Day 7 or 8,and Day 14.

Postmortem Observations

At study termination, each animal was euthanized by an injection of Fatal Plus (Vortech Pharmaceuticals, Dearborn, Michigan 48126). All study animals were subjected to gross necropsy and all abnormalities were recorded.

Statistical Analysis

In order to calculate a mean exposure, the Mean Value Theorem of Calculus was used to properly weight the concentration, since the concentrations could not be measured continuously (see Table 5). This method weights concentrations based on the time span of each concentration. A concentration can be calculated for each minute, which better represents the exposure concentration received by each animal.

C. Results:

1. Exposure Conditions:

For the 2.39 mg/L dose group, over the 4 hour exposure period the Mean Temperature was 72°F, the Relative Humidity was 95% and the Air Flow was 153 ppm. For the 2.83 mg/L dose group, the Mean Temperature was 71°F, the Relative Humidity was 95% and the Air Flow was 153 ppm.

For the 2.39 mg/L dose group, over the 4 hour exposure period the Mean Exposure Concentration was 2.39 mg/L and the Nominal Concentration was 12.3 mg/L. For the 2.83 mg/L dose group, the Mean Exposure Concentration was 2.83 mg/L and the Nominal Concentration was 8.79 mg/L.

The Mass Median Aerodynamic Diameter (MMAD) for the 2.39 mg/L (2390 mg/m^3) dose group was 2.894 µm and for the 2.83 mg/L (2830 mg/m^3) dose group was 5.919 µm. **For the 2.83 mg/L dose, the MMAD was rather high (>5.0 µm); however, the lower dose of 2.39 mg/L has an acceptable MMAD.**

2. Mortality/Estimated LC_{50}

No mortality was noted in the study; therefore the LC_{50} is greater than 2.39 mg/L.

3. Body Weights

Two low dose and all high dose females lost weight during the first week of the study (group mean and individual animal data).

4. Clinical Signs

The 2.39 and 2.83 mg/L dose group exhibited fur coated with feces/urine at the 0.5, 1.0, 2.5, 4.5, and 6.0 hour time points with the high dose animals also at the 1 day time point. Both dose groups also exhibited a decrease in activity at the 4.5, 6.0 hour and the 1 day time points in all animals as well as piloerection in nearly all animals at the 4.5, 6.0 hour and 1, 2, and 3 day time points. Ptosis was observed in the low dose group at 4.5, 6.0 hour and 1 day time points and at 4.5 and 6.0 hour time points in the high dose group. The investigators provided group summary and individual animal data.

5. Necropsy Findings

Gross necropsy revealed no treatment related observations (individual animal data were provided).

CYROMAZINE 21-DAY DERMAL TOXICITY-RABBIT

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/6/96
Senior Pharmacologist, Review Section I, TBII (7509C)
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. J M Ioannou 8/8/96
Section Head, Review Section I, TBII (7509C)

DATA EVALUATION RECORD

Study Type: 21-Day Dermal Toxicity
Species: Rabbit Guideline: §82-2

EPA Numbers: EPA MRID# 44060902
EPA Pesticide Chemical Code 121301
Toxicology Chemical No. 167B
EPA DP Barcode D220406
EPA Submission Barcode S495938

Test Material: Cyromazine Technical

Title of Report: CYROMAZINE TECHNICAL
21-DAY DERMAL TOXICITY STUDY IN RABBITS

Sponsor: Agricultural Division, CIBA-GEIGY Corporation, P.O. Box 18300, Greensboro, NC 27419

Testing Facility: Ciba-Geigy Pharmaceuticals Research, Toxicology/Pathology Division, Chemical Evaluation Subdivision, Safety Evaluation Facility, Summit, New Jersey 07901

Study Number: Toxicology/Pathology Report No. 152-84; MIN 842009

Author(s): S. Johnson, M.S., C.N. Tai, M.S., R. Katz, Ph.D.

Report Issued: August 16, 1984

Executive Summary: In a 21-day dermal toxicity study (MRID# 44060902), HAR:PF/CF (NZW) BR Albino Rabbits (5/sex/dose) from H.A.R.E. Rabbits for Research (531 Burnt Meadow Road, Hewitt, N.J. 07421) were exposed to either 0, or 2010 mg/kg/day Cyromazine Technical (96% a.i.).

No treatment related systemic toxicity was noted. **The Systemic Toxicity NOEL is equal to or greater than 2010 mg/kg/day and the Systemic Toxicity LOEL is greater than 2010 mg/kg/day.**

Dermal irritation was slightly increased over control. **The Dermal Toxicity NOEL is less than 2010 mg/kg/day and the Dermal Toxicity LOEL is equal to or less than 2010 mg/kg/day.**

The study is classified as Acceptable and satisfies the guideline requirement (§82-2) for a 21-day dermal toxicity study in rabbits.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, a CERTIFICATION OF GOOD LABORAOTRY PRACTICES statement, and a QUALITY ASSURANCE UNIT STATEMENT was provided.

A. Materials and Methods:

1. Test compound: Cyromazine Technical
Description - white, crystalline solid
Batch FL 821921
Purity - 96% a.i.
Contaminants - none reported

2. Vehicle(s): Purified water USP from
TPSS, CIBA-GEIGY Pharmaceuticals

3. Test animals: Species: Albino Rabbit
Strain: HAR:PF/CF (NZW) BR
Age: not provided
Weight:males:2.73-3.46 kg;females:2.79-3.41 kg
Source: H.A.R.E. Rabbits for Research, 531 Burnt Meadow Road, Hewitt, N.J. 07421

4. Animal Husbandry (scanned from the investigators report, page 12)

Pretreatment: The animals were acclimated to the SEF environment, diet, and handling for 25 days prior to study initiation.

Housing: The animals were housed individually in stainless steel cages (with flat expanded metal flooring) suspended on racks kept in a sanitized room maintained at a mean daily temperature of 65 + 5°F, a relative humidity of 50 + 20%, and having an artificial light cycle of 12 hours.

Diet: Certified Purina Rabbit Chow (#5322), analyzed by the vendor for nutrients and suspected environmental contaminants, was given to the animals ad libitum and in excess. Drinking water was given ad libitum by an automatic delivery system and was monitored periodically for potability and suspected environmental contaminants as outlined in the SOP ' s .

B. Study Design

This study was designed to assess the toxicity potential of Cyromazine Technical when applied dermally for 21 days.

1. Protocol (scanned from pages 13-15 of the investigators report)

EXPERIMENTAL DESIGN: Animal Selection and Distribution: Normal, healthy rabbits that passed a physical examination, including ocular, were distributed into 1 of 2 groups/sex according to randomization numbers generated by the Statistics Department. The females were nulliparous and non-pregnant.

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21-DAY DERMAL TOXICITY-RABBIT

Dosing Regimens:

Group No.	No. of Rabbits	Sex	Rabbit No.	Accession No.	Dose (mg/kg)
1	5	M	1-5	38931-38935	0 (4 ml purified water)
2	5	M	6-10	38936-38940	2010
1	5	F	11-15	38941-38945	0 (4 ml purified water)
2	5	F	16-20	38946-38950	2010

Skin Preparation: At least 24 hours before the first treatment, and as needed thereafter during the study, the dorsal and flank areas of each rabbit were clipped free of hair with an Oster clipper (head type: ANG-RA). The test was done on healthy, intact skin

Test Substance Preparation: Finely powdered Cyromazine was accurately weighed and stored in plastic bottles for handling. Immediately prior to application, Cyromazine was mixed with 4 ml purified water USP to form a thick, smooth slurry.

Test and Control Substance Application: The test substance, as a thick slurry, was applied topically, once daily, to a shaved area no less than 10% of the total body surface area[1], and spread uniformly with a glass rod. The control groups were treated with an equal volume of purified water USP. Calculations[2] of test substance to be applied were based on the predicted mid-week individual body weight. Each test site was covered with a porous gauze dressing which was secured in place with non-irritating tape. A plastic collar was secured around the neck of each rabbit before being returned to individual cages. At the end of each daily exposure, all wrapping materials were removed and the test sites rinsed with purified water USP to remove excess test substance. The test sites were wiped with gauze and each animal returned to its cage.

[1]Meek's equation, Appendix III

[2]Calculations: The total amount of test substance applied was calculated as follows:

$$\text{Total Test Substance (mg)} = \text{Dose (mg/kg)} \times \text{Body Wt. (predicted mid-week) (kg)}$$

Duration of Treatment: The rabbits were exposed to the test or control substance for 6 hours daily for 15 days, excluding weekends (5 consecutive days per week for 3 consecutive weeks).

OBSERVATIONS AND RECORDS:

Physical Examinations: Physical examinations were conducted on all rabbits during the predose period to select normal, healthy animals and weekly thereafter.

Ocular Examinations: Ophthalmologic examinations were conducted on both eyes of each rabbit during the predose period and at study termination.

Dermal Examination and Grading: Dermal reactions were graded according to the classifications given in Appendix II. Evaluations were conducted once daily immediately prior to the application of test substance. A final dermal evaluation was conducted on June 4, 1984.

Clinical Signs: Each animal was monitored daily (at least twice a.m. and p.m.) for appearance, mortality, toxicologic, and/or pharmacologic overt effects. On weekends or holidays observations were made only once daily.

Food Consumption and Body Weights: Both parameters were recorded weekly. Body weights were also recorded at study initiation.

Clinical Laboratory Tests: Non-fasted rabbits were bled from the auricular vessels. Serum was used for biochemistry; blood for hematology was collected with EDTA. The following tests were conducted on all animals during the predose period and at study termination (before the predose tests were performed, a health screen study was conducted in which those tests indicated by an asterisk (*) were performed):

Hematology

RBC Count*
Hematocrit*
Hemoglobin
Red Cell Morphology
Reticulocyte Count
WBC Count*
Differential*
Platelet Count
Clotting Time

Biochemistry

Total Protein*
Albumin*
A/G ratio
Glucose
BUN*
Total Bilirubin
Creatinine*
Phosphorous (Inorg.)
Na^+
K^+
Ca^{++}
Cl^-
SGPT*
γ-GT
SGOT
Alkaline Phosphatase

[the investigators did not measure cholesterol as required for subchronic studies; however, this will not have any impact on the study interpretation]
References for all clinical laboratory tests are presented in Appendix I.

Postmortem Examinations: Necropsies were performed on every animal as scheduled. Detailed gross postmortem examinations were made on the treated and untreated skin, all orifices, external and selected cut surfaces of brain and spinal cord, the thoracic and abdominal viscera, and the cervical tissues and organs. The following minimum list of tissues was harvested by Pathology from each animal and placed in 10% neutral buffered formalin:

All gross lesions
Liver
Skin (treated and reference untreated area)
All tissue masses
Kidneys
Brain

Histopathology: For each animal, histopathological examinations were performed on all tissues listed above.
[the investigators did not histologically examine the following

tissues: Aorta, Salivary glands, Heart, Esophagus, Bone marrow, Stomach, Lymph nodes, Pituitary, Duodenum, Jejunum, Thymus, Ileum, Adrenal gland, Cecum, Colon, Urinary bladder, Rectum, Testes. Parathyroids, Thyroids, Gall bladder, Pancreas, Ovaries, Trachea, Uterus, and Lung; however, this tissues were adequately examined in other subchronic and chronic toxicity studies with Cyromazine and therefore the lack of information here would have little impact on the study]

Organ Weights: The organs listed below were weighed for every animal at scheduled terminal necropsies (paired organs were weighed as pairs).

Pituitary (fixed weight)
Heart
Brain, including brain stem.
Adrenals
Kidneys
Testes/Ovaries
Liver

Statistical Analysis: See Appendix IV [of study report]

Records: All data, records, and a copy of the final report were filed in the SEF Archives, CIBA-GEIGY Pharmaceuticals Research, Summit, N.J. 07901. The following minimum records and specimens were maintained:

- Amendments
- Animal Purchase Records and Accountability
- Body Weights
- Chemical Analyses (water, food)
- Circumstances That May Compromise the Integrity of Data
- Clinical Laboratory Test Results
- Clinical Signs
- Correspondence
- Dermal Examinations
- Environmental Records (temperature/humidity)
- Examinations (physicals/ocular/skin reactions)
- Food Consumption
- Mortality Diary
- Necropsy Records
- Organ Weights
- Protocol
- Records of Animal Transfer to Pathology
- Specimens, Permanent (tissues, slides)
- Test and Control Substance Accountability

C. RESULTS:

1. Observations:

No animals died during the course of the study (the investigators supplied individual animal data). No treatment related clinical observations (other than dermal) were noted in the study (no individual animal data for clinical signs, other than dermal were provided).

2. Dermal Observations:

Slight erythema was observed in control and treated animals with the treated animal slightly greater than control. Mean skin reaction scores were as follows: control males - 0.03; control females - 0.04; dosed males - 0.77; treated females - 0.27.

3. Body weight

The investigators provided group mean and statistical analysis, graphed group mean and individual animal data. No treatment related effects were noted. Weight gains were as follows: control males - 0.28 g; control females - 0.41 g; treated males - 0.31 g; treated females - 0.49 g.

4. Food consumption

The investigators provided group mean and statistical analysis, graphed group mean (growth curves) and individual animal data. No treatment related effects were noted. Mean daily food consumption is as follows: control males - 158.9 g; control females - 167.0 g; treated males - 157.9 g; treated females - 167.4 g.

5. Ophthalmological Examination

No treatment related effects were noted.

6. Hematology and Clinical Analysis

a. Hematology

No treatment related effects were noted (the investigators supplied group summary and statistical analysis and individual animal data).

b. Clinical Chemistry

Treated females has an increase ($p < 0.01$) in total bilirubin when compared to control (control 0.126±0.004, treated 0.158±0.012, no other treatment related effects were noted (the investigators supplied group summary and statistical analysis and individual animal data), the biological relevance of this observation is unknown since no supportive pathology was noted and according to the investigators the absolute value for this parameter was within biologically acceptable limits.

7. Sacrifice and Pathology

a. Organ weight

The investigators supplied group summary and statistical analysis and individual animal data. The following table presents the organ weight data (data from Tables on pages 75-84):

Organ Weight Data

		Males	
Organ		**Control**	**2010 mg/kg/day**
Adrenals	absolute	0.274±0.013	0.318*±0.012(16)[1]
	relative/bw	0.0081±0.000	0.0091±0.000(12)
Kidneys	absolute	19.6±0.33	19.6±0.41
	relative/bw	0.579±0.014	0.562±0.014
Liver	absolute	120.8±6.81	132.8±5.57
	relative/bw	3.590±0.288	3.808±0.107
Pituitary	absolute	0.032±0.004	0.034±0.004
	relative/bw	0.001±0.000	0.001±0.000
Testes	absolute	6.9±0.47	6.8±0.46
	relative/bw	0.206±0.021	0.195±0.014
Heart	absolute	7.1±0.25	7.7*±0.07(8)
	relative/bw	0.208±0.007	0.221±0.004(6)
Brain	absolute	10.0±0.14	9.6±0.22
	relative/bw	0.296±0.013	0.275±0.006

Continued

Organ Weight Data continued

Females

Organ		Control	2010 mg/kg/day
Adrenals	absolute	0.260±0.016	0.198*±0.010(24)
	relative/bw	0.0075±0.001	0.0056*±0.001(25)
Kidneys	absolute	18.2±0.68	20.3±0.96
	relative/bw	0.520±0.018	0.573±0.033
Liver	absolute	103.9±4.77	125.7**±4.34(20)
	relative/bw	2.974±0.149	3.535*±0.120(18)
Pituitary	absolute	0.038±0.002	0.040±0.0002
	relative/bw	0.001±0.000	0.001±0.000
Ovaries	absolute	0.266±0.018	0.336±0.078
	relative/bw	0.008±0.001	0.009±0.002
Heart	absolute	7.8±0.41	7.8±0.34
	relative/bw	0.224±0.012	0.219±0.007
Brain	absolute	9.9±0.27	9.8±0.16
	relative/bw	0.282±0.008	0.276±0.006

* = $p < 0.05$; ** = $p < 0.01$; [1] = percent difference from control

Males had increased absolute but not relative adrenal and heart weights; females had decreased absolute and relative to body weight adrenal weights and increased absolute and relative to body weight liver weights. There was no related pathology noted; therefore the biological relevance of these observations is unknown.

b. Gross Pathology

No treatment related effects were noted (the investigators provided individual animal data).

c. Microscopic Pathology

No treatment related effects were noted (the investigators provided individual animal data).

D. Discussion

No systemic toxicity related to treatment with Cyromazine Technical was noted at the single dose tested of 2010 mg/kg/day. Dermal irritation was slightly increased over control.

CYROMAZINE

21-DAY DERMAL TOXICITY-RABBIT

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/6/96
Senior Pharmacologist, Review Section I, TBII (7509C)
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Yiannakis M. Ioannou 8/8/96
Section Head, Review Section I, TBII (7509C)

DATA EVALUATION RECORD

Study Type: 21-Day Dermal Toxicity
Species: Rabbit Guideline: §82-2

EPA Numbers: EPA MRID# 44060901
EPA Pesticide Chemical Code 121301
Toxicology Chemical No. 167B
EPA DP Barcode D220406
EPA Submission Barcode S495398

Test Material: Cyromazine Technical (CGA 72662)

Title of Report: CYROMAZINE TECH (CGA 72662)
21-DAY DERMAL TOXICITY STUDY IN RABBITS

Sponsor: CIBA-GEIGY Corporation, Agricultural Division, P.O. Box 18300, Greensboro, NC 27419-8300

Testing Facility: STILLMEADOW, Inc., 12852 Park One Drive, Sugar Land, Texas 77478

Study Number: Laboratory Study Number 3805-85

Author(s): Janice O. Kuhn, Ph.D.

Report Issued: May 18, 1992

Executive Summary: In a 21-day dermal toxicity study (MRID# 44060901), New Zealand White Rabbits (5-7 males/dose; 5 females/dose) from Ray Nichols Rabbitry (Lumberton, Texas) were exposed to either 0, 50, 200, or 2000 mg/kg/day Cyromazine Technical (94.6% a.i.; Batch FL 850478).

No treatment related systemic toxicity was noted. **The Systemic Toxicity NOEL is equal to or greater than 2000 mg/kg/day and the Systemic Toxicity LOEL is greater than 2000 mg/kg/day.**

No dermal irritation was noted. **The Dermal Toxicity NOEL is equal to greater than 2000 mg/kg/day and the Dermal Toxicity LOEL is greater than 2000 mg/kg/day.**

The study is classified as Acceptable and satisfies the guideline requirement (§82-2) for a 21-day dermal toxicity study in rabbits.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, statement of CERTIFICATION OF GOOD LABORATORY PRACTICES, STILLMEADOW, Inc. GLP Compliance Statement, an EPA Flagging Statement (the submitter stated that the study neither meets nor exceeds the applicable criteria) and an Affirmation of Quality Assurance was provided.

A. Materials and Methods:

1. **Test compound:** CYROMAZINE TECH (CGA 72662)
   Description - very fine, yellow granules
   Batch FL 850478
   Purity - 94.6% a.i.
   Contaminants - none reported

2. **Vehicle(s):** Deionized water

3. **Test animals:** Species: Rabbit
   Strain: New Zealand White
   Age: young adult
   Weight: males:2.100-3.025 kg;females:2.200-3.025 kg
   Source: Ray Nichols Rabbitry, Lumberton, Texas
   Acclimation Period: 30 days

4. **Animal Husbandry** (scanned from the investigators report, page 12)

   Cage Type: Suspended, wire bottom, galvanized steel
   Housing: One pa cage
   Transfer to Clean Cages: Weekly
   Litter Pan Lining: Paper
   Litter Pan Lining Change: Daily
   Food: Purina Rabbit Chow; available ad libitum
   Water Type: Tap; available ad libitum
   Water System: Automatic
   Light Cycle: 12 hours light, 12 hours dark; 6:30-6:30 PM; controlled automatically
   Air Exchange: 13 changes per hour
   Temperature Mean: 21.7°C
   Temperature Range: 20.6 - 23.3°C
   Relative Humidity Mean: 70.1 %
   Relativity Humidity Range: 57 - 79%

B. Study Design

This study was designed to assess the toxicity potential of Cyromazine Technical when applied dermally for 21 days.

1. Protocol (scanned from pages 12-16 of the investigators report)

Experimental Design
Pretest

Twenty-nine male and thirty female albino rabbits were released from quarantine prior to testing. Appearance and behavior during the acclimation period were factors used to select healthy animals for testing. A complete ophthalmologic examination was conducted on twenty-eight males and twenty-eight females. Body weights and food consumption were recorded for these animals. Hematology and clinical chemistry parameters were also determined for these animals.

On Day 0, after all pretest examinations were completed, each animal was evaluated for inclusion in the study. Twenty males and twenty females were selected for testing. The decision to include was based on body weight, body weight gain, general health, ophthalmologic exam, unhemolyzed blood samples, and hematology and clinical chemistry values.

Randomization

A computer-generated random number list was produced for selection of animals and the first five acceptable (after pretest evaluation) males and females were assigned to Group I, the next five males and females to Group II, etc. Animals were exchanged between the groups in order to obtain similar mean starting weights for all male groups and all female groups.

On Day 0, animals were assigned to each of four test groups (day of initial dosing considered Day 1). There were three treatment groups (50.0 mg/kg, 500.0 mg/kg, and 2000 mg/kg) and a vehicle control group (deionized water). Because of mortality, additional males were added to the study on Day 6; one male and two males were added to Groups II and III, respectively. These additional three animals were treated for three full weeks and were sacrificed five days after the rest of the animals.

Treatment

The animals were prepared prior to the initial treatment by clipping the back of the trunk of each animal free of hair to expose approximately 10% of the total body surface area. Clipping was repeated on Days 3, 7, 10, 14, and 17 within twenty-four hours prior to dosing to ensure intimate contact of the test material with the skin and to facilitate the scoring of dermal reactions.

Animals were treated five times weekly for three weeks. Treatment days were Days 1, 2, 3, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, and 21. The three additional animals were treated on Days 6, 7, 8, 9, 10, 13, 14, 15, 16, 17, 20, 21, 22, 23, and 24. On each treatment day, the back of each animal was moistened with 3.0 ml of deionized water. The appropriate amount of test material was applied evenly over the back of each animal in Test Groups II, III, and IV. A 4 x 8 inch surgical gauze patch (two layers thick) was then placed over the exposure area of each animal and was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was then loosely wrapped with a semi-permeable dressing (orthopedic stockinette). To secure the wrappings in place, the edges of the dressing were wrapped with non-irritating adhesive tape.

Six hours after each treatment, the wrappings, gauze, and tape were removed from each animal and the skin was gently washed with room temperature tap water to remove remaining test material. The animals were dried with a clean cloth and returned to their cages.

Observations

Observations for skin irritation were made immediately prior to each application of the test material. The scoring scale used is presented in Appendix D. Observations for mortality were made twice daily and observations for pharmacologic and/or toxicologic signs were made at least once daily. Individual body weights and food consumption were determined prior to the initial dosing, weekly throughout the study, and at termination of the study or at the time of discovery after death.

Hematology

Parameters for each animal were determined during the pretest week prior to the initial dosing and at termination of the study. The animals were fasted overnight prior to blood collection. Hematology determinations included total and differential leukocyte counts, erythrocyte count, hemoglobin, hematocrit, prothrombin time, platelet count, and reticulocyte count (for Group I and Group IV animals). There were no prestudy hematology data collected from one male from each of Groups II and III. The methods used for hematology determinations are presented in the Table 4 Legend.

Clinical Chemistry

Clinical blood chemistry values were determined prior to the initial dosing and at termination of the study. Parameters measured were fasting glucose, blood urea nitrogen, creatinine, total bilirubin, albumin, total protein, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), serum alkaline phosphatase, gamma-glutamyl transpeptidase, inorganic phosphorus, calcium, chloride, sodium, and potassium. The methods used for these determinations are presented in the Table 5 Legend.

Necropsy

A gross necropsy examination was conducted on each animal at termination of the study or at the time of discovery after death. Organ weights were determined for the liver, kidneys, brain with brainstem, heart, gonads, adrenal glands, and pituitary gland. Prior to being weighed, the organs were carefully trimmed of fat and any contiguous tissue. The following tissues were excised and fixed in 10% neutral buffered formalin for subsequent histopathological examination: two sections of treated skin from the exposure area, a section of untreated skin from the abdominal area, liver, kidneys, lungs with trachea (lungs were inflated with 10% neutral buffered formalin prior to fixation), and all gross lesions (including some apparently normal contiguous tissue).

Histopathology

Histopathologic examinations were performed on sections of treated skin, untreated skin, liver, kidneys, lungs, and on all gross lesions. The tissues were fixed, processed by an Ultratechnicon (Technicon, Inc.), embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin. Two sections were taken from each specimen of treated skin and one section was taken from each specimen of untreated skin; three sections were prepared from the liver, one cross-section was taken from each kidney, and two sections were taken from the lungs.

Statistics

All statistics and calculations were performed by computer with appropriate statistical software.

Hematology values (except for differential leukocyte counts), all clinical chemistry values, and Day 21 body weights were analyzed by Analysis of Variance (ANOVA). Statistical differences found by ANOVA were further analyzed by Dunnett's test and/or Student's t-test. If any parameter for a treatment group was found significantly different from the control for the terminal values, then the corresponding baseline values were also analyzed by the Analysis of Variance. Organ to body weight ratios and differential leukocyte counts were analyzed by the Kruskal-Wallis test (1). Ryan's procedure for the Mann-Whitney U test (1) was used to elicit differences indicated by the Kruskal-Wallis test. All statistical analyses were tested for significance at 0.05 and 0.01 alpha levels. If $p > 0.05$, the difference was judged non-significant.

C. RESULTS:

1. Observations:

Six animals died during the course of the study, 1 control male (day 20), 1 low dose female (day 21), 2 mid dose males (days 4 and 7), and 2 mid dose females (days 7 and 11), the investigators supplied individual animal data. No treatment related clinical observations were noted in the study (group summary and individual animal data for clinical signs were provided). Changes in bowel and bladder function were reported by the investigators for all groups which were attributed to handling, other signs exhibited no dose response.

2. Dermal Observations:

No observable skin irritation was noted.

3. Body weight

The investigators provided group mean and individual animal data. No treatment related effects were noted. Body weight gains (calculated by the reviewer from mean data) for the treatment period (days 0-21) were as follows:

	Control	LDT	MDT	HDT
Males	0.390(14.57) [1]	0.375(14.51)	0.210(8.69)	0.285(11.10)
Females	0.405(15.62)	0.155(5.67)	0.377(14.14)	0.335(13.27)

[1] = % body weight gain

4. Food consumption

The investigators provided group mean and individual animal data. No treatment related effects were noted.

5. Hematology and Clinical Analysis

a. Hematology

No treatment related effects were noted (the investigators supplied group summary and individual animal data).

b. Clinical Chemistry

according to the investigators the absolute value for this parameter was within biologically acceptable limits.

7. Sacrifice and Pathology

a. Organ weight

No treatment related effects were noted (the investigators supplied group summary and individual animal data.

b. Gross Pathology

No treatment related effects were noted (the investigators provided individual animal data).

c. Microscopic Pathology

No treatment related effects were noted (the investigators provided individual animal data); however, there was evidence of a low grade infection or parasite migration in the liver, kidneys, and lungs in animals of all groups. Since there was a lack of toxicity noted, this observation did not affect the outcome of the study.

D. Discussion

No systemic toxicity or dermal irritation related to treatment with Cyromazine Technical was noted at the dose levels tested.